Avian Vacuolar Myelinopathy

Photo by Tom Murphy

Linking Invasive Aquatic Plants, a Novel Cyanobacterial Species and Avian Vacuolar Myelinopathy in Southeastern Reservoirs

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John Grizzle

- The primary birds affected are American coots (1000's) and Bald Eagles (at least 100)

- Also affected are Mallards, Ring-necked ducks, Buffleheads, American wigeon, Canada geese, Great Horned owls, and Killdeer

Photos by

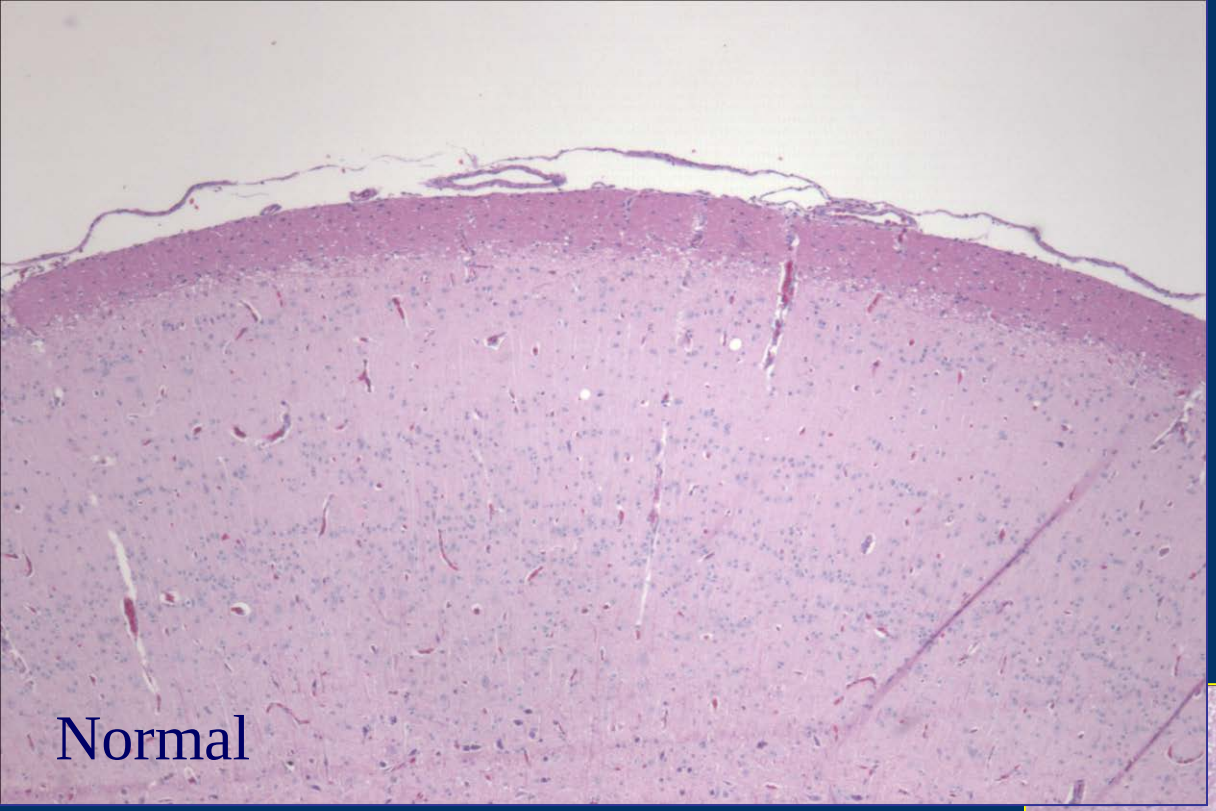
Tom Murphy

Emergent Eagle disease

First documented occurrence:
DeGray Lake, Arkansas----November 1994

- 29 Bald Eagles were found dead or dying
- Eagles and coots neurologically impaired

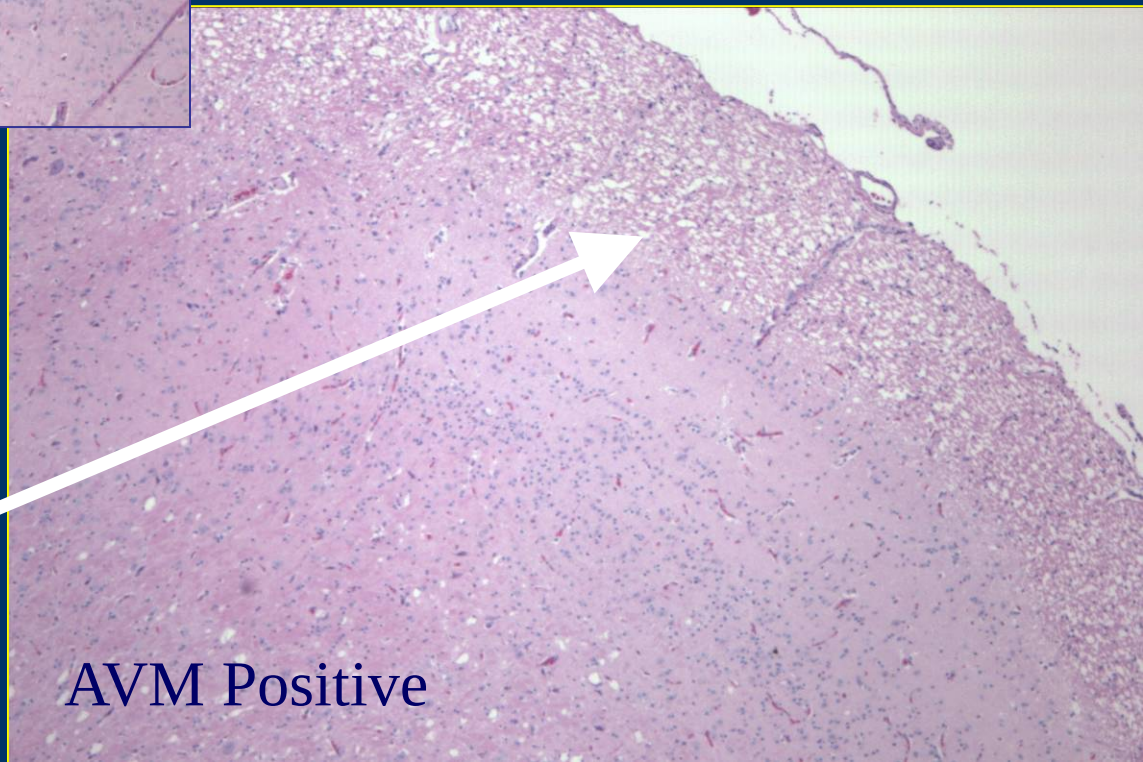
Diagnosis



Normal

No consistent gross abnormalities in eagles or coots

Lesions are present in the white matter of the brain tissue



AVM Positive

AVM confirmed sites within in southeastern United States

http://www.mvk.usace.army.mil/offices/od/odm/avm/avm_main.htm

- Deaths occur from October to March while birds overwinter on these reservoirs

Extensive diagnostic examinations

No infectious disease agents including:

- Viruses
- Bacteria
- Parasites
- Prions

No known toxins that cause brain lesions including:

- Pharmaceuticals
- Organic metals
- Plant toxins

- **Coots and other waterfowl contract disease on site within as few as 5 days**

National Wildlife Health Center, USGS
US Fish and Wildlife Service

Rocke, T. E., N. J. Thomas, T. Augspurger, K. Miller. 2002. Epizootiologic studies of Avian Vacuolar Myelinopathy in waterbirds. J. Wildlife Dis. 38:678-684.

AVM is not contagious.

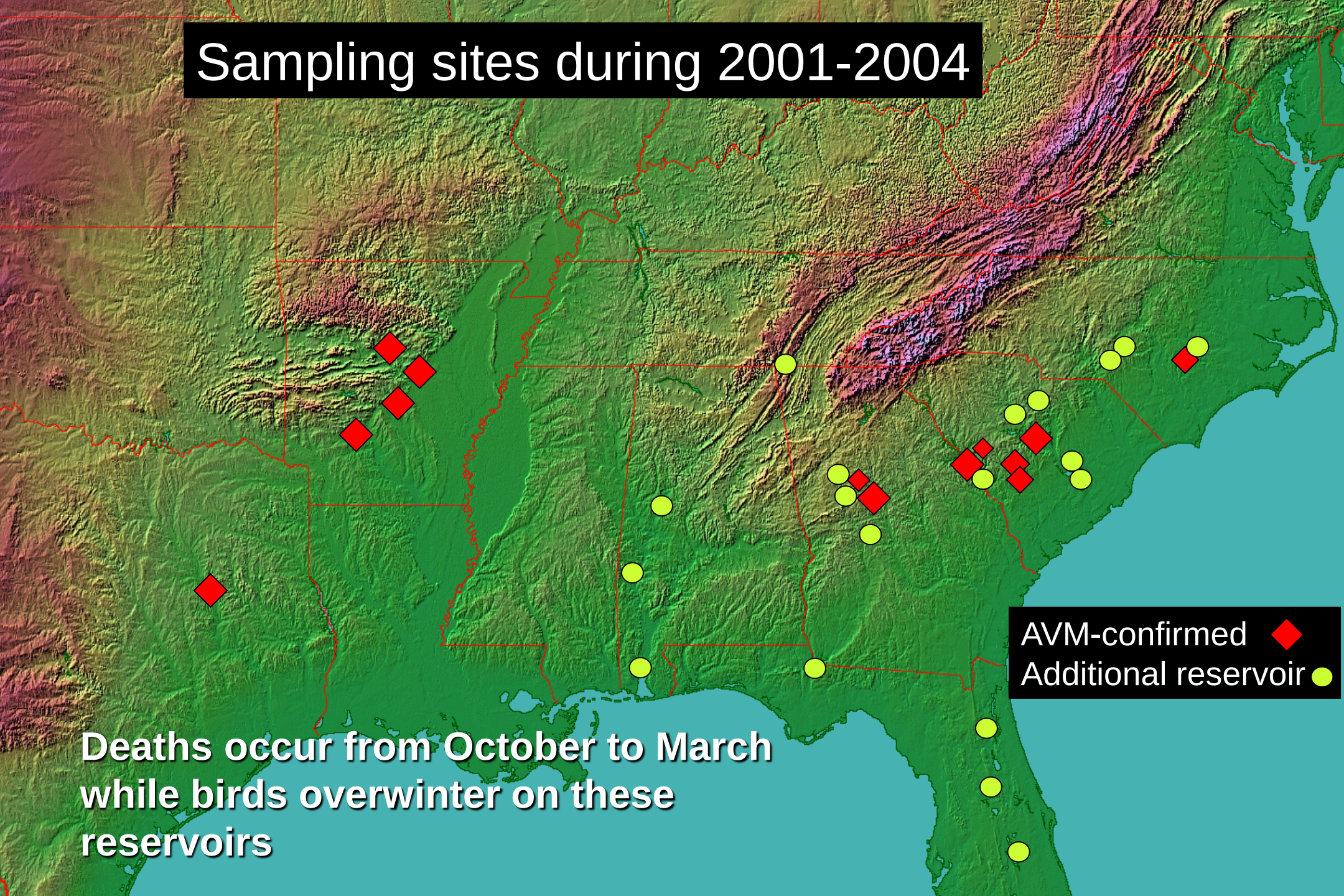
North Carolina State University

Larsen, R. S., F. B. Nutter, T. Augspurger, T. E. Rocke, L. Tomlinson, N. J. Thomas, M. K. Stoskopf. 2002. Clinical features of avian vacuolar myelinopathy in American coots. JAVMA 221:80-85.

- **Eagles and other predatory birds contract disease by ingesting affected birds.**

Southeastern Cooperative Wildlife Disease Study (SCWCS)
College of Veterinary Medicine
The University of Georgia

Fischer, J., L. A. Lewis-Weis, C. M. Tate. 2003. Experimental Vacuolar Myelinopathy in Red-tailed Hawks. J. Wildlife Dis. 39:400-406.


Sampling sites during 2001-2004
AVM-confirmed
Additional reservoir
Deaths occur from October to March while birds overwinter on these reservoirs

Three invasive aquatic species dominant AVM reservoirs

Hydrilla
(Hydrilla verticillata)

10/13

ARKANSAS
DeGray Lake
Lake Ouachita

TEXAS
Sam Rayburn Reservoir

SOUTH CAROLINA/GEORGIA
Lake Murray
Davis Pond
J. Strom Thurmond Reservoir
Lake Juliette
Emerald Lake

NORTH CAROLINA
Woodlake
Coachmans Trail

Photo by Tom Murphy
SCDNR

Brazilian elodea
(Egeria densa)

3/13

ARKANSAS
DeGray Lake
Lake Ouachita

GEORGIA
Lake Juliette

Photo by W.T. Haller
Univ. of Florida

Eurasian watermilfoil
(Myriophyllum spicatum)

4/13

ARKANSAS
Lake Hamilton

GEORGIA
Lake Juliette

SOUTH CAROLINA
SRS- L Lake and Par Pond

Photo by Steve deKozlowski
SCDNR

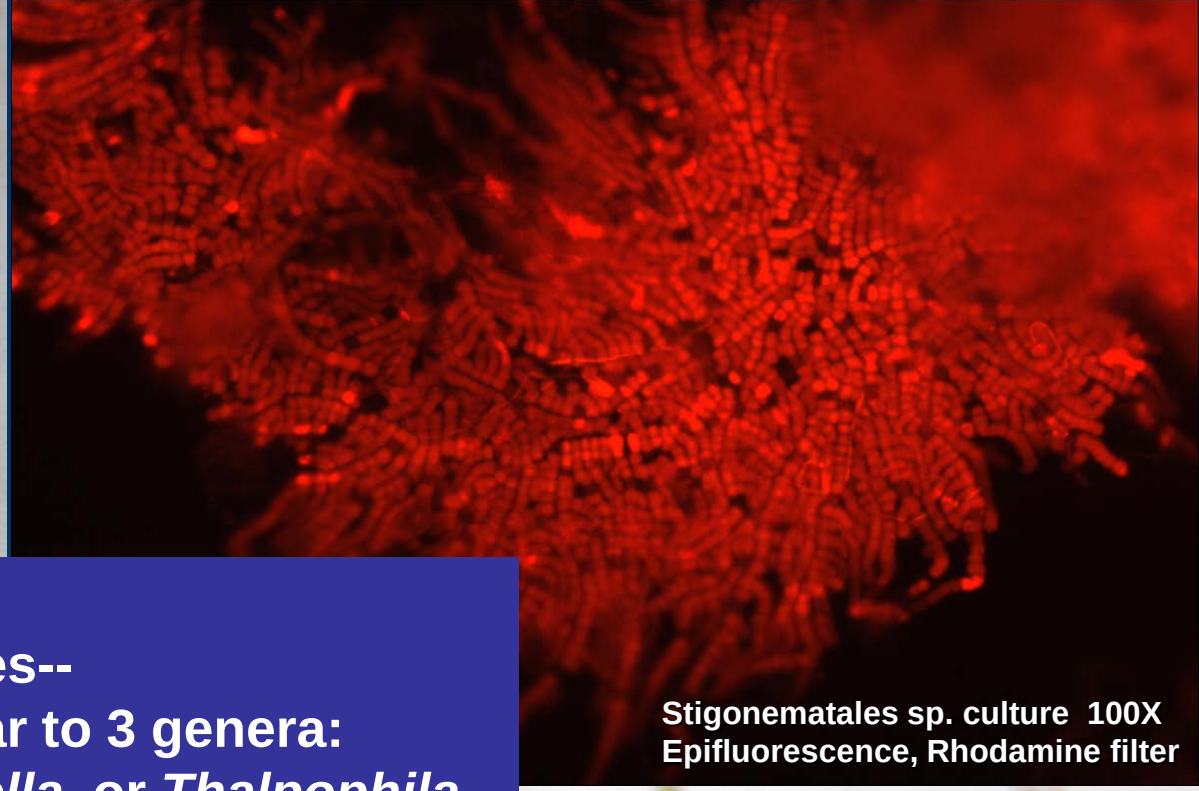
Avian Vacuolar Myelinopathy

Current working hypothesis---Food chain linkage

Stigonematales species that grows on aquatic plants produces the neurotoxic AVM agent

Aquatic plants with toxic algal epiphytes are consumed by Coots

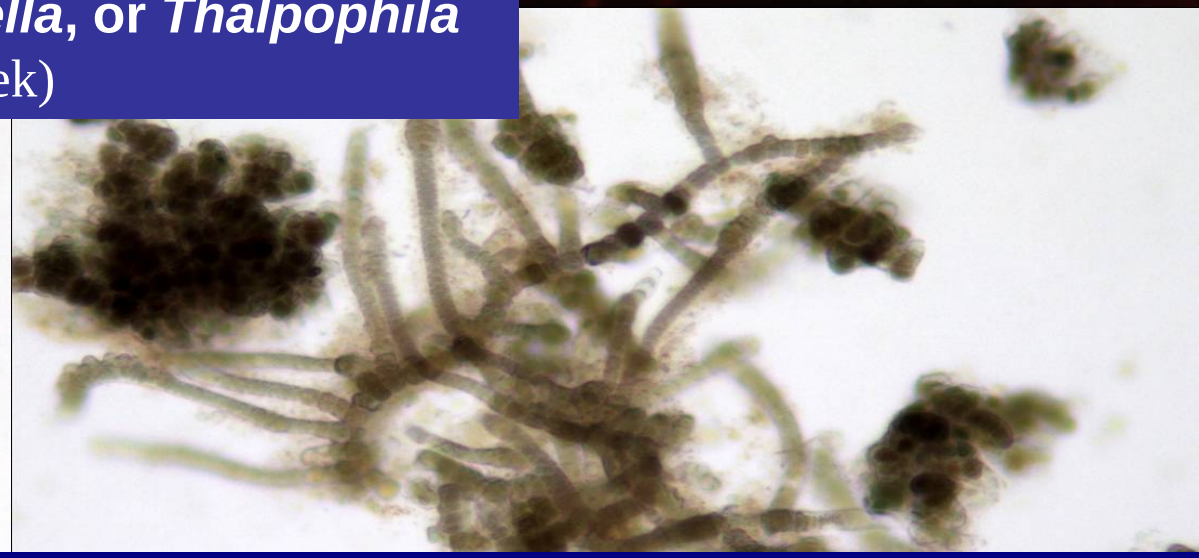
Sick coots are consumed by Eagles



Stigonematales sp. culture 100X
Epifluorescence, Rhodamine filter

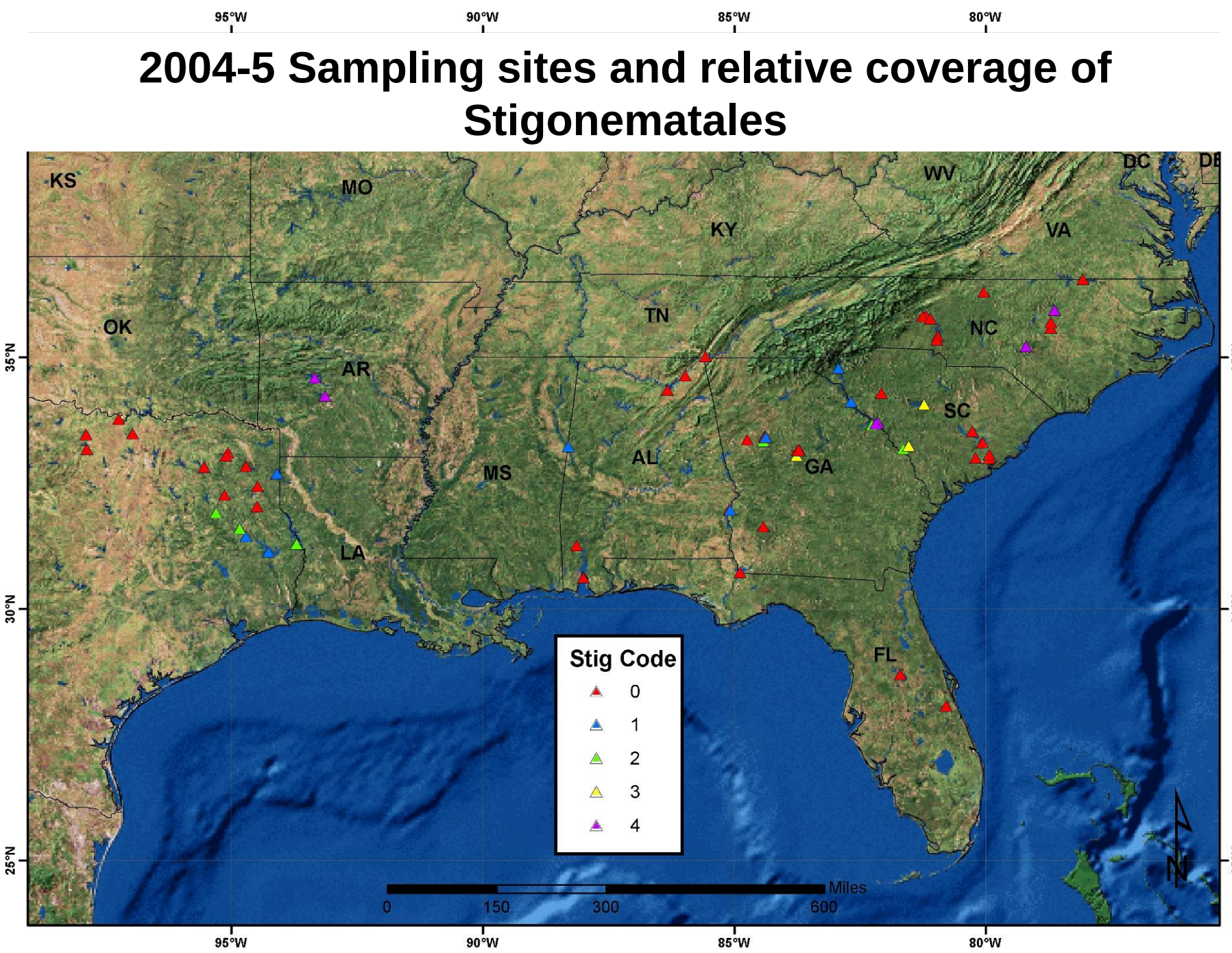
--Prime Suspect--

Stigonematales species--
Morphologically similar to 3 genera:
Hapalosiphon*, *Fisherella*, or *Thalpophila
(Pers. Comm. Jiri Kormarek)



Williams, S.K., S.B. Wilde, J. Kempton, and Alan J. Lewitus. (in prep) A novel epiphytic cyanobacterium associated with reservoirs affected by Avian Vacuolar Myelinopathy. To be submitted to Phycologia.

2004-5 Sampling sites and relative coverage of Stigonematales




Lake Horton
December 2005
Eagle, Canada Geese, Coots
Bufflehead and Ruddy duck

Woodlake, North Carolina confirmed 1998

J Strom Thurmond Reservoir

J. Strom Thurmond Reservoir

Davis Pond

Davis Pond Sentinel Trial

October 29 - December 9, 2003

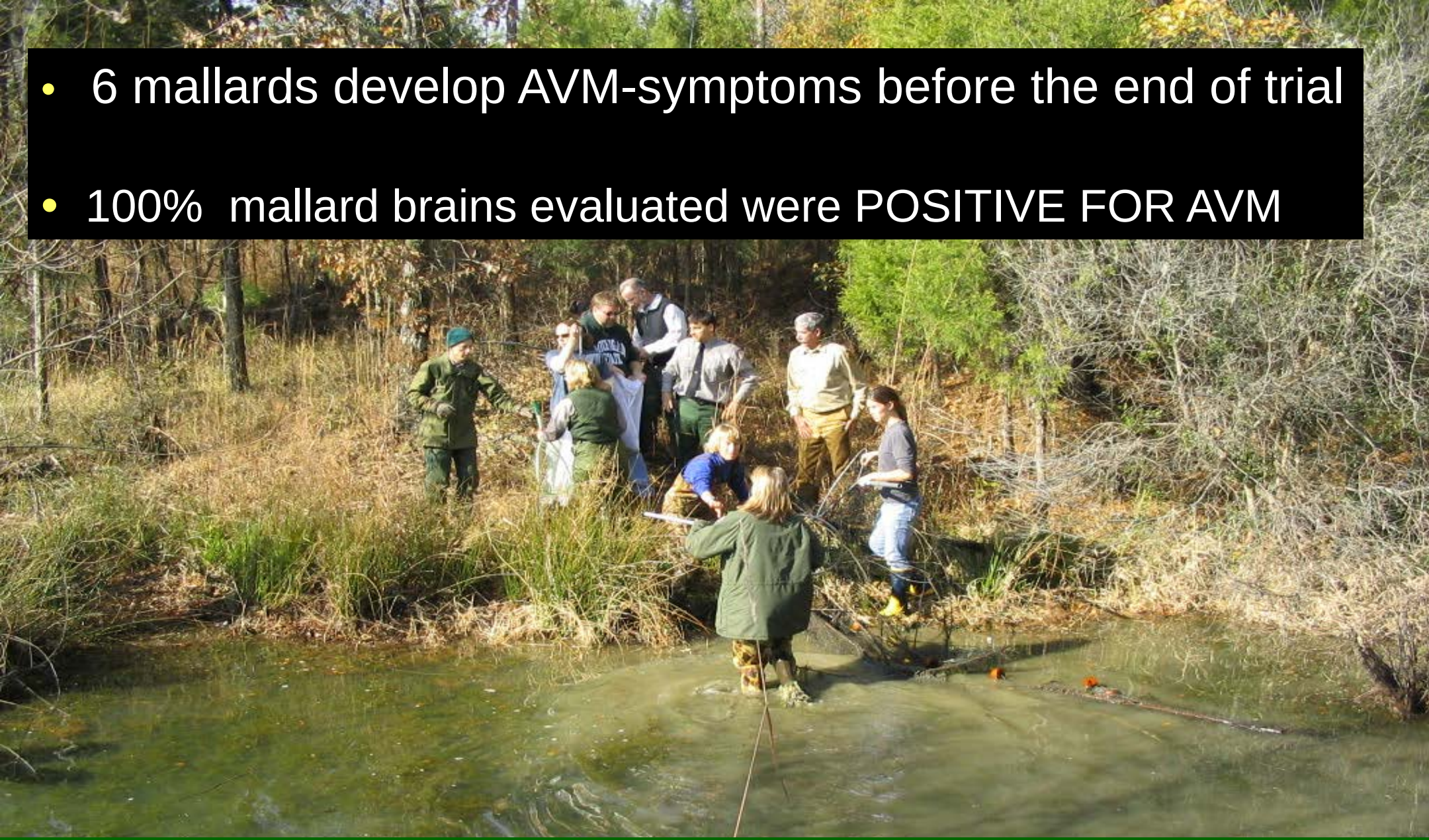
- **1st occurrence of hydrilla in pond**
  --Summer 2003

- **Initial survey finds Stigonematales species**
  ABUNDANT

- 20 farm-raised juvenile male mallards obtained from Whistling Wings, WI
- Each bird was wing-clipped, banded and weighed

- Mallards were released into the 10 acre farm pond for the 6 week trial

- 6 mallards develop AVM-symptoms before the end of trial
- 100% mallard brains evaluated were POSITIVE FOR AVM



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 (AVM) Linked to Exotic Aquatic Plants and a Novel Cyanobacterial Species. Environ. Toxicol. 20:348-353.

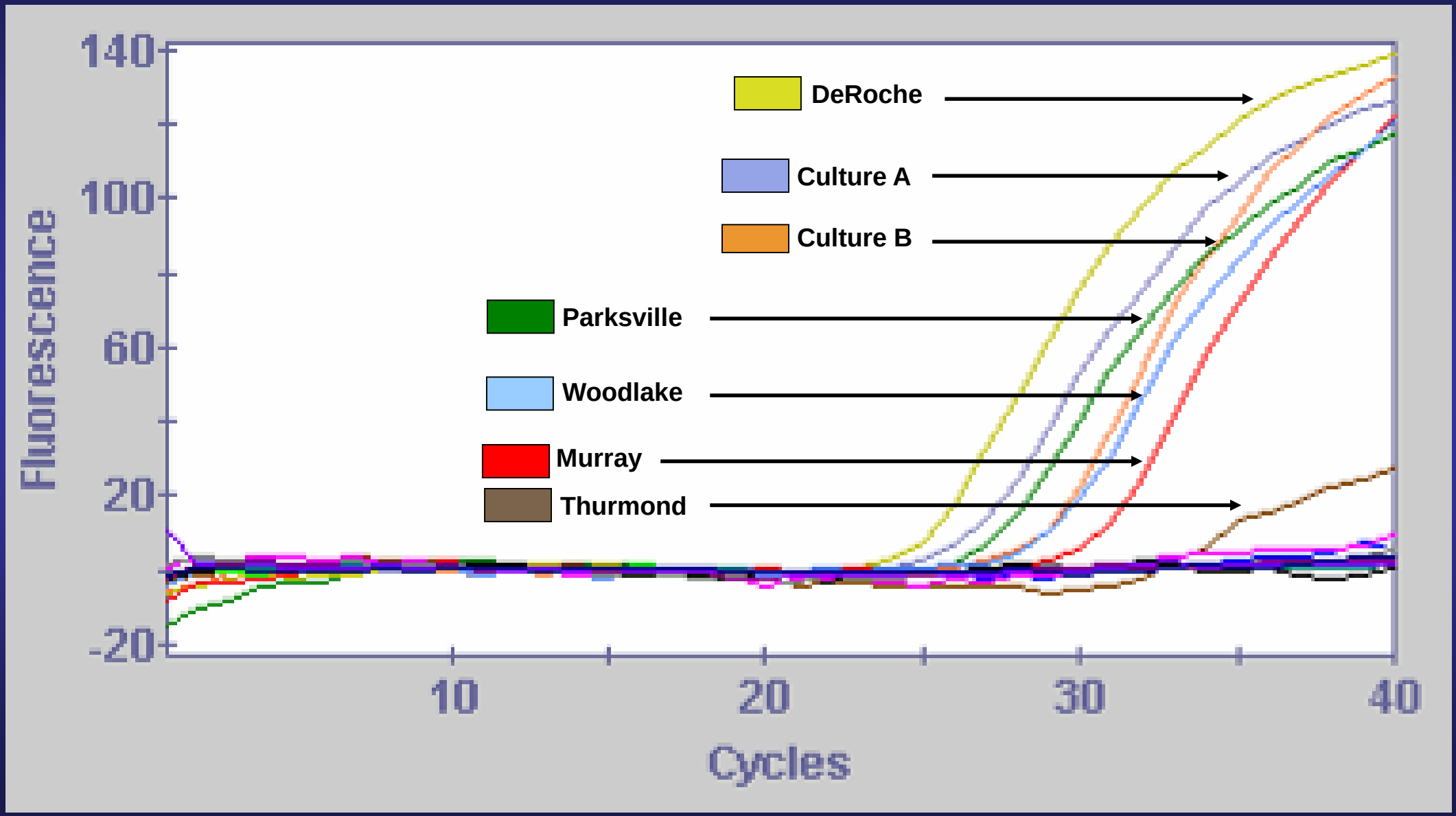
Genetic Analysis and Culturing an Epiphytic Cyanobacterial Species Associated with AVM Bird Deaths

Sarah Williams

Prime suspect

- Abundant colonies of an undescribed epiphytic cyanobacterium
- Initially identified as *Hapalosiphon fontinalis*
- Now known to be a new closely related species in the order Stigonematales
- Submitted the 16S sequence to GenBank - AY785313
- Created a Real-Time PCR assay specific to the *Stigonematales* species.

Real Time-PCR



Culture Progress

- Field samples of the targeted cyanobacterium have been cultured
- Experimenting with several media to retain/induce toxin production
- $BG\text{-}11_0$ + Hydrilla extract
- BG-11 results in rapid growth

Initial Feeding Trial

- Culture material was grown in BG-11_0 at 27C for six weeks
- Cold shocked before harvesting
- 6 coots were gavaged whole cell material followed by a rinse of BG-11_0 media
- Ducks were gavaged daily for 13 days at Clemson University
- None of the coots became symptomatic or had brain lesions

Cyanobacteria Research

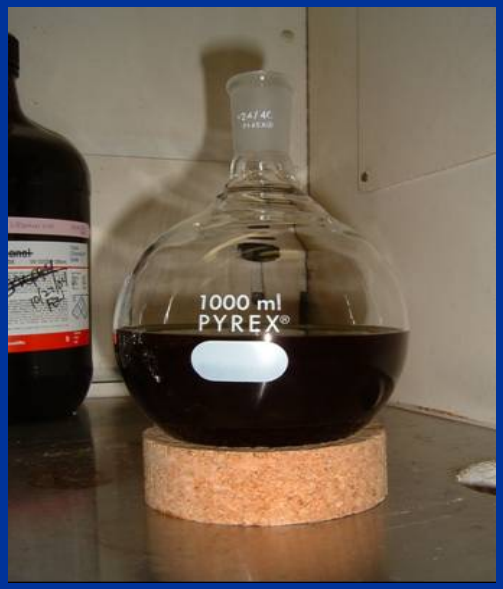
- Culture strains lose toxicity
- Evidence of certain environmental triggers for toxin production
- Looking at environmental conditions when birds become positive
- Started new strains from 2005-2006 sampling season

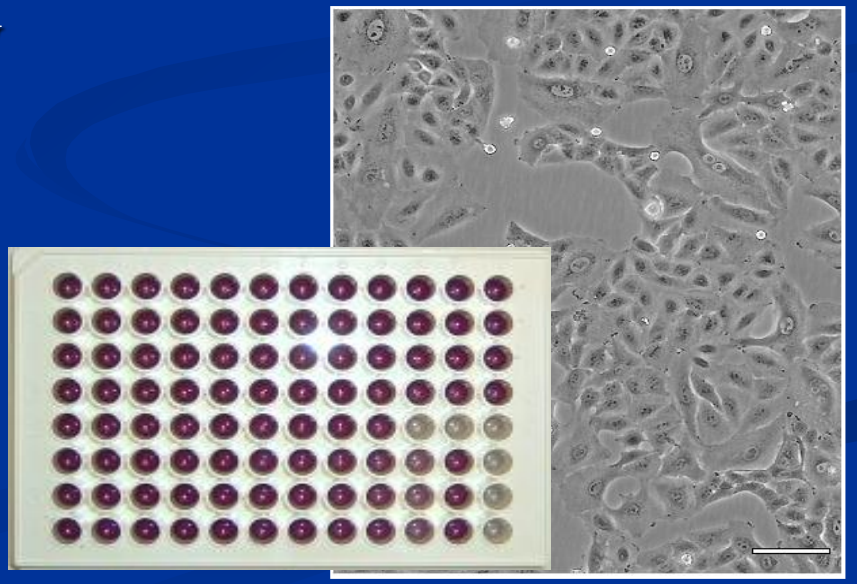
Future Directions

- Improve the Real-Time PCR assay to yield quantitative results for suspect cyanobacterium
- Continue to investigate environmental conditions that induce toxin production
- Develop toxin positive laboratory cultures

Development of an Extraction Method and a Cell Bioassay for Study of the Putative AVM Toxin

Faith Wiley





Rationale: Cell Bioassay

in vivo

-time-consuming (weeks)

-labor-intensive

-large amount of test material (kg/animal)

-slow results (days-weeks)

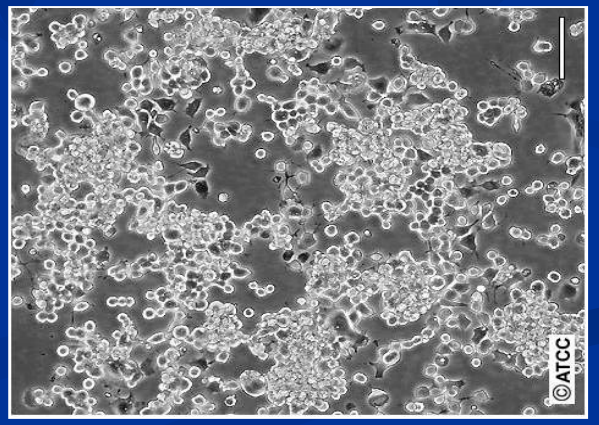
in vitro

- quick tests (days)

- less labor-intensive

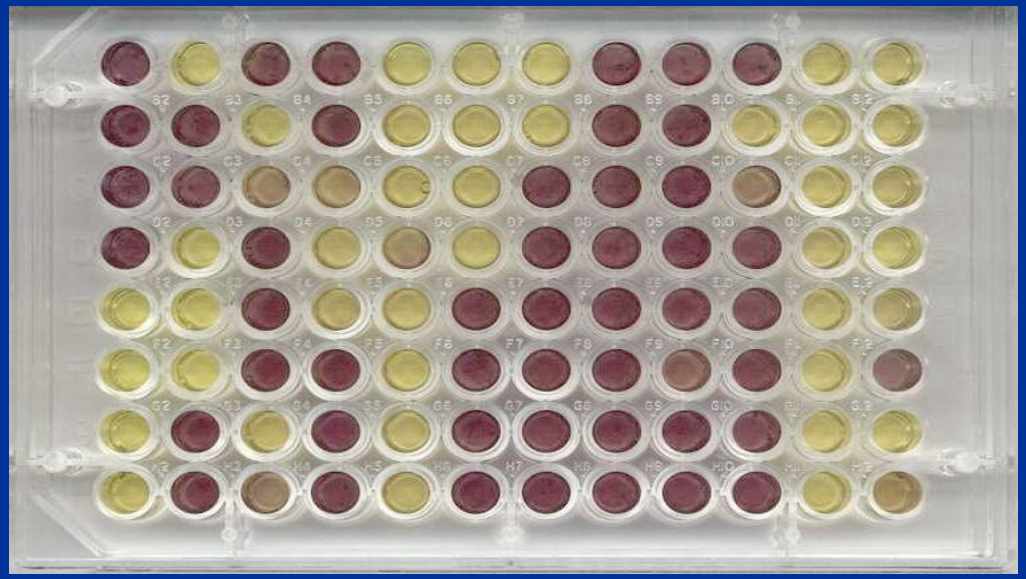
-minimal amount of test material (mg-µg/assay)

-quick results (hours)



Assessing cytotoxicity

- High-throughput 96-well format: Essential for testing hundreds of samples/fractions
- Mitochondrial dye (MTT) - colorimetric endpoint
- Total toxicity response



Live cells will take up the MTT dye and precipitate purple formazan crystals in mitochondria

Purple → live cells, no toxicity

Yellow → dead cells, toxic

Hydrilla Extraction
Basic Study Design

JSTL
4 bins

>20 kg ww

L Marion
4 bins

Dry Weight of each bin ~ 1kg

Freeze-dry, grind to coarse powder

Extract hydrilla using a gradient of non-polar to polar solvents

Filter and Concentrate

Non-Polar

Hexane

Acetone

Methanol

Water

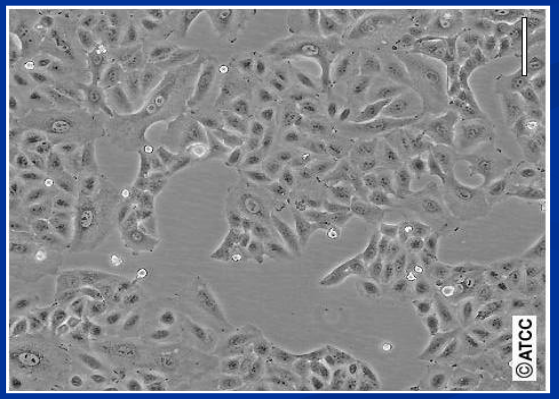
Polar

Hydrilla Extraction
Basic Study Design

Hexane | Acetone | Methanol | Water

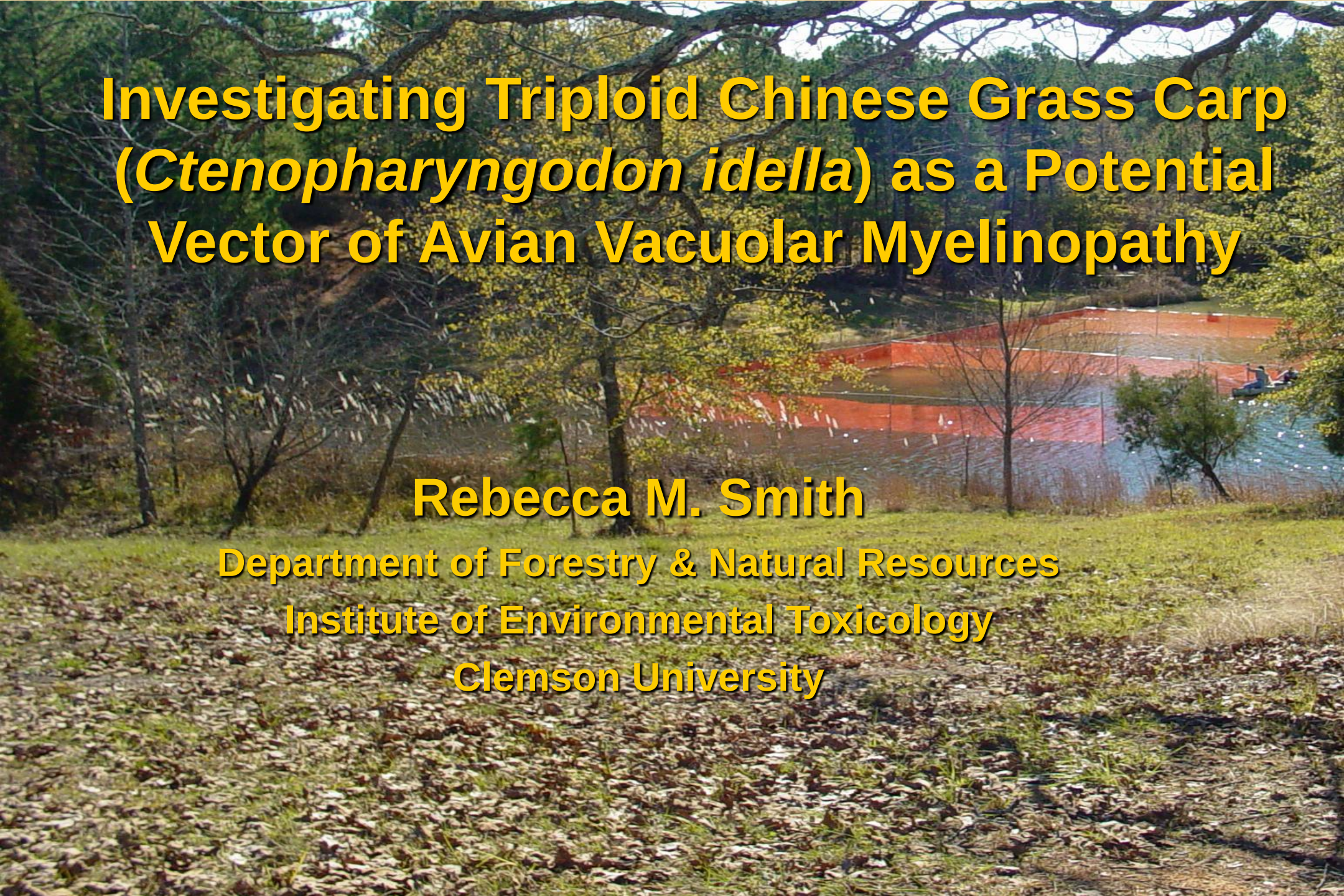
90% | 10%

In vivo Mallard Bioassay



In vitro Cell Bioassay

Confirm presence or absence of toxin



Investigating Triploid Chinese Grass Carp (*Ctenopharyngodon idella*) as a Potential Vector of Avian Vacuolar Myelinopathy

Rebecca M. Smith

Department of Forestry & Natural Resources

Institute of Environmental Toxicology

Clemson University

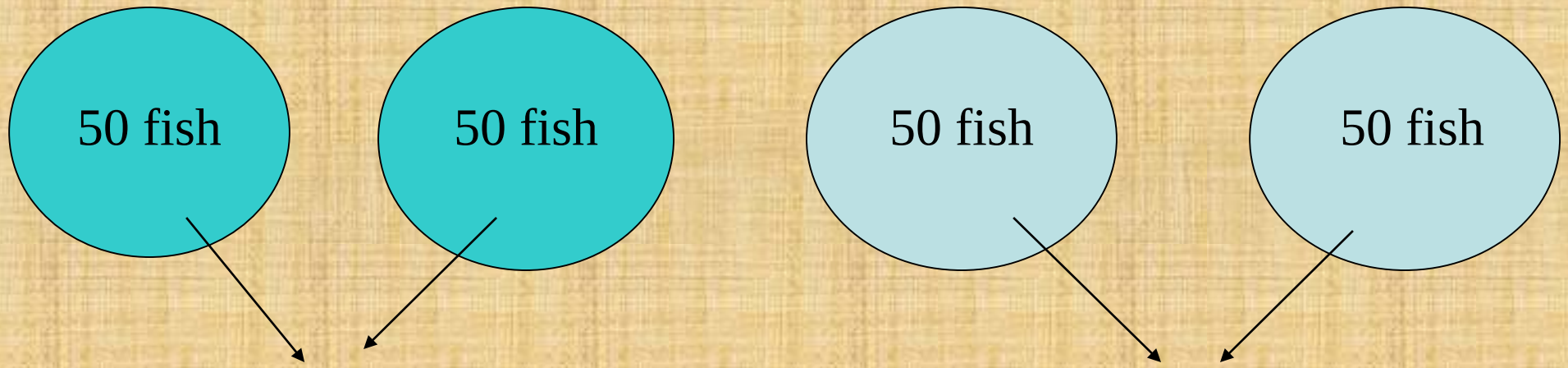
Field Trial
Grass Carp Stocking, October 2004

Photo credit: Larry McCord

Field Trial:
Carp Specimen Collections

- December 2004 subset of 25 carp removed from enclosures:
  - brains preserved for histological analysis
  - carcasses bagged, labeled, placed on ice for transport
  - carcasses frozen for feeding trial

Grass Carp Laboratory Feeding Trial



Treatment Groups:

Received hydrilla with associated suspect cyanobacterium

Control Groups:

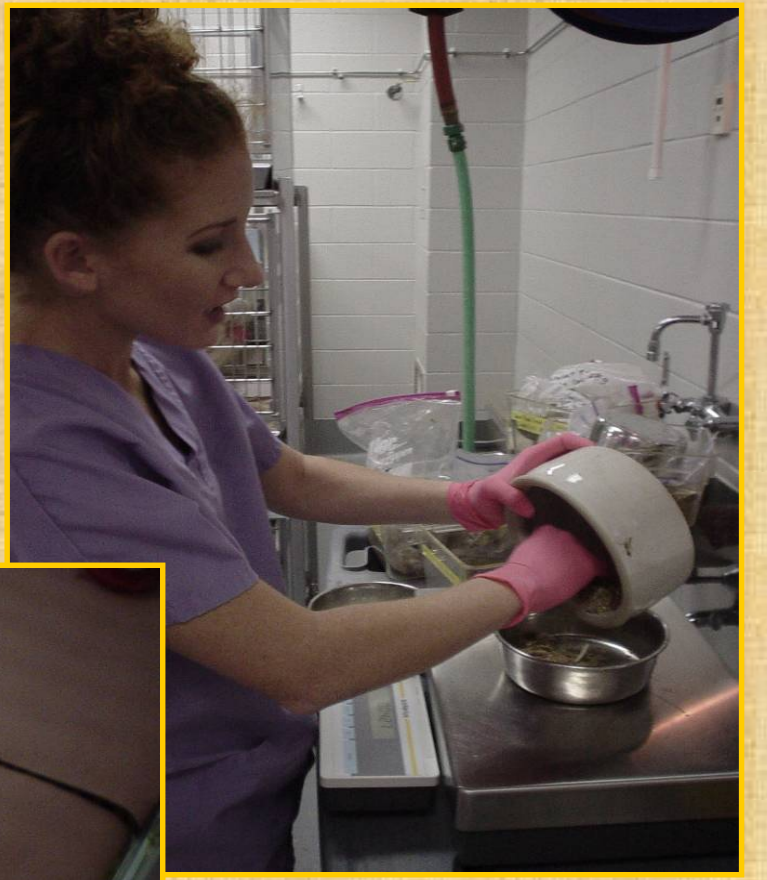
Received hydrilla without suspect cyanobacterium

For treatment and control groups, hydrilla was weighed in and out as needed

Phase II: Food Chain Linkage- Chicken Feeding Trial

400 g basal diet
50-600g respective grass carp tissues:
- GI tract
- Fillet
- Whole fish

Weighed feed in & out twice daily



27 birds total; treatment and control groups for respective grass carp tissue fractions

Experiment Summary

Field Grass Carp Trial:

Carp: Lesion formation in treatment fish

Sentinel Mallards- **6/10 AVM positive**

Lab Grass Carp Trial:

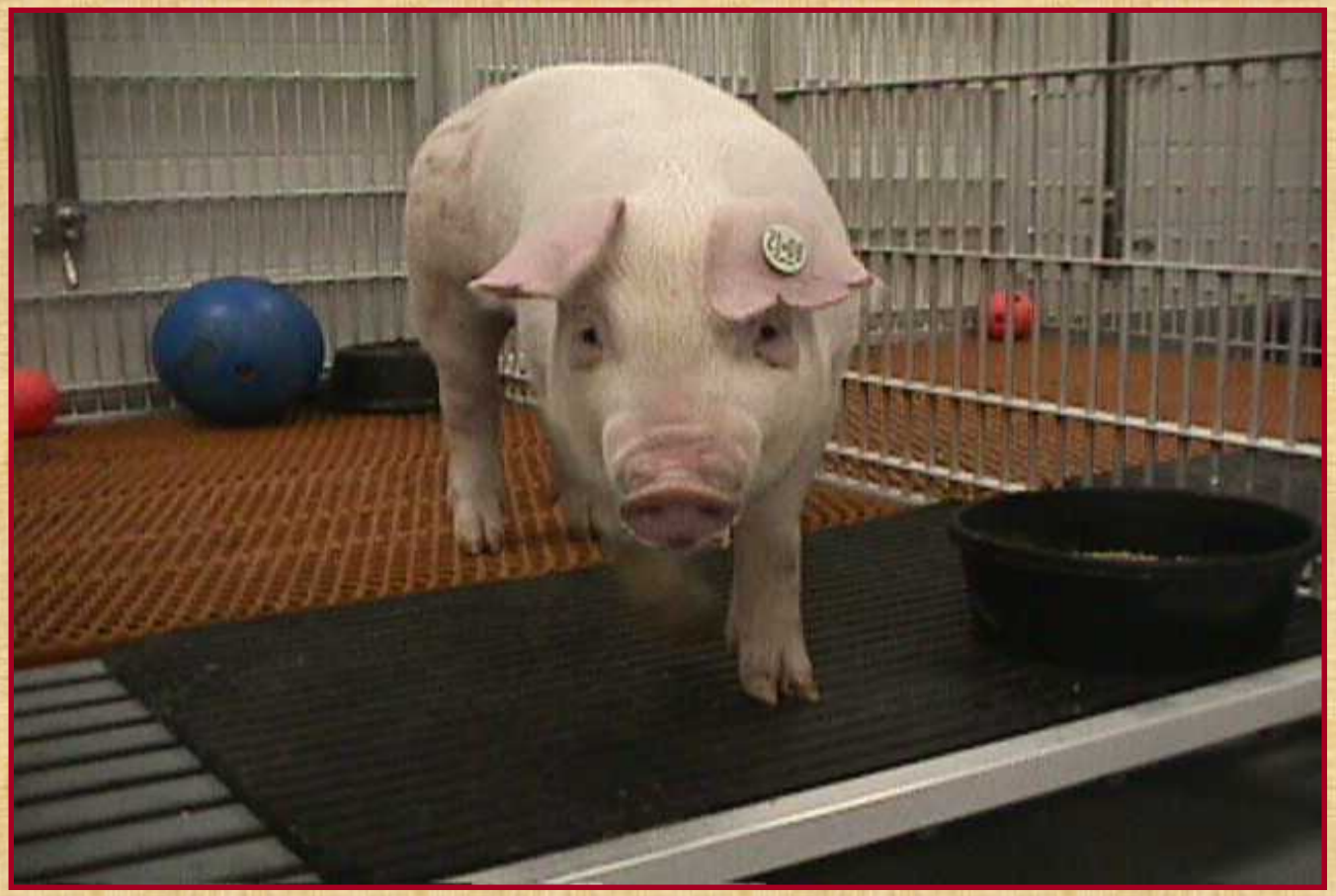
Carp: Lesion formation in treatment fish

Sentinel Mallards- **6/15 AVM positive**

Chicken Trial:

Results No Lesions

Mammalian Toxicity: Fall 2005



- 5 week study
- Five 6-week old pigs fed vegetation with confirmed presence of suspect cyanobacterium
- Vegetation toxicity will be illustrated in upcoming mallard trial
- Results pending histological analysis

Acknowledgements

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