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SCWDS BRIEFS

A Quarterly Newsletter from the

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Wildlife Restoration Program: 75 Years of Success

The Wildlife Restoration Program, created through the Pittman-Robertson (P-R) Act in 1937, is celebrating 75 years of success in wildlife management this year. Senator Key Pittman of Nevada and Representative A. Willis Robertson of Virginia were the primary sponsors of the bill in the U.S. Congress. Funded by the Federal Firearms and Ammunition Excise Tax on hunting and shooting equipment and supplies, the Wildlife Restoration Program provides money to states to restore, conserve, manage, and enhance wild birds and mammals and their habitats. Through 2011, this program provided more than $6 billion to state fish and wildlife management agencies for conservation.

Federal agencies collect the excise taxes and import duties from partners in the hunting and shooting sports industries. The tax is 11% on sporting arms, ammunition, bows, and archery accessories, and it is 10% on pistols and revolvers. These federal funds are allocated to states through a formula based on each state's land and water area, as well as the number of paid hunting license holders. Importantly, states can receive P-R funding only if their laws prohibit diversion of hunting license revenues to any purpose other than administration of the state fish and wildlife management agency. Previously, license dollars sometimes were diverted to state general revenue funds where they could be used for non-conservation issues, such as road construction or public education.

Wildlife Restoration Program funds may be used for wildlife research, property acquisition, managing land and waters as wildlife habitat, hunter education programs, and the construction and operation of shooting ranges. The states provide only 25% of the funding for eligible projects supported by the program. In other words each state dollar, typically obtained through hunting license sales, is matched by three P-R dollars. Thus, hunters are supporting wildlife conservation through the purchase of hunting licenses as well as equipment and ammunition they use.

The Pittman-Robertson Act was signed into law by President Franklin Roosevelt on September 2, 1937. At that time, many wildlife populations in the United States had dwindled to frighteningly low numbers. The Wildlife Restoration Program has helped bring back populations of white-tailed deer, elk, pronghorn antelope, wild turkey, and other species to abundance. While the Wildlife Restoration Program primarily enhances game species management, it has had much broader impacts: Acquiring and managing land for game species provides habitat for wildlife in general, thus benefiting non-game species, as well as the "non-consumptive" wildlife recreationists, such as hikers and bird watchers, who enjoy them.

The amount of P-R funds raised has risen steadily since its inception. For fiscal year 2011, the apportionment was nearly $400,000,000. The consistent availability of funds allows long-term projects to be seen through to completion. Early examples of Wildlife Restoration Program projects include re-establishment of white-tailed deer on nearly 30 million acres of range and wild turkey on 20 million acres in Alabama; acquisition of nearly 10,000 acres of land in Connecticut, including key wetlands along Long Island Sound and the Connecticut River; and the

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purchase of 57,000 acres of wildlife habitat in Kansas. Recent examples include purchase and maintenance of wildlife habitat in Pennsylvania, development of a shooting range in Georgia, studies estimating bear populations in Wisconsin, and a statewide survey of wildlife in Alabama. Several states use Wildlife Restoration Program dollars to cover their annual SCWDS membership.

The partnership between state and federal agencies, conservation groups, sportsmen and women, and the hunting and shooting industries is the element that is critical to the continuing success of the Wildlife Restoration Program. The Pittman-Robertson Act was a ground-breaking step for wildlife conservation and was used as a model for the Sport Fish Restoration Program, supported through an excise tax on fishing equipment that began under the Dingell-Johnson Act in 1950. Through excise taxes and license revenues, sportsmen and women have contributed more than $12 billion to conservation through the Wildlife and Sport Fish Restoration Programs (WSFRP), and annually they provide more than 80% of the funding for most state fish and wildlife agencies. So, if you have enjoyed natural areas and wildlife, hunting, fishing, birding, or other outdoor recreation, you should thank the WSFRP. Better yet, thank a hunter or an angler. And there's nothing stopping you from supporting conservation, even if you don't hunt or fish: You can always buy a license. (Prepared by Anne Nemeth-Wild, Iowa State University)

AIV in Minnesota Ducks: The Whole Shebang

Wild ducks are a known reservoir for low pathogenic avian influenza viruses (AIV). However, limited attention has been directed to developing AIV surveillance strategies to efficiently provide representative isolates for influenza databases or to detect new viruses. This information gap was evaluated in a collaborative project entitled "Influenza A Viruses in Ducks in Northwestern Minnesota: Fine Scale Spatial and Temporal Variation in Prevalence and Subtype Diversity."

More than 4800 ducks were sampled in 2007 and 2008 at study sites in northwestern Minnesota during the known peak of AIV prevalence in staging waterfowl populations in late summer and early fall. Prevalence varied between years with viruses isolated from 9% and 18% of the ducks sampled in 2007 and 2008, respectively. Overall, 660 AIV were isolated representing all North American HA and NA subtypes except H9. (This does not include H14 and H15 viruses that are uncommon or never reported in North America or H13 and H16 viruses that are associated with gulls but are not seen in ducks.) These results suggest that most of the subtype diversity seen in North American ducks can be detected within a limited area such as northwestern Minnesota. The reason for this success, however, relates to sampling intensity. Even though overall AIV prevalence was relatively high, prevalence estimates for individual subtypes was extremely low, ranging from a high of 4.6% for H3N8 viruses in 2008 to <0.5% for such subtypes as H5N2, H7N3, H8N4, and H12N5. These results suggest that attempts to detect specific subtypes (H5N1, [<0.1% in this study]) or novel viruses such as Eurasian highly pathogenic H5N1 (not detected in any North American surveillance to date), need to consider a sampling framework that can detect viruses that may be at extremely low prevalence.

There are several other sampling considerations highlighted in this study that can be used to make current surveillance attempts more efficient, or to aid in designing future surveillance strategies to detect novel viruses. For example, peak prevalence in ducks in this area of Minnesota occurred earlier than peak subtype diversity. This probably relates to increasing subtype diversity over time due to the AIV reassortment events that commonly occur in ducks, and to introductions of new subtypes with arriving migrants. These results indicate that to fully describe the diversity of viruses moving though a specific area, sampling strategies need to take temporal variation into account; in this case subtype diversity changed over a two month period. In addition, surveillance efficiency can be optimized by targeting certain species and age cohorts. Although ten duck species were sampled in this study, we learned all we needed to know by examining the data from juvenile mallards: We found all HA and NA subtypes and identified the AIV prevalence trends in that data set. If we had targeted only juvenile mallards initially, sampling and testing would have been reduced by 53%.

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Results of the study were published in PLos One 6(9) e24010. The project was funded by the National Institutes of Health (NIH) Centers for Influenza Research and Surveillance Network and a cooperative agreement between SCWDS and the USDA's Southeast Poultry Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture. Coauthors included collaborators from the University of Minnesota; Agassiz National Wildlife Refuge, United States Fish and Wildlife Service; Wetland Wildlife Populations and Research Group, Minnesota Department of Natural Resources; and the Southeast Poultry Research Laboratory. (Prepared by Ben Wilcox and David Stallknecht)

Another Vector-borne Virus Hits European Livestock

German dairy farmers and veterinarians began reporting nonspecific clinical signs of fever, diarrhea, decreased milk production, anorexia, and loss of condition in adult cattle in the summer of 2011. In November 2011, farmers reported abortions and stillbirths associated with congenital malformations, primarily in sheep, but occasionally in cattle and goats. The causative virus was isolated in late 2011 by the Friedrich-Loeffler Institut in Germany and provisionally named Schmallenberg virus (SBV) for the German town where it was first observed. Genetic testing revealed that SBV belongs to the *Bunyaviridae* family, genus *Orthobunyavirus*. It is closely related to viruses in the Simbo serogroup, including Aino, Akabane, and Shamonda viruses, which have been observed in cattle in Japan. Akabane virus can infect domestic and wild ruminants and swine; however, SBV has not been reported in European wildlife.

To date, Schmallenberg virus has affected domestic ruminants on more than 2,100 farms in Belgium, England, France, Germany, Italy, Luxembourg, the Netherlands, and Spain. Most affected operations have been sheep farms (85%), but SBV also has been detected in cattle (11%), goat (4%), and bison farms. Morbidity in affected herds ranged from 20-70% over 2-3 weeks, but animals usually recovered in a few days. The risk to humans is regarded as low or negligible, but cannot be excluded entirely.

Although other viruses belonging to the family *Bunyaviridae* occur in Africa, Oceania, and Asia, none had been reported previously in ruminants in Europe. Like other orthobunyaviruses, SBV is a vector-borne disease spread by biting midges (*Culicoides* spp.) and mosquitos; however, transmission across the placenta may occur and result in congenital malformations. The role of insects in SBV epidemiology was confirmed in October 2011 by the detection of the virus in two *Culicoides* species in Belgium. Interestingly, the spread of SBV in Europe has been similar to that of another emerging virus vectored by *Culicoides* midges: bluetongue-8.

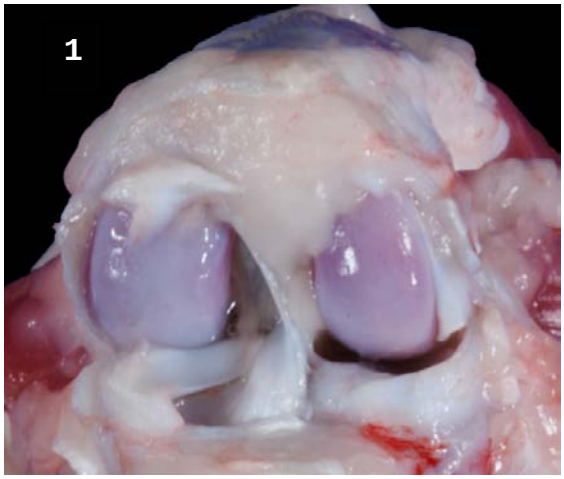
Infection with other orthobunyaviruses typically causes only mild disease in adult ruminants. However, infection of pregnant females can result in premature births, congenital problems in offspring, and reproductive problems. The presentation of SBV in adult cattle is nonspecific. In adult sheep and goats, an increased incidence of stillbirths or abortions may be the only clue the herd is infected with SBV. Arthrogryposis (joint contractures), and hydranencephaly (the presence of fluid in the skull rather than brain tissue) are the primary findings in aborted or stillborn small ruminant fetuses or neonates, although scoliosis (curved spine), and other congenital joint and neurological abnormalities may be present.

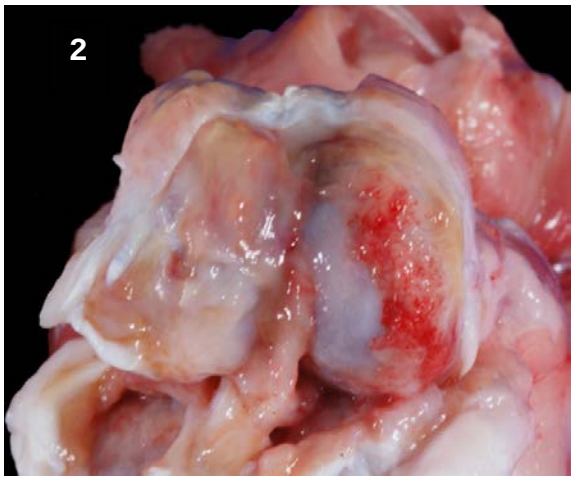
Diagnostic tests have been developed for SBV and include real-time reverse transcriptase PCR (rRT-PCR) and virus neutralization (VN), in addition to virus isolation. Although it has not been reported in the United States, testing for SBV is available at the USDA-APHIS National Veterinary Services Laboratories in Ames, Iowa. Sample submissions should include serum from reproductively symptomatic adult animals, as well as brain, serum, and heart blood from aborted fetuses or stillborn neonates.

Additional information on SBV can be accessed at http://www.aphis.usda.gov/animal_health/animal_diseases/schmallenberg/index.shtml (Prepared by Koren Moore Custer, UGA College of Veterinary Medicine)

Polyarthritis in Raccoons

Since late 2010, SCWDS has received raccoons with polyarthritis from rehabilitation facilities in Kentucky and Virginia. The animals had multiple swollen joints, often with abscesses in adjacent tissues, and soft tissues associated with the lesions were inflamed and contained purulent material. Within affected joints, synovial structures were inflamed and necrotic with fibrinous exudate. A normal (Figure 1) and arthritic stifle (Figure 2) from the same raccoon are shown below.





Typical rule-outs for polyarthritis in wild and domestic animals include sepsis (overwhelming bacteria in the blood stream), some tick-borne diseases, *Mycoplasma* sp. infection, trauma, and some immune-mediated diseases. In free-ranging animals with multiple swollen joints and no signs of trauma, sepsis is the most likely cause; however, in these cases there was no evidence of sepsis or trauma.

Samples submitted for culture of *Mycoplasma* spp., aerobic, and anaerobic bacteria tested negative. With PCR, all but one of the samples from affected raccoons tested positive for *Mycoplasma* sp., and the sequences from PCR positives were identical. Comparison with *Mycoplasma* sp. isolates in GenBank® strongly suggested that this was an undescribed *Mycoplasma* sp. that was most closely related to *Mycoplasma* spp. found in the upper respiratory tract of bottle-nosed dolphins, *Mycoplasma equigenitalium* associated with congenital disorders and infertility in horses, and *Mycoplasma elephantis*.

Polyarthritis associated with *Mycoplasma* spp. has been reported in the literature from many domestic animals and humans. Similar diseases have been reported infrequently from wildlife. A mycoplasmal epiphysitis/periostitis syndrome was observed in raccoons in Ontario, and a similar disease was described in striped skunks from Cape Cod, Massachusetts. Although *Mycoplasma* sp. is associated with disease in raccoons we examined, the significance of this disease for free-ranging populations is unknown. Thus far we have only seen this polyarthritis syndrome in juvenile raccoons from wildlife rehabilitation facilities. (Incidentally, one of these raccoons was rabies positive, highlighting the risk of rehabilitating known rabies vectors.) The risk factors and epidemiology of mycoplasmal arthritis in raccoons could be closely associated with wildlife rehabilitation, or the disease may occur naturally but has not been fully described. (Prepared by Brandon Munk)

Recent EHDV Studies at SCWDS: New and Old Viruses

Over the past 20 years we have isolated close to 800 epizootic hemorrhagic disease (EHD) and bluetongue (BT) viruses, and thanks to support from Mossy Oak® and the Safari Club International Foundation, we recently had the opportunity to put these viruses to good use. During the last 10 years, exotic EHD and BT viruses have been isolated consistently in the United States, and in 2007, the largest recorded orbivirus outbreak in wild ungulates was documented. Although such events suggest that the epidemiology of these viruses in North America may be changing, the factors responsible for large-scale regional outbreaks and successful introductions of exotic viruses

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are complex and poorly defined. Furthermore, when a vector-borne virus is involved, epidemiological changes may involve the host, virus, vectors, or any combination of them.

During the 2007 outbreak in the eastern United States, EHDV-2 was the primary virus associated with mortality in white-tailed deer. The unprecedented number of EHDV-2 isolates that we obtained provided a unique opportunity to study factors that may precipitate EHD outbreaks in wild ruminants. One important question relates to the source of viruses responsible for such outbreaks. It is unknown whether outbreaks are caused by a few viral genotypes spreading radially from endemic foci or by multiple, geographically-confined genotypes that cumulatively cause a large mosaic of discrete outbreaks. To investigate this question, SCWDS did a genetic analysis of representative isolates from different geographical regions by sequencing the VP2 gene. The VP2 gene encodes the major antigen that is exposed on the surface of the virus and is responsible for binding to and entering mammalian cells, as well as inducing neutralizing antibodies in the host. The VP2 gene is the most variable gene and is an ideal candidate for genetic analysis of the outbreak.

Numerous different genotypes were identified that appeared to be localized in their distribution to a single state or to a region within a state (see genotypes C and E, Figure 1). Additionally, based on the distribution of these various genotypes, there may be geographical barriers, such as the Appalachian

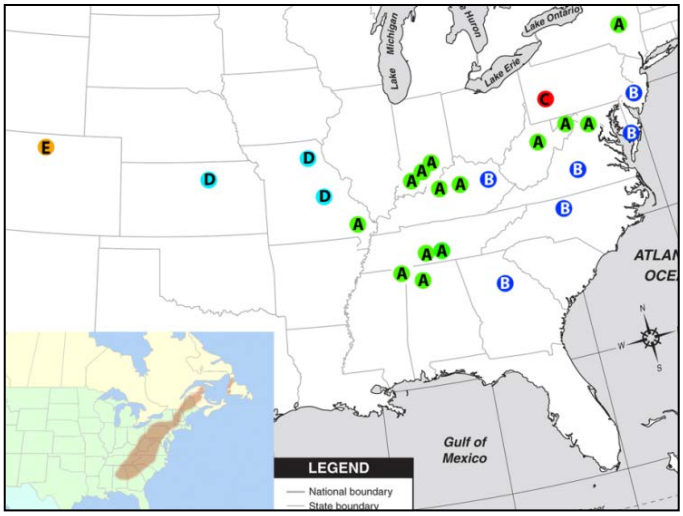

Figure 1

Mountains, which limit their distribution. However, certain EHDV-2 genotypes were much more geographically widespread than others (see genotypes A, B, and D, Figure 1), suggesting that specific genotypes may predominate during outbreaks.

It will take additional studies to determine whether some of these widespread genotypes have a fitness advantage over other more geographically-confined genotypes. This could occur via better replication or more efficient transmission in or between the ruminant host and vectors, or via widespread dispersion contingent upon other factors, such as wind dispersal of vectors carrying the virus.

In a second study, SCWDS, in collaboration with the Arthropod-Borne Animal Disease Research Unit, USDA-ARS, Manhattan, KS, evaluated the effects of temperature on EHDV replication in the vector, *Culicoides sonorensis*. Viruses used in this study included indigenous EHDV-1 and EHDV-2, and EHDV-6 (Indiana), which was first detected in the United States in 2006. Experimental results demonstrated a clear temperature-dependent relationship with the rate of replication for all three viruses: as temperature increased from 20ºC to 30ºC, the viral titers in infected midges increased and this increase appeared earlier after feeding. For instance, midges with sufficient virus to theoretically infect a deer were consistently present at day 12-16, day 6, and day 3-4 for 20ºC, 25ºC, and 30ºC, respectively. This is significant because these results suggest that increased temperature may provide more infected vectors in less time, potentially resulting in enhanced virus transmission.

Another significant part of this study relates to EHDV-6 replication in this North American vector system. The EHDV-6 replicated as efficiently as endemic North American EHDV-1 and EHDV-2 suggesting that this exotic virus can adapt to the HD vector, *Culicoides sonorensis.* Based on previous SCWDS research, this adaptation probably is facilitated by a reassortment event where genes were exchanged between an exotic EHDV-6 and an endemic EHDV-2. In this case, EHDV-6 (Indiana) obtained the VP7 gene that encodes a protein necessary for replication in the vector from an indigenous EHDV-2 virus. Based on the additional analysis of VP7 genes from more

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recent North American EHDV-6 isolates, such genetic reassortment is continuing as this virus spreads across the United States.

We are grateful to Mossy Oak® and the Safari Club International Foundation for their generous support of these studies to better understand the epidemiology of hemorrhagic disease, the most significant viral disease of white-tailed deer. (Prepared by Andrew Allison, Mark Ruder, and Dave Stallknecht)

Borrelia in African Penguins

In the summer of 2010, two veterinary students (Michaelle Purdee and Claire Rice) traveled to South Africa to work on a study aimed at better understanding tick-borne pathogens in African penguins (*Spheniscus demersus*) and other seabirds. This is part of a collaborative project between Dr. Michael Yabsley, Dr. Liz Horne at Penguins East Cape Marine Bird Rehabilitation Center, and Dr. Nola Parsons at Southern African Foundation for the Conservation of Coastal Birds. The African penguin is the only penguin species that breeds in Africa and is endangered. Several diseases, including avian malaria, babesiosis, and aspergillosis, are common in some African penguin populations.

This study resulted in the finding of a novel *Borrelia* species, possibly representing the fourth report of a relapsing fever *Borrelia* sp. in an avian host. A review of data from all penguins at the two centers since 2002 indicated that *Borrelia* was present in 115 of 8,343 (1.4%) penguins. Prevalence rates were significantly higher among chicks and juveniles compared with adults, and for samples taken during the summer months of October to February compared with winter months. Higher prevalence rates in summer are similar to those of another tick-borne pathogen (*Babesia piercei*) of penguins.

The majority of infected birds were released without antibiotic therapy, suggesting the *Borrelia* infections were not causing disease that warranted treatment. However, at least one penguin is believed to have died of borreliosis because it had gross and microscopic lesions consistent with those seen in other avian species and bats. This penguin had a history of unsteady gate, circling, and torticollis. It was given supportive care and antibiotics, but died four days after arrival at the facility. Grossly, the penguin had an enlarged spleen and liver. Histologically, there was pulmonary edema, chronic meningoencephalitis, and lesions in the spleen suggestive of a chronic, blood-borne disease process. Although uncommon in birds, relapsing fever in domestic chickens and an owl were characterized by similar lesions.

Genetically, the novel *Borrelia* sp. from the African penguin was most closely related to a relapsing fever *Borrelia* found in soft ticks (*Carios sawaii*) from a colony of Swinhoe's storm petrels (*Oceanodroma monorhis*) and streaked shearwaters (*Calonectris leucomelas*) in Japan. Interestingly, ticks from this Japanese bird colony have been associated with an avian disease that resembles relapsing fever in humans.

The zoonotic potential of the *Borrelia* species detected in African penguins is unknown, but many relapsing fever *Borrelia* species are zoonotic pathogens. Relapsing fever in humans is characterized by episodes of fever, headaches, muscle and joint aches that last 2-9 days and may recur up to ten times, typically with 2-4 afebrile days between episodes. Tick-borne relapsing fever (TBRF) occurs in discrete locations around the world, including mountainous areas of the western United States, and is transmitted via bites from soft ticks of rodents. Louse-borne relapsing fever (LBRF) is transmitted by the human body louse and typically is found in densely crowded settings, such as refugee camps, in developing regions of the world. Several different *Borrelia* spp. cause TBRF, and LBRF is caused by *B. recurrentis*. Definitive diagnosis is based on observing *Borellia* spirochetes in blood smears or culturing the organism from blood taken during febrile periods. Many antibiotics are effective but physicians typically prescribe tetracyclines. Most patients recover fully within a few days of appropriate treatment.

Our collaborators in South Africa have developed a network of researchers interested in investigating threats to the African penguin. In the next year, SCWDS researchers will work to genetically classify *Babesia* species that have been detected in African penguins, Cape Cormorants, and Gape Gannets. (Prepared by Michael Yabsley)

Accolades

In recent months, several of our very deserving folks have been recognized with various awards. In May, The University of Georgia (UGA) held the first annual Three Minute Thesis competition. This event originally was developed by the University of Queensland and is conducted to develop academic, presentation, and research communication skills, as well as to support the development of students' capacity to effectively explain their research in language appropriate to a non-specialist audience. Students across the UGA campus participated, and SCWDS-affiliated students received the top two prizes: April Conway, a PhD student of Dr. Sonia Hernandez, presented her research on pygmy hippos, and Elizabeth "Liz" Gleim, a PhD student of Dr. Michael Yabsley, discussed her research on the effects of prescribed fire on tick populations.

Liz also was selected as the 2012 Byrd-Dunn Award winner at the annual meeting of the Southeastern Society of Parasitologists. This award is given to the graduate student who provides the best oral presentation on his or her research findings. Liz's talk was on the impact of imported red fire ants on the survival of two common tick species in Georgia.

Barbara Shock, a PhD student of Dr. Yabsley who is working on disease issues of the Florida puma, was this year's winner of the Shikar Safari Club Foundation Scholarship. This scholarship is open to undergraduates or graduate students who hunt. The winner is selected from a nation-wide pool of students based on an essay on wildlife and land management, grades, leadership abilities, and if applicable, a research project.

Barbara also is this year's winner of the Wildlife Disease Association Graduate Student Scholarship that will be awarded at the annual meeting of the Wildlife Disease Association in Lyon, France. This scholarship acknowledges outstanding academic and research accomplishment, productivity, and future potential in pursuit of new knowledge in wildlife health.

Kerrie Anne Loyd, an MS student of Dr. Hernandez who works on issues surrounding feral cats and wildlife, was the 2012 recipient of the E.L. Cheatum Award. This award is given to a wildlife graduate student who exemplifies integrity, objectivity, leadership, vision and an appreciation for a broad interdisciplinary approach.

One of our undergraduate researchers, Luben Raytchev, has received several awards and honors. Luben was selected as a UGA Center of Undergraduate Research Opportunities (CURO) Summer Scholar based on his research on the parasites of aquatic turtles. He is interested in the effects basking behavior may have on parasite infections. Based on his initial findings and his proposal to expand the study, he was awarded the 2011-2012 Joshua Laerum Academic Support Award for Undergraduate Students. Luben also has been accepted to a study-abroad program at Oxford University, where he will spend his Fall semester before returning to SCWDS in the spring of 2013 to complete his research.

Earlier this year, Dr. Michael Yabsley received the UGA Student Government Association Recognition for Student Mentoring. In addition, Michael was the 2012 recipient of the CURO Undergraduate Research Mentoring Award at the Early Career Level, which is given to faculty who show excellence in mentoring undergraduates in research. Current and previous undergraduate students from UGA nominated him for the CURO award and he feels honored and humbled. (Prepared by Michael Yabsley and John Fischer)

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