ASSESSMENT OF THE POTENTIAL FOR AN AVIAN VACUOLAR MYELINOPATHY OCCURRENCE IN NEW HANOVER COUNTY PONDS

A research project in partial fulfillment of the requirements for the Post-Baccalaureate Certificate in Environmental Studies University of North Carolina at Wilmington

Spring 2006

By
Adam B. Poore

Table of Contents

Abstract{ TC "**Abstract**" \f C \l "1" }

Avian Vacuolar Myelinopathy (AVM) is a neurological disease affecting both herbivorous water birds and their avian predators. The disease was first reported during the winter of 1994 - 1995 when twenty-nine bald eagles (*Haliaeetus leucocephalus*) were found dead at DeGray Lake in Arkansas. Two years later during the winter of 1996 - 1997, twenty-six more eagles were confirmed dead due to AVM as well as a number of American coots (*Fulica americana*). To date, AVM has been confirmed in 11 reservoirs in Arkansas, Texas, North Carolina, South Carolina, and Georgia and has caused the death of up to 100 bald eagles, thousands of American coots, and numerous other waterfowl. Clinical signs of affected birds include the inability to fly, swim, or walk correctly and may eventually lead to paralysis and death. The disease affects the nervous system forming vacuoles within the myelin sheath causing lesions which disrupt normal nerve function. Although the etiology of the disease remains unknown recent studies have narrowed the causative agent to a neurotoxin produced by an unknown species of epiphytic cyanobacteria of the order Stigonematales. The bacteria have been found associated with the invasive aquatic plant species hydrilla (*Hydrilla verticillata*), Brazilian elodea (*Egeria densa*), and Eurasian watermilfoil (*Myriophyllum spicatum*). An infestation of at least one of the three species has been documented in all reservoirs associated with AVM outbreaks.

The purpose of this research is to explore the history of AVM and determine the potential for an AVM occurrence in New Hanover County, North Carolina. Fifty-six ponds from 35.30 ha to 0.13 ha have been surveyed for the presence of invasive submerged aquatic vegetation, suspect cyanobacteria, and avian wildlife. Although several sites contained abundant avian species susceptible to the disease including the

American coot (*Fulica americana*), Canada goose (*Branta canadensis*), and mallard duck (*Anas platyrhynchos*), no site showed infestations of the three types of vegetation present at other AVM positive locations. The results suggest there is no immediate threat of an AVM occurrence in any of the 56 ponds surveyed. However, a significant infestation of preferred aquatic vegetation capable of supporting the suspect bacteria may produce a future occurrence of AVM in several of the larger ponds. An infestation of *Ceratophyllum* at Airlie Lake should be further investigated at the microscopic level for presence of the suspect bacteria. Effective management of AVM depends on the ability of future research to determine the definitive cause of the disease.

Introduction{ TC "**Introduction**" \f C \l "1" }

Avian Vacuolar Myelinopathy{ TC "**Avian Vacuolar Myelinopathy**" \f C \l "2" }

An emerging neurological disease affecting herbivorous water birds and their avian predators has gained increasing attention since its discovery in Arkansas in 1994. To date, thousands of American coots (*Fulica Americana*) and up to 100 bald eagles (*Haliaeetus leucocephalus*) across five states have been killed from the mysterious disease known as avian vacuolar myelinopathy (AVM). The definitive cause of the disease has not been confirmed although recent studies have narrowed the causative agent to a neurotoxin produced by an unknown species of epiphytic cyanobacteria found associated with invasive submerged aquatic vegetation (Wilde et al., 2005). The purpose of this research is to explore the history of avian vacuolar myelinopathy and determine the potential for an AVM occurrence in New Hanover County, North Carolina.

During the winter (November to January) of 1994 - 1995, twenty-nine bald eagles were reported dead in the vicinity of DeGray Lake, Arkansas. Two years later during the same months of 1996 - 1997 twenty-six more eagles were found dead near the man-made reservoir. The affected eagles appeared intoxicated and demonstrated a lack of coordination, over flying perches and colliding with objects. Numerous American coots, a migratory duck like bird that feed on vegetation, insects, crustaceans, mollusks, and small fish, also demonstrated impaired flight and difficulty walking during the mortality events (Thomas et al., 1998).

Puzzled scientists examined the affected eagles and coots from the two winters to determine their cause of death. "All eagles examined and 62/77 (81%) coots had widespread, bilaterally symmetrical vacuolation of the white matter of the central nervous system" (Thomas et al., 1998) (Figure 1). The formation of vacuoles, or vacant cavities, occurring within the myelin strands disrupts normal nerve function and supports field observations of loss of coordination among affected birds. In 1998 Thomas et al. documented "the first known occurrence of a spongiform myelinopathy of unknown etiology in two species of wild birds" (1998).

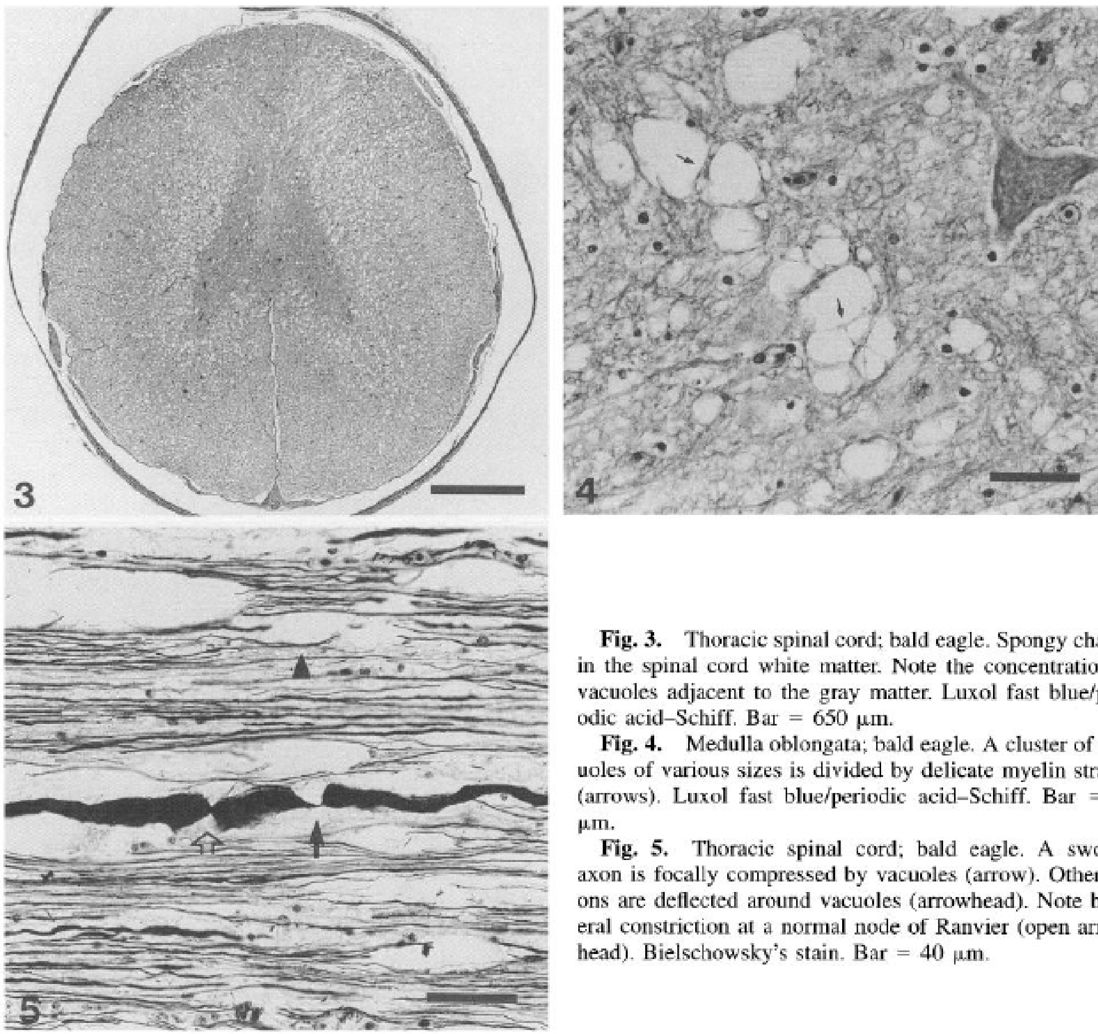

Fig. 3. Thoracic spinal cord; bald eagle. Spongy change in the spinal cord white matter. Note the concentration of vacuoles adjacent to the gray matter. Luxol fast blue/periodic acid–Schiff. Bar = 650 μm.

Fig. 4. Medulla oblongata; bald eagle. A cluster of vacuoles of various sizes is divided by delicate myelin strands (arrows). Luxol fast blue/periodic acid–Schiff. Bar = 40 μm.

Fig. 5. Thoracic spinal cord; bald eagle. A swollen axon is focally compressed by vacuoles (arrow). Other axons are deflected around vacuoles (arrowhead). Note bilateral constriction at a normal node of Ranvier (open arrowhead). Bielschowsky's stain. Bar = 40 μm.

Figure 1: Light microscopy images of vacuolation within a bald eagles nervous system. From (Thomas et al., 1998)

Similar spongiform myelinopathies in mammals are usually the result of exposure to toxic chemicals. However, "substances known to cause intramyelinic lesions in domestic animals and humans, including triethyltin, the rodenticide bromethalin, and the antituberculosis drug isonicotinic acid hydrazide, have not been identified in AVM-affected birds" (Birrenkott et al., 2004). There are also two plant species, *Stypandra imbricata* and *Heliochrysum argyrosphaerum*, known to produce similar lesions although they are not found in North America (Birrenkott et al., 2004). Scientists faced the difficult challenge of discovering an unknown agent responsible for causing the disease.

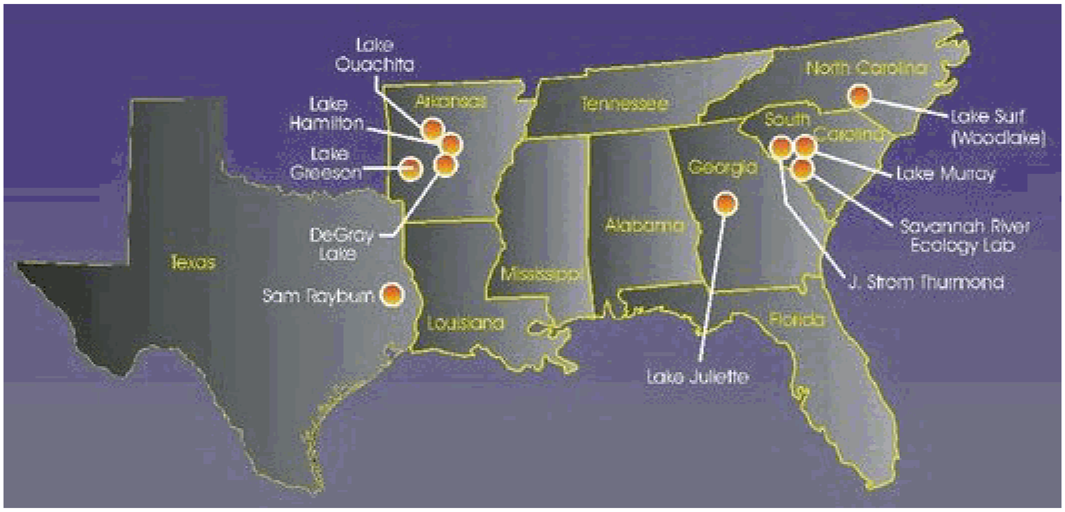

Figure 2: Map showing AVM positive locations. From http://www.aphis.usda.gov/vs/ceah/cei/taf/emergingdiseasenotice_files/avm_0101.htm

By 2002, avian vacuolar myelinopathy outbreaks in American coots had spread to lakes in Arkansas, North Carolina, South Carolina, and Georgia (Rocke et al., 2002). In 2003, Augspurger et al. documented the disease in other species including mallards (*Anas platyrhynchos*), ring-necked ducks (*Aythya collaris*), and buffleheads (*Bucephala albeola*) on a 460 ha impoundment called Lake Surf (Woodlake) in North Carolina.

Fisher et al. (2002) also identified AVM in Canada geese (*Branta canadensis*), two great horned owls (*Bubo virginianus*), and a killdeer (*Charadrius vociferous*) at J. Strom Thurmond Lake on the border of South Carolina and Georgia. "All the lakes where AVM-affected birds have been found are wintering sites for migratory populations and are man-made reservoirs, but they vary in age, size depth, surrounding natural habitat, and land use practices" (Rocke et al., 2002) (Figure 2).

Researchers continued to search for the cause of AVM while focusing on how the disease was being spread between species and locations. Studies by Rocke et al. (2002) were conducted to determine whether migratory coots contracted the disease at the site of diagnosis or if the birds brought the disease from another location, and to confirm the seasonality of the occurrences. Healthy birds were released on Lake Surf, North Carolina which had a history of AVM outbreaks every year since 1997. Healthy coots and mallards released on the lake were diagnosed with AVM within only 5-17 days. The finding confirmed that the causative agent of AVM is site-specific and acquired by the birds upon arrival to the site. Also, only those birds released on the lake during November developed signs of AVM. This suggested that contraction of the disease is seasonal and that the causative agent may only be present at lethal levels during the winter months. Rocke et al. (2002) concluded that the source of the disease is most likely a naturally occurring chemical toxin.

Rocke et al. (2002) also noted difficulty in diagnosing affected coots. Some birds that showed no clinical signs of the disease were found with severe brain lesions associated with AVM. The finding complicates management of AVM since many affected birds may go undiagnosed. In a similar study of American coots on Lake Surf, North Carolina, Larsen et al. (2002) discovered that some birds affected with AVM were able to regain neurological function despite having vacuoles present. Therefore, not all cases of AVM result in fatality.

The research by Rocke et al. (2002) showed that American coots could contract the disease when released on a site with AVM present, but not how bald eagles became affected. Bald eagles are known to be opportunistic feeders, preying on fish, water birds, and carrion (Guilfoyle et al., 2000). American coots affected with AVM may resemble sick or injured prey and become targeted as a food source by eagles. Dead coots may also provide an abundant food source during severe outbreaks (Fisher et al., 2003). In 2003, Fisher et al. published a study confirming the hypothesis that bald eagles were acquiring AVM from the ingestion of affected coots. During the study American coots confirmed AVM positive were fed to red-tailed hawks (*Buteo jamaicensis*). Although no hawks developed signs of neurological dysfunction during the study, "microscopic lesions of vacuolar myelinopathy were present in all five hawks that received tissues from coots with AVM" (Fisher et al., 2003). The study is significant because it demonstrated that AVM can be acquired through ingestion of affected coots by birds of prey under laboratory conditions. The results suggest that other birds of prey may be susceptible to the disease in addition to bald eagles (Fisher et al., 2003).

In order to determine how AVM was spreading between other species of birds, Larsen et al. (2003) conducted a study that attempted to transmit AVM to mallard ducks. "Four methods of exposure were investigated, including direct exposure to affected birds, ingestion of water, ingestion of aquatic plants, and ingestion of sediment from a lake (Lake Surf, NC) where affected birds were present," (Larsen et al., 2003). During the direct exposure trial, healthy mallards were housed in close proximity with AVM positive coots. However, none of the mallard ducks contracted the disease. The invasive aquatic plant species hydrilla (*Hydrilla verticillata*) was chosen in the plant feeding trial because hydrilla was the dominant submerged plant at Lake Surf, NC and the preferred food of American coots. Surprisingly, no mallards participating in any of the four exposures contracted AVM. Larsen et al. (2003) considered several explanations for their inability to transmit the disease including the possibility that the samples collected for the exposure trials either did not contain the causative agent or it was not present in high enough amounts to induce AVM. The hypothesis suggests that affected coots may be present at an AVM site for some time after the causative agent is no longer active.

Questions also remained about the susceptibility of mammals and humans to AVM. In 2004, Lewis-Weis et al. documented an attempt to transmit AVM to domestic chicken and swine. Swine that were fed tissues from AVM positive coots and those fed hydrilla material collected during an AVM outbreak did not develop the disease suggesting that mammals may not be susceptible, or may require higher exposure rates. Chickens however did develop AVM from both of the feeding trials. That led Lewis-Weis et al. (2004) to propose that the causative agent is most likely located in the material associated with the hydrilla collected from the AVM positive site.

While researchers continued to search for the agent responsible for causing AVM without success, Birrenkott et al. (2004) investigated the hypothesis that waterfowl were contracting AVM by ingestion of some factor associated with aquatic plants (Dodder et al., 2003). Their study discovered two types of cyanobacteria, *Pseudanabaena catenata* and an unknown species of Stigonematales, associated with hydrilla samples taken from the J. Strom Thurmond Lake during an AVM outbreak in November of 2001. Both species have the potential to produce harmful toxins and dominated the hydrilla surface during October and November of 2001 (the unknown species of Stigonematales covered greater than 90%). More importantly "the presence of the unknown Stigonematalan species and *P. catenata* was documented at all locations that have had cases of AVM" (Birrenkott et al., 2004). This led Birrenkott et al. (2004) to believe they had found the source of the disease.

To test their hypothesis, Birrenkott et al. (2004) fed mallard ducks hydrilla collected from J. Strom Thurmond Lake at the time of the AVM outbreak. The hydrilla material contained both *P. catenata* and the unknown species of Stigonematales. Six of the nine ducks under study developed lesions associated with AVM. Birrenkott et al. (2004) believed the mallards may have contracted the disease from a toxin produced by the unknown Stigonematalan species because of its abundance during AVM outbreaks. The research successfully established the first cause-effect link between AVM and aquatic vegetation (Birrenkott et al., 2004).

Wilde et al. (2005) continued research of the unknown suspect cyanobacteria with compelling results. Surveys were conducted of all ponds with documented cases of AVM and several AVM negative ponds for aquatic vegetation and the unknown species of bacteria. Hydrilla (*Hydrilla verticillata*), Brazilian elodea (*Egeria densa*), and Eurasian watermilfoil (*Myriophyllum spicatum*) were the most abundant aquatic plant species capable of supporting cyanobacteria in the ponds containing AVM. Of the AVM negative ponds sampled, the unknown species of Stigonematales was rare (Figure 4). However, among the AVM positive reservoirs sampled, the suspect species covered up to

95% of the invasive aquatic weeds (Figure 3). Wilde et al. (2005) also conducted a study in which they released 20 mallard ducks onto a small pond (1.6 ha) containing a dense infestation of hydrilla with a 50% to 95% coverage of the unknown Stigonematalan species. Fifteen of the twenty ducks released on the pond developed AVM within 6 weeks.

Figure 3: "Percentage of coverage of suspect Stigonematales species on three invasive aquatic plants in confirmed AVM reservoirs." From (Wilde et al., 2005)

Map Number	Year AVM Confirmed	AVM Reservoirs	Size (ha)	*Hydrilla verticillata*	*Egeria densa*	*Myriophyllum spicatum*
1	1994	DeGray,* AR	5,585	25–95	25–75	
2	1996	Quachita,* AR	16,212	25–95		10–25
3	1997	Hamilton, AR	2,938			10–25
4	1998	Thurmond,* SC/GA	28,328	25–95		
5	1998	Juliette,* GA	1,416	25–50	10–25	10–25
6	1998	Woodlake,* NC	457	25–50		
7	1998	Par Pond, SC	1,068			10–25
8	1998	L Lake, SC	418			10–25
9	1999	Murray, SC	20,558	0–25		
10	1999	Sam Rayburn, TX	46,337	0–10		
11	2003	Davis Pond, SC	2	25–95		
12	2003	Emerald Lake, GA	4	25–75		

* High (>20) eagle and waterfowl mortality reported from these sites.

Figure 4: "Percentage of coverage of suspect Stigonematales species on three different invasive aquatic plants in reservoirs where AVM has not been documented." From (Wilde et al., 2005)

Map Letter	Additional Reservoirs	Size (ha)	*Hydrilla verticillata*	*Egeria densa*	*Myriophyllum spicatum*
a	Back River Reservoir, SC	1,124	0		
b	Potato Creek Embayment, SC	655	0		
c	Lake Keowee, SC	7,487	0–20		
d	Lake Russell, SC/GA	10,704	0–20	0–10	
e	Stevens Creek Reservoir, SC	324		0	
f	Mtn. Island Reservoir, NC	1,828	0		
g	Harris Lake, NC	1,675	0		
h	Lake Norman, NC	13,142	0–5		
i	Nickajack Reservoir, TN	4,197	0		
j	Lake Seminole, FL	15,176	0		
k	Smith Reservoir, GA	325	0–2		
l	Lake Horton, GA	320	0		
m	Lake Worth, GA	567	0		

The conclusions made by Wilde et al. (2005) provide the basis for this study. "We hypothesized that three elements are needed to produce AVM: an abundance of preferred aquatic vegetation (e.g. hydrilla), an abundance of the suspect Stigonematalan species growing on the available substrate, and herbivores waterfowl" (Wilde et al. 2005). Until the study by Wilde et al. (2005), AVM had only been documented in reservoirs greater than 450 ha. The research recognized the potential for AVM to exist in many of the smaller ponds located throughout the southeast. "It is therefore important to determine the potential of this disease in ponds because the mortality may go undetected in these small, isolated systems" (Wilde et al., 2005). In response, this research has surveyed 56 ponds from 35.30 ha to 0.13 ha for the presence of invasive submerged aquatic vegetation, suspect cyanobacteria, and avian wildlife in New Hanover County, North Carolina.

Avian Wildlife{ TC "**Avian Wildlife**" \f C \l "2" **}**

Haliaeetus leucocephalus* (Bald Eagle){** TC "Haliaeetus leucocephalus* (Bald Eagle)**" \f C \l "3" **}**

The bald eagle is widely recognized as the national symbol of the United States. The species thrived during the late 1700s, when population estimates were as high as 25,000 to 75,000 in the United States alone. Significant mortalities from hunters and landowners resulted in the passage of the Bald Eagle Protection Act in 1940 (Guilfoyle et al., 2000). However, extensive use of pesticides such as DDT continued to reduce populations. Eagles that consumed prey which had been exposed to the toxic substances produced soft-shelled eggs that were crushed by the nesting female (Bald eagle fact sheet, 2005). “By 1963, only 417 breeding pairs remained in the lower 48 states” (Guilfoyle et al., 2000).

Bans on the use of pesticides and passage of the Endangered Species Act in 1973 helped populations grow to 5,800 breeding pairs by 2000 (Guilfoyle et al., 2000). Efforts to expand populations in North Carolina experienced success when the “first post-DDT wild bald eagle nest was documented just seven miles from Lake Mattamuskeet” in 1984 (Bald eagle fact sheet, 2005). In 2004 there were at least 60 active nesting sites that produced 80 young eagles in North Carolina (Figure 5). Continued population growth rates may result in the removal of the bald eagle from the threatened and endangered species list (Bald eagle fact sheet, 2005).

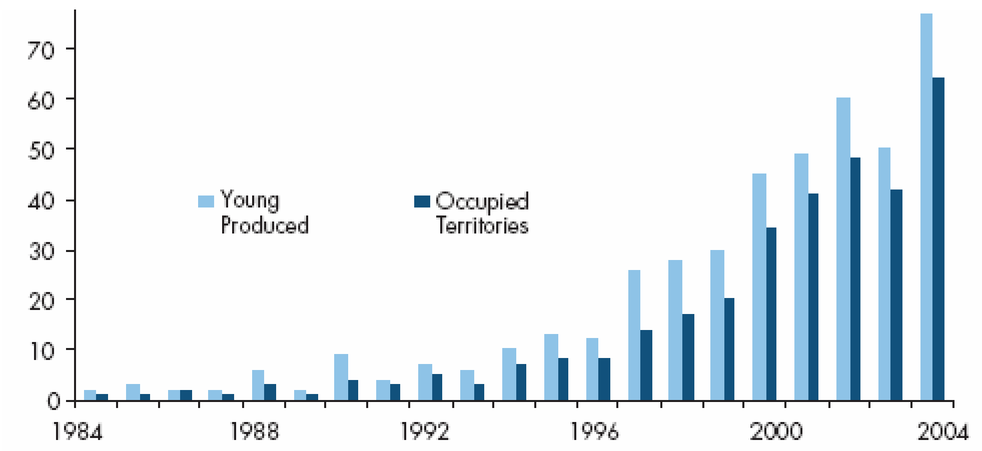

Figure 5: Growth rates of North Carolina bald populations as a result of the North Carolina Bald Eagle Project. From (Bald eagle fact sheet, 2005)

Most bald eagle nests are located less than half a mile from a body of water where they primarily feed on fish. As opportunistic feeders, they also feed on waterfowl and other vertebrates whether alive or dead. During the winter when food supplies diminish, eagles migrate south from Canada into the United States, although some populations in the far north or extreme south remain at their nesting site year round. Nests weighing hundreds of pounds are built high in mature trees and are used for many years. Mature eagles usually return to their site of birth to breed. Eagles live approximately 30 years in the wild and are capable of producing 1 to 3 eggs per year. However, approximately 50% to 70% of newborn eagles do not survive the first year (Guilfoyle et al., 2000). "Loss and degradation of habitat remains the largest obstacle to maintaining and increasing viable bald eagle populations" (Guilfoyle et al., 2000). Although the bald eagle population increases 8.5% every year, an AVM outbreak can set the population back by decades (Murphy, unpubl. data). During the AVM outbreak at DeGray Lake, Arkansas, 30% to 65% of the wintering eagle population may have been killed from the disease (Thomas et al., 1998).

Fulica americana* (American coot){** TC "Fulica americana* (American coot)**" \f C \l "3" **}**

American coots are migratory freshwater birds that can be found in lakes, ponds, and marshes. They spend summers throughout northern United States and Canada and winters as far south as California and Florida. Coots live approximately 9 years in the wild and are the only rail species which congregate in flocks. Their main predators are the osprey and bald eagle (Bridgman, 2003). Coots are omnivorous, consuming small animals and insects, although their primary diet consists of submerged aquatic vegetation which they dive for in shallow water. At Lake Surf, North Carolina, coots have demonstrated a preference for hydrilla, consuming it in large quantities and have been observed stripping material (presumably algae) from the plants surface (Rocke et al., 2005).

Invasive Aquatic Weeds{ TC "**Invasive Aquatic Weeds**" \f C \l "2" **}**

Aquatic macrophytes provide food and shelter for a variety of animals and help control water chemistry (Everitt et al., 1999). However, the spread of invasive, non-native plant species can have negative impacts on “water-based navigation, water quality and supply, hydropower, irrigation, fisheries, recreation, native vegetation, and wildlife” (Jakubauskas et al., 2002). Perhaps the most devastating characteristic for the purpose of this research is the ability of aquatic macrophytes to “harbor the vector of human and animal diseases” (Kay, 1992).

Hydrilla verticillata* (hydrilla){** TC "Hydrilla verticillata* (hydrilla)**" \f C \l "3" **}**

One of the most common, rapidly spreading aquatic weeds is *hydrilla verticillata*, or hydrilla. Native to Africa, hydrilla was first introduced to the United States as an aquarium plant but was found growing wild in Florida in 1960. Since then hydrilla has spread as far north as Maine and west through Texas into California. In North Carolina, hydrilla was first discovered in Raleigh’s Umstead Park in 1980. By 1990 there were 51 known infestations, mostly in the Neuse River basin area of Raleigh (Kay, 1992). In 2002 hydrilla had spread throughout the triangle and into the Catawba River basin near Lake Norman. Hydrilla has been reported as far west as Asheville and east to Wilmington in Burnt Mill Creek. “The explosive growth rate of this weed and its competitiveness with native vegetation makes hydrilla the most serious weed threat in North Carolina’s inland waters” (Kay, 1992).

Langeland (1996) describes hydrilla as “the perfect aquatic weed” because of its “extensive adaptive attributes.” It is capable of surviving in a wide range of temperatures, soils, and chemical environments allowing it to out compete most species of native and non-native submerged vegetation. Hydrilla can carry out photosynthesis in less light than other species allowing it to grow to deeper depths, forming dense mats when reaching the surface which block light penetration for competing species. At the surface, hydrilla is capable of modifying its metabolism to continue photosynthesis at increased temperatures and oxygen levels (Langeland, 1996; Kay, 1992).

Hydrilla grows in up to 30 feet of water (depending on clarity) and is rooted to the bottom by rhizomes. Stems form branches near the surface and can grow up to 18 feet long, or can also survive as fragments floating in the water column if broken. Leaves grow on the stems in whorls of 3-8 with a jagged edge that helps distinguish the plant from its close resemblance to Brazilian and American elodea (*Egeria densa* and *Elodea canadensis*) (Langeland, 1996; Kay, 1992) (Figure 6).

There are two types of hydrilla found in the United States, both monoecious and dioecious. The monoecious type produces both male and female flowers on the same plant and is found from North Carolina to Washington, D.C. Female flowers grow on the surface while male flowers are produced at the leaf axil and are released to the surface when mature. The dioecious type of hydrilla produces male and female flowers on separate plants. However, only the female plant has been found in the United States. The female, dioecious plant has been reported in Burnt Mill Creek in New Hanover County (Kay, 1992).

Hydrilla can reproduce by fragmentation or by the formation of tubers, turions, and seeds. Broken stem fragments are capable of surviving days before forming new roots upon contact with the bottom. Tubers, small bean-shaped structures, are produced on the rhizomes at the base of the plant and are released in the mud at the end of the growing season (late fall) (Figure 6). They produce new plants in the spring or can remain dormant in the sediment for many years and are capable of surviving adverse conditions including freezing and herbicides (Hodson et al., 1984). At the leaf axil, a smaller structure is formed similar to the tubers called turions. When the vegetation dies in the winter and breaks free from the bottom, turions are transported by the floating mat. The turions fall from the dying plants and form new infestations. Seed production in hydrilla most likely contributes little to the plants reproduction given its successful vegetative means mentioned above (Langeland, 1996; Kay, 1992).

Figure 6: Image and drawing of Hydrilla (*hydrilla verticillata*). From http://www.ecy.wa.gov/programs/wq/plants/weeds/hydrilla.html

Human activities provide the dominant means by which hydrilla is spread. Stem fragments can become attached to boat motors, trailers, or other equipment and carried from one body of water to another. Mechanical means of control can increase fragmentation providing distribution downstream by wind and currents. Hydrilla can also be spread by animals although there is some debate to what extent. Tubers and turions will survive ingestion and regurgitation by animals, but it is not believed the structures will survive digestion. Seeds of other plant species however are capable of being passed through animals and producing plants. The seeds of the monoecious type of hydrilla may therefore be potentially spread long distances by consumption (Langeland, 1996; Kay 1992).

Millions of dollars are spent annually by water resource managers in an effort to control the rapid spread of hydrilla (Jakubauskas et al., 2002). There are several options available to water resource managers including physical, mechanical, biological, and chemical controls. Physical methods, such as lowering water levels, and mechanical means of removing the weed, which often increases fragmentation, have little success. Chemical herbicides are costly, usually temporary, and often require multiple applications to eradicate the weed. An effective means of biological control often used in North Carolina is the grass carp (*Ctenopharyngodon idella*). Grass carp demonstrate a preference for hydrilla as a food source and can survive up to ten years (Kay, 1992).

Egeria densa* (Brazilian elodea){** TC "Egeria densa* (Brazilian elodea)**" \f C \l "3" **}**

Brazilian elodea appears remarkably similar to hydrilla. Also a submerged perennial plant, stems rooted in the bottom grow to the surface producing dense mats with broad leaves in whorls of 4 to 8. The leaves have serrated edges, although much less defined than the leaves of hydrilla making this a good characteristic for distinction. Introduced as an aquarium plant under the name Anacharis from Brazil in the late 1800s the plant has spread rapidly throughout North America. Unlike hydrilla, only dioecious plants exits in the wild and no seeds or female flowers have ever been reported in the United States. Brazilian elodea also lacks special structures like the rhizomes or tubers found on hydrilla but spreads rapidly through fragmentation. The plant is capable of producing dense stands that restrict water flow, trap sediments, and disturb water quality and the economy. The grass carp show a high preference for elodea as a food source and provide an effective method of biological control (Technical Information about *Egeria densa* (Brazilian elodea), 2003).

Myriophyllum spicatum* (Eurasian watermilfoil){** TC "Myriophyllum spicatum* (Eurasian watermilfoil)**" \f C \l "3" **}**

Eurasian watermilfoil is another non-native aquatic plant species introduced to the United States as an aquarium plant that adversely impacts aquatic ecosystems. Native to Europe and Asia, it was first discovered in Washington, D.C. in the 1940s and has since spread throughout the U.S. and Canada. Similar to hydrilla, Eurasian watermilfoil is highly competitive and grows tall stems from the bottom which branch out to form dense mats at the surface. The leaves are thin and feather-like, arranged in whorls of 4 around the stem. Watermilfoil produces seeds but like hydrilla reproduces primarily by vegetative means. The plant spreads rapidly by fragmentation but does not produce turions. It was first discovered in North Carolina on Currituck Sound in 1965 occupying approximately 40 hectares. After nine years, 32,000 hectares of the sound were infested. Eurasian watermilfoil can be managed by similar means as hydrilla although it is a less desirable food source for grass carp. Millions of dollars are spent annually to control the weed (Technical Information about *Myriophyllum spicatum* (Eurasian Water milfoil), 2003).

Cyanobacteria{ TC "**Cyanobacteria**" \f C \l "2" **}**

Cyanobacteria are commonly referred to as blue-green algae because of their close similarity to plants. They contain chlorophyll and release oxygen during photosynthesis. However, with the invention of the electron microscope came the realization that the “blue-green algae” are structurally similar to prokaryotic bacteria, lacking a nucleus and chloroplasts. Cyanobacteria are considered to be the first photosynthetic organism on the planet, supplying oxygen to the atmosphere and beginning the “evolution of the living world we now inhabit” (Canter-Lund et al., 1995).

Thousands of species of cyanobacteria exist in different forms, often in symbiotic relationships with plants (Canter-Lund et al., 1995). Cyanobacteria are known to produce a wide range of toxins responsible for both human and animal illnesses including microcystins, saxitoxins, and anaoxins (the later two are both neurotoxins) (Codd et al., 2005). Research of cyanotoxins began in the 1960s and continues to expand in an effort to achieve better management (Chorus, 2005). Recent examples of animals affected by harmful algal blooms include fish, dogs, cattle, and flamingos. Because blooms can produce extremely high local concentrations of toxins, they “may include products or components that are only injurious at higher concentrations” (Codd et al., 2005).

The suspect bacteria in the case of avian vacuolar myelinopathy is a filamentous form found attached to submerged aquatic vegetation. Although the species is morphologically similar to several other cyanobacteria, DNA testing has determined it to be a new species of Stigonematales. The bacteria grow as small filaments of a few cells that branch off to form large colonies of thousands of cells which can be seen by the naked eye as blue-green spots on the underside of leaves (Wilde et al., 2005) (Figure 7). Wilde et al. (2005) believes that this new species of cyanobacteria is producing an unknown secondary metabolite which acts as a neurotoxin in the affected bird species.

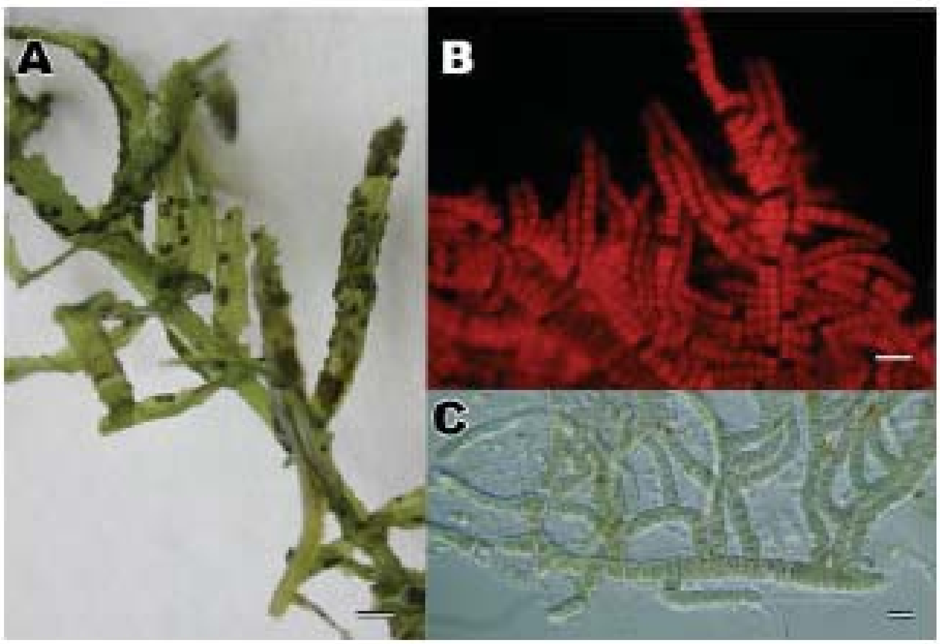

Figure 7: "Macro- and microscopic images of the unknown Stigonematales species suspected of producing the AVM agent. (A) Colonies visible to the unaided eye are growing on stems and the undersides of hydrilla leaves (scale bar, 1 mm); (B) Epifluorescent image of colonies (using Rhod-amine Red filter set) showing phycobiliprotein fluorescence (scale bar, 10 μm); (C) Differential interference contrast image of the colonies (scale bar, 10 μm)" From (Wilde et al., 2005)

Methods{ TC "Methods" \f C \l "1" }

Pond Locations{ TC "Pond Locations" \f C \l "2" }

Remote sensing and image analysis techniques were used to locate and calculate the area of the ponds surveyed. Google Earth software (2005 DigitalGlobe imagery) was used to locate 56 New Hanover County ponds based on size and accessibility (Figure 8). Extremely small ponds and ponds with no visible means of access were not marked for inspection. A point near the center of each pond was marked to obtain a latitude/longitude coordinate in degrees.

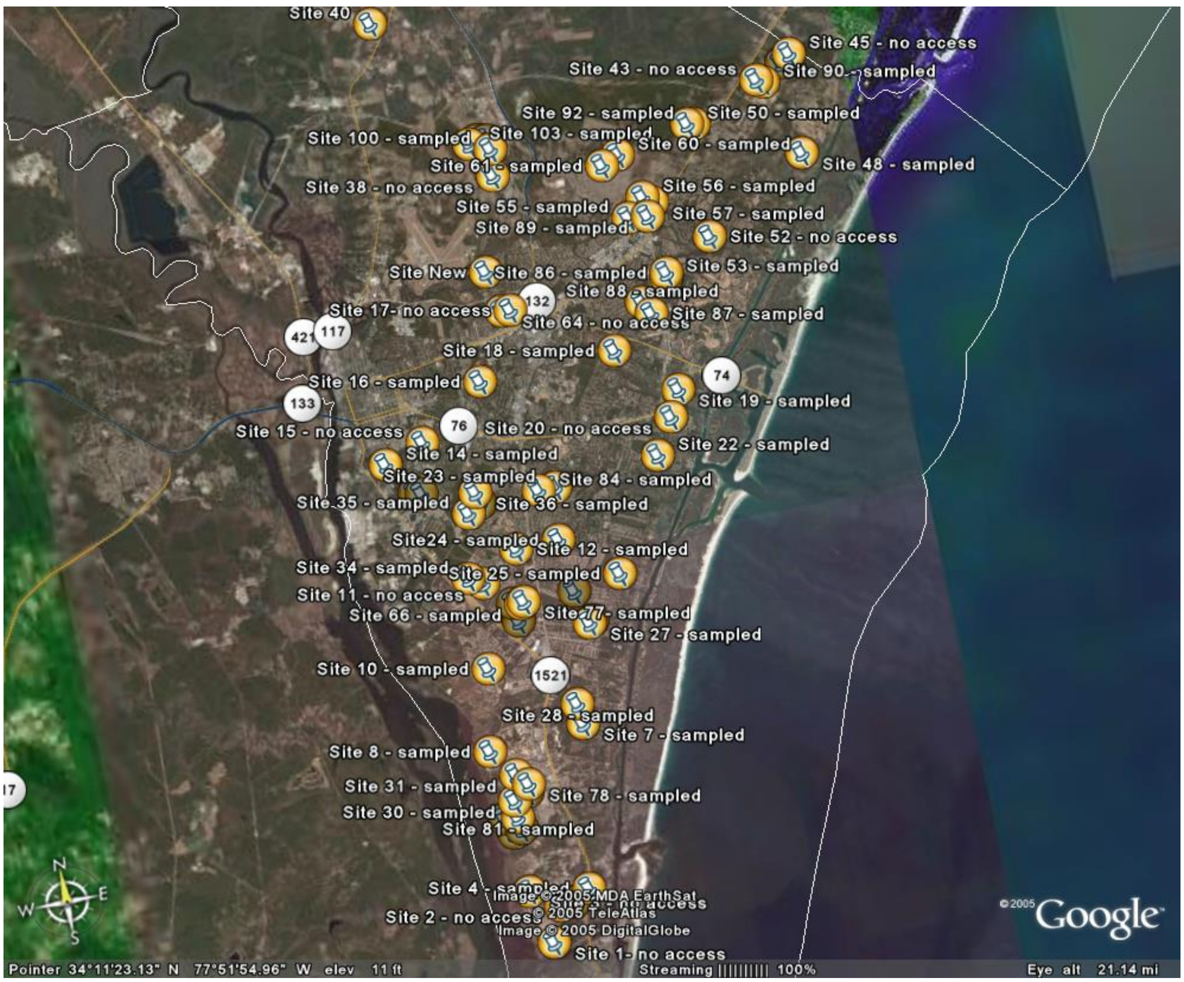

Figure 8: Google Earth imagery of pond locations in New Hanover County, N.C.

Pond Areas{ TC "**Pond Areas**" \f C \l "2" **}**

ArcView GIS image analysis software was used to determine the area of the ponds surveyed. First, a shape file was created containing points marking the relative location of the ponds using a rectified image of New Hanover County roads, hydroline topography, and images of the ponds captured from Google Earth. The shape file was then used to determine which aerial photos available from the New Hanover County website would be needed to include all 56 ponds. Thirty-seven 2002 color aerial photo tiles approximately 4 mb each were required. Next, a shape file was created using the polygon tool to outline the perimeter of each pond (Figure 9). For ponds that contained large islands, the area of the island was subtracted from the total area of the water boundary. Once outlined, a database was created displaying the area of each polygon in square feet and converted to acres and hectares (Table 1).

Figure 9: Site 3, a natural pond and Sites 16, 12, 23, and 15 (left to right), residential retention ponds with polygon shape files for area measurements

Sampling Techniques{ TC "**Sampling Techniques**" \f C \l "2" **}**

All ponds were surveyed during October and November of 2005. A visual inspection of submerged aquatic vegetation was made at each pond. A tool was also used to determine the presence or absence of vegetation and collect samples. The tool consisted of the head of a dethatching rake with the handle removed. A twenty-five foot rope was attached to the head of the rake, allowing it to be thrown approximately twenty feet from shore and retrieved along the bottom (Figure 10). This technique was used from shore at as many locations as each site allowed, not exceeding five locations for any one site. Samples resembling hydrilla, Eurasian watermilfoil, or Brazilian elodea where placed in a freezer bag filled with water from the sampled site. The samples were taken to UNCW for positive identification within five days of collection.

Figure 10: Tool for sampling submerged aquatic vegetation

Avian Wildlife Surveys{ TC "**Avian Wildlife Surveys**" \f C \l "2" **}**

An observation of avian wildlife present at each site was also made. Waterfowl and birds of prey were the focus of the survey. Two field guides were used to identify wildlife to the species level when possible (Sibley, 2003; Udvardy, 1977). At no site were observations made for longer than approximately 45 minutes.

Results{ TC "**Results**" \f C \l "1" }

Pond Locations{ TC "**Pond Locations**" \f C \l "2" }

The 56 ponds chosen for analysis were located throughout New Hanover County. Ponds are classified as either a natural pond (NP), residential retention pond (RP), or commercial retention pond (CP) unless a formal name is given such as Greenfield Lake. Three ponds are described as (NP?) because they resemble natural ponds but their origin is unknown.

Pond Areas{ TC "**Pond Areas**" \f C \l "2" }

Area measurements made of the 56 ponds surveyed ranged from 87.24 acres (35.30 ha) to 0.31 acres (0.13 ha). Natural ponds tended to have the largest area while man-made residential and commercial retention ponds were smaller. Eight of the 56 ponds surveyed were constructed post-2002 and therefore were not present on the latest aerial photography of New Hanover County for analysis. Human error may have contributed to a slight discrepancy between actual pond area and the results obtained from remote sensing analysis. Shoreline vegetation, shadows, and water levels at the time the photos were taken made it more difficult to determine the land-water boundary in some cases. Errors in area measurements are not considered to be significant for the purpose of this study.

Aquatic Vegetation{ TC "**Aquatic Vegetation**" \f C \l "2" }

No pond surveyed contained the invasive plant species of interest; hydrilla, Brazilian elodea, or Eurasian watermilfoil. Emergent species were observed at several locations. Also abundant were various forms of eukaryotic green algae (commonly known as pond scum) found both submerged and floating on the surface. One species of submerged aquatic vegetation with feather-like leaves similar to Eurasian watermilfoil was found at Airlie Lake, and was positively identified as *Ceratophyllum*, or coontail. Approximately two-thirds of the sites surveyed contained no vegetation.

Avian Wildlife{ TC "**Avian Wildlife**" \f C \l "2" **}**

Various species of birds were observed at 17 of the 57 sites including the American coot (*Fulica americana*), Canada goose (*Branta canadensis*), snow goose (*Chen caerulescens*), mallard duck (*Anas platyrhynchos*), great blue heron (*Ardea herodias*), white ibis (*Eudocimus albus*), great egret (*Ardea alba*), mute swan (*Cygnus olor*), redhead (*Aythya valisineria*), turkey vulture (*Cathartes aura*), and assorted diving ducks, gulls, and domestic waterfowl. Canada geese and ducks were the most numerous type of birds sighted, often in flocks of 20 or more and on ponds as small as 0.5 hectares. American coots, the species most susceptible to AVM were only observed at Site 1, Greenfield Lake (Figure 11).

Figure 11: Photo of American coots on Greenfield Lake (Site 1), taken on 1-25-2006

Table 1: Results of ponds surveyed greater than 1 hectare

Site	Date Surveyed	Description	Coordinates	Area (acres)	Area (hectares)	Vegetation	Avian Wildlife
1	11/17/2005 01/25/2006	Greenfield Lake	34°12'37.78"N 77°56'14.15"W	87.24	35.30	emergent, green algae	**AC**, CG, MD, BH, GE, RH, TV, DD, Various ducks, gulls, and domestic waterfowl
2	10/14/2005	Randall Pkwy Reservoir	34°13'48.13"N 77°53'51.35"W	22.94	9.28	emergent	BH, CG, DD, Various ducks
3	10/19/2005	NP	34° 7'3.09"N 77°55'17.03"W	14.03	5.68	no access	none
4	10/19/2005	Silver Lake	34° 8'34.50"N 77°54'56.31"W	10.68	4.32	emergent, green algae	CG
5	10/19/2005	NP?	34° 5'30.36"N 77°55'6.76"W	9.40	3.81	no access	no access
6	11/3/2005	Airlie Lake	34°12'57.56"N 77°49'40.20"W	8.21	3.32	*Ceratophyllum* (coontail)	CG, WI, MD, BH, GE, S
7	11/3/2005	NP	34°13'53.42"N 77°50'51.51"W	7.85	3.18	green algae	none
8	11/3/2005	RP	34°17'25.28"N 77°49'55.81"W	7.79	3.15	none	none
9	11/9/2005	NP?	34°16'48.36"N 77°45'59.53"W	7.05	2.86	none	CG, WI, MD, DD
10	10/14/2005	CP	34°11'27.22"N 77°54'40.58"W	4.65	1.88	green algae	DD
11	11/3/2005	RP	34°15'2.44"N 77°49'25.94"W	3.35	1.36	emergent, green algae	none
12	11/17/2005	RP	34°18'11.42"N 77°52'51.56"W	3.16	1.28	none	CG
13	10/19/2005	CP	34° 6'12.59"N 77°54'39.40"W	3.16	1.28	none	none
14	10/19/2005	RP	34° 9'40.77"N 77°52'46.67"W	2.91	1.18	water lily (unknown species), green algae	none
15	11/17/2005	RP	34°17'58.48"N 77°52'37.06"W	2.69	1.09	none	none
16	11/17/2005	RP	34°18'8.18"N 77°53'2.84"W	2.68	1.09	none	CG

NP = Natural Pond
RP = Residential Retention Pond
CP = Commercial Retention Pond

BH = Blue Heron
WI = White Ibis
DD = Diving Duck
GE = Egret
AC = American Coot
TV = Turkey Vulture

CG = Canada Goose
MD = Mallard Duck
SG = Snow Goose
S = Swan
RH = Redhead

Table 2: Results of ponds surveyed between 1 and 0.4 hectares

Site	Date Surveyed	Description	Coordinates	Area (acres)	Area (hectares)	Vegetation	Avian Wildlife
17	11/3/2005	RP	34°17'16.78"N 77°50'18.58"W	2.45	0.99	none	none
18	10/14/2005	RP	34°10'38.70"N 77°53'49.29"W	2.44	0.99	none	none
19	11/9/2005	NP?	34°17'44.15"N 77°48'18.90"W	2.42	0.98	none	none
20	10/19/2005	CP	34° 6'5.30"N 77°54'57.38"W	2.40	0.97	none	none
21	11/3/2005	RP	34°11'36.70"N 77°52'43.06"W	2.35	0.95	none	MD
22	11/3/2005	CP	34°16'14.56"N 77°49'58.65"W	2.09	0.84	none	MD
23	11/17/2005	RP	34°18'9.35"N 77°52'39.90"W	2.04	0.83	none	none
24	10/19/2005	CP	34° 5'46.42"N 77°54'57.91"W	1.86	0.75	none	none
25	10/19/2005	RP	34° 7'15.10"N 77°53'9.81"W	1.73	0.70	green algae	none
26	10/14/2005	RP	34°10'16.13"N 77°54'56.41"W	1.55	0.63	green algae	none
27	10/19/2005	RP	34° 4'14.70"N 77°53'45.53"W	1.45	0.59	water lilly (unknown species)	none
28	10/14/2005	RP	34°12'0.91"N 77°55'44.29"W	1.33	0.54	emergent	MD
29	10/14/2005	RP	34°11'36.06"N 77°54'23.38"W	1.26	0.51	green algae	none
30	10/19/2005	RP	34° 7'37.52"N 77°53'11.95"W	1.23	0.50	none	CG
31	11/3/2005	RP	34°16'26.53"N 77°49'24.46"W	1.21	0.49	none	none
32	10/14/2005	CP	34°11'48.71"N 77°54'26.88"W	1.19	0.48	emergent	DD
33	11/3/2005	RP	34°16'34.20"N 77°49'35.26"W	1.16	0.47	none	none
34	10/19/2005	RP	34° 9'5.51"N 77°52'30.93"W	1.09	0.44	emergent	BH
35	11/3/2005	CP	34°16'12.09"N 77°49'33.57"W	1.01	0.41	none	none

NP = Natural Pond
RP = Residential Retention Pond
CP = Commercial Retention Pond

BH = Blue Heron
MD = Mallard Duck

CG = Canada Goose
DD = Diving Duck

Table 3: Results of ponds surveyed less than 0.4 hectares or unavailable

Site	Date Surveyed	Description	Coordinates	Area (acres)	Area (hectares)	Vegetation	Avian Wildlife
36	10/19/2005	RP	34° 9'2.83"N 77°52'34.89"W	0.95	0.39	emergent, green algae	none
37	10/14/2005	RP	34° 9'38.06"N 77°53'57.51"W	0.95	0.39	green algae	none
38	10/14/2005	RP	34°11'58.74"N 77°55'35.60"W	0.95	0.39	emergent	none
39	10/19/2005	RP	34° 9'50.25"N 77°51'43.29"W	0.95	0.38	none	none
40	10/14/2005	RP	34° 9'40.62"N 77°54'8.01"W	0.87	0.35	water lily (unknown species), green algae	BH, SG
41	11/3/2005	RP	34°15'6.94"N 77°49'24.65"W	0.81	0.33	none	none
42	11/3/2005	RP	34°10'39.65"N 77°52'52.82"W	0.79	0.32	green algae	none
43	11/3/2005	CP	34°11'37.46"N 77°53'5.34"W	0.72	0.29	green algae	none
44	11/3/2005	RP	34°11'50.44"N 77°50'23.89"W	0.68	0.28	none	none
45	11/9/2005	NP?	34°17'42.67"N 77°48'10.29"W	0.67	0.27	none	none
46	10/19/2005	RP	34° 5'47.89"N 77°55'15.13"W	0.65	0.26	none	none
47	11/9/2005	RP	34°17'41.62"N 77°48'14.91"W	0.31	0.13	none	none
48	10/14/2005	CP	34°14'59.41"N 77°52'53.94"W	unavailable	unavailable	no access	CG, DD
49	10/14/2005	CP	34°15'0.22"N 77°53'5.27"W	unavailable	unavailable	no access	none
50	10/14/2005	RP	34° 9'19.91"N 77°54'5.05"W	unavailable	unavailable	none	CG
51	10/14/2005	RP	34° 9'40.07"N 77°53'53.50"W	unavailable	unavailable	emergent	none
52	10/19/2005	RP	34° 6'32.55"N 77°54'49.19"W	unavailable	unavailable	emergent, green algae	MD, DD
53	10/19/2005	RP	34° 6'20.71"N 77°54'35.22"W	unavailable	unavailable	none	none
54	11/3/2005	CP	34°14'25.65"N 77°49'54.41"W	unavailable	unavailable	none	CG, S
55	11/3/2005	CP	34°14'40.09"N 77°50'3.77"W	unavailable	unavailable	none	none
56	11/9/2005	RP	34°18'13.47"N 77°46'30.53"W	unavailable	unavailable	none	none

NP = Natural Pond
RP = Residential Retention Pond
CP = Commercial Retention Pond

BH = Blue Heron
MD = Mallard Duck
SG = Snow Goose

CG = Canada Goose
DD = Diving Duck
S = Swan

Discussion{ TC "**Discussion**" \f C \l "1" }

The results suggest that none of the 56 ponds surveyed will currently produce an avian vacuolar myelinopathy occurrence based on the hypothesis presented by Wilde et al. (2005). The hypothesis states "that three elements are needed to produce AVM: an abundance of preferred aquatic vegetation (e.g. hydrilla), an abundance of the suspect Stigonematalan species growing on the available substrate, and herbivores waterfowl" (Wilde et al. 2005). Although several sites contained abundant avian species susceptible to the disease, no site showed infestations of the three types of preferred vegetation present at other AVM positive locations (hydrilla, Eurasian watermilfoil, or Brazilian elodea). Wayne Batten, director of the Pender County Cooperative Extension, reports that there are currently no known infestations of hydrilla in the New Hanover County area. The Burnt Mill Creek (Randall Parkway Reservoir, Site 2) infestation was removed several years ago using a combination of herbicides and grass carp. However, the results do suggest that the potential for a future AVM occurrence exists at several sites.

Airlie Lake (Site 6) may provide the greatest risk of an AVM outbreak due to the large amount of aquatic vegetation found there. At 3.32 ha and home to a significant population of water birds, the majority of the lake appears to be infested by *Ceratophyllum*, or coontail (Figure 12). Wilde et al. (2005) documented the suspect Stigonematales bacteria growing on *Ceratophyllum demersum* and other native species including Illinois pondweed, bladderwort, lemon bacopa (*Bacopa caroliniana*), fragrant water lily (*Nymphaea odorata*), and watershield (*Brasenia schreberi*) in AVM positive reservoirs. "However, the Stigonematales species was denser and more prevalent on hydrilla and egeria leaves, and these plants made up orders of magnitude more biomass (and substrate for epiphytic growth) than native aquatic plants" (Wilde et al., 2005). No samples of *Ceratophyllum* taken from Airlie Lake had visible colonies of cyanobacteria attached. However, the ability of *Ceratophyllum* to support the suspect Stigonematalan species warrants further investigations in order to determine the full potential of an AVM occurrence at Airlie Lake. A more detailed survey should include an examination of the types of bacteria present at the microscopic level.

Figure 12: Photo of *Ceratophyllum* taken from Airlie Lake (Site 6) on 11-3-2005

Sites 14, 27, and 40 contained various species of water lilies although they were not taken to UNCW for positive identification. Because the Stigonematales species has been documented in association with fragrant water lily (*Nymphaea odorata*), it is possible that the three plants may be capable of harboring the disease (Wilde et al. 2005). However, due to the small amounts of plant material, small pond areas (< 1.18 ha), and lack of susceptible birds it is not believed that the three ponds containing the lilies present a significant threat of producing the disease.

Greenfield Lake, the largest pond surveyed in New Hanover County at 35.30 hectares, contained the highest number of susceptible birds including the American coot. Although no submerged aquatic vegetation was detected at the lake, an infestation of hydrilla or other invasive species could spread rapidly through the system. A significant infestation could harbor the suspect Stigonematalan species in large enough quantities to potentially produce AVM in the numerous birds present. Greenfield Lake was the only site included in this survey which contained American coots, and is considered the most likely source of an AVM outbreak if a submerged aquatic vegetation infestation occurs. The lake is managed by the city of Wilmington who contracts a private company to maintain the site.

Several other ponds may potentially produce an AVM occurrence based on size and abundance of avian wildlife as well. Sites 2, 4, 6, and 9 all contained several species susceptible to AVM such as Canada geese and mallard ducks, and are larger than the smallest pond (1.6 ha) with a documented AVM occurrence (Wilde et al. 2005). The sizes range from 9.28 ha to 2.86 ha respectively. Like Greenfield Lake, it is assumed that a significant infestation of preferred aquatic vegetation could support the suspect bacteria and produce an occurrence of AVM.

Although Wilde et al. (2005) discovered AVM on a 1.6 ha pond, it appears that ponds much smaller than that lack the resources necessary to pose a serious threat of producing AVM. Many of the ponds surveyed smaller than 1.0 ha lacked the presence of water birds or vegetation. Also, natural ponds tended to be larger than the commercial or residential retention ponds. Most retention ponds lacked any type of vegetation because they are often managed by environmental companies that remove vegetation to maintain the ponds function. Natural systems and those unmanaged by private owners are considered to have a greater potential for containing submerged aquatic vegetation capable of supporting the suspect bacteria.

The 56 ponds surveyed are estimated to represent 1/5 of the total ponds in New Hanover County. Many of the ponds not included in this survey were either too small for consideration or not accessible. Numerous ponds are located on golf courses within gated communities or on private land. Site 5 for example is listed in the results table because it is a natural pond (NP?) of considerable size (3.81 ha) although I was unable to gain close access to sample vegetation or observe the avian wildlife (Figure 13). In order to fully understand the potential for an avian vacuolar myelinopathy occurrence in New Hanover County, all ponds greater than a certain size, perhaps 1.0 ha, should be surveyed.

Figure 13: Google Earth image of Site 5

Current studies are underway which will help determine the definitive cause of AVM. Research has identified the genetic code of the new species of cyanobacteria believed to be responsible for causing AVM. The bacteria has been cultured and fed directly to American coots without producing lesions in the birds. However, the strains may lose toxicity or require environmental triggers not occurring under laboratory conditions to cause the disease. Results from a second culture trial are pending (Williams, unpubl. data).

Another study has examined the use of grass carp for managing hydrilla infestations as a potential carrier of AVM. Carp fed hydrilla with the associated Stigonematalan species of bacteria developed lesions in the optic tectum similar to those seen in affected birds. When the fish were fed to mallard ducks, the ducks developed AVM (Smith, unpubl. data). The results may complicate aquatic weed management, since the grass carp is the current preferred method of control.

Researchers are also developing methods for extracting the toxins produced by the suspect bacteria (Wiley, unpubl. data). However, Birrenkott et al. (2004) notes that recognizing the cyanobacterial toxin responsible for causing the disease can be difficult.

> "Although it is poorly understood what factors cause cyanobacteria to produce toxins, toxin production may be regulated by genetics and influenced by environmental changes. In a bloom of cyanobacteria that consists of one species it is possible to have strains that are toxic and strains that are nontoxic, and cyanobacteria can produce more than one toxin simultaneously" (Birrenkott et al, 2004).

Future research should continue to focus on finding the neurotoxin responsible for causing AVM, with emphasis on the genetic origin and physiology of the suspect Stigonematales species. Once the toxin is discovered, the environmental factors responsible for controlling its production should be examined. Assuming the suspect cyanobacteria is responsible, a database of sites infested with hydrilla or similar aquatic weeds known to support the bacteria is necessary to control its spread. This study has attempted to provide information for that database.

Research should also focus on the diseases ability to spread from site to site. Stoskopf (unpubl. data) reports that some birds diagnosed with brain lesions associated with AVM have been able to regain all or partial nerve function under laboratory conditions. A certain percentage of birds in the wild may theoretically contract AVM and regain motor function under mild environmental conditions. The affected birds may then be capable of migrating long distances with AVM lesions but normal function. AVM could then spread at the new site from the affected bird, making it impossible to determine the original source (location) of the disease.

It is possible that there are many undocumented cases of AVM because of public unawareness and difficulties associated with recognizing the disease. David Allen, coastal regional manager of faunal diversity for the N.C. Wildlife Resources Commission reports that there are currently no reports of bald eagles nesting in New Hanover County, or eagle fatalities related to the disease. There is one confirmed nest in neighboring Pender County and it is possible that an existing nest in New Hanover has not been spotted. Assuming eagle populations continue to rise, future nests are likely which will increase the need for greater public awareness of recognizing AVM.

It should be noted that until the definitive causative agent of AVM is determined, the results of this study may be inadequate in predicting the potential for an AVM occurrence in New Hanover County. This study is based on a hypothesis suggested by Wilde et al. (2005) because of the strong correlation between water birds, invasive aquatic weeds, a novel species of cyanobacteria, and cases of AVM resulting from their study. The possibility exists that the causative agent is not associated with the Stigonematalan species of bacteria, or that the agent can exist without the presence of invasive aquatic vegetation and therefore caution should be exercised when interpreting this study. However, the rapid spread of invasive aquatic vegetation has caused numerous harmful impacts on the natural environment and the ability of that vegetation to harbor bacteria potentially capable of killing large numbers of avian wildlife, including the bald eagle, demands more attention from the public and resource managers.

Acknowledgements{ TC "**Acknowledgements**" \f C \l "1" }

I would like to thank Dr. Lawrence B. Cahoon, professor of biology and marine science at UNCW; Dr. Jeffery M. Hill, graduate program coordinator of environmental studies at UNCW; Dr. Gregory T. Chandler, assistant professor of biology at UNCW; Ken Manuel, Duke Energy; Rob Emens, N.C. Division of Water Resources; David Allen, N.C. Wildlife Resources Commission; and Wayne Batten, N.C. Pender County Cooperative Extension Director.

Literature Cited{ TC "Literature Cited" \f C \l "1" }

AUGSPURGER, T., J. R. FISHER, N. J. THOMAS, L. SILEO, R. E. BRANNIAN, K. J. G. MILLER, AND T. E. ROCKE. 2003. Vacuolar myelinopathy in waterfowl from a North Carolina impoundment. Journal of Wildlife Diseases 39: 412-417.

BIRRENKOTT, A. H., S. B. WILDE, J. J. HAINS, J. R. FISHER, T. M. MURPHY, C. P. HOPE, P. G. PARNELL, AND W. W. 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.

BRIDGMAN, A. 2003. American coots. Available from the Animal Diversity Website via the Internet (http://animaldiversity.ummz.umich.edu/site/accounts/information/Fulica_americana.html).

CANTER-LUND, H., AND J. W. LUND. 1995. Freshwater algae: their microscopic world explored. Biopress Limited, Bristol, England, 360 pp.

CHORUS, I. 2005. Water safety plans: a better regulatory approach to prevent human exposure to harmful cyanobacteria. *In* Harmful cyanobacteria, J. Huisman, H. C. P. Matthijs, and P. M. Visser (eds.). Springer, Dordrecht, Netherlands, pp. 201-227.

CODD, G. A., J. LINDSAY, F. M. YOUNG, L. F. MORRISON, AND J. S. METCALF. 2005. Harmful cyanobacteria: from mass mortalities to management measures. *In* Harmful cyanobacteria, J. Huisman, H. C. P. Matthijs, and P. M. Visser (eds.). Springer, Dordrecht, Netherlands, pp. 1-23.

DODDER, N. G., B. STRANDBERG, T. AUGSPURGER, AND R. A. HITES. 2003. Lipophilic organic compounds in lake sediment and American coot (*Fulica Americana*) tissues, both affected and unaffected by avian vacuolar myelinopathy. The Science of the Total Environment 311:81-89.

EVERITT, J. H., C. YANG, D. E. ESCOBAR, C. F. WEBSTER, R. I. LONARD, AND M. R. DAVIS. 1999. Using remote sensing and spatial information technologies to detect and map two aquatic macrophytes. Journal of Aquatic Plant Management 37: 71-80.

FISHER, J. R., L. A. LEWIS, T. AUGSPURGER, AND T. E. ROCKE. 2002. Avian vacuolar myelinopathy: a newly recognized fatal neurological disease of eagles, waterfowl, and other birds. Transactions of the North American Wildlife and Natural Resources Conference 67: 51-61.

FISHER, J. R., L. A. LEWIS-WEIS, AND C. M. TATE. 2003. Experimental vacuolar myelinopathy in red-tailed hawks. Journal of Wildlife Diseases 39: 400-406.

GUILFOYLE, M. P., D. E. EVANS, R. A. FISHER, AND C. O. MARTIN. 2000. Riparian raptors on USACE projects: bald eagle (*Haliaeetus leucocephalus*). EMRRP Technical Notes Collection (ERDC TN-EMRRP-SI-12), U.S. Army Engineer Research and Development Center, Vicksburg, MS. Available via the Internet (www.wes.army.mil/el/emrrp).

HODSON, R. G., G. J. DAVIS, K. E. LANGELAND. 1984. Hydrilla management in North Carolina. Water Resources Research Institute of the University of North Carolina. Raleigh, North Carolina, 46 pp.

JAKUBAUSKAS M. E., D. L. PETERSON, S. W. CAMPBELL, F. DENOYELLES JR., S. D. CAMPBELL, D. PENNY. 2002. Mapping and monitoring invasive aquatic plant obstructions in navigable waterways using satellite multispectral imagery. Proceedings from the Pecora 15/Land Satellite Information IV/ISPRS Commission 1/FIEOS Conference. From the International Society for Photogrammetry and Remote Sensing Website via the Internet (http://www.isprs.org/commission1/proceedings/paper/00058.pdf).

KAY, S. H. 1992. Hydrilla: a rapidly spreading aquatic weed in North Carolina. N.C. Cooperative Extension Service, North Carolina State University. Raleigh, North Carolina, 12 pp.

LANGELAND, K. A. 1996. *Hydrilla verticillata*, “The perfect aquatic weed.” Castanea 61: 293-304

LARSON, R. S., F. B. NUTTER, T. AUGSPURGER, T. E. ROCKE, L. TOMLINSON, N. J. THOMAS, AND M. K. STOSKOPF. 2002. Clinical features of avian vacuolar myelinopathy in American coots. Journal of American Veterinary Medical Association 221: 80-85.

LARSON, R. S., F. B. NUTTER, T. AUGSPURGER, T. E. ROCKE, N. J. THOMAS, AND M. K. STOSKOPF. 2003. Failure to transmit avian vacuolar myelinopathy to mallard ducks. Journal of Wildlife Diseases 39: 707-711.

LEWIS-WEIS, L. A., R. W. GERHOLD, AND J. R. FISHER. 2004. Attempts to reproduce vacuolar myelinopathy in domestic swine and chickens. Journal of Wildlife Diseases 40: 476-484.

MURPHY, T. Unpublished data. North Carolina Avian Vacuolar Myelinopathy Forum, October 6, 2005. Tom Murphy is an employee of the US Fish and Wildlife Service.

ROCKE, T. E., N. J. THOMAS, T. AUGSPURGER, AND K. MILLER. 2002. Epizootiologic studies of avian vacuolar myelinopathy in waterbirds. Journal of Wildlife Diseases 38: 678-684.

ROCKE, T. E., N. J. THOMAS, C. U. METEYER, C. F. QUIST, J. R. FISHER, T. AUGSPURGER, AND S. E. WARD. 2005. Attempts to identify the source of avian vacuolar myelinopathy for waterbirds. Journal of Wildlife Diseases 41: 163-170.

SIBLEY, D. A. 2003. The Sibley field guide to birds of eastern North America. Alfred A. Knopf, Inc. New York, 431 pp.

SMITH, R.Unpublished data. North Carolina Avian Vacuolar Myelinopathy Forum, October 6, 2005. Rebecca Smith is a graduate student at Clemson University.

STOSKOPF, M. Unpublished data. North Carolina Avian Vacuolar Myelinopathy Forum, October 6, 2005. Dr. Michael Stoskopf is a professor at the NCSU College of Veterinary Medicine.

THOMAS, N. J., C. U. MYETEYER, AND L. SILEO. 1998. Epizootic vacuolar myelinopathy of the central nervous system of bald eagles (*Haliaeetus leucocephalus*) and American coots (*Fulica Americana*). Veterinary Pathology 35: 479-487.

UDVARDY, M. D. F. 1977. The Audubon Society field guide to North American birds. Alfred A. Knopf, Inc. New York, 854 pp.

WILDE, S. B., T. M. MURPHY, C. P. HOPE, S. K. HABRUN, J. KEMPTON, A. BIRRENKOTT, F. WILEY, W. W. BOWERMAN, AND A. J. LEWITUS. 2005. Avian vacuolar myelinopathy linked to exotic aquatic plants and a novel cyanobacterial species. Environmental Toxicology 20: 348-353.

WILEY, F. Unpublished data. North Carolina Avian Vacuolar Myelinopathy Forum, October 6, 2005. Faith Wiley is a graduate student at Clemson University.

WILLIAMS, S. Unpublished data. North Carolina Avian Vacuolar Myelinopathy Forum, October 6, 2005. Sarah Williams is a graduate student at the University of Charleston.

2003. Technical information about *Myriophyllum spicatum* (Eurasian Watermilfoil). From the Washington State Department of Ecology Water Quality Program Website via the Internet (http://www.ecy.wa.gov/programs/wq/plants/weeds/aqua004.html).

2003. Technical information about *Egeria densa* (Brazilian elodea). From the Washington State Department of Ecology Water Quality Program Website via the Internet (http://www.ecy.wa.gov/programs/wq/plants/weeds/aqua002.html).

2005. Bald eagle fact sheet. From the North Carolina Wildlife Resources Commission Website via the Internet (http://www.ncwildlife.org/pg07_wildlifespeciescon/nongame_baldeagle_hires.pdf).