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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:
Blue tit *(Cyanistes caeruleus L.).*
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Left:
Panther (*Puma concolor*).
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JOURNAL OF WILDLIFE REHABILITATION

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Importance of Research and Data Collection

One of the items in this issue of the JWR concerns changes to the U.S. Federal annual report forms. On the surface, you may find this a dry and uninteresting topic, especially if you live outside the United States. However, it brings up some interesting aspects of wildlife rehabilitation that concern all rehabilitators in and out of the United States.

A few questions to consider: What is our place in the larger conservation community? How can we effect change for individuals and species? How can we share our experiences with our colleagues? I posit that one answer to all of these questions is data collection and first-hand observation. Harden and Russell (see p. 29 this issue) state "It is of great value to the [U.S Fish and Wildlife] Service to track diseases that are causing mortality in wild bird populations. The activities of the rehabilitation community allow us to provide important support data for this effort." The benefit goes far beyond the U.S. Fish and Wildlife Service (USFWS); I argue that the USFWS is just a handy place for compiling the data from a certain region, that of the United States. To be truly effective, we must, whenever possible, contribute to studies and databases of wildlife data, regardless of our official reporting requirements. For example, Dr. Jennifer Siembieda (2009) employed data from wildlife centers to look at the occurrence of zoonotic disease, from avian influenza to phocine distemper virus; results from the latter helping to discover the cause of a mass stranding in the late 2000s. West Nile Virus was continuously found to be identified in wildlife rehabilitation centers before other detection methods had results (Nemeth et al. 2007).

Without wildlife rehabilitators working with scientists and sharing this important information, our observations and the impact of animals admitted to wildlife centers will not have a broader impact on conservation activities.

Section E of the new annual report form 3-202-4 requests optional data on confirmed disease and contaminants. As Harden and Russell point out, the confirmation of lead poisoning, West Nile virus, and other diseases and contaminants can exert quite a hardship on a rehabilitator, especially a small home rehabilitator who must rely on outside vets and labs. But confirmation is important for good data and, thus, good research.

How can we as rehabilitators access confirmation without going beyond our meager budgets? I am calling for all rehabilitators to submit the tools they use, be it creating a relationship with a lab, finding low-cost machines, or other creative answers that innovative people have discovered. Please email me at director@theiwrc.org or call me at 866–871-1869 with your thoughts, ideas, and solutions. I will publish them on the IWRC website so we can share our resolutions with each other and increase the data we collect for the good of the species under our care.

Kai Williams
Executive Director, IWRC

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South Mississippi Rescuers Helping Hundreds of Animals After Isaac

BILOXI, Mississippi, USA (September 6, 2012)—Animal rescuers spent several hours recently trying to rescue a young deer from the waters around Cable Bridge in Pass Christian, Mississippi. Flooding from Hurricane Isaac had trapped it there and the current was swift. Teams from two south Mississippi wildlife rescue groups—Wild at Heart Rescue and Wildlife Rehabilitation and Nature Preservation Society—as well as the ASPCA's water rescue team and Harrison County animal control officers, rescued the fawn. It suffered some spinal damage that caused nerve problems in its back leg, but it's being treated and is expected to recover.

The rescue groups along the coast, and the network of veterinarians who work with them, have been saving animals large and small since the storm. The groups have helped hundreds of animals in the days since Isaac including a variety of bird species, opossums, squirrels, deer, and others. The dozens of pelicans rescued have been weather-beaten and exhausted from battling high winds and waters. Many had problems from exposure to the elements. The squirrels have broken legs from trees falling on them. Turtles have been found with a variety of traumas.

Missy Dubuisson, founder and rehabilitator at Wild at Heart Rescue, explained that many of the surviving animals seem to have a remarkable knack for bounding back.

But not all. Wildlife experts say it's hard to know how many pelicans and other birds have died, but there have been reports of many being found on the beaches, along with thousands of dead nutria.

Alison Sharpe, director of Wildlife Care and Rescue Center in Ocean Springs, said her group has come across several birds not typically seen in the area including about four greater shearwaters, which are recovering, and one badly injured Wilson's storm petrel which died shortly after the group received it. There were also several tern species rescued including sandwich, royal, and a couple of least terns.

OBITUARY

***Diana Oakes Conger**, Director of Last Chance Wildlife Center in Thurmont, Maryland, passed away Wednesday 25 July 2012 at home on her beloved mountain, following a lengthy illness. She served the wildlife rehabilitation community for years on a local, state, national, and international level. All who knew her will long remember her gracious, generous, and humble heart.*

PHOTO © EVA MIZER. USED WITH PERMISSION.

Florida Wildlife Officials Prepare to Smooth Path for Panther

TAMPA, Florida, USA (September 6, 2012)—Because biologists say the rebounding Florida panther has filled nearly all of the available habitat in the southwest part of the state, wildlife officials have told their staff to begin working on expanding the population into central Florida.

The first step is a meeting with big landowners and community groups to prepare them for life with the state's biggest predator. Panthers once ranged across the entire Southeast but, since the 1970s, Florida's official state animal has been confined primarily to the wilderness south of the Caloosahatchee River near Fort Myers.

For the past 35 years, the federal plan for saving the panther from extinction has called for creating at least two more panther colonies… somewhere. Possibly even outside the state. All three populations need to have at least 240 panthers to be viable, according to the plan. But no other state that has appropriate panther habitat wants to take the big cats. Federal and state officials had shied away from controversy and relocating the species in other parts of Florida. Now the Florida Fish and Wildlife Conservation Commission is ready to move ahead.

Wildlife Commissioner Liesa Priddy, appointed to the commission by Governor Rick Scott nine months ago, understands what the FFWCC is up against since she and her husband run a cattle ranch near Immokalee that lost at least six calves to panthers this year. The commission is likely to offer various incentives for large landowners to preserve their land as panther habitat and set up a series of steps to deal with potential conflicts.

Rabies Vaccine "Baits" to Target Raccoons

BANGOR, Maine, USA (August 14, 2012)—Northern Maine continues to see fewer cases of wildlife-related rabies than other parts of the state, and health officials want to keep it that way. The U.S. Department of Agriculture's (USDA) Animal and Plant Health Inspection Service (APHIS) is teaming up with the Maine Center for Disease Control and Prevention and the state Agriculture Department to distribute 125,000 oral rabies vaccination baits in northeast Aroostook County.

The distribution area covers 900 square miles, and the program specifically targets raccoons; the fish–meal-coated bait pellets will be dropped by air and from the ground. Rabies is more prevalent in southern portions of the state where, according to Dr. Stephen Sears, state epidemiologist, 60 cases already have been reported this year.

CONTINUED FROM PREVIOUS PAGE

This is the ninth year the USDA has dispensed the oral vaccines, and Sears said this new batch is targeted toward raccoons that may not have eaten any in previous years. Foxes, which are also a rabies vector species, more than likely will consume some of the vaccine as well. Domestic dogs and cats may be attracted to the bait, but this vaccine has been shown to be safe in more than 60 different species, although Sears admits dogs that consume large numbers of baits may experience an upset stomach.

Five-year Action Plan to Reduce Human–Grizzly Conflicts in Banff

BANFF, Alberta, Canada (September 2, 2012)—University of Alberta biologist Colleen Cassady St. Clair and her graduate student, Benjamin Dorsey, are trying to get a feel for the increasingly constrained life of grizzly bears in Banff National Park. To do so, they take off their boots, roll up their pants, and step barefoot onto an electrified mat straddling the Canadian Pacific Railway track, then quickly jump off again as a jolt runs up their legs. The human–wildlife conflict specialist approves—intense but fleeting pain is just what she's after.

Kris McCleary, a science advisor at Parks Canada, says rail tracks are the number-one source of mortality for grizzlies in the park. The bears have become habituated to feeding on grain that spills out of railcars hauling wheat, canola, and lentils from the prairie to the port in Vancouver. Twelve grizzlies have been killed on the tracks in Banff and nearby Yoho National Park since 2000, ten of them since 2005.

Canadian Pacific Railway has spent CA$20 million to reduce grain spillage, including a vacuum truck dispatched to suck up grain spotted on the tracks. Nearly all of the 6,300 leaking grain railcars have been repaired or replaced, cutting spillage by 60–80% since 2008. But with their super-sensitive noses, the bears can smell the grain and will root around to get every kernel. The scarcity of food—mostly berries, roots, and the occasional ground squirrel or elk—makes the grain especially attractive. Banff's grizzlies are "food stressed" and known to have one of the lowest reproduction rates for bears.

Banff's situation is unique, with a small, isolated population living in a landscape visited by three million human tourists a year and crisscrossed by the TransCanada Highway and the Canadian Pacific tracks. Parks Canada has spent tens of millions of dollars in Banff to get wildlife off the highway, installing fencing and building overpasses and underpasses that bears and other species have learned to use to avoid the road. But the fencing has reduced the amount of roadkill available to the bears and, in some cases, the tunnels appear to be funneling bears onto the railway.

In Whistler, British Columbia, Cassady St. Clair and her students used whistles and slingshots to teach bears to avoid people. They've also used aversion conditioning on elk near Edmonton, and now they're hoping to teach Banff's grizzlies to avoid trains [by] using bells, electro-mats, and other behavior modification techniques.

Wyoming Wolves to Lose Endangered Species Act Protection

JACKSON, Wyoming, USA (August 31, 2012)—Wyoming's gray wolves, the last state in the northern Rockies where the animals are federally protected, will lose endangered species status at the end of September, opening them to unregulated killing in most of the state, the U.S. government said. The planned delisting of Wyoming's estimated 350 wolves caps a steady progression of diminishing federal safeguards for a predator once hunted, trapped, and poisoned to the brink of extinction throughout most of the continental U.S.

Texas Landowners, Hunters Face New Restrictions in Effort to Contain Chronic Wasting Disease

AUSTIN, Texas, USA (September 1, 2012)—Thanks to two positive tests from 32 mule deer collected in the desert near New Mexico this past summer, Texas is now officially one of 21 states with a Chronic Wasting Disease (CWD) problem. Texas Parks and Wildlife Department (TPWD) staff have proposed a series of stringent rules for hunters and landowners in the Trans-Pecos and the western Panhandle.

According to Mitch Lockwood, the big game program director who made the rules presentation to the TPW Commission, the quickest way for CWD to move in an area is in a trailer taking deer down the highway. Texas, which allows high-fencing, also allows landowners to capture bucks on their property and put them in pens with a controlled number of does for breeding, and subsequent hunting, purposes. Bucks, does, and fawns are released back onto the ranch once the holding period is finished.

TPWD would establish a Containment Zone in the area where deer with CWD were collected, and hunters in that zone would have mandatory check stations where TPWD personnel can collect samples to be tested for CWD. However, check stations in the high-risk area would be voluntary.

Oiled Birds Found in Louisiana in Isaac's Wake

HOUSTON, Texas, USA (September 4, 2012)—The U.S. Coast Guard and state officials in Louisiana are evaluating the environmental impact of Hurricane Isaac on the area. Wildlife management teams recovered three birds covered in oil and continued to search for other affected wildlife. The teams have investigated about 90 reports of pollution directly linked to the hurricane. ■

Coccidiosis as a Cause of Death in Orphaned American Robins: A Pilot Study in Assessing the Effectiveness of Ponazuril as a Means of Reducing Mortality Rate

Shangzhe Xie and Jennifer Nevis

ABSTRACT: A pilot study was conducted in 2011 to assess the effectiveness of ponazuril as a means of reducing the mortality rate due to *Eimeria* sp. infections in orphaned American robins (*Turdus migratorius*) being rehabilitated for release back into the wild. Eight robins were determined to be eligible for the pilot study. Four robins in Group A received ponazuril orally, one dose of 30 mg/kg for 2 days in a row; this dose was repeated 1 wk later. Robins in Group B received a compounding mix that excluded ponazuril. There was no statistically significant difference between the mean number of days where the fecal coccidia count was less than 100/g of feces ($P = 0.46482$; 10% confidence level) in robins belonging to the ponazuril group when compared to robins belonging to the control group. The mortality rate was 50% for robins in each group. It was concluded that the use of ponazuril at 30 mg/kg, once daily, orally for 2 consecutive days and repeated 7 days later, was not effective in reducing the mortality rate in rehabilitated orphan American robins affected by coccidiosis. Further studies are warranted that investigate the use of ponazuril as an anti-coccidial for avian species.

KEY WORDS: Avian coccidiosis, anti-coccidial drugs, ponazuril, robins.

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Introduction

American robins (*Turdus migratorius*) are a common species presented to Willowbrook Wildlife Center during spring and early summer. They are commonly seen and heard early in the morning in spring and summer and tend to gather in roaming flocks where food is available in the fall and winter (Kaufman 1996). The female does most of the nest-building, incubation, and feeding of the young, and the young leave the nest about 14–16 days after hatching (Kaufman 1996).

PHOTO © SCOTT MEYER (EDGEPLOT@FLICKR.COM). USED WITH PERMISSION.

Most of the robins are presented to Willowbrook Wildlife Center as apparently healthy orphans and are housed in groups according to age. The birds are hand-fed until they are old enough to be moved into bigger cages. However, one of the most common causes of death during this period of rehabilitation at Willowbrook Wildlife Center is coccidiosis.

Coccidiosis is caused by protozoa (eukaryotic organisms) of the phylum Apicomplexa (Page and Haddad 1995). In the infective stages, these single-celled, obligate intracellular parasites enter host cells using a characteristic apical complex of organelles (Page and Haddad 1995). Destruction of the host cells occur after invasion, resulting in a myriad of diseases in avian species (Page and Haddad 1995).

There are several common routes of transmission for coccidia oocysts including dried feces, debris from nests, footwear, and contaminated water (Krautwald-Junghanns *et al.* 2009). Enclosed sporozites are released into the intestinal lumen after the ingestion of coccidial oocysts, where they penetrate epithelial cells lining the small intestinal villi (Krautwald-Junghanns *et al.* 2009).

Traditional methods of control and treatment of coccidiosis include anti-coccidial drugs in water or feed (or both), vaccinations, and prevention of crowding and stress (Yabsley 2008). Vaccination is the least-feasible option because of the high cost of producing such vaccines and the difficulty in vaccinating large numbers of wild birds (Yabsley 2008). Moreover, coccidial vaccines have low efficacy, as they need to include

J. Wildlife Rehab. 32(3): 7–10.

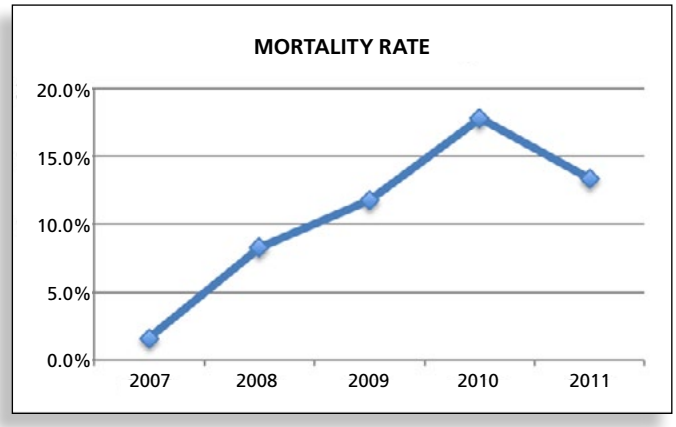

FIGURE 1. Estimated mortality rate, from all causes, of orphaned robins brought to Willowbrook Wildlife Center for rehabilitation from 2007–2011 (2007, *n* = 127; 2008, *n* = 169; 2009, *n* = 170; 2010, *n* = 135; and 2011, *n* = 165).

relevant antigens from different developmental stages (Innes and Vermeulen 2006) because of the highly variable response of the avian immune system to coccidial antigens (Horak *et al.* 2006).

Toltrazuril is a coccidiocidal drug that functions by interfering with nuclear division and mitochondrial activity as well as by damaging wall-forming bodies II formed in the microgamete stage of coccidia (Krautwald-Junghanns *et al.* 2009). Additionally, toltrazuril also causes swelling of coccidial endoplasmic reticulum, resulting in vacuolization in all intracellular developmental stages of coccidia (Krautwald-Junghanns *et al.* 2009). Ponazuril is the metabolic derivative of toltrazuril, which is available in other countries as an anti-coccidial drug.

Several treatment and prophylactic regimes have been tried at Willowbrook Wildlife Center to reduce the mortality induced by coccidiosis, but none of them have been particularly effective. In 2008, oral albendazole was used as a treatment at 55 mg/kg once a day (q.d.) on the first day followed by 25 mg/kg q.d. for the next 9 days. In 2009, amprolium (1 tsp of 96 mg/ml solution in 5 gal of water) and sulfamethazine (2 tbls of 125 mg/ml solution per 1 gal water) in water were used as a preventative measure, but mortality rates remained high and the survivors had to be treated with the same regime of albendazole as in 2008. In 2010, ponazuril was used at 30 mg/kg q.d. for 10 days together with fenbendazole at 50 mg/kg q.d. for 5 days.

The aim of this study was to determine whether a treatment–prophylactic regime using oral ponazuril would be effective in reducing the mortality rate of rehabilitated, orphaned robins at Willowbrook Wildlife Center. It was hypothesized that if ponazuril is useful for controlling coccidial infections in other species of animals, then it could be a useful drug for controlling coccidia infections in wild avian species such as the American robin.

MATERIALS AND METHODS

Candidates for the ponazuril trial were selected from all orphaned robins (*n* = 165) presented to Willowbrook Wildlife Center between June–August 2011 that tested positive for coccidia on a fecal flotation test, the test-of-choice for the diagnosis of coccidiosis (Krautwald-Junghanns *et al.* 2009). Candidates had to be fledglings or older with a body condition score of at least 2, normal plumage and mentation, and eating voluntarily when presented with food before they were considered suitable for admission into the study. A total of eight robins with coccidia infections were determined to be eligible for the study. The intention was that more eligible robins be admitted into a later phase of the study, but a decision was made to discontinue the trial when four of the eight initial candidates still eventually succumbed to coccidiosis, as detailed later in this paper. Eligible candidates were marked with 2 leg bands instead of the Center's normal 1 leg band, housed in individual cages that were cleaned daily, and were randomly allocated into Group A or Group B.

A fecal sample from one robin that was positive for coccidia was sent to Antech Diagnostic Laboratory (Irvine, California, USA) for identification of the coccidia; it was confirmed to be of the genus *Eimeria.* Four robins in Group A received ponazuril compounded in SyrSpend® (Roadrunner Pharmacy, Phoenix, Arizona, USA) orally at a dose of 30 mg/kg q.d. for 2 days in a row and the same dose was repeated 1 wk later. Robins in Group B received the compounding mix SyrSpend (Roadrunner Pharmacy), excluding ponazuril, once daily for 2 days in a row; this was also repeated 1 wk later.

Feces from all robins were collected around 9:00 am and coccidial counts were performed daily. The collection time was kept as consistent as possible to avoid inaccuracies caused by the shedding rhythms of *Eimeria* sp. oocysts (Lopez *et al.* 2007). The initial plan was to count all the coccidia on a slide made by spinning down 1 g of feces and placing a cover slip on the spun-down sample for 5 min before transferring it onto a slide. However, there were samples that had excessive numbers of coccidia that made this method impractical. For these samples, the total count from 10 high-power (×1,000) fields was recorded instead.

The daily coccidia count from every robin in the study was then tabulated and the number of days where the coccidia count was greater than 100/g of feces was counted. The mean number of days where the coccidia count was greater than 100/g of feces was then compared between the control group and the ponazuril group using a Student's *t*-test.

Robins were monitored throughout the trial for appetite and weight gain. Exclusion criteria included presence of signs of poor health. However, all robins in the study remained healthy and within defined weight ranges, and none of them had to be withdrawn from the study. At the end of the study, the robins were placed into communal cages with the general population of orphaned robins being rehabilitated. Two out of four robins in each group died, among other deaths in the general population and, therefore, no further robins were admitted into the study. One robin that died in each group was sent out for necropsy to determine possible causes of death.

All orphaned robins in the Center during 2011, including those in the trial, were eventually treated with ponazuril at 30 mg/kg q.d. and fenbendazole at 50 mg/kg q.d. for 5 consecutive days in an attempt to reduce their overall parasitic load further

before their release back into the wild.

To determine if the different treatment protocols for coccidiosis from 2007 to 2011 made a difference to the survival rate of rehabilitated orphaned robins, the mortality rate of orphaned robins admitted for rehabilitation each year (2007, n = 127; 2008, n = 169; 2009, n = 170; 2010, n = 135; and 2011, n = 165) was also estimated based on the number of robins that were released back into the wild after staying at Willowbrook Wildlife Center for 21 or more days (n = 684) out of the total number of robins that stayed for 21 or more days (n = 766). This precluded birds that died from trauma or other causes (not coccidiosis) shortly after admission.

RESULTS

The four robins in the control group had a coccidia count greater than 100/g of feces on days 8, 10, 4, and 5, respectively, out of the 2 wk, resulting in a mean of 6.75 days and a standard deviation (SD) of 2.75. The four robins in the ponazuril group had a coccidia count greater than 100/g of feces on day 6, 11, 0, and 1, respectively, out of the 2 wk, mean = 4.5 days, SD = 5.07. The mean number of days where the coccidia count was greater than 100/g was not statistically different (P = 0.46482, df 1; 10% confidence level) in robins belonging to the ponazuril group as compared to robins belonging to the control group.

The mortality rate for robins in each group was the same at a 50% confidence level, and necropsy revealed severe intestinal coccidial infection in 2 robins. The robin that died who belonged to the ponazuril group also had a marked multifocal histiocytic hepatitis of unknown cause.

DISCUSSION AND CONCLUSION

Eimeria species are particularly problematic in young birds that are placed under stressful conditions while their immune system is not yet fully developed, and are then exposed to a high inoculation dose of *Eimeria* without having been previously exposed to coccidia (Yabsley 2008). Orphaned American robins being rehabilitated at Willowbrook Wildlife Center fulfill all of these criteria.

There have also been limited studies assessing the efficacy of conveniently available anti-coccidial drugs (Yabsley 2008). Gerhold *et al.* (2011) assessed the efficacy of amprolium, clopidol, diclazuril, decoquinate, lasalocid, monensin, narasin/nicarbazin, robenidine, roxarsone, sulfadimethoxine/ormetoprin, salinomycin, semduramicin, and zoalene against *Eimeria* in northern bobwhites (*Colinus virginianus*). They concluded that clopidol, decoquinate, diclazuril, lasalocid, narasin/nicarbazin, robenidine, sulfadimethoxine/ormetoprin, and zoalene were effective; monensin, salinomycin, semduramicin, or a roxarsone/semduramicin combination were only marginally effective; and amprolium, roxarsone, and zoalene were ineffective at controlling *Eimeria* in bobwhites. These conclusions were made based on the weight gain, gross intestinal lesions, severity of diarrhea, and feed conversion ratio (Gerhold *et al.* 2011). The extrapolation of studies such as Gerhold *et al.* (2011) to *Eimeria* infections in other avian species may be difficult because each species of *Eimeria* varies in susceptibility to these anti-coccidial drugs (Yabsley 2008). This is reflected by the fact that the use of trimethoprim-sulfamethoxazole (same group of drugs as sulfadimethoxine-ormetoprin) and ponazuril (same group of drug as diclazuril) was not effective in reducing the mortality rate in rehabilitated orphaned robins at Willowbrook Wildlife Center, which increased from 1.6% in 2007 to 17.8% in 2010. It is believed that a major portion of this increase in mortality rate can be attributed to coccidiosis because many of the robins had heavy coccidial loads and were in poor body condition prior to death.

In this study, we have attempted to isolate the data from all orphaned robins received by the Center to reflect only the group of robins most affected by coccidiosis. Most robins are admitted as very young birds and appear to be healthy. As stated earlier, when we began the ponzuril trial, 165 orphaned robins had been brought to the Center from June–August 2011. The robins are hand-raised before being introduced into cages of increasing size before they are eventually released into the wild. Some orphaned robins brought for rehabilitation die from other causes such as trauma, severe malnutrition before presentation, and so forth. The exact cause of death can be difficult to ascertain for some robins. The majority have a zero to light coccidial load at the beginning and only really start to experience die-offs when they are introduced to the largest cage right before their anticipated release. Post-mortems on these robins usually reveal nothing except for coccidiosis.

This study has shown that, although the number of days where the coccidia count was greater than 100/g of feces was not different in robins treated with oral ponazuril at 30 mg/kg q.d. for 2 consecutive days and repeated 7 days later than it was in birds receiving the compounding mix, and that the mortality rate remained the same.

The dead robins from each group that were submitted for gross necropsy also had a similar extent of intestinal coccidial infection, and the robin that was in the ponazuril group had significant inflammation in the liver that was not found in the robin from the control group. The intestinal changes were similar with those found in captive Nashville warblers (*Vermivora ruficapilla*), except that the predominant cells within the intestinal walls were lymphocytes (89%) in Nashville warblers (Swayne *et al.* 1991) whereas there was a mix of macrophages and lymphocytes in the American robins we necropsied.

The estimated mortality rate from all causes of rehabilitated orphan robins at Willowbrook Wildlife Center showed a steady increase from 1.6% in 2007, 8.3% in 2008, 11.8% in 2009, to 17.8% in 2010 before decreasing slightly to 13.3% in 2011. This is summarized in Figure 1. We believe that coccidiosis is a major contributing factor to the increase in mortality rate from 2007 to 2011, and the situation could continue to worsen until an effective treatment and preventative regime is put in place.

The conclusion from the results of this study is, therefore, that the use of ponazuril at 30 mg/kg q.d. orally for 2 consecutive days, repeated 7 days later, was not effective in reducing the

mortality rate in rehabilitated orphaned American robins affected by coccidiosis. Future studies to evaluate ponazuril as a means of control of coccidiosis in American robins or other avian species should include pharmacodynamics and pharmacokinetics studies to determine an effective dose rate against the most common individual *Eimeria* species. In the meantime, husbandry changes to reduce crowding, contamination of the environment, and stress appear to confer the best chance of survival for orphaned American robins against coccidiosis at Willowbrook Wildlife Center.

ACKNOWLEDGMENTS

We would like to thank all staff and volunteers, in particular our summer interns Ellen Haynes and Jennifer Fleming Jones, for their help in the care of the robins and the collection of samples and data. We would also like to thank Roadrunner Pharmacy for providing the compounded ponazuril and placebo used in the study.

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Trap–Vaccinate–Release Program to Control Raccoon Rabies, New York, USA

Sally Slavinski, Lee Humberg, Martin Lowney, Richard Simon, Neil Calvanese, Brooke Bregman, Daniel Kass, and William Oleszko

ABSTRACT: In 2009, an outbreak of raccoon rabies in Central Park in New York City [Manhattan], New York, United States infected 133 raccoons. Five persons and 2 dogs were exposed but did not become infected. A trap–vaccinate–release program vaccinated ~500 raccoons and contributed to the end of the epizootic.

KEY WORDS: Epizootic, raccoon rabies, trap–vaccinate–release

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Introduction

Central Park [New York City, New York, United States], described as an oasis in the midst of an urban jungle [Manhattan], spans 843 acres. Raccoons thrive in Central Park, an ideal habitat with an abundance of human refuse as food. Although not actually counted, the estimated raccoon population in the park is ~500. Each year, Central Park receives >25 million visitors, offering ample opportunity for humans and off-leash dogs to be exposed to raccoons.

On 27 August 2009, a sick raccoon collected from Central Park in Manhattan tested positive for rabies virus, marking the emergence of an enzootic of raccoon rabies in Central Park. From December 2009 through December 2011, rabies test results for 133 raccoons collected in or near Central Park were also positive (Fig. 1). The New York City Department of Health and Mental Hygiene (DOHMH) quickly assembled a task force with the objective of developing a response plan. The task force comprised members of the New York City DOHMH, the U.S. Department of Agriculture Wildlife Services, the Central Park Conservancy, the New York City Department of Parks and Recreation, the New York State Department of Health, New York City Animal Care and Control, and the New York State Department of Environmental Conservation. A trap–vaccinate–release (TVR) plan was developed and implemented.

The Program

The TVR program goals were to reduce transmission of rabies among raccoons and prevent human and pet exposure to rabid raccoons. The few examples of raccoon rabies epizootics in similar settings often used a point-infection-control approach: oral rabies

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vaccine, depopulation of up to 80% of the raccoons, and TVR (Brown and Rupprecht 1990; Rosatte, MacDonald *et al.* 2007; Rosatte, Sobey *et al.* 2007; Rosatte, Tinline *et al.*2007, Rosatte *et al.* 2009; Sobey *et al.* 2010).

For the Central Park outbreak, oral rabies vaccine was ruled out because of the small but potential risk for vaccinia infections of humans (Rupprecht *et al.* 2001; CDC 2009), given the large volume of park visitors and poor raccoon seroconversion rates (9%–61%) (Roscoe *et al.* 1998; USDA 2001; Ohio Department of Health 2001; Boulanger *et al.* 2008; Slate *et al.* 2008; Brown *et al.* 2011). Depopulation was also eliminated because it would have overwhelmed the animal shelter system with demand for humane euthanasia and decapitations, and because a national animal welfare organization and the public voiced opposition. Thus, the task force chose TVR.

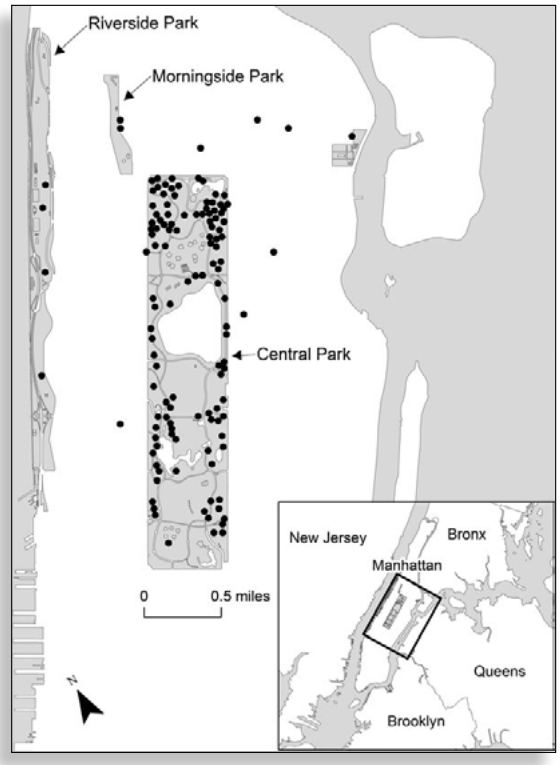


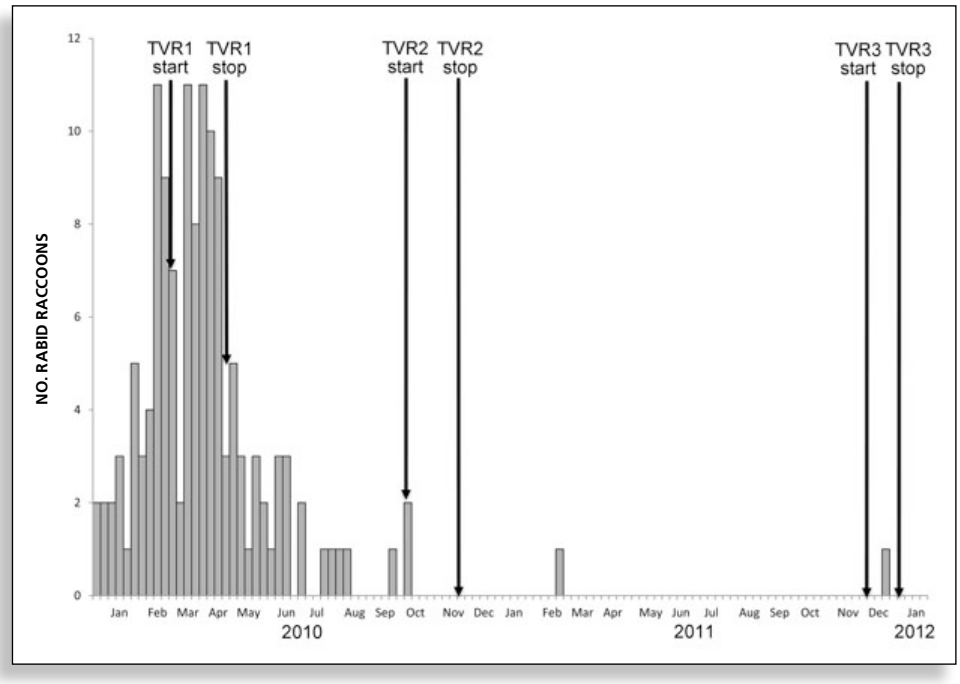
FIGURE 1. Location of rabid raccoons in and around Central Park, New York City, New York, United States, December 1, 2009–December 1, 2011. Each dot represents a rabid raccoon.

The makeup of Central Park and the surrounding Manhattan area creates a fishbowl-style habitat; inside the park are acres of ideal living habitat surrounded by a mass of concrete, roadways, vehicles, and pedestrians which contain the raccoons. Central Park and two small parks in close proximity were targeted for three rounds of TVR. The public was notified through press releases, posters, flyers, electronic messaging, and the New York City 311 telephone information service. Community boards, political leaders, and human and animal health communities were notified directly. The DOHMH website was kept updated.

The three rounds of TVR were conducted: February 16–April 7, 2010, September 20–November 5, 2010, and November 28–December 16, 2011. Trapping efforts were focused in Central Park followed by Morningside and Riverside Parks. Humane cage traps baited with marshmallows and anise oil as a scent attractant were placed in sites that were off limits or of limited access to the public and their dogs. Each trap had a rabies warning sign with emergency contact information.

Trapped raccoons were visually assessed for evidence of injury, illness, or death. Raccoons that were ill or injured were humanely euthanized and, along with those found dead, were submitted for rabies testing at the DOHMH Public Health Laboratory. Healthy raccoons were immobilized in the trap by a squeeze comb, given 1 ml of rabies vaccine in a thigh muscle, identified by placement of an ear tag, and then released at the capture site. Healthy, tagged raccoons that were later recaptured during the same round of TVR were released, but those recaptured during a subsequent TVR round were revaccinated.

FIGURE 2. Number of rabid raccoons, Manhattan, New York, USA, by week, during and after the epizootic in Central Park and the corresponding dates of the 3 rounds of the trap–vaccinate–release program (TVR).



During each round of TVR, 26–73 traps were set per night, resulting in 3,822 trap-nights (Table 1). A total of 1,129 raccoons were trapped (range 0–34/night), of which 484 raccoons were vaccinated and 112 were revaccinated. Among 232 raccoons, there were 586 instances of recapture (median 1, range 1–9).

During round one, of 460 raccoons trapped, 237 were deemed

healthy and were processed and released (Table 1). Of 58 raccoons deemed unhealthy or found dead, 11 were rabies positive; of these 0/6 were dead, 5/8 were sick, and 6/44 were injured. During round two, of 459 raccoons trapped, 148 were newly vaccinated, tagged, and released and 68 were revaccinated. One injured raccoon was euthanized; the rabies test result was negative. None were found dead or sick. During round three, of 210 raccoons trapped, 99 were newly vaccinated, tagged, and released and 44 were revaccinated. One sick raccoon was euthanized; the rabies test result was positive.

TABLE 1. RESULTS OF RACCOON TRAP–VACCINATE–RELEASE PROGRAM, NEW YORK, NEW YORK, USA, 2010.

ROUND	TRAP NIGHTS	NO. TRAPPED	NO. VACCINATED AND RELEASED	NO. RECAPTURED	NO. SICK, INJURED, OR DEAD
1	1,697	460	237	165	58
2	1,409	459*	148	307	1
3	716	210	99	114	1
TOTAL	3,822	1,129	484	586	60

*Three raccoons were submitted for rabies testing after human exposures.

Among the vaccinated raccoons, rabies later developed in 14. The time between vaccination and recapture was 1–26 days (mean 10 days), suggesting that they were probably incubating the virus at the time of vaccination.

Exposures (confirmed or possible raccoon bites, contact with raccoon saliva) were identified for 5 persons and 2 dogs, all during January–June, 2010. Each person received rabies post-exposure prophylaxis. Each dog was currently vaccinated against rabies, received a booster dose, and was monitored. Rabies did not develop in these persons or dogs.

At the peak of the epizootic, 11 rabid raccoons were reported per week. By April, the epizootic started to decline (Fig. 2), probably attributable to the natural depopulation resulting from the rapid spread of the virus and to the population immunity resulting from TVR. The last cases of rabid raccoons were reported on February 2 and December 9, 2011.

Conclusions

TVR seems to have effectively stemmed this epizootic of rabies in an established raccoon population. Critical to its success was the collaboration among federal, state, and local agencies and the private organizations responsible for park stewardship and animal control. This example suggests that a TVR program tailored to the geography, scope, and specifics of an epizootic in an urban area can successfully immunize a large population of raccoons and limit the potential for human and pet exposure to rabies virus.

Ongoing surveillance suggests that raccoon rabies has been successfully controlled in Manhattan. Had TVR not been implemented, the epizootic would probably have reached a state of continuous, low-level enzootic activity. Given the natural border around Manhattan, it is unknown how rabies was initially introduced, but theories include illegal release of a raccoon or a raccoon entry by bridge, tunnel, or even vehicle.

Annual use of TVR is not likely. However, after the immunized raccoon population declines and subsequent generations of susceptible animals predominate, another large epizootic could occur. Given the favorable park environment in which raccoon numbers can grow almost unchecked, population control should be explored as another way to prevent a recurrent epizootic with a similar explosive pattern. Public health and wildlife officials, along with academicians, should continue to explore efforts to develop safe, effective, and acceptable population control measures to help manage the unchecked growth of wildlife supported by urban centers.

Acknowledgments

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Incubation Temperature Affects Growth and Energy Metabolism in Blue Tit Nestlings

Andreas Nord and Jan-Åke Nilsson

ABSTRACT: Because the maintenance of proper developmental temperatures during avian incubation is costly to parents, embryos of many species experience pronounced variation in incubation temperature. However, the effects of such temperature variation on nestling development remain relatively unexplored. To investigate this, we artificially incubated wild blue tit (*Cyanistes caeruleus L.*) clutches at 35.0°, 36.5°, or 38.0°C for two-thirds of the incubation period. We returned clutches to their original nests before hatching and subsequently recorded nestling growth and resting metabolic rate (RMR). The length of the incubation period decreased with temperature, whereas hatching success increased. Nestlings from the lowest incubation temperature group had shorter tarsus lengths at 2 wk of age, but body mass and wing length were not affected by temperature. In addition, nestlings from the lowest temperature group had a significantly higher RMR compared with mid- and high-temperature nestlings, which may partly explain observed size differences between the groups. These findings suggest that nest microclimate can influence nestling phenotype, but whether observed differences carry over to later life-history stages remains unknown.

KEY WORDS: Egg temperature, embryonic development, epigenetic temperature adaptation, growth trajectories, RMR

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Introduction

Avian incubation normally involves a substantial increase in parental effort (Williams 1996; Tinbergen and Williams 2002), which varies depending on the physical attributes of the incubation environment (Williams 1996; Thomson *et al.* 1998). Parents use more energy when incubating enlarged clutches or at low ambient temperatures (Biebach 1979, 1981, 1984; Vleck 1981; Haftorn and Reinertsen 1985; Weathers 1985; de Heij *et al.* 2007), and they invest less energy in incubation when costs are relieved (Bryan and Bryant 1999; Cresswell *et al.* 2004; Pérez *et al.* 2008; Ardia *et al.* 2009; D'Alba *et al.* 2009). Consequently, ambient conditions are predicted to interact with intrinsic properties of the nest or clutch [or both] in determining the amount of energy required for incubation (e.g., Moreno and Sanz 1994; but see Engstrand *et al.* 2002; de Heij *et al.* 2008 for evidence of no such effects).

In its simplest form, incubation is little but a transfer of heat from parents to eggs (Deeming 2008). However, since the maintenance of incubation temperature can be energetically costly (Niizuma et al. 2005; Ardia and Clotfelter 2007; Ardia *et al.* 2009), parents may need to trade off investment in egg heating for self-maintenance. This is sometimes reflected as a reduction in incubation temperature when environmental conditions deteriorate (Haftorn 1983; Nord *et al.* 2010). Such variation in incubation investment may be directly harmful for developing young because high and stable incubation temperatures are prerequisites for normal embryonic growth and maturation (Webb 1987; Nilsson 2006). Yet, surprisingly little is known about how avian development is affected by variation in embryonic environment. The data at hand originate largely from studies on poultry, where low incubation temperatures have long been known to result in abnormal embryonic growth and increased *in ovo* mortality (Lundy 1969). Likewise,

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suboptimal incubation temperatures reduce neonatal body mass and produce chicks with lower weight gain potential in early life (Joseph *et al.* 2006) and may also alter the relative timing of onset of physiological regulatory systems (Black and Burggren 2004a, 2004b). Considerably less is known about temperature effects on embryonic development in nonpoultry species. Periodic cooling of zebra finch (*Taeniopygia guttata*) eggs reduced the efficiency of embryonic tissue synthesis (Olson *et al.* 2006) and also resulted in a decreased embryonic body condition before hatching (Olson *et al.* 2008). These findings are largely corroborated by work on wild species. In wood ducks (*Aix sponsa*), eggs incubated in low temperatures took longer to hatch and produced hatchlings with reduced protein mass (Hepp *et al.* 2006). Similarly, experimentally reduced developmental temperatures in the Australian brush turkey *(Alectura lathami*) prolonged the incubation period but also increased the amount of energy needed for development (Booth 1987), which resulted in lower residual yolk reserves at hatch (Eiby and Booth 2008). However, since no studies on wild species have extended beyond the actual hatching event, it remains unclear whether observed phenotypic consequences of sub-optimal developmental conditions persist into the post-hatching period and even into adult life, thereby potentially impinging on individual fitness.

We experimentally manipulated incubation temperature within the natural range of variation in a free-ranging blue tit (*Cyanistes caeruleus L.*) population in southern Sweden. By applying a novel experimental design where field-collected clutches were artificially incubated in the lab and then returned to their respective nests of origin shortly before hatching, we were able to unambiguously assess whether variation in post-hatching nestling phenotypes are best explained by embryonic developmental conditions or by parental behaviors after hatching. Our overall aim was to quantify the pre- and post-hatching developmental consequences of variation in egg temperature with respect to both longitudinal growth and physiological maturation. In addition, developmental temperature can affect embryonic energy use (Booth 1987; Eiby and Booth 2008) and metabolic rate (Booth 1987; Olson *et al.* 2006). We were thus interested to see whether this effect, if present, persisted during the nestling stage, which could indicate that variation in incubation intensity may permanently modify the metabolic phenotype. This would offer important insights into the causes of inter-individual variation in metabolic rate and its subsequent fitness consequences. We hypothesized that the length of the incubation period and embryonic mortality would vary inversely with egg temperature, and that nestlings hatching from eggs incubated at lower temperatures would be smaller and grow less efficiently than nestlings from higher temperatures. To the best of our knowledge, this is the first attempt to relate a quantitative measure of the embryonic environmental conditions to subsequent post-hatching growth and development in birds.

Materials and Methods

The experiment was conducted in a nest-box breeding population of blue tits from April to June, 2008 to 2010, in the Revinge area, ~20 km east of the city of Lund in south-central Sweden (55°42'N, 13°28'E). The study area, which consists of small deciduous woodlots and groves interspersed among pastures and arable fields, contains ~500 nest boxes scattered over 64 km^2, which have been monitored yearly since 1982.

Egg collection and incubation

We visited nests at least once weekly during nest building and egg laying to determine clutch initiation date; from the 10th egg onward (assuming 1 egg was laid per day), we visited every other day to determine clutch size. Incubation was arbitrarily defined to start at the day of clutch completion (incubation day 0). Two days later (i.e., on incubation day 2), we substituted the entire clutch with warm (~35°C) clay dummy eggs that were similar in size and color to the original eggs. Incubating females were temporarily removed from the nest and were held in the hand while egg substitution proceeded. The whole operation took <1 min to perform, after which females were put back on the eggs. Clutches were uniquely marked with a permanent, nontoxic, felt-tipped pen and transported to a nearby field station (transportation time ≤40 min) for incubation in artificial incubators (Ruvmax, Ödskölt, Sweden). We randomly assigned clutches to one of three incubation temperatures: 1) 35.0°C ("low temperature" $n^{2008} = 19$, $n^{2009} = 19$, $n^{2010} = 20$); 2) 36.5°C ("mid temperature" $n^{2008} = 21$, $n^{2009} = 19$, $n^{2010} = 21$); and 3) 38.0°C ("high temperature" $n^{2008} = 20$, $n^{2009} = 19$, $n^{2010} = 21$). These temperatures are within the natural range of variation of closely related species of similar size for which incubation temperature has been measured in the wild (Haftorn 1988). Clutch size did not differ between treatments (35.0°C: 11.3 ± 0.19 eggs; 36.5°C: 11.1 ± 0.17 eggs; 38.0°C: 11.0 ± 0.16; $P = 0.4$). One incubator was used for each treatment, and we used the same three incubators during the course of the study. We changed the incubator–treatment combination between years so that treatment was not replicated within an incubator. The incubators were kept indoors in a completely dark room with stable temperature. We monitored relative humidity (RH) inside the incubators using a standard hygrometer (Clas Ohlson, Insjön, Sweden) and maintained RH at a constant level of 70% throughout all treatments.

We installed incubators approximately 1 wk before collection of the first clutch for temperature calibration. We measured temperature at the position of the eggs in 24-hr cycles using a small temperature data logger (iButton DS1922-L, Maxim Integrated Products, Sunnyvale, California, USA; accuracy, ± 0.5°C) with a sampling interval of 1 min and a resolution of 0.0625°C. We then calculated the temperature difference between the temperature logger and the desired treatment temperature (to the closest 0.1°C) and adjusted the incubator settings accordingly until no temperature deviance was recorded. The data loggers were also left in place when egg collection had begun, and temperatures were evaluated once daily and adjusted when necessary. However, temperature remained relatively constant after the initial calibration (mean temperature deviance ± SE, for 35°C: 0.020 ± 0.017°C;

for 36.5°C: 0.030 ± 0.017°C; for 38.0°C: 0.030 ± 0.018°C), thus necessitating only minor adjustments.

On day 10 of incubation, clutches were transferred back to their original nests. In cases where nests had been deserted or predated during the incubation period (in 2008, 5 nests; in 2009, 14 nests; in 2010, 12 nests), eggs were fostered to a replacement nest at the same stage (±1 day) and with the same clutch size (±2 eggs) as the original nest.

Sampling of nestlings and adults

Starting on day 11, we checked nests daily for hatched eggs. On nestling day 2 (day of hatching = 0), we measured brood mass (to the closest 0.1 g) using a Pesola spring scale (Pesola, Baar, Switzerland). We returned to record nestling body mass and tarsus length (to the closest 0.1 mm) on day 6. We also banded nestlings with a uniquely numbered aluminum ring and collected any unhatched eggs. On day 14 we measured nestling mass (g) and tarsus and wing length (to the closest 0.1 and 0.5 mm, respectively). Throughout the study, all measurements on nestlings were made by the same person.

To subsequently monitor nest provisioning, in 2009 and 2010 we caught, measured (mass, tarsus, and wing lengths), and equipped parents with a unique passive integrated transponder (PIT) tag glued to two plastic rings on day 6. All provisioning parents were caught in 2009, but two nests were left undisturbed in 2010 because of inclement weather. In all but four cases (two in each year), parents resumed normal provisioning behaviors immediately after capture. On day 8 we attached a circular antenna connected to a data logger (Trovan, AEG ID, Ulm, Germany) powered by a 12-V 72-Ah marine battery (Biltema, Helsingborg, Sweden) around the nest-box entrance hole. The logger automatically stored the unique PIT tag number together with the time of entry each time a parent entered the nest until day 10, when the measuring period was finished and all equipment was removed.

Metabolic measurements

We measured nestling resting metabolic rate (RMR) by means of flow-through respirometry during the night between days 14 and 15. Between one and four nestlings (depending on the number of synchronous nests [for 38.0°C, 62 nestlings from 34 broods; for 36.5°C, 67 nestlings from 37 broods; for 35.0°C, 69 nestlings from 37 broods]) were randomly collected from their nest box after 2,000 hours. Parental nest provisioning is negligible after this time (A. Nord, pers. obs.). Nestlings were weighed and placed singly into a 0.6-L sealed metabolic chamber and placed in a dark, temperature-controlled cabinet (Heraeus Vötsch BK600, Vötsch Industrietechnik, Balingen, Germany) at 25°C, that is, within their thermo-neutral zone (Gavrilov and Dolnik 1985). A measurement session ended between 0600 and 0700 hr the ensuing morning, at which point nestlings were weighed and transported back to their original nests. We used the nestlings' morning mass values in all analyses, as these were gathered closest to the time when actual RMR was recorded (A. Nord, pers. obs.).

The respirometer consisted of one block with eight parallel channels with identical setups, one of which was left empty for baselining. We were thus able to measure seven birds per night. Each chamber was connected to a PP2 bench pump (type UNMP830 KVDC-B; Sable Systems International, Las Vegas, Nevada, USA) that was positioned downstream of the birds and consequently pulled air from the chambers. Channels were measured sequentially for 13 min each and were separated temporally by 2 min of flushing, during which time no data were collected. A baseline was recorded at the start and the end of each measurement cycle. Switching was maintained by a RM8 Intelligent Multiplexer (Sable Systems).

Oxygen and carbon dioxide concentrations of effluent sample air scrubbed on Drierite (W. A. Hammond Drierite Company, Xenia, Ohio, USA) were analyzed sequentially by a CA-10A carbon dioxide analyzer (Sable Systems) and an FC-10A oxygen analyzer (Sable Systems) and automatically registered on a computer connected to the machinery via a UI2 Data Acquisition Interface (Sable Systems) every second during a measurement cycle. Oxygen concentration was calibrated against outside air to 20.95% O_2 before each measurement series. Flow rate was set at 166 ml min^{-1} (10 L hr^{-1}) and was recorded continuously by a FlowBar8 Multichannel Mass Flow Meter (Sable Systems). It should be noted that the system did not have a loop that regulated flow, and this caused a slight drift in flow rate with as night progressed (≤10 ml min, ≤1 during a full measurement series). However, this variation did not affect the estimated metabolic rates, as the close monitoring of flow rate allowed calculations to be made on the actual flow at the time of sampling. Calculations of oxygen consumption were performed using ExpeData 1.1.9 for Windows. We checked all channels manually for drift before analyses were performed, and this confirmed that the oxygen consumption was stable for the full length of the cycle in all cases. Oxygen consumption (ml O_2 min^{-1}) was defined as the difference in oxygen concentration between effluent sample air and reference air from the empty metabolic chamber according to equation C in an article by Hill (1972). Because of the possible malfunction of the carbon dioxide analyzer, we conservatively considered the fraction of CO_2 in reference air to be 0.0005 during all calculations, which is a reasonable assumption for indoor conditions (Lighton 2008). The value of oxygen consumption used in analyses was taken as the single lowest value from 7-min running averages for a full measurement session. Oxygen consumption was converted to metabolic rates assuming an energy equivalence of 20 J $(ml\ O_2)^{-1}$.

Statistical analyses

All statistical tests were made using R, version 2.12.0 for Windows [The R Project for Statistical Computing]. Broods that were predated, deserted, or provisioned by one parent only (as determined by PIT tag records and repeated observations at the nest) were excluded from the data set. Because no adults bred in experimental nests in more than 1 yr, and because only 13 nest boxes were included more than once during the study, we did not account for

potential variance inflation due to nest-box identity in the analyses. The length of the incubation period (from onset to the first signs of hatching) and hatching success (the proportion of the clutch that had hatched by nestling day 6) were analyzed in identical linear models, with treatment and year as factors and clutch size as a covariate. Data for hatching success were arcsine–square-root-transformed before analyses to meet assumptions of normality (Sokal and Rohlf 1995). We included data from 60 (20 in each year) unmanipulated nests (hereafter referred to as control nests) as a reference point in models for length of the incubation period and hatching success. Thus, the treatment factor had four levels in these models as compared with three in all other analyses. Mean nestling mass (m_{brood}/n_{brood}) on day 2 was analyzed with a general linear model with experimental treatment and year as factors and laying date (i.e., the day on which the first egg was laid) and laying date-2 as covariates. We analyzed variation in nestling biometrics (mass, and tarsus and wing lengths) on days 6 and 14, respectively, using linear mixed-effects models fitted with restricted maximum-likelihood methods (using the lme function in the nlme package), with treatment and year as fixed factors, laying date and laying date-2 as covariates, and nesting attempt (defined as the specific nest by year combination) as a random factor. Nestling RMR was analyzed in a linear mixed model with the main effects of treatment and year as fixed effects and mass, laying date, and laying date-2 as covariates. Nesting attempt and respirometer channel were included as random effects. We analyzed variation in nest provisioning rates, in terms of feedings per nestling and unit time for each parent, using a linear mixed model with treatment, year, and sex as fixed factors, laying date and laying date-2 as covariates, and nesting attempt as a random factor. The full model also included the two-way interactions between the experimental treatment and sex. Models were reduced by backward elimination of nonsignificant terms ($P > 0.05$; Seber and Lee 2003) until only significant variables remained. Differences between groups were compared following the Tukey method, with *P*-values adjusted for unbalanced multiple comparisons, using the glht function in the multcomp package. Significances of random factors were assessed by comparing the restricted log-likelihood ratio of the reduced and saturated models to a ×2 distribution with one degree of freedom (Sokal and Rohlf 1995). All means are reported with their standard errors, and all significances except for the restricted log-likelihood ratio tests are two-tailed. For simplicity, only final models are presented in Results.

Results

Incubation period and hatching success

Experimental manipulation of incubation temperature affected the length of the incubation period in the predicted direction (Table 1; Fig. 1). Low-temperature clutches required 1.7 and 1.2 more days to hatch than did high- and mid-temperature clutches, respectively ($P < 0.001$ in both cases), and high-temperature clutches hatched about 0.6 days earlier than did mid-temperature clutches ($P = 0.023$). In addition, the high- and mid-temperature

TABLE 1: TEST STATISTICS, DEGREES OF FREEDOM, AND THE CORRESPONDING *P* VALUES DERIVED FROM MARGINAL ANOVA TABLES FOR FINAL MODELS, AND PARAMETER ESTIMATES FOR SIGNIFICANT TERMS (*P*<.05)

PARAMETER	ESTIMATE (SE)	DF	*F* OR Λ[b]	*P*
INCUBATION PERIOD				
TREATMENT:				
CONTROL [AB]	12.95 (.16)	3, 207	25.28	<.001
38.0°C [A]	12.52 (.12)			
36.5°C [B]	13.13 (.15)			
35.0°C [C]	14.30 (.16)			
YEAR:				
2008 [A]	12.91 (.13)	2, 207	8.89	<.001
2009 [A]	13.07 (.16)			
2010 [B]	13.58 (.15)			
HATCHING SUCCESS:				
TREATMENT:				
CONTROL [A]	1.30 (.035)	3, 210	30.74	<.001
38.0°C [A]	1.30 (.035)			
36.5°C [B]	1.11 (.037)			
35.0°C [C]	.75 (.045)			
MEAN MASS, DAY 2:				
YEAR:				
2008 [A]	1.98 (.048)	2, 147	7.16	.0011
2009 [A]	1.98 (.036)			
2010 [B]	1.78 (.042)			
MEAN MASS, DAY 6:				
YEAR:				
2008 [A]	6.00 (.095)	2, 136	9.49	<.001
2009 [A]	5.87 (.10)			
2010 [B]	5.40 (.10)			
LAYING DATE	.053 (.014)	1, 136	12.98	<.001
NESTING ATTEMPT (RANDOM)		1	120.63	<.001
MEAN MASS, DAY 6\14:				
YEAR:				
2008 [A]	11.60 (.099)	2, 111	10.92	<.001
2009 [A]	11.84 (.11)			
2010 [B]	11.13 (.11)			
LAYING DATE	.065 (.0196)	1, 110	16.13	<.001
NESTING ATTEMPT (RANDOM)		1	281.50	<.001
TARSUS LENGTH, DAY 6:				
LAYING DATE	.056 (.016)	1, 138	11.38	<.001
NESTING ATTEMPT (RANDOM)		1	174.79	<.001
TARSUS LENGTH, DAY 14:				
TREATMENT:				
38.0°C [A]	18.58 (.071)	2, 110	11.15	<.001
36.5°C [B]	18.41 (.070)			
35.0°C [C]	18.10 (.074)			
LAYING DATE	.032 (.010)	1, 110	9.62	.0076
NESTING ATTEMPT (RANDOM)		1	193.01	<.001
WING LENGTH, DAY 14:				
YEAR:				
2008 [A]	42.83 (.29)	2, 110	1,278	<.001
2009 [A]	42.34 (.33)			
2010 [B]	40.42 (.32)			
LAYING DATE	.17 (.048)	1, 110	16.63	<.001
NESTING ATTEMPT (RANDOM)		1	121.88	<.001
RESTING METABOLIC RATE:				
TREATMENT:				
38.0°C [A]	32.023 (1.38)	2, 194	5.65	.0041
36.5°C [B]	32.30 (1.37)			
35.0°C [C]	34.62 (1.37)			
MASS	2.00 (.36)	1, 194	30.59	<.001
NESTING ATTEMPT (RANDOM)		1	119.16	<.001
RESPIROMETER CHANNEL (RANDOM)		1	45.40	<.001
NESTLING PROVISIONING:				
YEAR:				
2009 [A]	1.62 (.070)	1, 71	7.95	.0080
2010 [B]	1.91 (.075)			
(LAYING DATE)2	-.0080 (.00029)	1, 71	7.97	.0080
NESTING ATTEMPT (RANDOM)		1	1.28	.26

TABLE 1: (CONTINUED)

PARAMETER	ESTIMATE (SE)	DF	*F* OR Λ[b]	*P*
RESTING METABOLIC RATE:				
TREATMENT:				
38.0°C [A]	32.023 (1.38)	2, 194	5.65	.0041
36.5°C [B]	32.30 (1.37)			
35.0°C [C]	34.62 (1.37)			
MASS	2.00 (.36)	1, 194	30.59	<.001
NESTING ATTEMPT (RANDOM)		1	119.16	<.001
RESPIROMETER CHANNEL (RANDOM)		1	45.40	<.001
NESTLING PROVISIONING:				
YEAR:				
2009 [A]	1.62 (.070)	1, 71	7.95	.0080
2010 [B]	1.91 (.075)			
LAYING DATE–2	-.0080 (.00029)	1, 71	7.97	.0080
NESTING ATTEMPT (RANDOM)		1	1.28	.26

[a]Note: For factors, estimates represent the fitted values (and their SEs) corrected for variation in the random factor (when applicable), with covariates fixed at their respective mean values. For continuous variables, estimates are the slope of the regression between the dependent variable and the covariate, with SE for the fit of the regression. Nonsignificant terms were removed from the original models by backward elimination as described in the main text. Shared letters within brackets indicate nonsignificant (P>.05) differences between treatment categories and years, respectively.

[b]For fixed effects, the test statistic is F; for random effects it is Λ.

clutches hatched as fast as the unmanipulated nests in the field (i.e., control nests), but the low-temperature nests lagged behind the controls by 1.3 days (P < 0.001). The incubation period also varied between study years, with nestlings hatching later in 2010 compared with both previous years (Table 1).

Patterns in hatching success followed those for incubation period. Hatching success was reduced by 30.7% and 29.2% in the low-temperature group compared with clutches exposed to the high- and mid-temperature incubations (P < 0.001 in both cases; Table 1, Fig. 2), but there was no difference in hatching success between groups exposed to the two highest temperatures. Clutches in control nests hatched significantly better than did those in all other treatments (38.0°C: 8.2%, P = 0.014; 36.5°C: 9.5%, P = 0.0043; 35.0°C: 41.3%, P < 0.001). However, the difference in absolute values between the controls and the mid- and high-temperature groups, respectively, was relatively small (Fig. 2).

Nestling morphology

The experimental treatment did not have a significant effect on nestling mass at any of the sampling occasions (days 2, 6, and 14; Table 1). However, nestlings were significantly heavier at all ages in the first 2 yr of the study (Table 1), and mass at days 6 and 14 also increased slightly with laying date (Table 1).

Tarsus length did not differ between temperature treatments at day 6; however, the experimental treatment significantly affected tarsus length at day 14 in the predicted direction (Table 1; Fig. 3). Specifically, mean tarsus length in chicks from the low-temperature group was reduced by 0.55 and 0.36 mm compared with that of high-temperature (P < 0.001) and mid-temperature chicks (P = 0.0020), respectively. Tarsus length did not differ

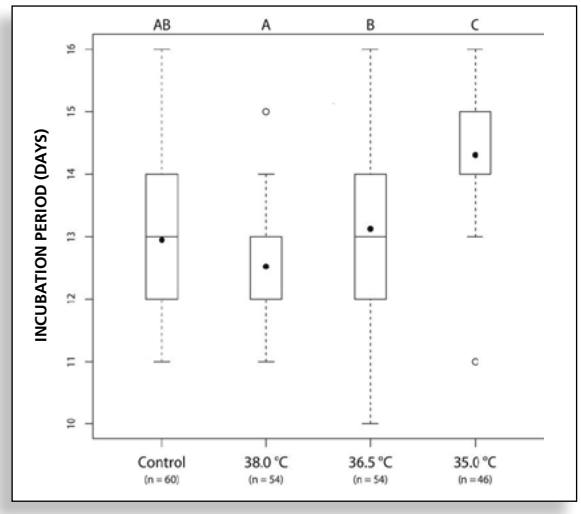

FIGURE 1. Length of the incubation period by experimental treatment for blue tit clutches exposed to different temperatures during artificial incubation in laboratory conditions. Clutches were returned to their original nests shortly before hatching. "Control" refers to randomly selected, unmanipulated nests in the wild. Boxes show medians and first and third quartiles. Whiskers extend to the last observations within 1.5 times the interquartile range (IQR). Open circles denote observations outside 1.5 times the IQR; filled circles within boxes show means. N values correspond to the number of broods. Shared letters above the boxes indicate nonsignificant differences (P > 0.05) between treatment categories.

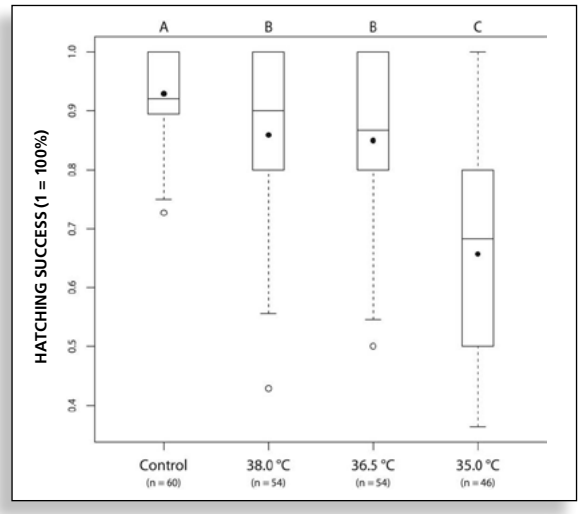

FIGURE 2. Hatching success (hatched divided by unhatched eggs) by experimental treatment for blue tit clutches that were incubated in laboratory conditions and subsequently returned to their original nests shortly before hatching. "Control" refers to randomly selected, unmanipulated nests in the wild. Calculations were performed on arcsine–square-root-transformed proportions, but untransformed values are illustrated for simplicity. Boxes show medians and first and third quartiles. Whiskers extend to the last observations within 1.5 times the interquartile range (IQR). Open circles denote observations outside 1.5 times the IQR. Filled circles within boxes show means. N values correspond to the number of broods. Shared letters above the boxes indicate nonsignificant differences (P > 0.05) between treatment categories.

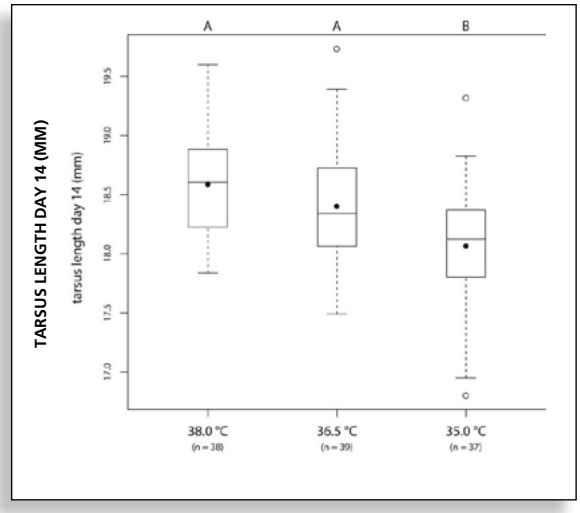

FIGURE 3. Tarsus length at 2 wk of age by experimental treatment for blue tit nestlings originating from clutches incubated in different temperatures. Illustrations are based on mean brood values. Boxes show medians and first and third quartiles. Whiskers extend to the last observations within 1.5 times the interquartile range (IQR). Open circles denote observations outside 1.5 times the IQR; filled circles within boxes show means. N values correspond to number of broods. Shared letters above the boxes indicate non-significant differences ($P > 0.05$) between treatment categories.

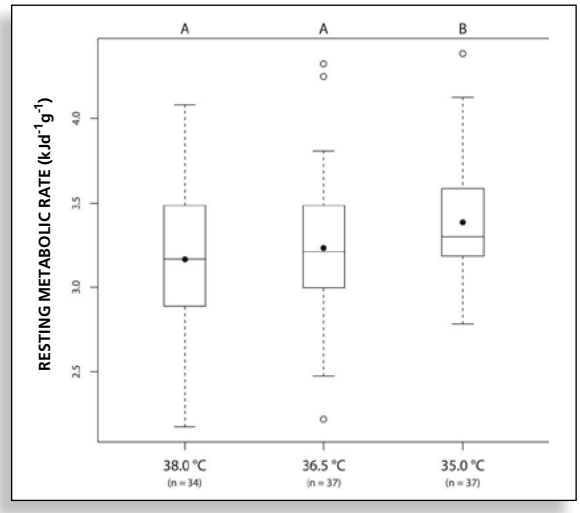

FIGURE 4. Mass-specific resting metabolic rate, measured at night by flow-through respirometry, of 14-day-old blue tit nestlings originating from clutches exposed to different incubation temperatures. Illustrations are based on mean brood values. Boxes show medians and first and third quartiles. Whiskers extend to the last observations within 1.5 times the interquartile range (IQR). Open circles denote observations outside 1.5 times the IQR; filled circles within boxes show means. N values correspond to number of broods. Shared letters above the boxes indicate nonsignificant differences ($P > 0.05$) between treatment categories.

between the latter treatments. In contrast, wing length at the same age was not affected by the experimental treatment but followed similar patterns as nestling mass. Thus, when controlling for the effect of laying date, wings were shorter in 2010 than in both of the previous years (Table 1).

Resting metabolic rate

Variation in incubation temperature significantly affected nestling RMR at 2 wk of age (i.e., 16–18 days after manipulation; Table 1, Fig. 4). When controlling for mass, chicks from the lowest temperature group experienced an increase in RMR of 8.1% ($P = 0.0069$) and 7.5% ($P = 0.016$) over that of high- and mid-temperature chicks, respectively. However, as above, we could not detect any differences between the two higher temperatures.

Nest provisioning rate

All nests, regardless of their respective temperature treatments, were provisioned at the same rate in both years. However, nest provisioning across treatments was higher in 2010 than in 2009 and it also increased slightly with laying date-2 (Table 1).

Discussion

By manipulating incubation temperature within the natural range of variation, we have shown that early developmental conditions affected both pre- and post-natal growth and physiology in blue tits. This was manifested as a prolonged incubation period and lower hatching success at low incubation temperatures, and as reduced nestling growth and increased RMR in broods from low incubation temperatures (i.e., 35.0°C), compared with nestlings from both the mid- (i.e., 36.5°C) and the high-temperature (i.e., 38.0°C) incubation groups. These observations corroborate recent experimental findings that support a potentially causal role of incubation temperature in determining nestling condition (Reid *et al.* 2002; Ardia and Clotfelter 2007; Nilsson *et al.* 2008; Pérez *et al.* 2008; Ardia *et al.* 2010). Some results also suggest that parents on the nest, via their direct influence on incubation temperature (Ardia and Clotfelter 2007; Ardia *et al.* 2009; Nord *et al.* 2010), can influence neonatal performance by altering embryonic investment. This is paralleled in some reptiles, which by nest site selection can alter the thermal environment of embryos with carryover effects on the hatchling phenotype (e.g., Blouin-Demers *et al.* 2004). At this point it should be noted that our experiment differs from natural incubation because constant incubation temperatures generally do not occur in nature (Deeming 2002), and it is likely that temperature variation, as such, may have developmental consequences. Nonetheless, our results are qualitatively similar to those of previous field studies (see above), and we feel confident that this work adequately reflects some of the possible developmental consequences of a suboptimal embryonic environment.

Effects of egg temperature on incubation period and hatching success

We found that incubation period was shorter for clutches incubated at higher temperatures. Evidence for a direct effect

of incubation temperature on incubation period from natural populations is scarce and largely restricted to correlative studies. For example, wood duck eggs naturally incubated at higher temperatures hatched faster (Hepp*et al.* 2006), and biparentally incubated starling (*Sturnus vulgaris*) clutches spent more time at higher temperatures and had shorter incubation periods than did clutches in which the female incubated alone (Reid *et al.* 2002). Similarly, Martin (2002) showed that incubation periods vary predictably with embryonic temperatures across species (but see Tieleman *et al.* 2004 for evidence of no such effects). Lengthy incubation periods can be ecologically costly in terms of an increased predation risk with age of the nest (Tombre and Erikstad 1996; Remes and Martin 2002). Such risks might be further exacerbated by physiological costs because the amount of energy required for embryonic maintenance processes increases rapidly with time in the egg (Booth 1987; Booth and Jones 2002). As a result, the residual yolk mass at hatching is often reduced in chicks that have experienced suboptimal embryonic conditions (Olson et al. 2006; Eiby and Booth 2009). The maintenance of proper incubation temperatures is, thus, presumably adaptive since this generally appears to decrease the incubation period.

Apart from requiring a longer time to hatch, eggs incubated at low temperatures showed a higher incidence of embryonic mortality. It is possible that the sustained hypometabolism at low incubation temperatures (Vleck and Vleck 1996) reduced the efficiency of nutrient uptake (cf. Feast *et al.* 1998; Olson *et al.* 2006), thereby resulting in a chronic nutritional stress that in the end may have been incompatible with embryonic survival. However, since exposure to low incubation temperatures can obstruct the development of muscle tissue (including that of the hatching muscle; Olson *et al.* 2008), it is perhaps also possible that embryos exposed to the lowest incubation temperature were physically incapable of hatching.

Our results indicate that the effect of incubation temperature on embryonic mortality was not necessarily linear because hatching success did not differ between high- and mid-temperature clutches (see also Eiby and Booth 2009). It thus seems plausible that embryonic development was relatively robust to temperature deviation within a given interval, delimited at the lower end by a threshold temperature of between 36.5° and 35.0°C. Sustained exposure to temperatures below this threshold seems to adversely affect embryonic development. High incubation temperatures may be equally detrimental for embryonic survival (Strausberger 1998; Moraes *et al.* 2004), but because we did not sample above 38.0°C (where embryonic survival was not negatively affected), we do not have enough information to speculate about the upper limit of temperature tolerance of blue tit embryos.

Effects of egg temperature on nestling morphology

Contrary to the results of previous studies (Hepp *et al.* 2006; Mortola 2006), we found no effect of incubation temperature on nestling body mass shortly after hatching (i.e., when nestlings were 2 days old). Because hatching seems to occur at a relative rather than an absolute age (Black and Burggren 2004a), this could potentially be explained if the effect of developmental temperature on embryonic growth delayed physical maturation (thereby extending the incubation period) without affecting neonate size or mass. However, periodic cooling of zebra finch eggs has previously been shown to reduce yolk assimilation efficiency (Olson *et al.* 2006), which suggests that this might not be the case. Because we did not assess nestling body composition, it is therefore possible that differences in residual yolk mass (e.g., Eiby and Booth 2009) or protein content (e.g., Hepp *et al.* 2006) at hatching could explain the absence of temperature effects on nestling mass. It should also be mentioned that, because of the rather imprecise measurements of nestling mass at day 2 (see Material and Methods), we might have been unable to detect phenotypic differences between treatments.

Nestlings from the different incubation temperatures did not differ in any of the biometric measurements at 6 days of age (although treatment means varied in the predicted direction). However, nestlings from the lowest incubation temperature were structurally smaller than mid- and high-temperature nestlings at 2 wk of age. Low temperature has been shown to reduce limb growth by constraining the efficiency of cartilage proliferation in laboratory reared mice (Serrat *et al.* 2008). Similarly, Hammond *et al.* (2007) showed that appendage growth *in ovo* was positively affected by high incubation temperatures in domestic fowl, and they attributed this to the higher levels of embryonic activity at high temperatures. However, it is unlikely that the difference in structural size that we observed in this study can be explained solely by temperature-related constraints on prenatal longitudinal growth because differences between treatments were not present when nestlings were 6 days old. This suggests that the main differences between treatments became apparent during the period of peak nestling growth rather than during embryonic and early postnatal development. Therefore, it seems likely that incubation temperature constrained nestling growth trajectories indirectly by affecting intrinsic physiological properties such as energy turnover rates. In line with this, we found that incubating blue tit eggs in suboptimal temperatures produced nestlings with higher RMR. As a result, low-temperature nestlings most likely had to trade off maintenance for growth to a larger extent than did nestlings from mid- and high temperatures, thereby explaining the observed reduction in structural size.

Despite the fact that low-temperature nestlings had shorter tarsi, the wing length and mass did not differ between treatments. This observation suggests that low-temperature nestlings traded off energy allocation to different growth compartments by investing more resources in body mass and feather growth. Predation risk is a major driving force in avian life-history evolution (Martin 1995) and, although it is sometimes dependent on nest site characteristics (Martin *et al.* 2000), we would expect predation risk to increase with nest age (Remes and Martin 2002). It is therefore possible that the reduced tarsus length in low-temperature nestlings can be explained if these nestlings traded off structural size for plumage

development (reflected as a higher wing-length to tarsus-length ratio), thereby reducing predation risk by mediating earlier fledging (cf. Nilsson and Svensson 1996).

Effects of egg temperature on RMR

This experiment affected nestling RMR in a nonlinear fashion, as nestlings from the low-temperature group had elevated RMRs compared with both mid- and high-temperature nestlings. Our experimental design does not allow us to separate the following two alternative hypotheses: 1) the effect of developmental temperature on metabolic rate is a physiological response to suboptimal embryonic conditions, without any positive fitness consequences; and 2) the observed pattern is of adaptive significance.

In precocial species, a reduction in temperature toward the end of incubation (when the thermoregulatory system begins to form) increases metabolic rate and improves cold tolerance in embryos and neonates, whereas an increase in temperature at this time produces the opposite effects (Nichelmann and Tzschentke 1999, 2002; Tzschentke 2007, 2008). It has been speculated that this epigenetic perinatal temperature adaptation (terminology sensu Tzschentke 2007) might serve as a way for parents to pre-adapt hatchlings to prevailing ambient conditions, and the effect of cold exposure during incubation on thermoregulatory capacity may persist throughout adult life (Shinder *et al.* 2009). There are indications that low incubation temperatures elevate embryonic metabolic rates in other bird species (in mallee fowl *Leipoa ocellata*, Booth 1987; in zebra finch, Olson *et al.* 2006), and a similar thermal acclimation of metabolic rate was recently described for a diverse array of reptiles (Du *et al.* 2010). However, whether this is a consequence of a suboptimal embryonic thermal environment or whether it functions as a pre-adaptation to low ambient temperatures is not known because in neither of the above cases were eggs allowed to hatch. Nonetheless, the existence of a similar mechanism in blue tits would provide a functional explanation for the higher RMR observed in nestlings from the low incubation temperature. The energetic cost of keeping eggs warm varies with ambient temperature (Ardia *et al.* 2009; Nord *et al.* 2010) and results in a corresponding variation in incubation temperatures (Haftorn 1983). Thus, prenatal temperature adaptation could potentially also account for some of the variation in metabolic rate between conspecific populations native to different latitudes (e.g., Broggi *et al.* 2004).

Alternatively, differences in RMR between treatments might have occurred if low-temperature nestlings up-regulated their metabolism to improve the rate of tissue synthesis, thus compensating for a potentially bad start. However, growth rates (gain per day) in the traits we measured were independent of incubation temperature. Still, suboptimal developmental conditions may retard the maturation of physiological regulatory systems or visceral organs independently of the longitudinal growth axis (Deeming and Ferguson 1989; Black and Burggren 2004a; Mortola 2006). Any compensatory growth in such traits would not have been detected by us. Regardless, this strategy would necessitate an increased energy intake to fuel the higher metabolic demands. Because parental feeding effort did not differ between treatments, we thus consider the compensatory growth hypothesis to be unlikely.

It is also possible that a nonrandom subset of eggs survived incubation in the lowest temperature. Because hatching success was markedly lower in the 35.0°C group, only embryos with certain characteristics, such as a higher metabolic rate, may have been able to withstand these conditions. If there was higher survival of embryos with high metabolic rate in the low-temperature group, we predicted that the within-brood metabolic phenotype would be less variable in this treatment. This was not the case. If anything, the repeatability (sensu Lessells and Boag 1987) of RMR was lower in low-temperature chicks (35°C: 0.034, $P = 0.053$; 36.5°C: 0.063, $P = 0.013$; 38.0°C: 0.090, $P < 0.001$). This suggests that egg survival was random also with the lowest temperature. However, these ideas remain untested because we do not have empirical data on embryonic metabolic rate and embryonic growth.

Conclusions

We have provided evidence of the effect of the embryonic environment on incubation period, hatching success, nestling morphology, and metabolic rate. However, the long-term effects of variation in incubation temperature remain unknown. Unfavorable conditions during early development can decrease reproductive success in adulthood (Gorman and Nager 2004; Naguib and Gil 2005), and nestling size at the time of fledging is often positively related to a variety of fitness-related traits such as survival and recruitment (McCarty 2001; Naef-Daenzer *et al.* 2001; Schwagmeyer and Mock 2008). Although nestlings can sometimes compensate for a bad start should conditions improve, such compensations can be energetically costly (Criscuolo *et al.* 2008) and result in reduced subsequent survival and reproductive output (Lindström 1999; Metcalfe and Monaghan 2001). Additionally, the extent of compensation need not always be complete (e.g., Schew and Ricklefs 1998), suggesting that intrinsic constraints may prevent a complete recovery from a sub-optimal developmental period. If that is the case, the offspring phenotype may be permanently altered by conditions experienced during early life. This remains speculative, as studies that relate the embryonic environment to neonatal phenotype, and then assess the relationship between phenotype and fitness, are largely absent. Thus, even though it seems likely that phenotypic consequences of a sub-optimal incubation environment may extend far beyond the nestling phase, studies explicitly assessing this relationship are currently highly warranted.

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Royal Physiographic Society, the Lund Animal Protection Foundation, the Helge Ax:son Johnson Foundation, and the Längman Cultural Foundation (to A.N.). All experimental protocols comply with national legislation and were approved by the Malmö/Lund Animal Care committee (permit M 94-07).

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Symposium Registration

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The International Wildlife Rehabilitation Council invites you to register now for the 2012 Education Symposium.

Field Trips for this year include:
International Conservation with Wisconsin Roots - Tour the International Crane Foundation and the Aldo Leopold Foundation.
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Symposium events will be held from Monday, 12th to Saturday, 17th November, 2012. Presentations will run from the Thursday to Saturday.

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'The Roadkill Experiment' and Teaching Ethics

By Deb Teachout, DVM

I'm always on the alert when I survey the news of the day for something related to animals and their welfare, so when I saw this video referenced in an

online article, I had to investigate. Perhaps you have already seen it as, within a few days, it went viral not only in the mainstream press but also in both animal and automobile blogs. Mark Rober, by day a mechanical engineer working for NASA's Jet Propulsion Laboratory and by nights and weekends a curious scientist with diverse interests, is the creator of "Roadkill Experiment." Why did he perform this experiment? In his words, "I read a long time ago that people will swerve more to hit turtles over snakes… as a firm believer in the scientific method, I decided to test this hypothesis." I have to admit, I didn't view this video immediately as I thought it would be gruesome, but when I found he used rubber animals (tarantula, snake, and turtle) and a leaf as a control, I watched. He chose a section of highway and systematically placed one of the animals or the leaf on the shoulder of the road.

He logged reactions for 1,000 drivers. His results? Six percent of drivers drove out of their lane to run over the rubber animal. That is, 60 motorists felt compelled to swerve out of their lane in order to hurt or kill a wild animal posing absolutely no threat to them. Nobody swerved out of their lane to hit the leaf. The breakdown: 3.2% of the drivers squashed the tarantula; 1.8% aimed at the snake; 1% veered toward the turtle. Compassion and ethical treatment of animals sadly appear to be absent in this subset of people.

It raises the question, "Can ethics or compassion actually be taught?" As wildlife rehabilitators, we certainly hope and believe that we can teach people to respect wildlife and to do the right thing when confronted with challenges involving wildlife (and to this I would include the challenge of someone's internal motivation to kill wildlife on the road); but, does the public's attitudes toward wildlife reflect or embrace our messages of ethics, compassion, and respect after we have delivered them?

There exists a parallel concern amongst veterinary college educators. The public has an emerging expectation that veterinarians are knowledgeable and competent in the areas of animal welfare and the ethical principles that guide the use of animals by society. In order to prepare veterinary students for that eventual role, educators must teach ethics and animal welfare. Surprisingly, these are new subjects in the veterinary curriculum. According to a recent study that took place at Michigan State University, where veterinary students were introduced to the subjects of animal welfare and ethics in a mandatory two-credit course, ethics can be successfully taught. The goal was to expose students to cases and situations in which they could practice recognizing ethical dilemmas, use a framework for ethical reasoning, and apply principles of animal welfare to their own decision-making processes (Abood and Siegford 2012). Most students at the end of the course felt it had improved their ability to identify, discuss, and process ethical dilemmas.

Belief in the ability to teach the humane treatment of all living creatures has been the lifelong work of Zoe Weil, president and co-founder of the Institute of Humane Education. In fact, through a partnership between the Institute for Humane Education and Valparaiso University, one can even obtain a graduate degree in humane education. It is the only program of its kind in the United States or Canada, but its existence and success provide testament that ethics and regard for the welfare of animals, people, and the planet are definitely teachable subjects. According to Zoe, the world becomes what you teach.

So, can we reach/teach the 6% that are likely responsible for at least some of the animals wildlife rehabilitators try to make whole again? (That is, outside of repeating Rober's experiment and hiding a sharp spike inside each rubber animal—not my idea, but I admit I kind of liked it.) Even though we may become angered by the 6% of motorists who swerve, remember there is proof that people can be educated in ethics and compassion.

Believe that the world becomes what you teach, because it does.

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Abood, S. K., and J. M. Siegford. 2012. Student perceptions of an animal welfare-and ethics course taught early in the veterinary curriculum. *Journal of Veterinary Medical Education* 39(2): 136–141.

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

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Education and Resources for Wildlife Conservation Worldwide

Explaining the New 3-202-4 Annual Report Forms Issued by the U. S. Fish and Wildlife Service

Janelle Harden and Anne Russell

Background

In September of last year (2011), the U. S. Fish and Wildlife Service (USFWS) posted a revised 3-202-4 form on their website. Rehabilitators were supposed to file their annual reports on the new form beginning with 2011 data; the form will be used until at least 28 February, 2014 (i.e., for your 2012 and 2013 data). Each year, most Service regions send their permittees an annual report reminder and a copy of the form or a link to the current digital (PDF) form, which can be downloaded from the Service's Permits page (see exact link in the next column). Some rehabilitators, however, were not aware that there had been revisions. There were several issues that caused a disconnect in the normal process.

First, although the forms issued in March of 2004 were "revised" in November of 2007 (USFWS 2007), no substantive changes were made from the prior version. By the time the 2007 form reached its expiration date of 30 November 2010, essentially the same form had been used for at least six (6) years. However, the Service did not revise the form for reports due in January of 2011—rehabilitators were told to continue using the old form. Therefore, most rehabilitators did not realize that a revised 3-202-4 had been issued (USFWS 2010) when they began preparing their 2011 annual reports.

Second, the Service did not issue a blanket notice to all rehabilitators, and different regions contacted their permit holders with different schedules and methods. Without getting an official notice that revisions had been made, most continued to use the same old form for their 2011 data. This was not a problem for the Service, as the primary difference in the old and new forms was a reduction in the information requested (for example, the new form no longer requests information on birds held over from the prior year or birds still pending on 12/31, *except* those held longer than 180 days). The new Section E information requested is entirely voluntary.

Using the new 3-202-4 Forms

This article was prepared after much consultation with the Service, specifically Susan Lawrence who is the U. S. Fish and Wildlife Service's National Migratory Bird Permits Coordinator in Arlington, Virginia. The authors have been working with Ms. Lawrence for several years because their organization, The RAVEN Project, is intricately linked with the annual report data required by the Service. The IWRC and the authors are grateful that Ms. Lawrence coordinated with us to produce this document.

The current 3-202-4 forms have two sections that appear to be entirely new, but actually only one is new and the other requests a reduced amount of information for what used to be the section (A) on birds held over. Data requested on one of the old sections is no longer required at all, and the sections of standard information that is still required are rearranged. For these reasons, the IWRC is providing this important information before you begin to prepare your 2012 reports (due this-coming January).

The remainder of this article compares the prior annual report form with the new version, which is provided as the last two pages of this document so that you can scan it as you read these instructions. (To obtain a blank form with which to file a 2012 report, please go to: http://www.fws.gov/forms/3-202-4.pdf). The exact instructions we have provided below were pulled from both the prior and the current report forms. We compare the old instructions for each section (highlighted in pale green) with the new (highlighted in a brighter green). Sections that are no longer required at all, or portions that are no longer required, are highlighted in tan.

The finer points are discussed in each report section below, but we do want to point out one thing that is important across the board. Please watch for the phrase "*individual* birds" in the majority of section instructions versus *categorized by species*, which is only allowed in your list of new acquisitions. In the 'individual' cases, you are required to itemize the requested information for each individual bird, not as bulk data for collective species.

Digital Format

The new 3-202-4 form is still in a digital format. You can type directly into the PDF form, but be aware of two things:

1. You need to be succinct because the rows/boxes will not expand to accommodate longer entries (as will Excel, for example).
2. A digital signature is not accepted; please print your report, sign it, and submit the printed annual report to your regional Migratory Bird Office.

As before, you do not *have* to use the on-line form, but you do have to provide the same information required by the form and in a format that clearly represents each Section A through E.

Section A

Section A of your annual report now collects data on new acquisitions—*this was Section B in the prior form*.

NO LONGER REQUIRED: Prior Section A

A. BIRDS HELD OVER. Please list individual birds that were held over from the last report year for continued care, and provide the following information. For DISPOSITION, check appropriate column. Also complete Section E for all transfers.

This information is no longer required anywhere within the 3-202-4 report form.

Prior Instructions:

B. NEW ACQUISITIONS. Please provide a summary of all migratory birds acquired during the report year, categorized by species. The quantity in the **Received** column should equal the sum of the quantities in the **Disposition** column. (For example: Robins: 14 - 10, 0, 1, 2, 1). Also complete section D and E for Pending and Transferred birds, respectively. All birds, including birds reported in C, D, and E, must be reported here.

New Instructions:

A. NEW ACQUISITIONS. Please provide a summary of all birds acquired during the report year, categorized by species. The quantity in the **Received** column should equal the sum of the quantities in the **Disposition** column. (For example: Robins: 14 - 10, 0, 1, 2, 1). Also complete sections B and D for Pending and Transferred birds, respectively. All birds, including birds reported in B, C, D, and E, must be reported here. **Please enter any Bald Eagle or Golden Eagles first**.

You may be aware that the Bald Eagle is no longer listed as a threatened species. However, both eagles also fall under the Bald and Golden Eagle Protection Act (16 U.S.C. 668-668c), and it is important to track data concerning these species. The Service is requesting that you list these two eagle species *first* in Section A. In other words, if you list new acquisitions in alphabetical order by common name, American Coot, American Robin, Ash-throated Flycatcher, and so forth would appear before the Bald Eagle, and the Golden Eagle would normally appear much further down the list. Therefore, remember to pull those species data out and report them in the first two rows.

The new acquisitions information still requires that the quantity for each type of disposition be entered along with the total number of each species received. The Service has added a column to accommodate birds that were Dead on Arrival (DoA).

Section B

Section B is a reduced version of the old Section D that required itemization of each bird still pending at year's end.

Instructions for New Section:

B. BIRDS HELD 180 DAYS OR LONGER ON 12/31. Please complete for any individual birds that you held 180 days or longer as of 12/31 of the report year.

We no longer have to list every acquisition still in our care on December 31st—only those that have reached 180+ days by the 31st. This would *not* include any bird held for 180 days during an earlier part of the calendar year (for example from February 3 to August 17) that had reached a final disposition prior to 12/31; that bird and disposition will appear in the new Section A where all acquisitions and dispositions are listed. In other words, Section B only lists birds held 180 days or longer and *still in your possession* as of 12/31.

Reminder:

As soon as you are aware that you intend to keep any bird longer than 180 days, regardless of the reason, at that point you are required to submit distinct paperwork that requests approval! In other words, no bird should appear in section B for which you have not already obtained authorization to keep it longer than 180 days. This situation falls under your Standard Conditions [for] Rehabilitation Permits (50 CFR 21.31):

> 1. With the exception of limited feathers for imping purposes (see condition 14), you may not hold migratory birds for more than 180 days without additional authorization from the issuing office.

Remember that birds held as foster parents remain under your rehabilitation permit and are, therefore, subject to the same Standard Condition. However, keeping a bird to foster conspecifics requires that you notify your permit officer *as soon as you know the bird will become a foster parent*—and will thus be kept for "more than 180 days"—so that you can obtain the required authorization. Section B is not the appropriate place to let the Service know you want to use a bird for fostering (i.e., 'after the fact'). However, if you have properly obtained permission to retain a new bird for fostering, do not include it in Section B if it has not yet reached 180 days as of 12/31.

The revised annual report form no longer requires that any bird be identified as a foster bird.

Section C

Section C is basically the same as it was in the prior form.

Prior Instructions:

C. REPORTED INJURIES. Please complete for any individual birds that were shot, poisoned (confirmed), electrocuted, trapped, or otherwise injured or killed as the result of a potentially criminal activity. (Such injuries should have been reported immediately). **DISPOSITION CODES:** R=Released; T=Transferred; P=Pending; E=Euthanized; D=Died.

New Instructions:

C. REPORTED INJURIES. Please complete for any individual birds received that were shot, poisoned (confirmed), electrocuted, trapped **(e.g., foot-hold)**, or otherwise injured or killed as the result of a potentially criminal activity. (Such injuries should have been reported immediately). **DISPOSITION CODES:** R=Released; T=Transferred; P=Pending; E=Euthanized; D=Died; **DoA=Dead on Arrival.**

The two phrases within the sentence that are **bold green** are the only changes in this section's instructions. These directions are self-explanatory, although one item should be clarified. "Confirmed" for poisonings means that you obtained written confirmation from a veterinarian or a laboratory; those are the only acceptable authorities.

Reminder:

Don't forget that "Such injuries *should have been reported immediately*" is another portion of your Standard Conditions (50 CFR 21.31):

> 11. You must immediately report to U.S. Fish and Wildlife Service Law Enforcement Office any birds (dead or alive) that appear to have been poisoned, shot, or otherwise injured as the result of criminal activity.

Section D

Section D of your annual report now collects Transfer data—*this was Section E in the prior form*.

NO LONGER REQUIRED: Prior Section D

> **D. STILL PENDING**. Please complete for any individual birds still held as of 12/31 of the report year. Please identify any birds you maintain as foster parents with a circled "F" next to their common name.
>
> ***Most of the information in the prior Section D is no longer required; "still held as of 12/31" is in Section B.***

Prior Instructions:

> **E. TRANSFERS**. Please complete for individual birds you transferred during the report year (1/1/10 - 12/31/2010). For **Permit Number or Address**, provide the permit number if applicable; if not applicable, provide address. For **Purpose of Transfer**, use the following codes: **R**=Released; **C**=Continued Care; **Live-E/S** =Live-Education or Scientific Purposes; **Dead-E/S**= Dead-Education or Scientific Purposes.

New Instructions:

> **D. TRANSFERS.** Please complete for individual LIVE birds you transferred during the report year (1/1 - 12/31). For **Name & Permit Number or Address**, provide the name & permit number if applicable; if not applicable, provide name & address. For **Purpose of Transfer**, use the following codes: R = Released; C = Continued Care; E/S = Education or Scientific Research permit; F/P = Falconry or Raptor Propagation permit; O = Other please enter permit type.

Concerning "Other," these are transfer to a Zoo or other exempt institution (such as a Museum), Native American Eagle Aviary permit, Game Bird Propagation permit, Waterfowl Sale and Disposal permit, or Special Purpose–Miscellaneous permit.

Section E

Section E is information that was not collected in prior years. (The old Section E collected the Transfer information just described for Section D in the new forms.)

Instructions for New Section:

> **E. OPTIONAL. - DISEASE & CONTAMINANTS. Providing the information requested below is voluntary**. Please complete for any individual birds received that were tested & were confirmed to have died of infectious disease such as West Nile virus (not parasites), or ingested contaminants such as sodium pentobarbital, carbofuran, or lead. **Note:** The FWS does not require testing of birds for disease or contaminants and the following information request should not be construed as a recommendation to do so. However, for any birds that you chose to have clinically tested that resulted in a confirmed diagnosis, please provide the requested information. Do not include data on birds you *suspect* succumbed as a result of disease or toxins but were not tested, or birds that were tested but results were inconclusive. *Thank you*.

It is of great value to the Service to track diseases that are causing mortality in wild bird populations. The activities of the rehabilitation community allow us to provide important support data for this effort. There are two phrases in the Section E instructions that might need clarification:

Concerning the "*not* parasites," the Service is interested in microparasites (endoparasites/internal; e.g., viruses and bacteria), not macroparasites (be they internal; e.g., helminths, protozoans, etc.; or ectoparasites/external; e.g., mites, flat flies, etc.).

Concerning the "confirmed diagnosis," this means *laboratory*-confirmed. If you reported any laboratory-confirmed poisonings in Section C. INJURIES, duplicate them here in this voluntary report section.

Conclusions

The data required on the revised 3-202-4 form with which you file your annual report will actually reduce the amount of time and information needed. Several things are no longer required information, and filling out the new Section E to track disease and contaminants is strictly optional. Beyond being a voluntary portion of your report, there are a limited number of rehabilitators or large organizations that have the facilities (or finances) to obtain laboratory confirmation on diseases or toxic contaminants. For those that do obtain such confirmation, sharing those data will be an important facet of the scientific nature our activities.

Your regional permit officer can assist you if any confusion arises when switching to the revised form. If you'd like clarification of any explanation in this article, please feel free to contact the authors (Janelle Harden jharden@nmia.com or Anne Russell ac.russell@hotmail.com).

Literature Cited

50 CFR 21.31. 2003. Standard Conditions Rehabilitation Permits. U. S. Fish and Wildlife Service. (12/3/2003).

16 U.S.C. 668-668c. 1940. Bald [and Golden] Eagle Protection Act.

U.S. Fish and Wildlife Service (USFWS). 2007. U.S. Fish and Wildlife Service – Migratory Bird Permit Office, Rehabilitation Annual Report. Form 3-202-4 Rev 11/2007, OMB No. 1018-0022. Expires 11/30/2010.

USFWS. 2010. U.S. Fish and Wildlife Service – Migratory Bird Permit Office, Rehabilitation Annual Report. Form 3-202-4 Rev 9/2010, OMB Control No. 1018-0022. Expires 02/28/2014.

IWRC member Janelle Harden is the Senior Editorial Assistant for the Journal of Wildlife Rehabilitation, and Anne Russell is the Data Manager for Wildlife Rescue, Inc. of New Mexico (WRINM) in Albuquerque. Janelle (since 1996) and Anne (beginning in 2004) have produced annual reports for WRINM. They have partnered in The RAVEN Project, which created the RAVEN Wildlife Rehabilitation Records System, a digital acquisition log that gathers rehabilitation data for annual reports as well as for species-specific analyses. They have worked with the U. S. Fish and Wildlife Service (Region 2), the New Mexico Department of Game and Fish, the Museum of Southwestern Biology (University of New Mexico), and rehabilitators in several countries while developing The RAVEN Project. ■

U.S. FISH & WILDLIFE SERVICE - MIGRATORY BIRD PERMIT OFFICE

(See attached addresses)

REHABILITATION ANNUAL REPORT - REPORT YEAR _____

Report Due: _____

PERMITTEE: _____ **PERMIT NUMBER:** _____

ADDRESS: _____ **PHONE NUMBER:** _____

_____ **E-Mail:** _____

City State Zip Code

Check here if reporting a change of name, address, or contact information

INSTRUCTIONS: Please type or print the information requested below for all migratory birds (see 50 CFR 10.13) held under your permit during the report year, and return the completed report to the above address by **January 31** of the following year. Use of this form is not mandatory, but the same information must be submitted. A supplemental sheet is available if needed. *Do not include species other than migratory birds in your report*. Filing an annual report is a condition of your permit. Failure to file a timely report could result in suspension of your permit. You must submit a report even if you had no activity during the year. **Make sure you sign the certification at the end of the form.** (Ref. 50 CFR parts 13 & 21)

DISPOSITION CODES: R=Released; T=Transferred; P=Pending; E=Euthanized; D=Died; DoA=Dead on Arrival

A. NEW ACQUISITIONS. Please provide a summary of all birds acquired during the report year, categorized by species. The quantity in the **Received** column should equal the sum of the quantities in the **Disposition** column. (For example: Robins: 14 - 10, 0, 1, 2, 1). Also complete sections B and D for Pending and Transferred birds, respectively. All birds, including birds reported in B, C, D, and E, must be reported here. **Please enter any Bald Eagle or Golden Eagles first.**

Common Name	Total Number Received	Disposition (enter quantity)					
		Released	Transferred	Pending	Euthanized	Died	DoA

B. BIRDS HELD 180 DAYS OR LONGER ON 12/31. Please complete for any individual birds that you held 180 days or longer as of 12/31 of the report year.

Common Name	Date Acquired	Nature of Injury	Proposed Disposition (check one)		
			R	T	E

CERTIFICATION: I certify that the above information is true and correct to the best of my knowledge. I understand that any false statement herein may subject me to the criminal penalties of 18 U.S.C. 1001.

Signature: _____ Date:

C. REPORTED INJURIES Please complete for any individual birds received that were shot, poisoned (confirmed), electrocuted, trapped (e.g., foot-hold), or otherwise injured or killed as the result of a potentially criminal activity. (Such injuries should have been reported immediately.) **DISPOSITION CODES:** R=Released; T=Transferred; P=Pending; E=Euthanized; D=Died; DoA=Dead on Arrival.

Common Name	Date Acquired	Cause/Nature of Injury	Disposition (check one)						Source of Bird (County & State)
			R	T	P	E	D	DoA	

D. TRANSFERS. Please complete for individual LIVE birds you transferred during the report year (1/1-12/31). For **Name & Permit Number or Address**, provide the name & permit number if applicable; if not applicable, provide name & address. For **Purpose of Transfer**, use the following codes: R = Released; **C** = Continued Care; **E/S** = Education or Scientific Research permit; **F/P**=Falconry or Raptor Propagation permit; O = Other please enter permit type.

Common Name	Transferred to (Recipient)			Purpose of Transfer
	Name	Name & Permit Number or Address	Date	

E. OPTIONAL. - DISEASE & CONTAMINANTS. Providing the information requested below is voluntary. Please complete for any individual birds received that were tested & were confirmed to have died of infectious disease such as West Nile virus (not parasites), or ingested contaminants such as sodium pentobarbital, carbofuran, or lead. **Note:** The FWS does not require testing of birds for disease or contaminants and the following information request should not be construed as a recommendation to do so. However, for any birds that you chose to have clinically tested that resulted in a confirmed diagnosis, please provide the requested information. Do not include data on birds you *suspect* succumbed as a result of disease or toxins but were not tested, or birds that were tested but results were inconclusive. *Thank you*.

Common Name	Date Acquir-ed	Name of Disease or Contaminant	Concentration of toxin, or if infectious disease, test used for diagnosis	Tissue Tested (e.g., blood/ bone/ brain/ liver/kidney/ GI tract contents)	Name of Lab & State	Source of Bird (County & State)

TAIL END

EASTERN GRAY KANGAROO (*MACROPUS GIGANTEUS*). PHOTO ©BU. CREATIVE COMMONS LICENSE.

"Enough waltzing, Matilda!"

Winning caption by Alex Almande, Omaha, Nebraska USA.

We've posted the next issue's Tail Ends photo on the web at:
www.theiwrc.org/journal-of-wildlife-rehabilitation/tailends
Submit your clever caption to jwr.editor@theiwrc.org by December 1.

INSTRUCTIONS FOR AUTHORS

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

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

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

Kieran Lindsey, Editor
jwr.editor@theiwrc.org

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

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

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

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

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

STYLE The JWR follows the Scientific Style and Format of the CBE Manual, 6th Edition, for Authors, Editors, and Publishers. The complete "JWR Author Instructions" document is available at:

http://www.theiwrc.org/journal/submissions.html

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

Wilson's Storm Petrel *(Oceanites oceanicus)*.

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