Report to the Fish and Wildlife Health Committee of the Association of Fish and Wildlife Agencies from USGS Science Centers
September 2009

Wildlife Highlights

Research progress on white-nose syndrome of bats (MA, NY, VT, NH, CT, NJ, PA, WV, VA)
Scientists continue to investigate the causes of bat white-nose syndrome (WNS), an emerging disease associated with the loss of an estimated half million insectivorous bats of 5 species in the eastern United States. Little brown bats and eastern pipistrelles have been particularly hard hit with as many as 90-100% of the local population wiped out at some winter hibernacula. Partnerships among state, Federal, academic, and non-profit organizations have formed to investigate the disease, its effects on bat populations, and management options. A rapid and specific test for detection of the WNS-associated fungus is under development. Data from an infection trial demonstrated that *G. destructans* is transmitted bat-to-bat and further experimentation is underway to determine whether the fungus is the sole causative agent of WNS. In addition, an environmental survey of caves was conducted to characterize the distribution of *G. destructans* in the eastern U.S. This study indicated that the fungus was present in cave sediments collected from hibernation sites within the WNS-infected region of the United States, indicating that the environment may play a role in the WNS transmission cycle. Other studies in which NWHC scientists are involved include summer bat surveys for evidence of latent infections, and evaluation of possible treatment or control options. Future WNS research directions include: determining the origin of *G. destructans*; predicting the potential for future WNS spread; investigating *G. destructans* pathogenesis mechanisms; developing strategies for WNS control and mitigation; and identifying bat survival strategies (e.g., are there resistant bats?). **Contacts:** David Blehert, National Wildlife Health Center, 608-270-2466, dblehert@usgs.gov; Anne Ballmann, 608-270-2445, aballmann@usgs.gov

Conservation/Ecological Support for WNS
The USGS Fort Collins Science Center (FORT) continues to collaborate with disease specialists and resource managers to understand the causes and conservation implications of white-nose syndrome to bats including synthesizing and reporting information on WNS to the scientific community and public (www.fort.usgs.gov/WNS); providing technical support on bat ecology to disease experts and resource managers; serving as a liaison between researchers in North America and those investigating observations of fungi on bats in Europe; and beginning this fall, developing and deploying video surveillance systems for use inside bat hibernation sites to investigate likely behavioral links between skin infection by *Geomyces destructans* and mortality associated with its presence. **Contacts:** Paul Cryan, Fort Collins Science Center, 970-226-9389, cryanp@usgs.gov

H5N1 Highly Pathogenic Avian Influenza
The Federal, State and Tribal partnership formed to develop and implement the National Interagency Early Detection System for Highly Pathogenic H5N1 Avian Influenza in Wild Migratory Birds has continued into its third year of surveillance. Birds have been tested from all 50 states and 6 freely-associated states and territories. While the surveillance focused on waterfowl, shorebirds, gulls and terns, a total of 284 species were sampled. So far, during the 2009 sampling year (April 1, 2009 – March 31, 2010), cooperating agencies collected and analyzed over 7,100 wild bird samples and the highly pathogenic avian influenza H5N1 virus was **not** detected. Since April 1, 2009, over 7,100 birds have been sampled for avian influenza at the NWHC. Of these, 49 have tested positive for avian influenza based on

molecular screening; 3 were H5 positive, but none were H5N1. No highly pathogenic avian influenza viruses have been detected so far. **Contact:** Scott Wright, National Wildlife Health Center, 608-270-2460, swright@usgs.gov

Oral vaccination of prairie dogs against plague via ingestion of vaccine-laden baits
Prevention of plague in wild rodents by immunization could reduce outbreaks of the disease. However, efficient large-scale immunization of free-ranging wildlife populations can only be achieved through voluntary consumption of vaccine. Poxviruses are ideal vectors for oral vaccines because they have a predilection for mucosal tissue in the mouth. In collaboration with scientists at the University of Wisconsin, Madison, the NWHC developed and tested plague vaccines using raccoonpox as a vector for two plague antigens. The vaccines were incorporated into palatable baits and offered several times over the course of several months to a group of 16 black-tailed prairie dogs (*Cynomys ludovicianus*) for voluntary consumption. Upon challenge with 70,000 cfu of virulent *Y. pestis*, the survival rate of the orally immunized group (94%) was significantly higher than the control group (7%). These findings demonstrate that oral immunization of prairie dogs with RCN-vectored vaccines can provide near full protection against challenge at dosages that simulate simultaneous delivery of the plague bacterium by numerous (3-10) flea bites. **Contact**: Tonie Rocke, National Wildlife Health Center, 608-270-2451, trocke@usgs.gov

Developing new animal models for CWD
Deer and elk are expensive and cumbersome experimental subjects, which has seriously hampered the rate of scientific discovery regarding CWD. Traditional lab mice are not good models for CWD, and genetically engineered mice can be problematic. We have discovered that meadow voles (*Microtus pennsylvanicus*) are extremely susceptible to CWD (100% penetrance via the intracerebral route) and are very promising wild-type models for CWD. We are currently using meadow voles in a series of CWD infectivity and bioassay studies. Additionally, we have determined that red-backed voles (*Myodes gapperi*), deer mice (*Peromyscus maniculatus*) and white-footed mice (*P. leucopus*) are all susceptible to CWD via intracerebral inoculation, but with longer incubation times than meadow voles. **Contact**: Bryan Richards, National Wildlife Health Center, 608-270-2485, brichards@usgs.gov

Quantitative epidemiology for CWD management
To design effective CWD management strategies for free-ranging herds, we need to understand the basic dynamics of CWD epidemics. Most of the data available on free-ranging herds comes from spatially referenced hunter-harvested samples that are tested for CWD. We have developed and applied sophisticated statistical epidemiological techniques to model and estimate the spatial-temporal dynamics of CWD. These analyses have helped explain the processes that drive the pattern of CWD prevalence typically seen on the landscape, and have suggested how management strategies might exploit these processes. An extensive manuscript detailing this new methodology has been submitted to Ecological Monographs and has been accepted for publication. **Contact:** Bryan Richards, National Wildlife Health Center, 608-270-2485, brichards@usgs.gov

Environmental Persistence of CWD
Deer have been shown to acquire CWD following habitation in environments previously contaminated with disease agent. Our research is focused on understanding the persistence of CWD in the environment, identifying reservoirs of infectivity and understanding routes by which deer acquire disease from the environment. More specifically, we are interested in how soil contributes to CWD transmission and how associated vegetation and microbes influence CWD infectivity on the landscape with respect to dissemination or degradation of agent. **Contact:** Bryan Richards, National Wildlife Health Center, 608-270-2485, brichards@usgs.gov

Remediation of CWD-contaminated sites
Anecdotal, epidemiological and controlled field experiments have all indicated that prions are stable in the environment and in soil. A goal of our research program is to identify and characterize biotic and abiotic means of degrading prions in the environment. We have found that certain lichens, common fungi-algae symbiotic organisms, contain a potent anti-prion activity that could influence CWD persistence on the landscape. Additionally, we have found that the common oxidative soil mineral birnessite (MnO2) is capable of degrading prions in *in vitro* experiments. We are pursuing each of these lines of study to try to achieve practical means of remediating CWD-contaminated sites. **Contact:** Bryan Richards, National Wildlife Health Center, 608-270-2485, brichards@usgs.gov

Brucellosis in the Greater Yellowstone Ecosystem (MT, WY, ID)
The USGS Northern Rocky Mountain Science Center hosted a conference on brucellosis related research in July 2009. The primary goals of the conference were to: 1) keep managers and researchers abreast of the ongoing and recently completed research projects, 2) provide a venue for discussion among researchers and managers, and 3) facilitate interactions across disciplines. The meeting consisted of 28 presentations and over 60 attendees from across the United States and Canada. In addition USGS scientists published a paper in Ecological Applications noting recent increases in brucellosis seroprevalence in Wyoming elk and assess the probable causes. **Contact**: Paul Cross: Northern Rocky Mountain Science Center, 406-994-6908, pcross@usgs.gov

Disease Investigations

Ranavirus confirmed in amphibians from several states (MA, TN, WY)
Several states had confirmed amphibian cases of ranavirus in the spring of 2009. The affected species included wood frogs, tiger salamanders, and marbled salamanders. The submitters from all 3 locations reported observations of amphibians with characteristic skin ulcerations or lesions. The infected tiger salmanders were collected from a reservoir in WY. The infected wood frog tadpoles were from a MA vernal pond that had previously experienced a ranavirus-associated mortality event in 2000 and 2001 resulting in a loss of >95% of the wood frog tadpoles and spotted salamander during those years. The ranavirus-infected marbled salamanders collected this spring were from a TN pond where ranavirus-infected amphibians also were recorded in 1999 and 2000 and amphibians with chytrid fungal infections were documented in 2001. **Contact:** Anne Ballmann, National Wildlife Health Center, 608-270-2445, aballmann@usgs.gov

Chytrid fungal infections in leopard frogs (NV, NY)
Chytridiomycosis, an epidermal infection caused by the pathogenic chytrid fungus, *Batrachochytrium dendrobatidis*, was detected in northern leopard frogs from NV and southern leopard frogs from NY. The northern leopard frog mortalities occurred during the winter of 2009 and were from captive reared populations that originated from the Truckee River where chytrid fungus was previously identified in the frog population. The southern leopard frog mortalities in the spring of 2009 were from a wild population in NY. This NY site was thoroughly surveyed this spring and researchers are confident that all the affected frogs were collected at that time. Clinical signs of chytridiomycosis include loss of righting reflex, lethargy, abnormal postures (e.g., frogs spreading legs away from body), and discolored or sloughing skin. Since chytrid fungus is most likely spread by direct contact between individuals or contact with infected water, frogs should not be moved from one area to another and should only be handled when necessary with clean equipment (gloves, sample bags, etc). **Contact:** Krysten Schuler, National Wildlife Health Center, 608-270-2447, kschuler@usgs.gov

Large mortality of Brandt's cormorants around San Francisco (CA)

Beginning in mid-April 2009, natural resource agencies, including National Oceanic and Atmospheric Administration (NOAA), California Department of Fish and Game (DFG), and U.S. Fish and Wildlife Service (USFWS) began receiving reports of dead and dying cormorants and other coastal birds in the Bay area. Dead Brandt's cormorants were found at a nesting colony on Alcatraz Island, and more were recovered on the coast from San Francisco Bay south to Monterey. In addition, dozens of sick cormorants were recovered by several local wildlife rehabilitation centers. Sick birds were found to be extremely emaciated. Brandt's cormorants and Western grebes were the primary species affected. Necropsy results from Brant's Cormorants sent to the USGS National Wildlife Center showed severe emaciation. Tests for domoic acid, a natural marine algae toxin fatal to birds, were negative, as were tests for Newcastle disease, avian influenza and West Nile virus. Researchers speculate that a strong upwelling may have averted a large amount of water and prey offshore. Since cormorants are near-shore feeders their prey base, including anchovies and juvenile sardines, may have been placed out of reach. **Contact:** Krysten Schuler, National Wildlife Health Center, 608-270-2447, kschuler@usgs.gov

Avian salmonellosis mortality confirmed in numerous states (AL, GA, ID, MD, ME, MI, MN, NY, NC, TN, VA, VT, WA, WI, WV)

Outbreaks of avian salmonellosis (*Salmonella typhimurium)* were confirmed in wild birds across the United States between February – May 2009. Suspected salmonellosis mortality also was reported from CA, UT, ME, and PA. Concerned citizens across the country reported finding dead or distressed wild birds near their homes and bird feeders. Public concern most likely was heightened as a result of concurrent yet unrelated Salmonella cases in humans and numerous product recalls. No evidence exists that the strains found in dead wild birds this year were the same strains of Salmonella that prompted the recalls in peanuts, pistachios, or wild bird seed. **Contact:** Anne Ballmann, National Wildlife Health Center, 608-270-2445, aballmann@usgs.gov

Bat mortality at summer roosts (CT, IN, NJ, CO, WA, TX, OR, UT)

The National Wildlife Health Center (NWHC) is investigating cases of higher than normal bat mortality at roost sites from multiple states this summer. Other states (MA, NH, WI) have received similar reports from the public and may reflect an increased awareness and population monitoring due to publicity about white-nose syndrome (WNS) in bats. In several cases, both adults and young pups are affected. Mortalities and submissions from the eastern and central US states have primarily in been big brown and little brown bats, whereas submissions from western states have included Townsend's big-eared, Brazilian free-tailed, Rafinesque's big-eared, and western long-eared bats. As observed in Summer 2008, some bats are roosting on outside walls during daylight hours and increased numbers of individuals are observed beneath maternity roosts. Anecdotal reports from several areas in the eastern US indicate a reduction in colony size compared to previous summers. Emaciation is a frequent finding from samples submitted to NWHC although trauma, predation, and rabies have also been identified as cause of death in several cases. Summer bats with evidence of moderate wing damage collected from areas where WNS was confirmed this past winter are being closely examined for the presence of *Geomyces destructans*, the fungus causing skin damage seen with this devastating disease. Thus far, however, there has been no evidence to support a link between these summer mortalities and WNS. **Contact:** Anne Ballmann, National Wildlife Health Center, 608-270-2445, aballmann@usgs.gov

Parasitic trematodes cause large-scale mortalities of waterbirds

Since 2002, up to 65,000 waterbirds have died along the Upper Mississippi River System (near La Crosse, WI) from disease caused by three parasitic trematodes that are carried by the invasive faucet snail. Birds can swallow lethal doses of the trematodes while feeding on the faucet snail or infected aquatic insect larvae. The Upper Mississippi River National Wildlife and Fish Refuge lies within the Mississippi Flyway, through which an estimated 40 percent of the continent's waterfowl migrate. Recently, bird mortality has spread south on the Refuge—about 125 miles downstream from Pool 7. Lesser scaup and American coots have had the largest mortalities. For lesser scaup, overall mortality is estimated at nearly

28,000–35,000 in Pool 7 and 20,000–25,000 in Pool 8. Other waterbirds affected include blue-winged teal, ring-necked duck, ruddy duck, bufflehead, redhead, Northern shoveler, mallard, American black duck, Northern pintail, gadwall, American wigeon, tundra swan, and herring gull. **Contact**: Rebecca Cole, National Wildlife Health Center, 608-270-2468, rcole@usgs.gov.

Amphibian Highlights

Non-lethal Sampling Methods for Detection of Pathogens among Captive and Wild Amphibians
In recent years, population declines and extirpations have been observed with alarming frequency among many species of amphibians worldwide. While habitat alteration and climate change are likely contributory factors in many, if not most, of such instances, infectious disease agents have also been identified in association with a number of amphibian die-offs. A number of other infectious agents including viral or mycotic pathogens, various systemic bacterial pathogens, and protozoan or metazoan parasites have also been recently associated with disease epizootics among different amphibian species. Reliable, non-lethal diagnostic methods that could be applied in studies, surveys, or disease investigations using wild amphibians in field or laboratory situations are needed. The objective of this on-going study is to develop a safe and effective non-lethal method to obtain tissue samples for the identification of systemic internal pathogens present among wild amphibian hosts, using ranavirus as a model infectious agent. **Contact:** Christine L. Densmore, Leetown Science Center, 304-724-4437, cdensmore@usgs.gov

Fisheries Highlights

Assessment of Health and Reproduction in Fishes from the Chesapeake Bay Drainage: Association with Habitat and Land Use
A high incidence of external lesions on bass and other fishes in the South Branch of the Potomac River (WV) raised concerns of management agencies as well as citizen's groups in recent years. In 2003, smallmouth bass were collected from sites along the South Branch of the Potomac, as well as, from reference or control sites in neighboring watersheds. Significant differences were observed in the prevalence of external lesions which included proliferative skin lesions (hyperplasia) and inflammatory responses to parasites. During this preliminary assessment, a high incidence of intersex was noted in male bass from a number of the sites (20-80%). Since then, scientists at LSC have been working with fishery biologists from the states of WV, VA, MD and PA to further explore the prevalence and severity of intersex in the upper Potomac River watershed. The work in FY2009 focused on 1) understanding the changes that may be occurring in the normal flora of the smallmouth bass mucus prior to and during fish kills in the Shenandoah and South Branch Potomac; 2) correlating the chemical and biological data collected at sites throughout the Potomac and other Chesapeake drainages to better understand sources of contaminants and landuse associated with reproductive endocrine disruption, specifically intersex in smallmouth bass and 3) sampling yellow perch from four tributaries (2 with good reproduction, 2 with moderate to poor reproduction) and assessing the reproductive endpoints that may help use understand the lack of recruitment in some tributaries. **Contact:** Vicki Blazer, Leetown Science Center, 304-724-4434, vblazer@usgs.gov.

Putative Bacterial Etiology of Lesions and Fish Kills in Virginia Rivers
The Virginia Department of Environmental Quality and the Department of Game and Inland Fisheries, along with their partners on the Shenandoah River Fish Kill Task Force (including the USGS), have researched putative causes of the fish kills that have occurred in the Shenandoah River watershed among smallmouth bass and redbreast sunfish since 2004. In previous work, *Aeromonas salmonicida* was identified as the principal pathogen isolated from the lesions of smallmouth bass, redbreast sunfish, and

rock bass from the Shenandoah, James, Cowpasture, and Jackson Rivers. In 2008, pre-kill microbial analyses were conducted from the South Branch of the Potomac River, the North and South Forks of the Shenandoah River, the James and Cowpasture Rivers when water temperatures approximated 10°C. As water temperatures climbed towards 15°C during late April, lesions and dead fish were reported from the North and South Fork of the Shenandoah River, the Cowpasture River, the James River, and the Jackson River. In each case, *Aeromonas salmonicida* was the principal pathogen isolated from lesions. Also, during this period a few fish were determined to be infected with Largemouth Bass Virus and neither of these latter two pathogens was considered prevalent enough to be the cause large-scale lesion development and mortality. Smallmouth bass from the Maury River (an unaffected river) were used in experimental challenges with smallmouth bass to fulfill Koch's postulates. In addition to these studies, additional field work was conducted to determine where reservoir of infections may exist in the natural environment. Those results are still pending analysis. **Contact:** Rocco Cipriano, Leetown Science Center, 304-724-4432, rcipriano@usgs.gov.

Etiology of Atlantic Salmon Egg Production Mortality and Implications for Restoration Programs in the Northeast

The USFWS Craig Brook (NFH) and Green Lake NFH were short approximately 400,000 fry for 2009 stocking in the Penobscot River Atlantic salmon recovery program in 2009. This resulted from egg mortality among approximately 40+ sea run females. Egg takes sustained poor eye-ups and there were many more complete family losses than normal. Although the losses have been most serious in the Penobscot River, other Downeast River stocks have also been affected. Externally and in all cases, *Pseudomonas fluorescens* was the predominant bacterium associated with the surface of all eggs. The results of assays conducted at the Leetown Science Center indicated that *P. fluorescens* was resistant to the germicidal activities of formalin. Consequently, the monoclonal nature of the bacterial flora on the surface of all eggs was not considered to be a function of disease but rather a result of the formalin treatments. The continued finding of *F. psychrophilum* within captive Atlantic salmon broodstock bears witness to its widespread and persistent prevalence, which presents additional concerns for Atlantic salmon restoration in New England. There is no reason to doubt that the bacterium does not persist within infected individuals after the egg has hatched. This was evident by the cases of sac fry and juvenile mortality continually diagnosed within restoration facilities. It appears that a significant portion of fry stocked into New England rivers may harbor the bacterium as a result of intraovum infection. The introduction of infected individuals may, therefore, have an adverse impact upon wild fish survival. **Contact:** Rocco Cipriano, Leetown Science Center, 304-724-4432, rcipriano@usgs.gov.

Estrogen disrupts expression of immune-related genes in largemouth bass

The co-occurrence of fish lesions and fish kills with a high incidence of intersex in fish from the Potomac and Shenandoah Rivers suggests that estrogen or estrogenic compounds, which may cause intersex, may also interfere with the expression of genes involved in innate immunity. Hepcidin is an antimicrobial peptide and iron-regulatory hormone that is conserved from fish to mammals. We identified two distinct hepcidin genes (*hep-1* and *hep-2*) in largemouth bass (*Micropterus salmoides*) and in smallmouth bass (*M. dolomieu*). In a laboratory study, we showed that estradiol reduced the constitutive expression of *hep-1* in the liver of largemouth bass. In addition, we showed that expression of a second hepcidin gene, *hep-2*, was induced in largemouth bass liver by exposure to bacteria, suggesting that this second hepcidin may have an antimicrobial function, and this induction was blocked by estradiol. Disruption of hepcidin expression, and possibly the expression of other immune-related genes, by estrogenic chemicals in the environment may make fish more susceptible to microbial infection and disease, providing a possible explanation for the coincidence of fish lesions and intersex in fish in the Chesapeake Bay Drainage rivers. The study, *Identification of centrarchid hepcidins and evidence that 17β-estradiol disrupts constitutive expression of hepcidin-1 and inducible expression of hepcidin-2 in largemouth bass (*Micropterus salmoides*)*, by USGS researchers Laura Robertson, Luke Iwanowicz, and Jamie Marie Marranca, was

published in the June 2009 issue of Fish & Shellfish Immunology. **Contact:** Laura Robertson, Leetown Science Center, 304-724-4579, lrobertson@usgs.gov.

Special Aquatic Animal Health Issue: Scientists from the USGS continue collaboration with the Department of Fisheries and Oceans, Canada, to produce a special issue in the Journal of Aquatic Animal Health, "Progress Toward Understanding the Complexities of Thiamin Deficiency in Aquatic Organisms," due out in September 2009, by D.C. Honeyfield, D.E. Tillitt and J.D. Fitzsimons. Thiamin deficiency causes fry and adult mortality, changes in behavior, and has biochemical and histological effects. Reported in this issue includes studies from the Great Lakes, Baltic Sea and lakes in Florida; laboratory and field investigations; salmonids, carp, alewife, gizzard shad and alligators. Although results reported in this issue and elsewhere increase knowledge about thiamin deficiency, impacts at the population level are yet to be determined. Thiamin deficiency poses significant problems for fishery management requiring development of strategies to address this complex nutrition and health puzzle. **Contact**: Donald Tillitt, Columbia Environmental Research Center, 573-876-1886, dtillitt@usgs.gov

Contaminants Highlights

Fish Health in the Shenandoah and James River Basins : Fish exhibiting external lesions, incidences of intersex, and death have recently been observed in the Shenandoah and James River Basins. These basins are characterized by widespread agriculture (intensive in some areas), several major industrial discharges, numerous sewage treatment plant discharges, and urban, transportation, and residential growth. Using state-of-the-art passive contaminant samplers, sites along the Shenandoah and James River Basins in Virginia were studied to identify chemicals that may have contributed to declining fish health. Chlorpyrifos, endosulfan, and lindane were the most commonly detected chlorinated pesticides. Atrazine was detected at concentrations much greater than other pesticides associated with agricultural use. Fragrance components, caffeine, the nicotine metabolite cotinine, prescription pharmaceuticals, and natural and synthetic hormones were detected at several sites where an elevated incidence of intersex in smallmouth bass populations occurred. **Contact**: David Alvarez, Columbia Environmental Research Center, 573-441-2970, dalvarez@usgs.gov