

U.S. Fish and Wildlife Service



**Avian Vacuolar Myelinopathy in the Southeast:
An Ecoepidemiological Assessment with Emphasis
on Lake Surf, North Carolina**

Final report: Off-Refuge Contaminant Study 4F33

May 2008

U.S. Fish and Wildlife Service
Ecological Services
Raleigh, North Carolina



Preface

Between 1995 and 2005, the U.S. Fish and Wildlife Service and partners investigated the occurrence and significance of avian vacuolar myelinopathy in North Carolina. One aspect of the work was a cooperative study on timing and causes of the disease, with a series of experiments conducted at, or with waterbirds or environmental samples collected from, Lake Surf (also known as Woodlake), near Vass, North Carolina. All study results have been published in peer reviewed journals, and this report merely synthesizes those publications which should be referenced for detailed methods and results. The work was coordinated by Tom Augspurger in the U.S. Fish and Wildlife Service's Raleigh Field Office and Dr. Tonie Rocke of the U.S. Geological Survey's National Wildlife Health Center in Madison, WI. Partners in different aspects of the work included the National Wildlife Health Center, Southeastern Cooperative Wildlife Disease Study, North Carolina State University, Indiana University, and the North Carolina Wildlife Resources Commission. Funding was provided by the U.S. Fish and Wildlife Service's Division of Environmental Contaminants (study identifiers 4F33 and 200040002.1).

Copies of each of the published papers (listed in Table 1) from this study, which document all aspects of methods and results, can be obtained from the U.S. Fish and Wildlife Service at the following address:

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Suggested citation: Augspurger TP. 2008. Avian Vacuolar Myelinopathy in the Southeast: An Ecoepidemiological Assessment with Emphasis on Lake Surf, North Carolina. Final Report: Off-Refuge Contaminant Study 4F33, U.S. Fish and Wildlife Service, Raleigh, NC.

Cover photo credit: Mallard with clinical signs of avian vacuolar myelinopathy at Lake Surf (Woodlake), North Carolina. Photo by John Fischer, Southeastern Cooperative Wildlife Disease Study

Avian Vacuolar Myelinopathy in the Southeast: An Ecoepidemiological Assessment with Emphasis on Lake Surf, North Carolina

Executive Summary

Between 2000 and 2005, the U.S. Fish and Wildlife Service (Service) and partners conducted an investigation of avian vacuolar myelinopathy (AVM), an unusual neurological disease which has killed more than 100 bald eagles (*Haliaeetus leucocephalus*) since and thousands of coots (*Fulica americana*) wintering in Arkansas, Georgia, North Carolina, and South Carolina. By light microscopy, affected birds show diffuse, spongy degeneration of central nervous system white matter; by electron microscopy, the lesion is characterized as vacuoles formed in the myelin sheaths. The study was conducted to elucidate the etiology of AVM with a focus on 1) pathway identification through field and lab trials to reproduce the disease; and 2) toxicant identification through chemical analyses of birds and other media at affected and unaffected sites. Lake Surf (also known as Woodlake), North Carolina, was the site for most of the work. We posed these specific study questions to address aspects of the disease not understood in 2000:

- Do birds arrive with the disease upon migration or get sick upon arrival?
- When is disease onset and what is its duration?
- Do birds with AVM always die, or can they get recover?
- What at the lakes with AVM-affected birds might be causing the disease?

Components of the study have been summarized in five peer reviewed publications (Larsen et al. 2002, 2003; Rocke et al. 2002, 2005; Dodder et al. 2003), and these papers provide detailed methods and study results. Some key findings include:

- Exposure to the causative agent of AVM is site-specific (birds get the disease locally)
- Exposure to the causative agent is seasonal, autumn to early winter
- Onset of disease can be rapid, within as little as 5 days post-exposure
- Mallards (*Anas platyrhynchos*) can be effectively used as sentinels to monitor the disease
- The severity of clinical signs does not appear closely linked with the severity of brain lesions; some birds with no clinical signs had lesions described as severe and several birds with obvious neurologic impairment had brain lesions described as mild
- Clinically-affected birds could regain function with supportive veterinary care
- AVM was not transmitted by direct contact between affected birds and healthy birds
- Feeding studies were inconclusive (although others have since experimentally reproduced the disease through feeding)
- Neither the sediments nor tissues analyzed show any compounds that are present in all of AVM positive lakes but not present (or of low abundance) in AVM negative lakes

This study and others have not defined a specific cause. Currently, the cause is thought to be a naturally produced toxin associated with something in or on aquatic plants at the affected sites. Results of our study have been routinely cited in the work of others, indicating that the cooperative assessment provided advances in understanding of the disease.

Key words: Avian vacuolar myelinopathy, North Carolina, American coot, *Fulica americana*, mallard, *Anas platyrhynchos*, sentinel

CONTENTS

PREFACE 2

EXECUTIVE SUMMARY 3

INTRODUCTION 5

OUTCOMES

 Findings 8

 Accomplishments and management recommendations. 14

REFERENCES 16

INTRODUCTION

A disease of unknown etiology is responsible for nervous system lesions leading to morbidity and mortality of birds in the southeastern United States. The disease is avian vacuolar myelinopathy (AVM), and it has been confirmed in five States. Extensive pathology pointed to a noninfectious disease with a toxicant as the most likely cause (Thomas et al. 1998). This investigation and others have still not defined a specific causative agent, but the disease has been experimentally reproduced through feeding studies (Fischer et al. 2003; Birrenkott et al. 2004) and the cause is thought to be a naturally produced toxin associated with something in or on aquatic plants at the affected sites (Wilde et al. 2005; Wiley et al. 2007; Williams et al. 2007), all of which are man-made impoundments or reservoirs.

This study was initiated in 2000 to expand efforts in two areas: 1) toxicant identification (by chemical analyses of environmental media at the sites and of tissues from affected birds); and 2) pathway identification (by field and laboratory efforts to reproduce the lesions).

The justification for the project was founded on the severity of impacts to migratory birds and indications of a toxicant mediated etiology. Each of these factors is described briefly below.

Severity of Impacts to Migratory Birds

AVM can cause significant impairment. Clinical signs of affected birds include those consistent with central nervous system impairment of motor function (uncoordination, abnormal movement and posture, weakness, paralysis). Affected birds have erratic flight or are unable to fly, may crash land, swim tipped to one side (Figure 1) with one or both legs or wings extended or be in the water on their back with their feet in the air. On land, birds stagger and have difficulty walking and may fall over, unable to right themselves (USGS 1999a). They may also be lethargic and remain away from a larger flock of the same species. The degree of impairment may make affected individuals more vulnerable to predation and environmental stressors, such as cold stress.

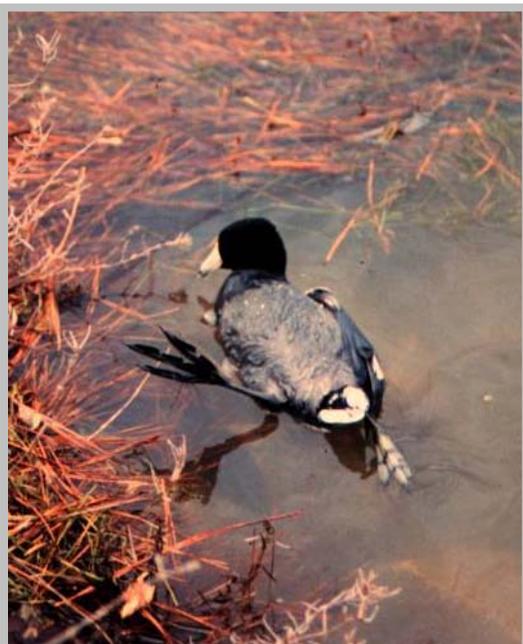


Figure 1. American coot (*Fulica americana*) with impaired motor function from Woodlake, North Carolina. Avian vacuolar myelinopathy can only be confirmed through microscopic examination of brain tissue, and this bird was diagnosed with the disease. Affected coots at this lake show a range of clinical signs with severe impairment such as this birds' inability to fly, inability to right itself (swimming tipped to one side or on its back with one or both legs or wings extended).

Photo: USFWS, Raleigh

AVM can also cause bird deaths. In the winters of 1994 and 1996, vacuolar myelinopathy killed at least 55 bald eagles (*Haliaeetus leucocephalus*) at three lakes in southwestern Arkansas, along with an unknown number of American coots (*Fulica americana*). Those events led to the first account of epizootic vacuolar myelinopathy (Thomas et al. 1998) which became known for a brief time as “coot and eagle brain lesion syndrome.” In 1999, pathologists confirmed that bald eagles collected from four new locations (Lake Surf, North Carolina; Aiken, South Carolina; and Strom Thurmond Lake and Lake Juliette, Georgia) died from the same brain disease (USGS 1999a, b). Dead coots have also been diagnosed with the disease in all these States. Total coot mortality throughout the region was unknown; documented mortality in North Carolina between 1990 and 1999 was >400 coots with the total estimated mortality in excess of 1,000.

At the time this investigation was proposed, vacuolar myelinopathy was also hypothesized as the likely cause of morbidity in several buffleheads (*Bucephala albeola*), one American widgeon (*Anas americana*), a northern shoveler (*Anas clypeata*) and mallard (*Anas platyrhynchos*) at the North Carolina lake based on field signs and preliminary pathology results. Subsequent confirmation of the disease in waterfowl (Augsburger et al 2003), owls, and shorebirds (Fischer et al. 2006) expanded the range of avian taxa impacted to five avian orders: gruiformes (rails, cranes coots, and allies); anseriformes (waterfowl); falconiformes (diurnal birds of prey); charadriiformes (shorebirds, gulls and alcids); and, strigiformes (owls).

Indications of a Site-specific Toxicant Mediated Etiology

Thomas et al. (1998) described lesions observed in epizootic AVM. By light microscopy, affected birds show striking, diffuse, spongy degeneration of central nervous system white matter; vacuolation is present in all myelinated central nervous system tissue. By electron microscopy, the lesion is characterized as intramyelinic vacuoles formed in the myelin sheaths by splitting of one or more myelin lamellae at the intraperiod line. Myelin is responsible for saltatory conduction of nerve impulses, and myelinopathy results in a perturbation of normal nerve function (Morell 1994). Peripheral myelinopathy has been observed in a few AVM-positive birds (Augsburger et al. 2003). Thus, the clinical signs of neurological impairment are consistent with observed histopathology.

Extensive pathology points to a toxicant as the most likely cause of these lesions. First, other disease agents known to affect birds, including bacteria, viruses or parasites, have largely been ruled out through extensive diagnostic testing and due to the absence of any inflammatory cell infiltration at the affected sites (Thomas et al. 1998). Second, the disease is characterized by primary demyelinating lesions; that is, damage is first seen in the myelin rather than the axon or perikaryon. As such, it is a true myelinopathy (i.e., breakdown of properly formed myelin) (Chang et al. 1992). The lesions in the brain and spinal cord resemble lesions naturally- and experimentally- induced by certain chemicals supporting the theory that the disease is caused by a neurotoxicant. Third, preliminary sentinel studies conducted at Lake Surf in 1998 indicate the onset of disease is very rapid and exposure to the agent is likely site-specific, i.e. likely occurs at the site of mortality.

While the actual toxicant is unknown, there are several compounds which are known to induce vacuolar myelinopathy in humans and lab animals (Morell 1994). The lesion has parallels to intoxications from triethyltin and hexachlorophene which preferentially affect myelin by vacuolation at the intraperiod line. Intramyelinic vacuolization of the brain and spinal cord with

minimal involvement of the peripheral nervous system was noted in mallards fed 50 ppm triethyltin (Fleming et al. 1991). Lesions in triethyltin intoxicated mammals include vacuolation characterized by splitting of myelin at the intraperiod line (Watanabe 1980; Morell 1994). Lesions in adult mammals with hexachlorophene neurotoxicity are similar to those reported for triethyltin toxicity and AVM, namely fluid accumulation at the intraperiod line (Morell 1994). Ducklings administered isoniazid (isonicotinic acid hydrazide) mixed with their feed exhibited central nervous system intramyelinic lesions characterized by vacuolation as a result of splitting at the intraperiod line. Signs of intoxication included tremors, incoordination, ataxia, inability to stand and lethargy (Carlton and Kreutzberg 1966; Lampert and Schochet 1968). A significant notation was lateral recumbency with paddling movements of the limbs and an inability to return to an upright position. These are common signs in moribund coots we've observed. In mammals, isoniazid appears to more significantly affect the peripheral nervous system and a primary axonopathy may confound the vacuolization seen in the myelin (Blakemore 1972; Schmued et al. 1996). Other compounds which induce lesions similar to those in AVM are cuprizone (Blakemore 1972), *Stypandra imbricata* toxin (Huxtable et al. 1980), *Helichrysum* (Van der Lugt et al. 1996), vigabatrin (Morell 1994), and acetyl ethyl tetramethyl tetralin (Spencer et al. 1980). Myelin is rich in lipids (70% dry weight) making it a prime site for accumulation of hydrophobic compounds (Morell 1994; Morell and Quarles 1999).

At the time this study was initiated, efforts to determine the cause of disease included epidemiological assessments (geographic extent, disease onset and duration, affected species), histopathology (disease progression, severity, and extent), and environmental chemistry / toxicology (analyses of environmental media and attempts to reproduce the lesion via field and laboratory exposures). This study was initiated in 2000 to expand efforts in toxicant and pathway identification. There were three overall objectives for this investigation:

- 1) Assess disease progression:** Using sentinel coots and mallards placed on a confirmed AVM lake and a paired reference location prior to the arrival of fall migrants, determine:
 - a) site specificity of AVM, time of onset, and mortality rate in relation to other epizootiologic information;
 - b) clinical progression of AVM and diagnostic criteria for sick birds;
- 2) Assess disease mechanisms:** Through feeding trials and observation, determine:
 - a) possible route of exposure by a series of controlled exposures of healthy coots and/or mallards to water, foods, and sediments collected from an affected site; and,
 - b) transmissibility of AVM by direct contact between affected birds and healthy birds of a susceptible species.
- 3) Work toward identification of toxicant:** Search for and identify organic compounds in affected sites through analyses of sediments from lakes where AVM has been documented in comparison to unaffected lakes. Compare chemicals in tissues of affected birds from Lake Surf, NC, to tissues from unaffected birds in other locations.

FINDINGS

Notification of funding was received by the Raleigh Field Office in April 2000. Work began with preparation of an intra-agency agreement to fund the National Wildlife Health Center's components of this project. We completed a cooperative agreement with the Indiana University School of Public and Environmental Affairs to cover the organic analytical chemistry. We also completed a cooperative agreement with the North Carolina State University College of Veterinary Medicine to cover the veterinary services associated with the transmissibility studies and feeding trials.

Work was either conducted at, or with material (affected birds, sediment, vegetation, etc.) collected from, Lake Surf (also called Woodlake), near Vass, in Moore County, North Carolina (35°14'N, 79°12'W) (Figure 2). Lake Surf is a 470 hectare impoundment created in 1973; land cover prior to impoundment was forested wetlands and the current shoreline consists of residential homes, golf courses, a dam, and pine forests. Vacuolar myelinopathy was documented in birds at this lake in 1997, 1998, and 1999. The disease may have a longer history at this location; in 1990, dead and moribund coots were observed with paralysis of the legs. A pathology report on an afflicted coot noted "softening and demyelination in the brain" which may have been a characterization of vacuolar myelinopathy (Augsburger et al. 2003). The lake's small size and easy access (its shoreline is almost entirely ringed by roads) supported its use as a basis of study.

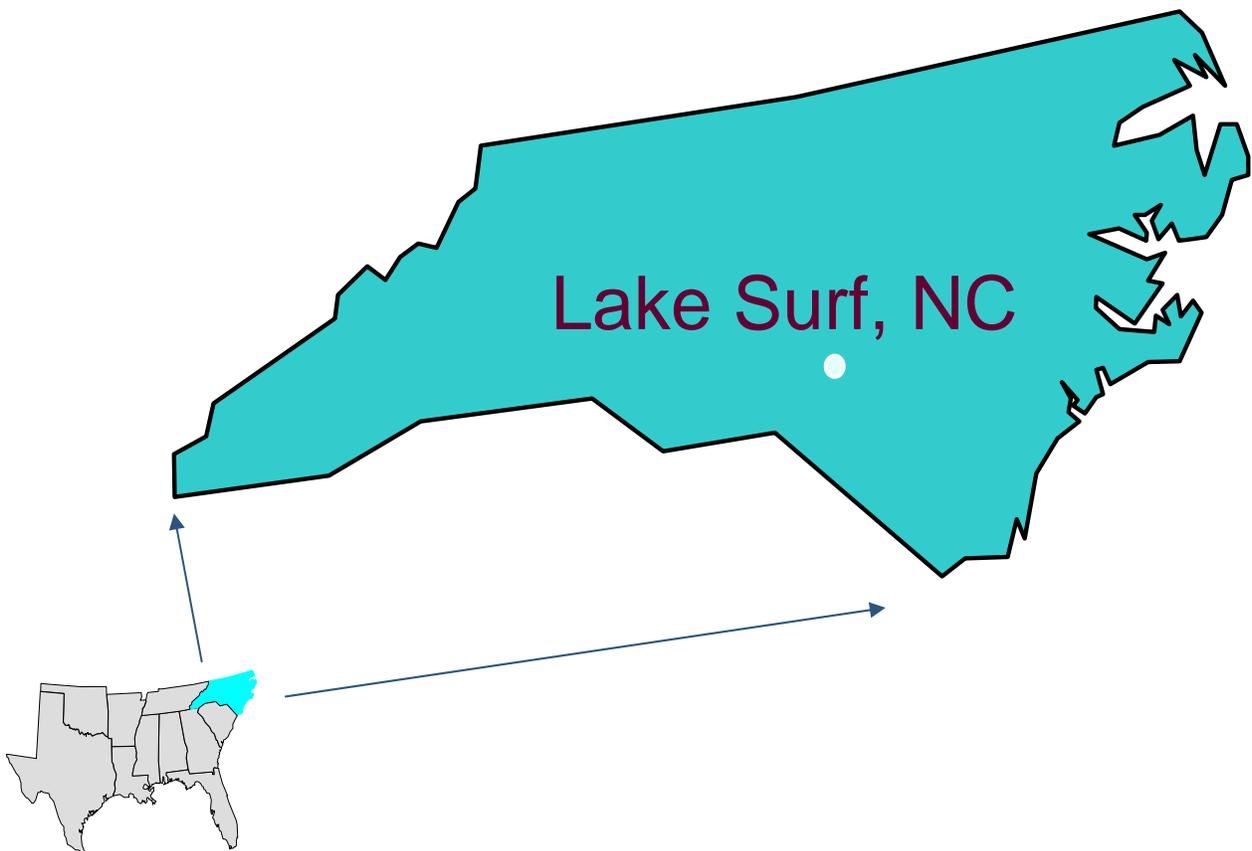


Figure 2. Lake Surf (also called Woodlake), near Vass, in Moore County, North Carolina

Five peer reviewed publications (Table 1) summarize the major components of the study. While readers are referred to those papers for the detailed methods and results, each aspect of the overall study is summarized below. There were four types of assessments: *Sentinel Bird Deployments*, *Neurological Evaluations*, *Feeding Studies*, and *Analytical Chemistry*.

Table 1. Publications^a associated with the U.S. Fish and Wildlife Service and partners' Environmental Contaminant Program study on avian vacuolar myelinopathy at Lake Surf (Woodlake), North Carolina

Dodder NG, B Strandberg, T Augspurger and RA Hites. 2003. Lipophilic organic compounds in lake sediment and American coot (*Fulica americana*) tissues, both affected and unaffected by avian vacuolar myelinopathy. *Science of the Total Environment* 311: 81-89.

Larsen RS, FB Nutter, T Augspurger, TE Rocke, L Tomlinson, NJ Thomas and MK Stoskopf. 2002. Clinical features of avian vacuolar myelinopathy in American coots. *Journal of the American Veterinary Medical Association* 221: 80-85.

Larsen RS, FB Nutter, T Augspurger, TE Rocke, NJ Thomas and MK Stoskopf. 2003. Failure to transmit avian vacuolar myelinopathy to mallard ducks. *Journal of Wildlife Diseases* 39: 707-711.

Rocke TE, NJ Thomas, T Augspurger and K Miller. 2002. Epizootiologic studies of avian vacuolar myelinopathy in waterbirds. *Journal of Wildlife Diseases* 38: 678-684.

Rocke TE, NJ Thomas, CU Meteyer, C Quist, J Fischer, T Augspurger and SE Ward. 2005. Attempts to identify the source of avian vacuolar myelinopathy for waterbirds. *Journal of Wildlife Diseases* 41: 163-170.

^a Copies can be obtained from the U.S. Fish and Wildlife Service in Raleigh, NC

Sentinel Bird Deployments

Deployment of sentinel birds was under the direction of Dr. Tonie Rocke (National Wildlife Health Center), whose previous experience with use of sentinel mallards in wildlife diseases work (Rocke et al. 1994, 1997) guided this effort. In mid- December 1999, 26 tagged and wing-clipped coots (collected in WI) and 40 tagged and wing-clipped game farm mallards (purchased from Whistling Wings in IL) were released on Woodlake (known to be AVM-positive that year) and 20 tagged and wing-clipped game farm mallards were released on Crystal Lake (reference lake). The sentinels were monitored for morbidity and mortality until early March when all birds that survived were captured for examination. In summer 2000, we released wing-clipped game farm mallards on Woodlake and Sharon Harris Reservoir (reference) for 6 weeks (Figure 3). We monitored the birds and collected several each week to obtain samples. This release was done to determine if AVM occurs at other times of the year. New groups of sentinel mallards were released at Woodlake and Lake Trace (reference lake) in November 2000 and January 2001.



Figure 3. Sentinel bird studies consisted of tagging and releasing healthy birds at reservoirs known to be AVM-positive and AVM-negative then periodically recapturing them to monitor disease prevalence and severity. Photos: USGS, NWHC

A manuscript on the sentinel studies (Rocke et al. 2002) was written for the *Journal of Wildlife Diseases*; the abstract and summary are excerpted here:

Between 1998 and 2001, wing clipped sentinel birds (wild American coots and/or game farm mallards) were released at Lake Surf, North Carolina, a lake with recurrent outbreaks of AVM, in order to gain a better understanding of the epizootiology of the disease. As early as 5-7 days post release, coot and mallard sentinels showed neurologic signs of disease and were confirmed with AVM upon histologic examination of their brains. Serial releases of sentinel mallards during the fall and winter of 2000-2001 demonstrated that exposure to the causative agent at a threshold sufficient to manifest disease was seasonal and occurred over a 2 month period or less, around November and December. Our findings that disease onset can be very rapid (5-7 days) and that exposure to the causative agent of AVM is site-specific, seasonal (late fall to early winter), and of relatively short duration (2 months or less) supports the hypothesis that the disease is caused by a chemical or toxin, most likely of natural origin.

Until this work, the hypothesis that birds arrived with the disease during migration had not been ruled out. Also, it is apparent that the time between exposure and the onset of disease symptoms or death can be relatively rapid. These findings in sentinels released in Lake Surf, and the absence of disease in either sentinels or wild birds in nearby reference lakes with similar populations of birds, suggest that the onset of the disease in migratory birds is tied to local conditions in the lake and not to events preceding or during migration.

The findings of our study also demonstrate the seasonality of exposure and occurrence of AVM. Sentinel mallards released in August 2000 and sampled periodically through the end of October did not develop signs of disease or brain lesions. Whereas, many of the mallards released in early November 2000 contracted the disease and became sick or died within 7-12 days post-release. Brain lesions were observed among apparently

healthy birds in this group recaptured as early as 6 days post-release and as long as 2 months post-release. However, sentinel mallards released in Lake Surf in early January 2001 and recaptured periodically through January and February did not contract the disease, as no lesions were detected in their brains. These findings suggest that the causative agent of AVM may persist in the lake and be available to birds at a level that causes disease for relatively short periods of time, up to several months, and is not present year round (Excerpted from Rocke et al. 2002).

Neurological Evaluations

The proximity of the North Carolina State University College of Veterinary Medicine to the study site provided an ideal opportunity to apply their expertise to better understanding AVM. Their Zoo, Wildlife, and Aquatic Medicine faculty and residents (primarily Dr. Michael Stoskopf and Dr. Scott Larsen) were involved in two aspects of the study including a neurological assessment of the affected birds to further refine the disease's character. That work was published in Larsen et al. (2002), the abstract of which is reprinted here:

Twenty-six American coots with AVM were evaluated by physical, neurologic, clinical pathologic, and gross and histologic postmortem examination and compared with 12 unaffected reference coots. Of the AVM-affected coots, 77% were found dead within 7 days of admission, but five survived > 21 days and showed signs of clinical recovery. Neurologic abnormalities included ataxia, decreased withdrawal reflexes, proprioceptive deficits, and head tremors, as well as absent pupillary light responses, anisocoria, blindness, nystagmus, and strabismus. Affected coots had relative lymphocytoses, elevated bile acid concentrations, and elevated lipemia index values. Vacuolation of CNS white matter was confirmed in all of the affected coots, but was not found in controls. This report documents clinical recovery of AVM-affected coots when supportive care is administered. Until the etiology is identified, caution should be exercised when rehabilitating and releasing coots thought to be affected by AVM (Excerpted from Larsen et al. 2002).

Environmental Chemistry

In 2000, we worked with partners (U.S. Army Corps of Engineers and Georgia Department of Natural Resources) to collect sediment from four AVM-positive (Lake Surf, NC; Lake Ouchita, AR; Strom Thurmond Reservoir, GA/SC; and Lake Juliette, GA) and two AVM-negative lakes (Harris Lake, NC and Lake Hamilton, AR). Determinations of disease presence or absence at the lakes was made from sampling coots as part of the Southeastern Cooperative Wildlife Disease Study's concurrent epidemiological assessments. Chemical analyses were led by Dr. Ron Hites of Indiana University who has expertise in environmental chemistry (Hites et al. 1993). Early results were provided in Strandberg et al. (2001) and detailed methods and results are in a *Science of the Total Environment* paper (Dodder et al. 2003); a summary is reprinted here:

With the goal of identifying the toxicant that causes AVM, we qualitatively analyzed sediments and American coot (*Fulica americana*) tissues from reservoirs that were affected and unaffected by AVM using high-resolution gas chromatographic low-resolution mass spectrometry. Polychlorinated biphenyls (PCBs), octachlorodibenzo-p-

dioxin, and biogenic and anthropogenic polycyclic aromatic hydrocarbons (such as retene) were the most abundant compounds in the sediment. Penta- and hexachlorobenzene, oxychlorane, p,p'-DDE, dieldrin, and polychlorinated biphenyls were the most abundant compounds in the avian tissues. None of these compounds were more abundant in the AVM affected sediments and tissues than in the unaffected media. Therefore, it is unlikely that any of these compounds are the cause of this avian disease.

The PAHs detected in the sediments were not present in the bird tissue samples presumably because they were readily metabolized. On the other hand, penta- and hexachlorobenzene (HCB) were present in most of the samples. In addition, the pesticide metabolites oxychlorane (from chlordane), p,p'-DDE (from DDT), and dieldrin (a metabolite of aldrin as well as an insecticide on its own) were found in the AVM positive and AVM negative birds. Penta- through decachlorinated PCBs were also observed in both sets of bird tissues. PCBs were the most abundant group of compounds found, which is a common observation. The brain tissues of presumed AVM positive and negative birds contained relatively less organochlorine contaminants than the other tissue types, perhaps due to the more polar nature of the lipids found in the brain versus adipose tissue. Although the organochlorine compounds found in these samples have been implicated in a number of wildlife diseases, they are probably not a cause of AVM. Our data show that these compounds are found at approximately equal abundances in the AVM positive and the AVM negative birds.

Neither the sediment data nor the tissue data show any compounds that are present in all of the AVM positive lakes but not present (or of low relative abundance) in the AVM negative lakes. This study was, therefore, unsuccessful in identifying a compound responsible for AVM. There are several reasons for this failure: The extraction procedures and GC/MS parameters were optimized to measure non-polar organic molecules with molecular weights between approximately m/z 40 to 600. This represents a very broad range of natural and anthropogenic compounds. However, these methods will not detect compounds such as proteins, metals, or polar organic compounds. A compound from one of these classes could be the neurotoxin or neurotoxicant responsible for AVM. Alternatively, the toxicant could be a non-polar organic compound present at such low concentrations that it cannot be detected in the presence of a background of abundant naturally occurring compounds (Excerpted from Dodder et al. 2003).

Feeding Studies

The hypothesis that the causative agent of AVM is a toxicant encountered at the reservoirs has led to experimental attempts to reproduce the disease in healthy birds exposed to items (food, sediment, water) from the lakes. Our study included feeding trials conducted by the National Wildlife Health Center and the North Carolina State University College of Veterinary Medicine. North Carolina State University's work was summarized in a progress report (Stoskopf et al. 2001) and was also published in the *Journal of Wildlife Diseases* (Larsen et al. 2003). The abstract of that paper is reprinted here:

Avian vacuolar myelinopathy (AVM) is a neurologic disease that has been diagnosed in free-ranging birds in the southeastern United States. Bald eagles (*Haliaeetus*

leucocephalus), American coots (*Fulica americana*), and mallards (*Anas platyrhynchos*) have been affected. Previous investigations have not determined the etiology of this disease. In November and December 2001, we attempted to induce AVM in game-farmed mallards through four, 7-day exposure trials. Mallards were housed in six groups of eight, with two of these groups serving as controls. One group was housed with AVM-affected coots, one group was tube fed daily with water from the lake where affected coots were captured, one group was tube fed daily with aquatic vegetation (*Hydrilla verticillata*) from the same lake and another group was tube fed daily with sediment from the lake. No ducks exhibited clinical neurologic abnormalities consistent with AVM and no evidence of AVM was present at histopathologic examination of brain tissue. Although limitations in sample size, quantity of individual doses, frequency of dose administration, duration of exposure, and timing of these trials restrict the interpretation of the findings, AVM was not readily transmitted by direct contact, water, hydrilla, or sediment in this investigation (From Larsen et al. 2003).

The final portion of this study was also published in the *Journal of Wildlife Diseases* (Rocke et al. 2005). This last set of feeding trial results is described here:

Attempts were made to reproduce avian vacuolar myelinopathy (AVM) in a number of test animals in order to determine the source of the causative agent for birds and to find a suitable animal model for future studies. Submerged vegetation, algae, invertebrates, forage fish, and sediments were collected from lakes with ongoing outbreaks of AVM and fed to American coots (*Fulica americana*), mallard ducks and ducklings (*Anas platyrhynchos*), quail (*Coturnix japonica*), and laboratory mice either via gavage or *ad libitum*. Tissues from AVM-affected coots with confirmed brain lesions were also fed to ducklings, kestrels (*Falco sparverius*) and American crows (*Corvus caurinus*). Two of four mallards that ingested one sample of *Hydrilla verticillata* along with its associated epiphytes developed brain lesions consistent with AVM, although none of these birds had clinical signs of disease. Ingestion of numerous other samples of *Hydrilla* from both affected lakes and a lake with no prior history of AVM, other materials (sediments, algae, fish, invertebrates, and water from affected lakes), or tissues from AVM-affected birds did not produce either clinical signs or brain lesions in any of the other test animals in our studies. These results suggest that waterbirds are most likely exposed to the causative agent of AVM while feeding on aquatic vegetation, but we do not believe the vegetation itself is the agent. We hypothesize that the causative agent of AVM may either be accumulated by aquatic vegetation, like *Hydrilla*, or produced by organisms associated with its external surfaces. Also two coots that ingested a sample of *Hydrilla* from another lake during an ongoing AVM outbreak in wild birds developed neurologic signs within nine days (ataxia, limb weakness, and incoordination), and one of two coots that ingested *Hydrilla* from the same site collected 13 days later became sick and died within 38 days. None of these three sick coots had definitive brain lesions consistent with AVM by light microscopy, however, some vacuolation was visible in 2 of these cases upon examination of the brain by electron microscopy. It is unclear if these birds died of AVM. Perhaps they did not ingest a dose sufficient to produce brain lesions. It is possible that a separate neurotoxic agent is responsible for the morbidity and mortality observed in these coots (Excerpted from Rocke et al. 2005).

ACCOMPLISHMENTS AND MANAGEMENT RECOMMENDATIONS

This study began in 2000, when answers to some relatively basic questions regarding AVM were not known, such as whether birds arrive at southeastern reservoirs with the disease upon migration or instead develop the disease after arrival. Also unknown was how long it takes for birds to develop the disease, whether birds with clinical signs can recover with supportive veterinary care, and what at the lakes with AVM affected birds might be causing the disease. The work documented through this study (Larsen et al. 2002, 2003; Rocke et al. 2002, 2005; Dodder et al. 2003) provided key findings on AVM:

- Exposure to the causative agent of AVM is site-specific (birds get the disease locally)
- Exposure to the causative agent is seasonal, autumn to early winter. Although affected birds are observed at the sites into late winter, those likely developed the in late fall / early winter
- Onset of disease can be rapid, within as little as 5 days post-exposure
- AVM was documented in several species, and mallards (*Anas platyrhynchos*) can be effectively used as sentinels to monitor the disease
- Many of the positive birds appeared “normal” (not all positive birds look sick)
- The severity of clinical signs does not appear closely linked with the severity of brain lesions, as coots and mallards with no detectable signs had lesions described as severe or marked and several birds with obvious neurologic impairment had brain lesions described as mild or moderate
- Clinically-affected birds could regain function with supportive veterinary care
- AVM was not transmitted by direct contact between affected birds and healthy birds
- Feeding studies were inconclusive (although others have since experimentally reproduced the disease through feeding)
- Neither the sediments nor tissues analyzed show any compounds that are present in all of AVM positive lakes but not present (or of low abundance) in AVM negative lakes

Our findings that exposure is site-specific, disease onset is rapid, and the agent may persist in the environment for only a few months at a level sufficient to induce disease in birds further supports the hypothesis that the cause of AVM is a chemical substance, most likely of natural origin (i.e., a toxin). That helps focus the search for its identity.

Results of our study provide a better scientific understanding of the disease, and each of the papers included recommendations for the path forward (e.g., more intensive sampling during the first wave of mortality in coots, additional feeding studies with vegetation collected early in disease outbreaks, biogeochemical site characterization of affected lakes in comparison with paired control lakes, bioassay-guided fractionation of tissue and vegetation extracts). Several of the recommendations have been followed by study cooperators or other colleagues. Table 2 is a list of the publications that reference one or more of the components of our study, an illustration of the cooperative approach taken and the utility of our results in fostering work of others.

The Southeastern Cooperative Wildlife Disease Study was the first to reproduce the disease experimentally (Fischer et al. 2003) by feeding AVM-positive coot tissues to red-tailed hawks. Researchers with Clemson University and the National Wildlife Health Center confirm that AVM lesions develop in some ducks following ingestion of aquatic vegetation (and associated

Table 2. Publications subsequently referencing the results of U.S. Fish and Wildlife Service and partners' studies of avian vacuolar myelinopathy in NC

Augspurger T, JR Fischer, NJ Thomas, L Sileo, RE Brannian, KJG Miller and TE Rocke. 2003. Vacuolar myelinopathy in waterfowl from a North Carolina impoundment. *Journal of Wildlife Diseases* 39: 412-417.

Birrenkott AH, SB Wilde, JJ Hains, JR Fischer, TM Murphy, CP Hope, PG Parnell and WW Bowerman. 2004. Establishing a food-chain link between aquatic plant material and avian vacuolar myelinopathy in mallards (*Anas Platyrhynchos*). *Journal of Wildlife Diseases* 40: 485-492

Fischer JR, LA Lewis, T Augspurger and TE Rocke. 2002. Avian vacuolar myelinopathy: A newly recognized fatal neurologic disease of eagles, waterfowl, and other birds. *Transactions of the 67th North American Wildlife and Natural Resources Conference* 67: 51-61.

Fischer JR, LA Lewis-Weis and CM Tate. 2003. Experimental vacuolar myelinopathy in red-tailed hawks. *Journal of Wildlife Diseases* 39: 400-406.

Fischer JR, LA Lewis-Weis, CM Tate, JK Gaydos, RW Gerhold and RH Poppenga. 2006. Avian vacuolar myelinopathy outbreaks at a southeastern reservoir. *Journal of Wildlife Diseases* 42: 501-510.

Friend M. 2006. Disease Emergence and Resurgence: The Wildlife-Human Connection. USGS Circular 1285, USGS, Reston, VA.

Lewis-Weis LA, RW Gerhold and JR Fischer. 2004. Attempts to reproduce myelinopathy in domestic swine and chickens. *Journal of Wildlife Diseases*: 476-484

Wilde SB, TM Murphy, CP Hope, SK Habrun, J Kempton, A Birrenkott, F Wiley, WW Bowerman, and AJ Lewitus. 2005. Avian vacuolar myelinopathy linked to exotic aquatic plants and a novel cyanobacterial species. *Environmental Toxicology* 20: 348-353.

Wiley FE, SB Wilde, AH Birrenkott, SK Williams, TM Murphy, CP Hope, WW Bowerman and JR Fischer. 2007. Investigation of the link between avian vacuolar myelinopathy and a novel species of cyanobacteria through laboratory feeding trials. *Journal of Wildlife Diseases* 43: 337-344.

Wiley F, M Twiner, T Leighfield, S Wilde, F Van Dolah, J Fischer, W Bowerman. 2008 in press. An extract of *Hydrilla verticillata* and associated epiphytes induces avian vacuolar myelinopathy in laboratory mallards. *Environmental Toxicology*

Williams SK, Kempton J, Wilde SB and A Lewitus. 2007. A novel epiphytic cyanobacterium associated with reservoirs affected by avian vacuolar myelinopathy. *Harmful Algae* 6: 343-353.

materials) collected from a reservoir during an AVM event (Birrenkott et al. 2004; Rocke et al. 2005). This, in combination with the results of Fischer et al. (2003), completes the food chain linkage of AVM from aquatic vegetation to waterbirds (i.e., coots and ducks) and then to raptors (i.e., bald eagles that feed on the waterbirds). The cause of AVM remains unknown, but data suggests the agent is either seasonally accumulated by aquatic vegetation or seasonally produced by one or more organisms associated with aquatic vegetation at affected sites. Recent work by others focuses on the association between AVM and a unique cyanobacteria species (*Stigonematales*) abundant on vegetation during AVM outbreaks (Wilde et al. 2005, Wiley et al. 2007, Williams et al. 2007), indicating either evidence that the novel species may be involved with AVM induction, or at the least it is a good predictor of AVM toxin presence in a system. Current research is also progressing on use of solvent fractionation and avian bioassays to identify chemical properties of the toxin, an important step in its ultimate identification (Wiley et al. in press).

More research is needed to identify the source and specific agent that causes AVM. Until the source and causative agent of AVM are discovered, the significance of the disease and potential wildlife management implications remain unknown. The disease remains a significant concern for local eagle impacts (Fischer et al. 2006).

Our expertise and equipment in the field, close to where the birds are affected, made us a valuable partner for scientists with a more national (National Wildlife Health Center) or regional (Southeastern Cooperative Wildlife Disease Study) focus. This study is an example of the value of the Service's Environmental Contaminants Program's field presence in facilitating wildlife assessment and management.

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