



**Report to the Fish and Wildlife Health Committee
of the Association of Fish and Wildlife Agencies
from USGS Science Centers
September 9, 2012**

Wildlife Highlights

Sylvatic plague vaccine for prairie dogs

Laboratory studies have demonstrated that oral vaccination of prairie dogs against plague using raccoon pox-vectored vaccine is feasible, resulting in significant protection against challenge with *Yersinia pestis*. Further laboratory studies to assess duration of immunity in prairie dogs and vaccine safety and efficacy in non-target species are ongoing. The Sylvatic Plague Vaccine (SPV) Subcommittee, under the direction of the Executive Committee of the Black-footed Ferret Recovery Implementation Team, is continuing its work to complete development and delivery of the sylvatic plague vaccine (SPV) as a management tool to combat plague in prairie dogs and promote the recovery of the black-footed ferret. Field trials to confirm the safety of the vaccine in non-target animals are underway. Field sites to assess vaccine efficacy in free-ranging prairie dogs are being selected for studies beginning in 2013. **Contact:** Tonie Rocke, National Wildlife Health Center, trocke@usgs.gov

North America bat white-nose syndrome update, Winter 2011/2012

Geomyces destructans, the fungus that causes fatal bat white-nose syndrome (WNS), continued to spread this past winter season. Most notably, WNS was confirmed in little brown bats at a hibernaculum west of the Mississippi River for the first time in Lincoln County, Missouri and the southern Appalachians of northern Alabama (Jackson County). Additionally, WNS was confirmed in federally listed, endangered gray bats in two counties (Hawkins and Montgomery) in Tennessee. While unusual mortality was not detected at these locations or among the gray bats, and mortality remains low along the western disease front, an estimated 5.5 million bats are believed to have died from WNS since it was first recognized five years ago near Albany, New York. Winter bat populations have been reduced more than 80% in the northeast and mid-Atlantic United States, although there are some early indications that bat populations may have stabilized at some of the original affected sites in New York. White-nose syndrome was also confirmed at the only known bat hibernaculum in Delaware for the first time this past winter, although *G. destructans* had been previously detected on bats returning early to known maternity roosts in April 2010 from this hibernaculum. WNS has now been confirmed in 19 states and 4 Canadian provinces, and it continues to expand into new counties and districts within the affected area. Iowa recently announced the detection of low levels of *G. destructans* DNA on a single big brown bat showing no clinical signs of disease in Jackson County. The viability of the fungus at the Iowa site is still unknown. To date, WNS has been confirmed in endangered gray bats (*Myotis grisescens*), little brown bats (*M. lucifugus*), Northern long-eared bats (*M. septentrionalis*), Eastern small-footed bats (*M. leibeei*), endangered Indiana bats (*M. sodalis*), tricolored bats (*Perimyotis subflavus*), and big brown bats (*Eptesicus fuscus*). To view a recent (June 2012) fact sheet on WNS that includes updates on collaborative WNS research activities by USGS, please visit http://www.nwhc.usgs.gov/publications/fact_sheets/pdfs/WNS_Factsheet_2012.pdf. **Contact:** David Blehert, National Wildlife Health Center, dblehert@usgs.gov

Avian cholera in California

Pasteurella multocida (avian cholera) epizootics were reported in various locations within nine California counties during January –April 2012. Cases were investigated and reported by the California Department of Fish and Game in partnership with USGS National Wildlife Health Center. The smallest event involved an estimated 75 dead wood ducks on a pond in Butte County. The largest event was at Tule Lake/Lower Klamath Lake NWR (Klamath Basin NWR Complex), where refuge staff and volunteers retrieved 3,908 dead birds between mid-February and late April. Staff members estimate this is approximately one-third of the birds that died during the event. The species most affected were snow goose, American coot, American widgeon, white-fronted goose, and northern pintail. This was the largest

outbreak of avian cholera the Klamath Basin NWR staff has seen since 2008 when an estimated 10,000 birds died due to avian cholera. **Contact:** Barbara Bodenstern, National Wildlife Health Center, bbodenstern@usgs.gov

Avian cholera in the Midwest

Avian cholera was the second leading cause of avian mortality events (after trematodiasis) reported to the USGS National Wildlife Health Center in the Central and Mississippi flyways during the first quarter of 2012. Greater and lesser snow geese and Ross's geese were the primary species involved in all four mortality events in the Midwest. The first 2012 cholera event in the Midwest occurred in Pike County, Missouri, at the beginning of February and involved less than ten geese. The other three events in the Midwest were reported shortly thereafter, first in northwestern Missouri (Holt County) followed by Iowa and Nebraska. The event in Iowa involved an estimated 150 geese in Fremont County in a wildlife management area. The last avian cholera event in that area was reported in 2003. The largest event in the Midwest in 2012 occurred in Nebraska across multiple Waterfowl Production Areas in Clay, Phelps, and Kearney Counties and involved almost 1,300 birds, primarily snow geese and Ross's geese, as well as a few other species of waterfowl, such as Northern pintails and mallards. The avian cholera mortality in 2012 in Nebraska was the highest for the state since 1999 when an estimated 1,400 died from this disease. **Contact:** LeAnn White, National Wildlife Health Center, clwhite@usgs.gov

Lake Michigan volunteer AMBLE (Avian Monitoring for Botulism Lakeshore Events) program

Scientists from the U.S. Geological Survey, the National Park Service, and the private sector are working together to explore the ecological pathways through which botulism toxin produced by a natural bacterium (*Clostridium botulinum*) is transported to birds. The help of volunteer beach monitors to record timing, numbers, and species of bird carcasses deposited on beaches is providing valuable information needed to better understand this important wildlife disease. AMBLE volunteers monitored a total of 17.2 miles of transects in 2011 and found 82 dead birds. Double-crested cormorants, ring-billed gulls, and herring gulls were the most frequently found carcasses. Botulism type E was confirmed in multiple species submitted to NWHC for testing, indicating that low-level avian mortality due to ingestion of botulinum toxin type E can occur absent of large die-off events. As of mid-August 2012, over 22 miles of transects are being covered and 47 dead birds have been reported. Continued monitoring of beaches will provide further insight into epidemiological patterns of avian botulism. More information about AMBLE can be found at <http://www.nwhc.usgs.gov/AMBLE/> **Contact:** Jennifer Chipault, National Wildlife Health Center, AMBLE@usgs.gov

Disease Investigations

Investigating alopecia in polar bears of South Beaufort Sea, Alaska

During March through May of 2012, the Polar Bear Project field capture crew of the USGS Alaska Science Center observed multiple cases of alopecia and skin lesions on bears captured at the boundary of the Southern Beaufort seas. The USGS Polar Bear Project and the USGS NWHC are investigating the occurrence of the alopecia and skin lesions on various samples that were collected from bears during the 2012 field season to determine the causative agent and the potential implications to polar bear health. Similar alopecia and skin lesions have previously been observed in 1999, when clinical signs were observed in approximately 19% of the bears captured by the USGS Polar Bear Project. No causative agents were identified at that time. **Contact:** Barbara Bodenstern, National Wildlife Health Center, bbodenstern@usgs.gov

Common murre winter mortalities, Alaska

Two common murre mortality events were reported during January through March in Skagway/Sitka Burroughs and Afognak Island, Alaska. Birds were found dead or weak and unable to fly or escape capture. An estimated 15 birds were found dead, primarily in Nahku Bay, Skagway. An estimated 250 were found in bays near on Afognak Island. Historically, common murre are not frequent visitors to Skagway bays, but this year there were 120 counted on the Christmas Bird Count. Severe winter weather with temperatures hovering around zero to 10 (F) and north winds (20 mph, with gusts to about 40) was reported in Skagway. The probable cause of death in specimens submitted to the NWHC was attributed to

emaciation and starvation. **Contact:** Barbara Bodenstein, National Wildlife Health Center, bbodenstein@usgs.gov

Toxoplasmosis in Hawaii, Hawaii

The cat parasite *Toxoplasma gondii* continues to intermittently kill endangered birds in Hawaii and the Pacific with recent cases in Hawaiian geese, Hawaiian coots (from Kauai) and purple capped fruit dove from American Samoa. This parasite occurs in pacific islands where feral cats are present. For more information, see <http://www.nwhc.usgs.gov/hfs/Toxoplasmosis.jsp> Contact: Thierry Work, 808-792-9520, Thierry_work@usgs.gov

Invasive corallimorph on Palmyra, Hawaii

In 2007, the NWHC Honolulu Field Station (HFS) documented a corallimorph (a type of anemone) colony smothering coral reefs surrounding a shipwreck at Palmyra Atoll NWR. In 2011, another survey showed that the infestation on the reef has increased from 1 to 3 square kilometers. Trials to eradicate this anemone were done in 2011 and are promising but labor intensive. We suspect metals leaching from the ship may be fueling the growth of the anemone, and DOI is looking at options of removing the wreck from the atoll. Contact: Thierry Work, 808-792-9520, Thierry_work@usgs.gov

Amphibians

Detection of sublethal effects of atrazine on amphibians in the Atchafalaya River Basin (ARB), Louisiana, United States

Atrazine, an herbicide known to cause health problems in vertebrates, is the most commonly detected pesticide in the Atchafalaya Basin and Mississippi River. The goal of this study was to determine whether a range of environmental concentrations of atrazine may be associated with molecular, cellular, and organ responses in amphibians exposed to waters containing atrazine in this extensive wetland ecosystem. The results of this study suggest that the sublethal effects observed in frogs at the cell and molecular level followed the gradient of atrazine concentrations measured in the ARB wetland ecosystem. **Contact:** Hardin Waddle, National Wetlands Research Center, waddleh@usgs.gov

Fisheries Highlights

Fish disease investigations in hatcheries and wild fish in Virginia rivers

Since 2007, Virginia has experienced a chronic disease among smallmouth bass (*Micropterus dolomieu*) and redbreast sunfish (*Lepomis auritus*) in the Shenandoah, Cowpasture, Jackson, and James Rivers. Occurrence of disease correlated with recovery of *Aeromonas salmonicida* subsp. *salmonicida*, characteristically a coldwater pathogen, from lesions of diseased fish. During 2010 - 2012, we evaluated the prevalence of infection at approximately 5 sites equidistantly spaced from source to their mouth in both the Shenandoah and James Rivers. Presence of the pathogen and the expression of disease correlated with those areas in each river system that had karst geology, characterized by coldwater, underground springs, which apparently provide coldwater refugia that are necessary for the persistence of *A. salmonicida* in an ecosystem that otherwise surpasses this bacterium's upper thermal tolerance. Stocked trout are also present in many of these areas. Because of this, attempts are currently underway to reduce and/or eliminate the amount of disease associated with *A. salmonicida* in the Virginia hatchery system, especially at the Marion, Wytheville, and Coursey Springs State Fish Culture Stations. **Contact:** Rocco Cipriano, Leetown Science Center, rcipriano@usgs.gov

Investigating the possible association of virus with orocutaneous lesions in brown bullhead

The observation of tumors in brown bullheads is currently used as a 'Beneficial Use Impairment (BUI)' in Great Lakes Areas of Concern. An increased incidence of hepatic neoplasms is associated with polyaromatic hydrocarbons (PAHs) and other contaminant exposure in this species. Likewise skin tumors, including papillomas and squamous cell carcinomas have been used as indicators of chemical exposure in bullhead and other species. Although no cause-and-effect correlations have been established for wild populations, papillomas have been experimentally induced in brown bullhead by repeated dosing

of the skin with sediment extracts that contained high levels of PAHs. While there is considerable evidence that contaminants are associated with skin tumors in the brown bullhead, biotic factors including viruses are known to induce tumors in mammals and lower vertebrates. If viruses are the cause of these tumors, they may not be indicators of contaminant exposure as currently employed. Given that management decisions are made based on the assumption that all skin tumors are associated with contaminant exposure, critical comprehensive investigation into this matter to determine if this is correct in all circumstances will be important for risk assessments. **Contact:** Luke Iwanowicz, Leetown Science Center, liwanowicz@usgs.gov

Novel fish virus highlighted at International Biology Symposium

Research from the Western Fisheries Research Center on the biomedical applications of a novel fish virus was outlined in an invited presentation before the 2012 World Congress of *In Vitro* Biology. The virus, termed cutthroat trout virus (CTV), was characterized by researchers at the WFRC and found to be closely related to Hepatitis E virus, the leading cause of viral hepatitis among humans in the developing world. Importantly, there is no cell culture system for Hepatitis E virus and only non-human primates are suitable as animal models. Thus, the ability of CTV to be grown *in vitro* using established fish cell lines provides an important tool for antiviral drug testing and opens the use of fish for studies on mechanisms of immunity that can assist in vaccine development. **Contact:** Jim Winton, Western Fisheries Research Center, jwinton@usgs.gov

Genomic characterization of a virus from fathead minnows

Scientists from the Western Fisheries Research Center and University of Arkansas reported the molecular characterization of a novel fish virus known as fathead minnow nidovirus. The complete genome of this unusual fish virus was sequenced and found to be unique, yet most similar to another virus found in a different fish species in Germany. Molecular based detection methods have been able to confirm several occurrences of fathead minnow nidovirus in other mid-west states. For more information, see: <http://www.ncbi.nlm.nih.gov/pubmed/22422065>. **Contact:** Bill Batts, Western Fisheries Research Center, bbatts@usgs.gov

Demonstration of a key facet of innate immunity to Francisella

Researchers from the USGS Western Fisheries Research Center and the University of Washington published their findings on the activation and regulation of inflammation in response to an emerging pathogen in fish. In the report, the scientists demonstrate the proteolytic processing of a key mediator of innate immunity during infection with the fish-specific pathogen, *Francisella noatunensis* in zebrafish. Importantly, this response is analogous to what occurs during *Francisella* infection in mammals (tularemia) thus demonstrating the utility of the zebrafish infection model not only for fish health but human health as well. For more information see: <http://iai.asm.org/content/80/8/2878.abstract> **Contact:** John Hansen, Western Fisheries Research Center, jhansen@usgs.gov

Potential biomarker for domoic acid exposure

Researchers from the Western Fisheries Research Center in collaboration with investigators from NOAA, The Marine Mammal Center, The University of California, and The University of Washington report their findings of chronic domoic acid exposures in zebrafish. The research consortium found that repeated, low-level exposures of domoic acid (a neurotoxin produced during harmful algal blooms) resulted in significant neurosensitivity in zebrafish. In addition, initial results from zebrafish that were later confirmed in Sea Lions, imply that vertebrates can mount an antibody response to domoic acid which could serve as a trackable biomarker for addressing chronic domoic acid exposures in wildlife and humans. Collectively, these findings could have profound implications for human populations that regularly consume fish or shellfish containing low-levels of domoic acid. Results appeared in the May 2nd release of PLoS One:

<http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0036213>. A summary of this work is available at: http://www.nwfsc.noaa.gov/features/new_biomarker/new-biomarker.cfm. **Contact:** John Hansen, Western Fisheries Research Center, jhansen@usgs.gov

Predictive factors for infection of fish with VHS Virus

Investigators at the Western Fisheries Research Center in collaboration with Cornell University determined the presence of viral hemorrhagic septicemia virus (VHSV) in yellow perch and round gobies in Lake Ontario and the St. Lawrence River. The potential predictive factors associated with infection were evaluated, showing that mature yellow perch were more likely to test positive than immature fish. Sequencing results increased the known genetic diversity of this important virus. This study provides the basis for more comprehensive monitoring and risk assessment of VHSV in wild fish populations. For more information, visit <http://www.sciencedirect.com/science/article/pii/S0380133012000081>. **Contact:** Bill Batts, Western Fisheries Research Center, bbatts@usgs.gov

Immunological tools for marine fish health

Researchers from the Western Fisheries Research Center and Marrowstone Marine Field Station reported the development of immunological markers for Pacific herring. Pacific herring are a key forage fish species and are highly susceptible to viral hemorrhagic septicemia virus (VHSV). Herring may contribute to the persistence and cycling of VHSV in the marine environment. This study first identified a number of Pacific herring immunological genes. Next, a suite of gene expression assays were developed and used to profile the herring anti-VHSV response. In addition, a set of monoclonal antibodies against herring IgM were created. These immunological tools will be useful for future studies to investigate antiviral immunity in Pacific herring populations. For more information: <http://www.ncbi.nlm.nih.gov/pubmed/22155011>. **Contact:** Maureen Purcell, Western Fisheries Research Center, mpurcell@usgs.gov

Effects of standard 3-pass electrofishing on fishes in coldwater streams

In this study we examined the effects of electrofishing on native, non-game fishes and in particular, those non-target species subjected to multiple electrical shocks routinely captured during depletion sampling programs. We included 675 fish of seven species in the experiment. We examined radiographs of 355 fish for spinal injuries and performed necropsies on 303 fish for hemorrhagic trauma in soft tissue. While rainbow trout and brook trout generally sustained the highest incidence and severity of injuries, those injuries were generally independent of the number of electrical shocks. The exception to this was the frequency of hemorrhagic trauma in rainbow trout which demonstrated a linear relationship ($p < 0.05$) with the number of shocks. Thirty-day post shock survival for the salmonids was better than 94% and for the non-salmonids, ranged from a low of 80% in fathead minnows to 100% survival in green sunfish and channel catfish. **Contact:** Frank M. Panek, Leetown Science Center, fpanek@usgs.gov

Application of biotechnology in addressing Asian carp management initiatives

Asian carp are nonindigenous in the U.S. and ecological and economic damage may result when populations become established in non-permitted habitats. A national Asian carp management and control plan (Plan) (Conover et al. 2007; asiancarp.us) spells out goals with specific strategies. The objective of this research employing flow cytometry (FCM) technology in assessing ploidy with Asian carp species was to develop protocols beneficial to the producers of triploid carp, as well as to address needs of natural resource managers. These advances make possible 1) accurate estimates of ploidy percentages of batches of larvae after yolk absorption, as verified in field grow-out trials; 2) ploidy determinations from dead fish through analysis of their eyes, within 2 weeks if maintained cooled or frozen with 20% methanol in buffer, and; 3) establishment of blood from Nile tilapia *Oreochromis niloticus* as an optimal internal control for assessing ploidy of field-caught or hatchery-produced triploids by virtue of their genome size of 2.40 pg as determined by FCM. Integrating FCM expertise within committees which coordinate and drive implementation efforts among partner agencies will promote attainment of the Plan goals of Asian carp management and control. **Contact:** Jill Jenkins, National Wetlands Research Center, jenkinsj@usgs.gov

Use of AQUI-S® 20E by in U.S. fishery management programs as an immediate release sedative

USGS scientists completed work to: characterize the response of freshwater fish to feeding stimuli after sedation by AQUI-S® 20E, estimate the number of fish sedated in fishery management activities, and estimate the number of fish that might be captured after sedation. Based on those data and the residue depletion data, the FDA concluded that the risk of human exposure to AQUI-S® 20E residues in fish immediately released after sedation during fishery management activities was best modeled using a

process to establish an Acute Reference Dose. Based on the USGS information and other publicly available information, FDA established a tolerance of 400 mg eugenol/kg fish tissue. As a result of the preceding work, the U.S. Fish and Wildlife Service Aquatic Animal Drug Approval Partnership (AADAP) program was successful in obtaining an amended authorization for its investigational new animal drug permit to allow immediate release of freshwater fish sedated with AQUI-S® 20E. Now participants in the AADAP study may use AQUI-S® 20E in the field and immediately release those fish back to the waters where they were captured instead of holding those fish for four days following sedation. **Contact:** Mark Gaikowski, Upper Midwest Environmental Sciences Center, 608-781-6284, mgaikowski@usgs.gov.

Contaminants Highlights

Zebrafish model for immune suppression by contaminants

Researchers at the USGS Western Fisheries Research Center and the Columbia Environmental Research Center are utilizing a zebrafish exposure model and accompanying genomic resources to examine the impact of estradiols and cadmium on innate immunity; the first line of immunity for all vertebrates. Fish were exposed to environmental concentrations of 17 α -ethinylestradiol or cadmium and then subjected to immune stimulation. Immune-relevant tissues were examined for differential gene expression using a genome-wide microarray for zebrafish. Results indicated that acute and sub-chronic exposures to 17 α -ethinylestradiol and cadmium have adverse impacts on immune function thus impacting overall fish health. **Contact:** John Hansen, Western Fisheries Research Center, 206-526-6282; jhansen@usgs.gov

Investigations of tissues obtained from field animals necessitate successful handling to maintain sample quality

Transfer of live tissues from remote locations is often impractical due to degradation during shipment. In this study, a pragmatic method was developed for assessing sperm chromatin structure by the use of fixed milt. Reproductive condition can be impacted by contaminants, so as part of a larger study in the Chesapeake Bay on female and male yellow perch (YP), *Perca flavescens*, a new biomarker assay for use with any species was developed for measuring DNA fragmentation. DNA fragmentation leads to effects in the individual, such as lessened fertility, and to the population, such as lower hatch rates. The YP were collected during spawning runs over three years from sites with varied land use characteristics impacting water quality among Chesapeake Bay tributaries, and several male andrology parameters were measured. **Contact:** Jill Jenkins, National Wetlands Research Center, jenkinsj@usgs.gov

Recent NWHC Publications of Interest

USGS research on avian influenza in seals

USGS NWHC microbiologist Hon Ip is a co-author of a paper on avian influenza recently published in MBio titled "Emergence of fatal avian influenza in New England harbor seals." The article reports the first finding of influenza A virus (subtype H3N8 of avian origin) isolated from seals. The seals died during an unusual mortality event in coastal New Hampshire. Mortality from influenza A infections have occurred in the past among free-ranging seal populations although this is the first report of the H3N8 subtype. The article can be accessed at <http://mbio.asm.org/content/3/4/e00166-12> This event was reported as a top story by the USGS, available at http://www.usgs.gov/blogs/features/usgs_top_story/mutated-flu-virus-kills-new-england-seal-pups/?from=title Diagnostic investigations continue on available samples. **Contact:** Anne Ballmann, National Wildlife Health Center, aballmann@usgs.gov

USGS circular on plague

Scientists at the National Wildlife Health Center recently released *Plague*, USGS Circular 1372. *Plague* offers readers an overview of this highly complex disease caused by the bacteria *Yersinia pestis*. The history of the disease, as well as information about *Yersinia pestis* and its transmission by fleas, is described. Tonie E. Rocke, the senior author, is a prominent researcher on oral vaccination of prairie dogs to prevent plague. She is currently working to transfer her success in the laboratory to the field to control plague in prairie dogs. Rachel C. Abbott is assisting Dr. Rocke in this process and will coordinate field

trials of the vaccine. More information about the publication, including a PDF, can be viewed at <http://pubs.usgs.gov/circ/1372/>

Wildlife health bulletin on surveillance for avian influenza

In May 2012, NWHC issued a Wildlife Health Bulletin entitled “NWHC Plan for Avian Influenza Surveillance of Wild Birds” that summarized the testing conducted during the surveillance program from 2006 to 2010, and explained that the NWHC is continuing surveillance for H5N1 highly pathogenic avian influenza by focusing on testing sick and dead migratory birds, particularly ducks, geese, and swans. The bulletin also reminds wildlife managers to be alert regarding wild bird morbidity and mortality events that meet certain criteria. The bulletin can be viewed at http://www.nwhc.usgs.gov/publications/wildlife_health_bulletins/WHB_2012-03_AI_Surveillance.pdf

WNS-Related Publications

USGS NWHC microbiologist David Blehert is the author of a paper on white-nose syndrome titled “**Fungal disease and the developing story of bat white-nose syndrome,**” published in PLoS Pathogens <http://www.plospathogens.org/article/info%3Adoi%2F10.1371%2Fjournal.ppat.1002779>

Frequent arousal from hibernation linked to severity of infection and mortality in bats with white-nose syndrome. PLoS One 7(6):e38920. Reeder, D. M., and others. This publication includes an appendix titled [Histologic Severity Scoring of WNS using Wing Membrane](#) developed by NWHC wildlife pathologist Carol Meteyer