South Carolina Algal Ecology Lab

AVM Updates--October 2006

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Avian Vacuolar Myelinopathy: Model Estimating Potential Distribution

Susan B. Wilde
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and
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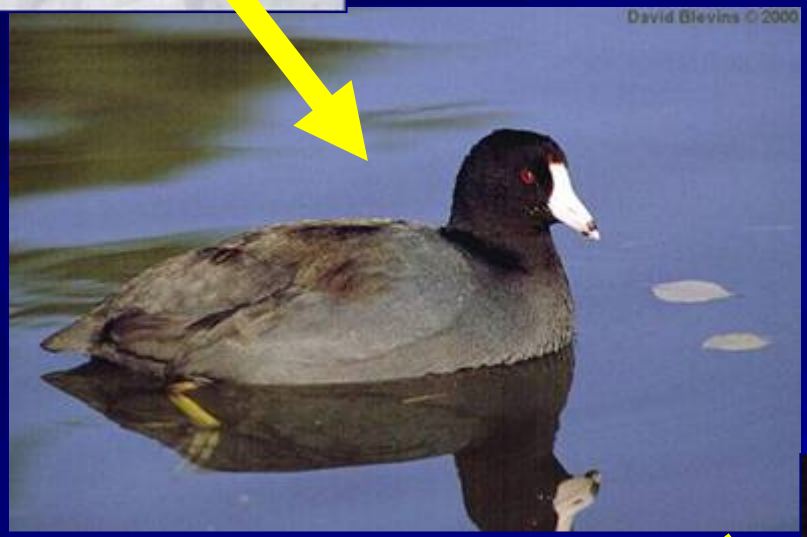
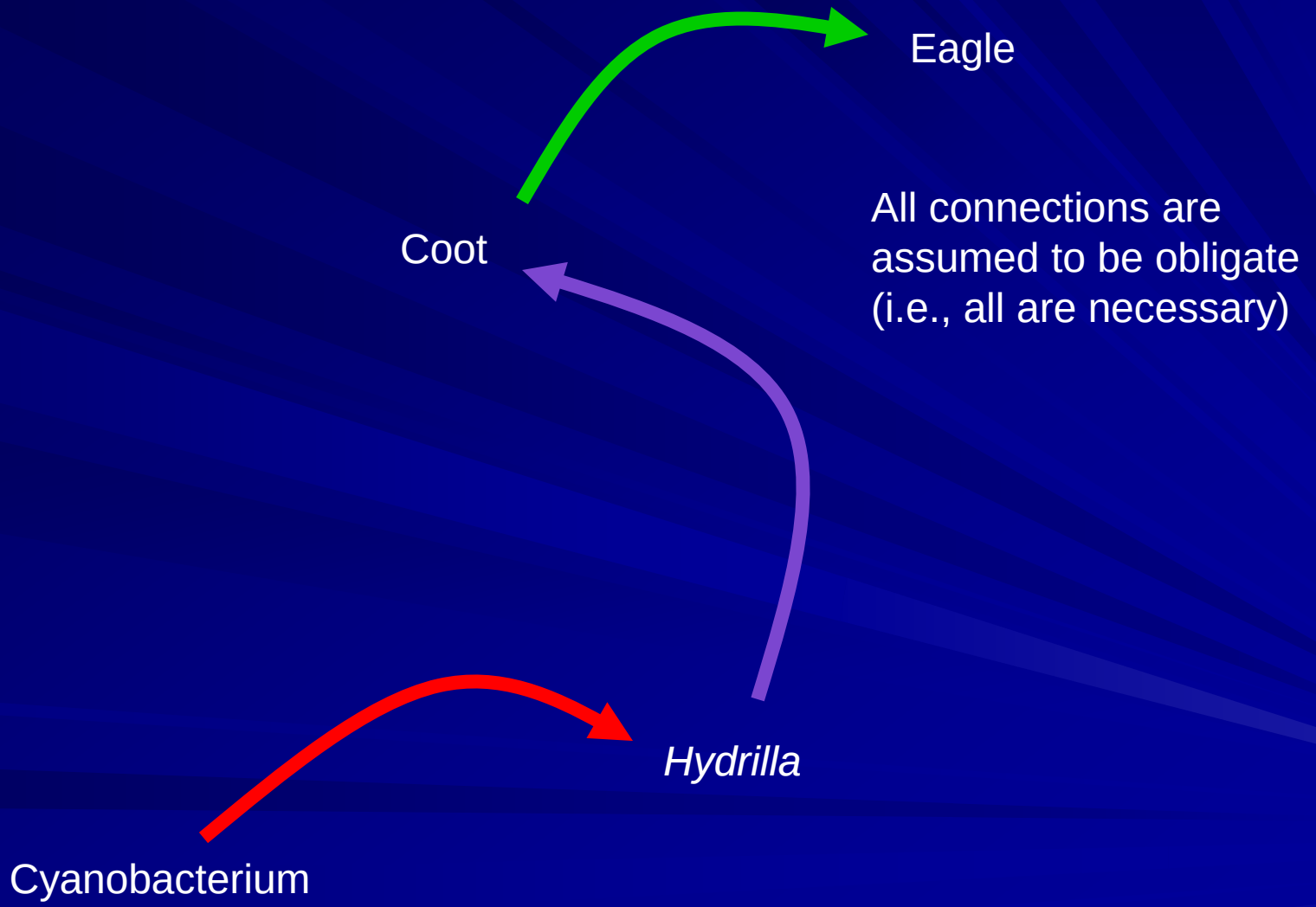
Potentially toxic cyanobacterial colonies on hydrilla and other aquatic plants in AVM sites



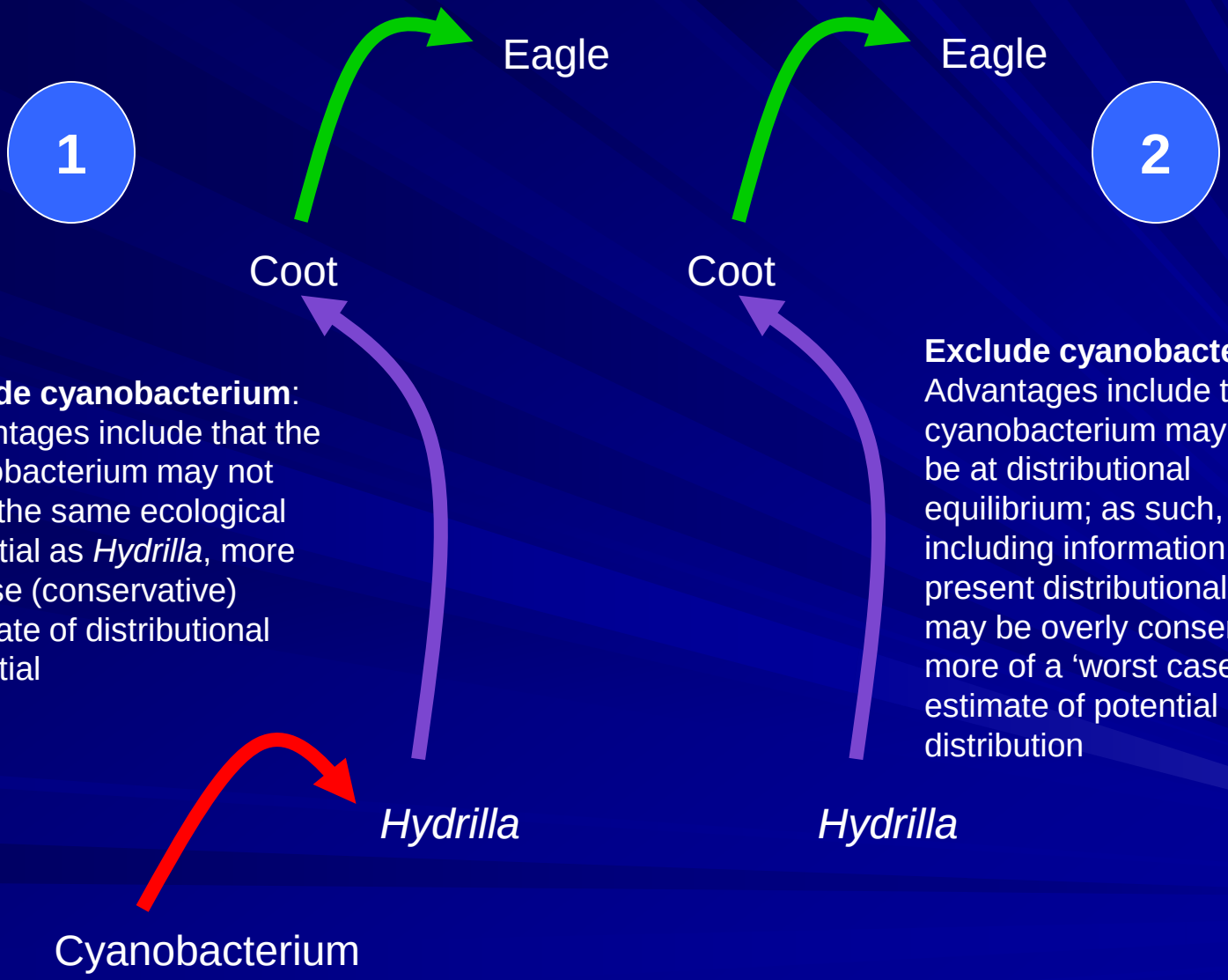
Aquatic plants and epiphytic algae are primary food source for coots

Sick waterfowl are consumed by Bald Eagles

Supposed Structure of Transmission



Two Simulations



Include cyanobacterium: Advantages include that the cyanobacterium may not have the same ecological potential as *Hydrilla*, more precise (conservative) estimate of distributional potential

Exclude cyanobacterium: Advantages include that the cyanobacterium may not yet be at distributional equilibrium; as such, including information from its present distributional area may be overly conservative; more of a 'worst case' estimate of potential distribution

Three invasive aquatic species dominant AVM reservoirs

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

Hydrilla *(Hydrilla verticillata)*

9/12

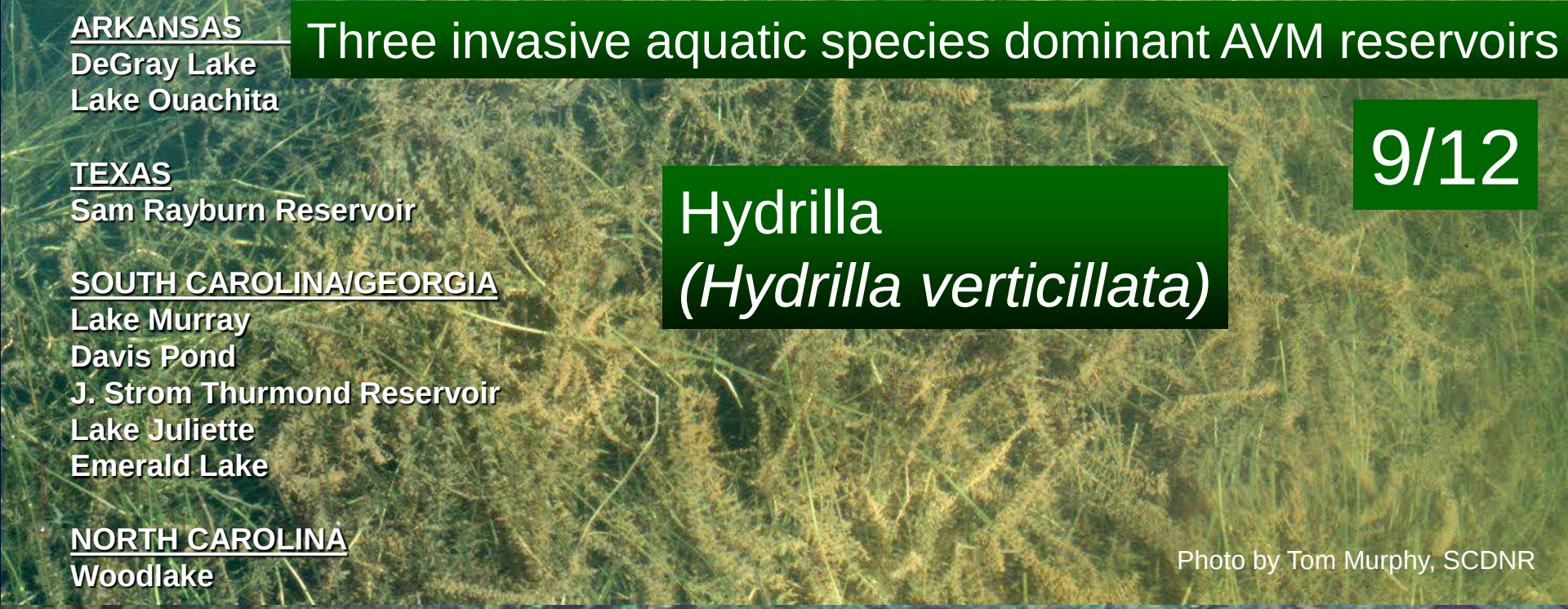


Photo by Tom Murphy, SCDNR

ARKANSAS
DeGray Lake
Lake Ouachita

GEORGIA
Lake Juliette

Brazilian elodea *(Egeria densa)*

3/12

Photo by W.T. Haller
Univ. of Florida

ARKANSAS
Lake Hamilton

GEORGIA
Lake Juliette

SOUTH CAROLINA
SRS- L Lake and Par Pond

Eurasian watermilfoil *(Myriophyllum spicatum)*

4/12

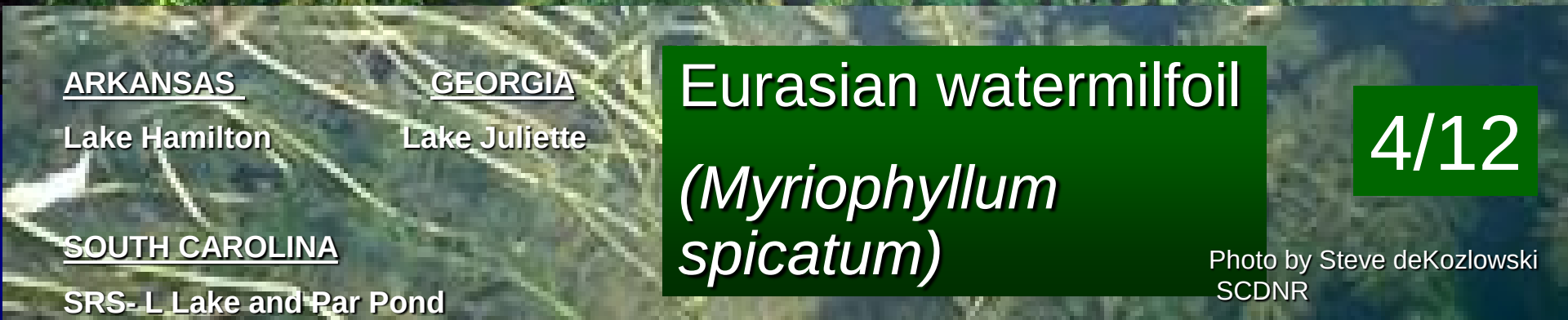
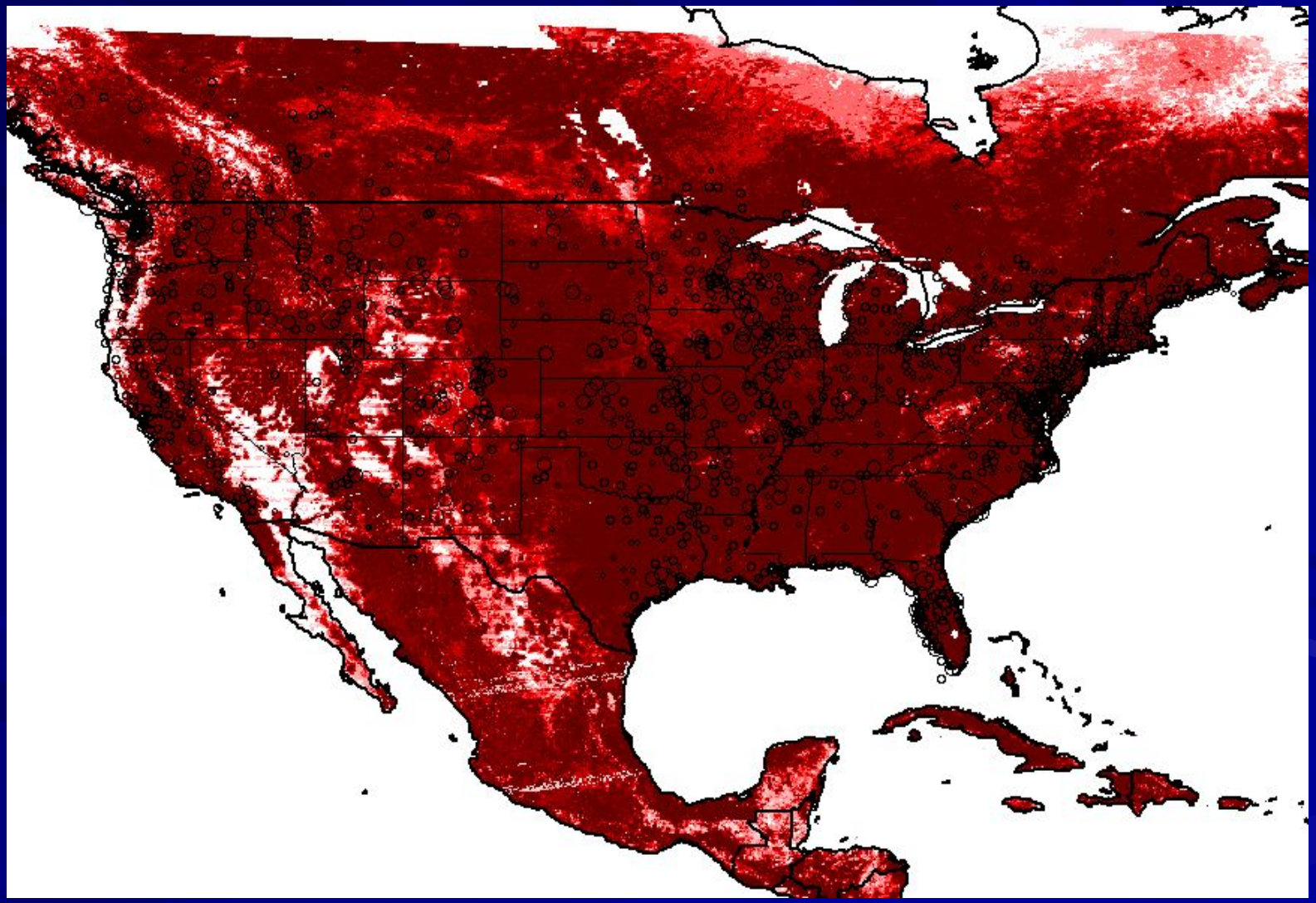


Photo by Steve deKozlowski
SCDNR

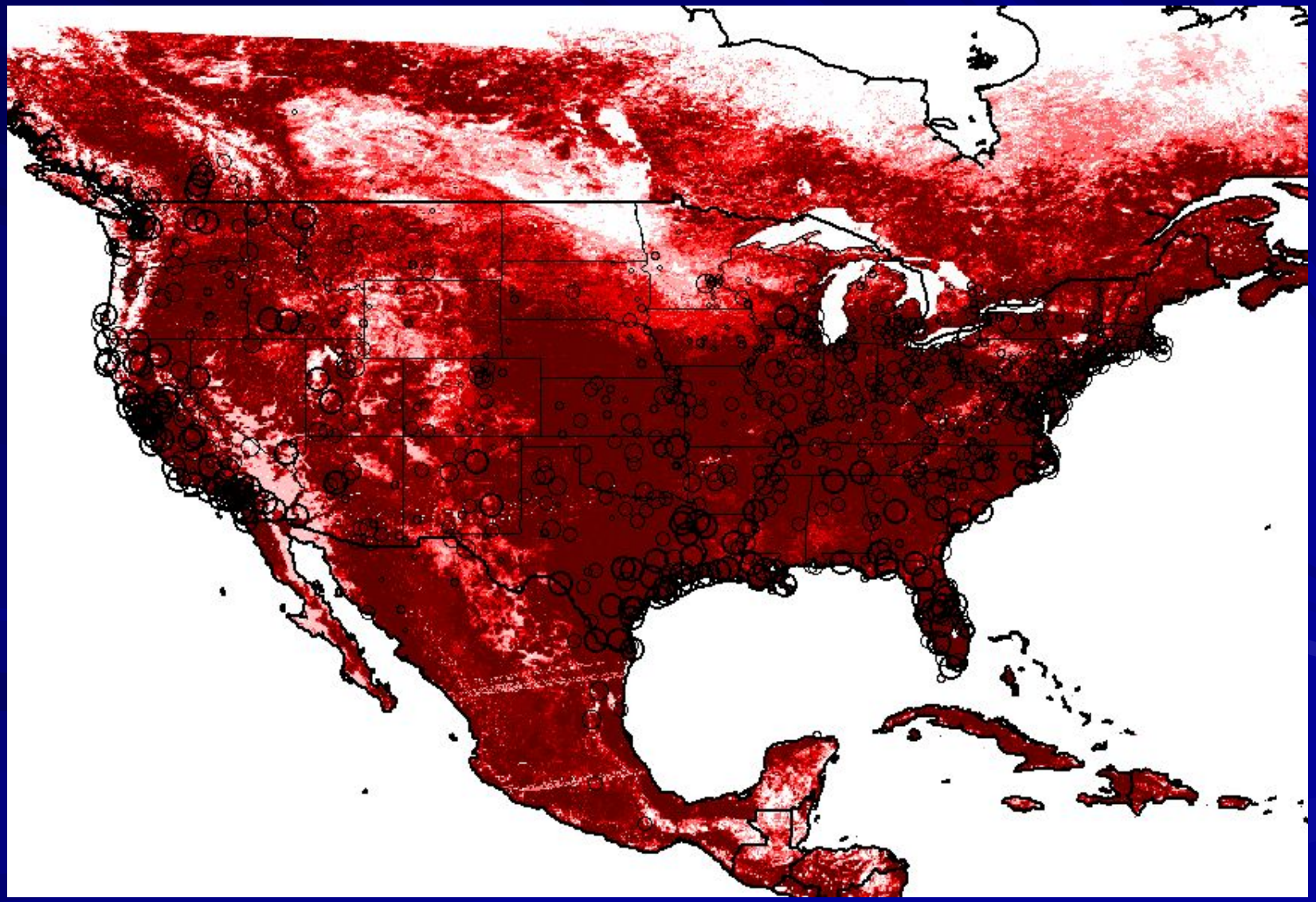
Eagle

Data from Christmas Bird Counts; used total observed over 1990-2000 to weight inclusion of sites in the Ecological Niche Model analysis.



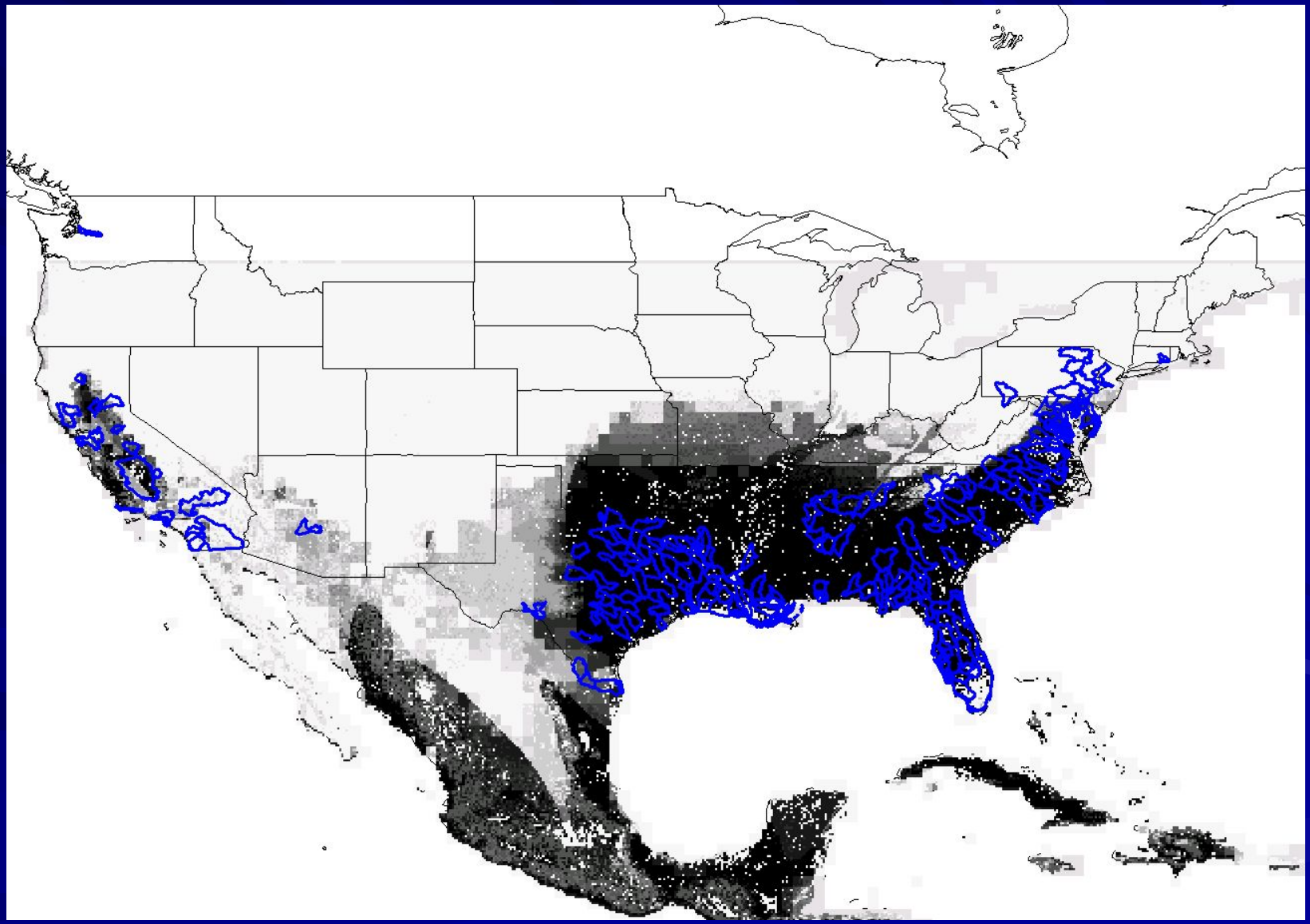
Coot

Data from Christmas Bird Counts; used total observed over 1990-2000 to weight inclusion of sites in the Ecological Niche Model analysis.



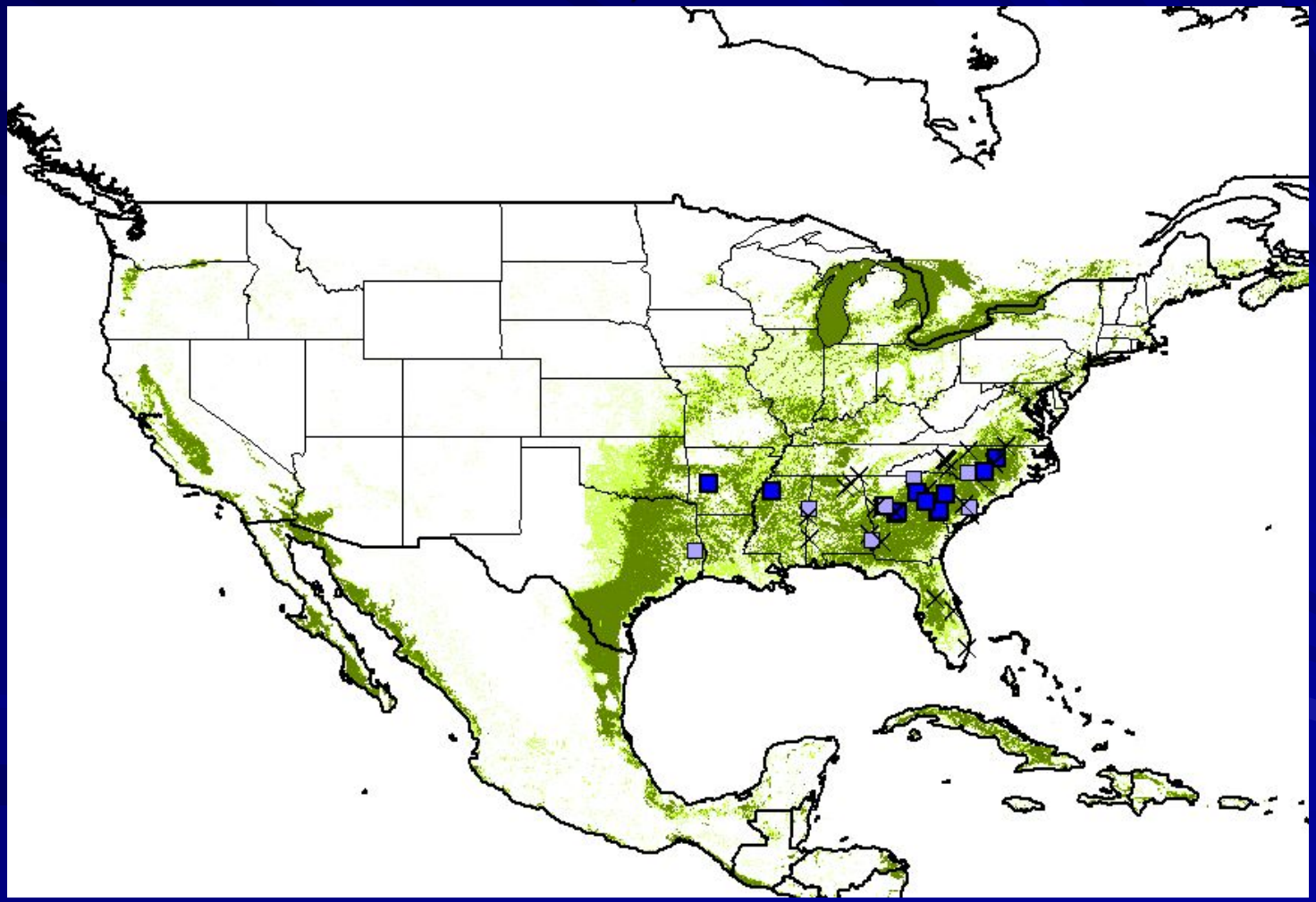
Hydrilla

Prediction based on native-range ecological characteristics, projected to North America (Peterson et al. *Weed Science* 2003). Blue polygons on North American map represent independent test occurrence data overlaid to show good correspondence.



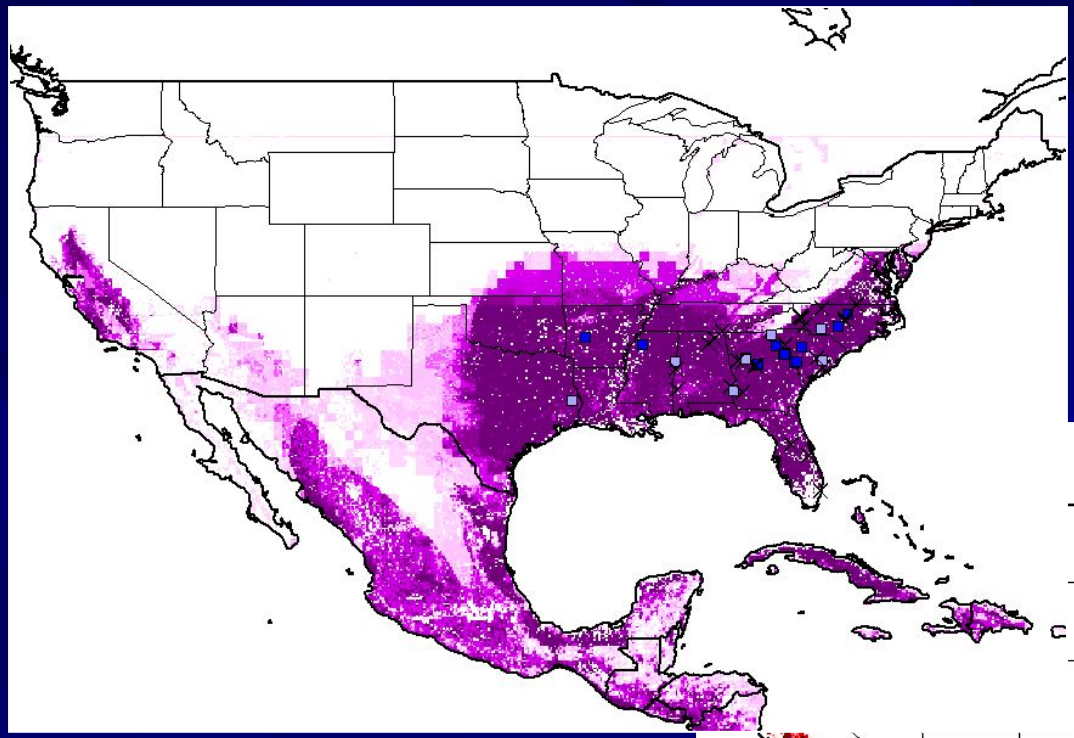
Cyanobacterium

Two distinct models developed: one based on high abundance situations (6-10 on SW's abundance scale), and the other on all detections

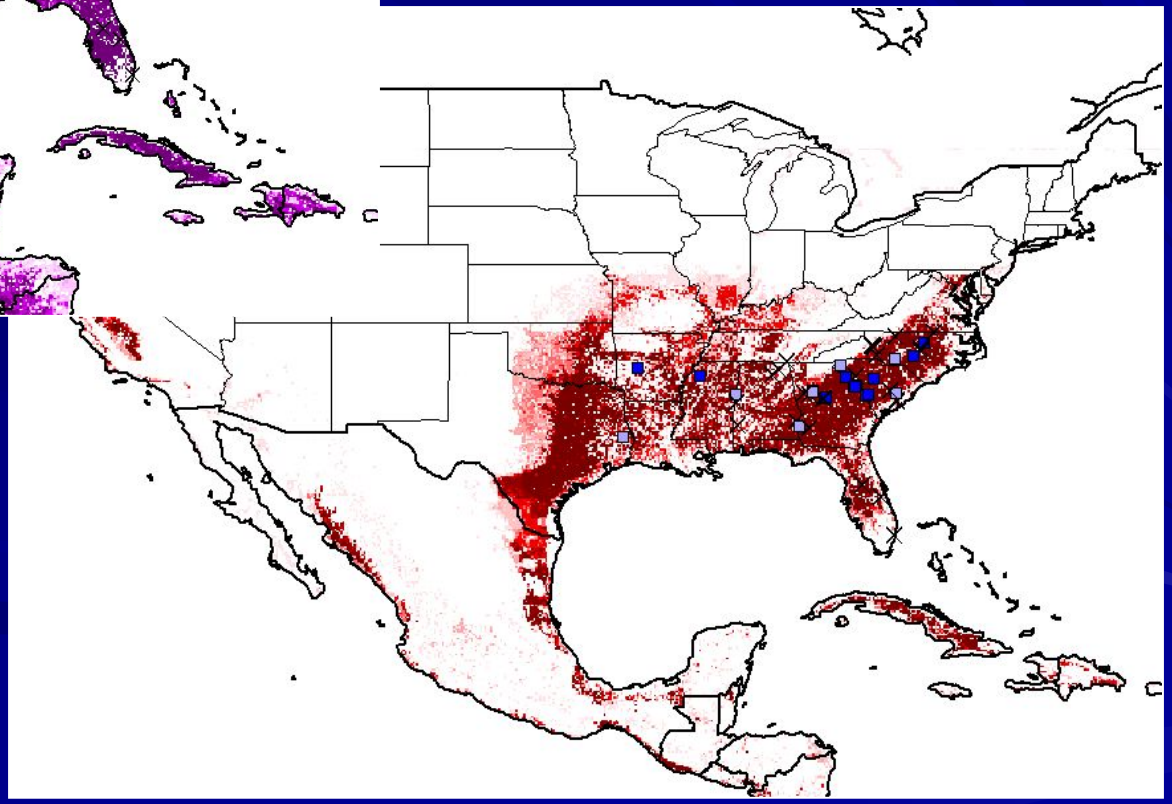


First slide is known occurrences at low (lt blue) and high (dk blue) abundances
Second slide is map based on hi abundances only; third is based on all known occurrences

Final Predictions

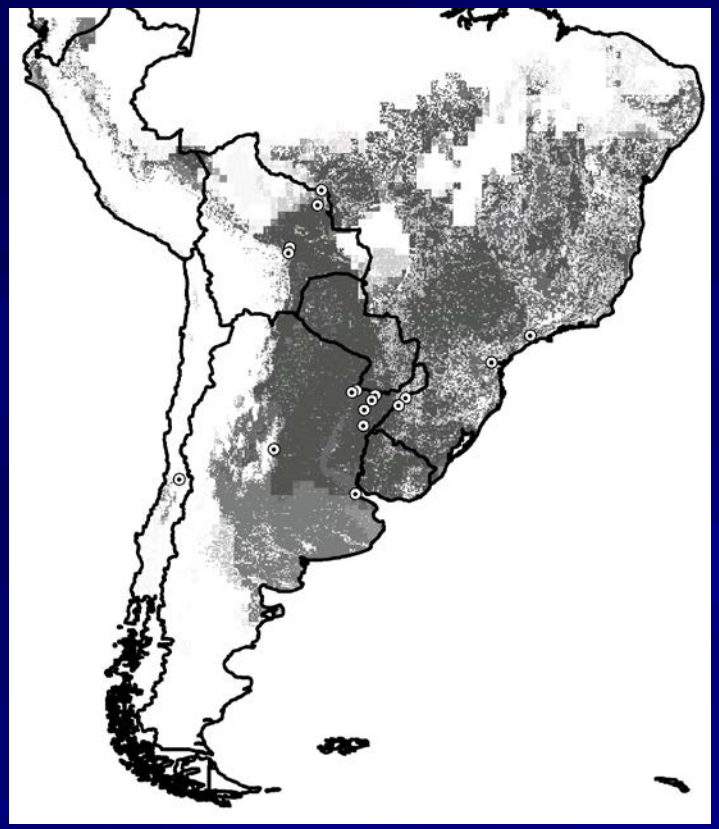


Worst-case prediction (i.e., ecological potential of cyanobacterium = that of *Hydrilla*



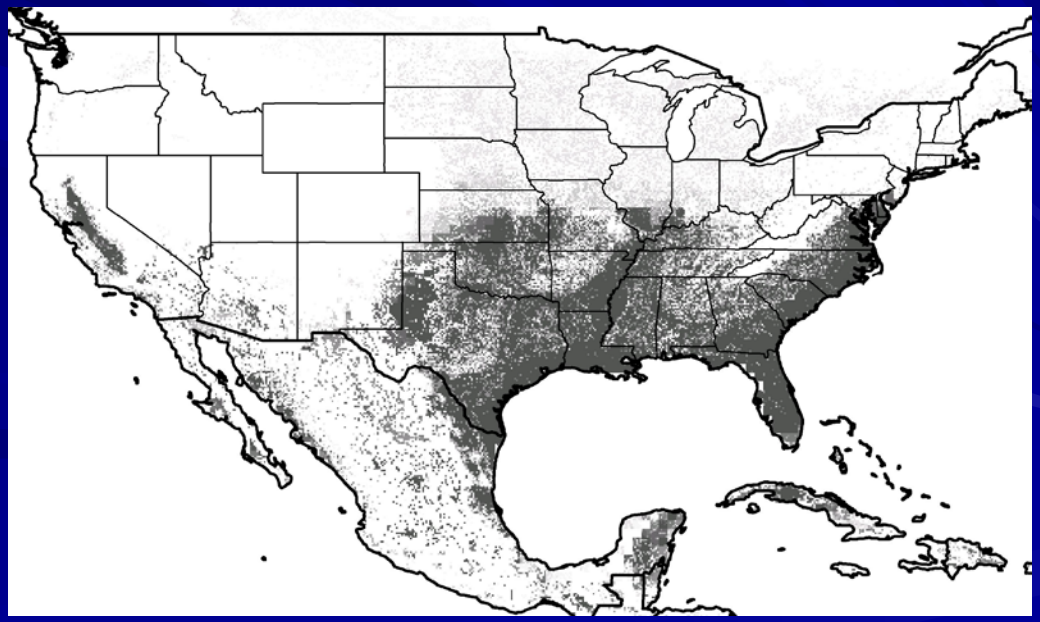
Better-case prediction (ecological potential of cyanobacterium is reflected in current distribution

Egeria densa

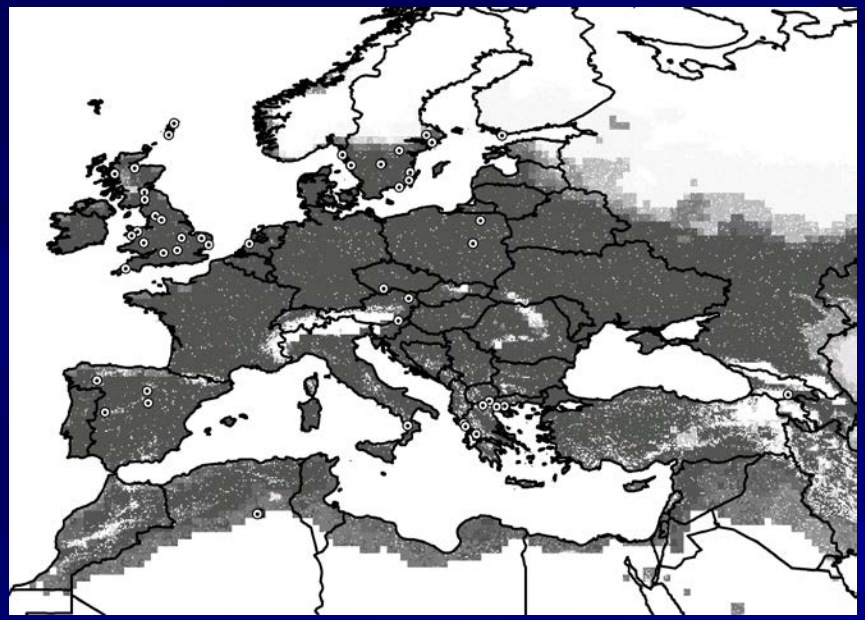


Native range

North American distribution

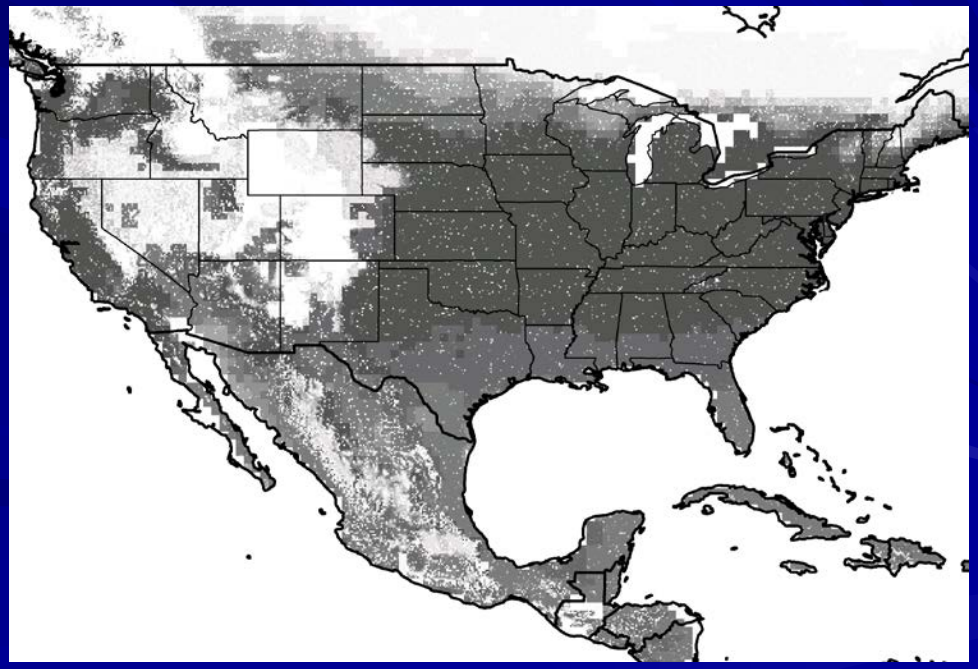


Myriophyllum spicatum

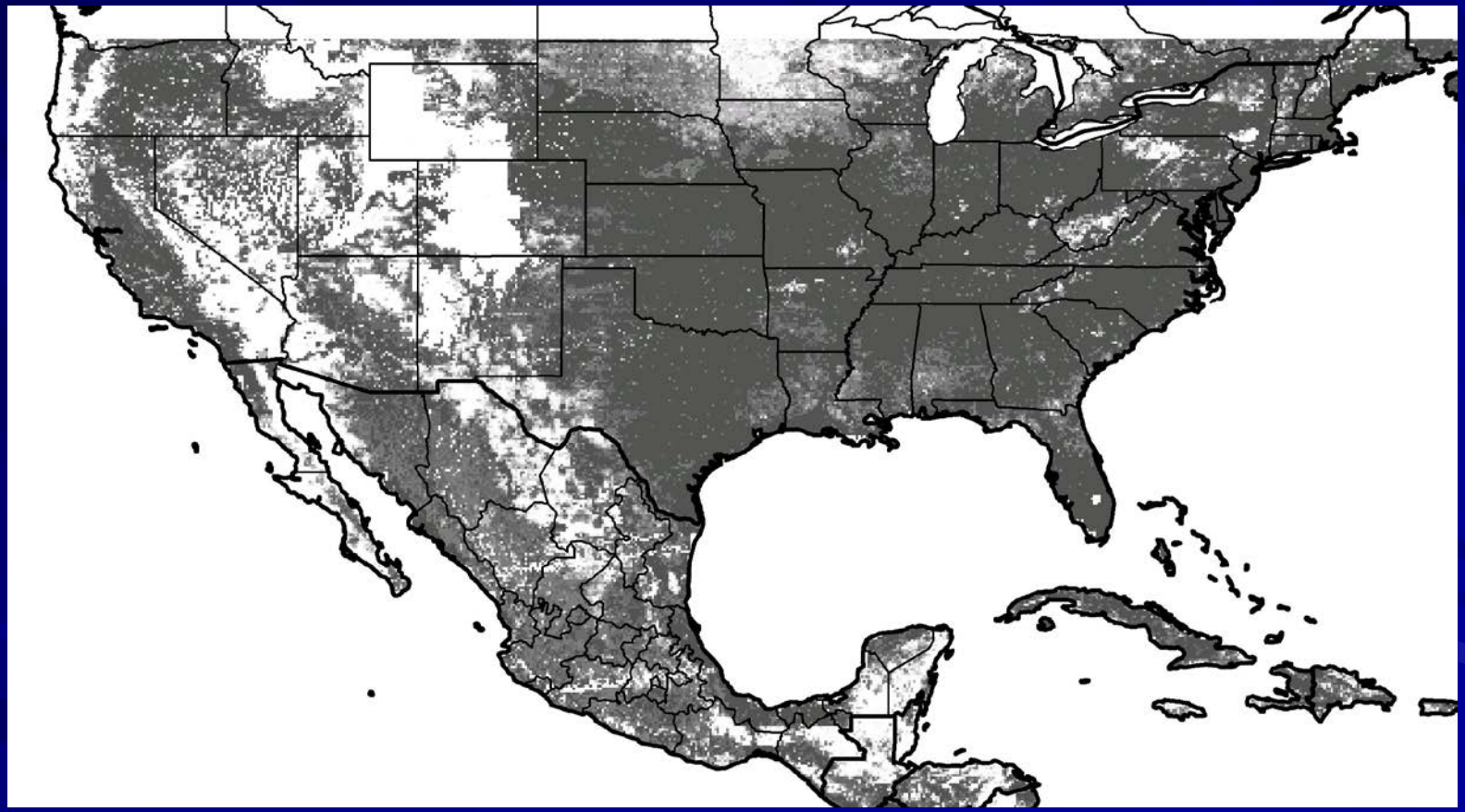


Native range

North American distribution



Hydrilla, Egeria, Milfoil Eagles & Coots



Fall 2006-2007 Research

More accurate AVM mortality assessment in waterfowl

Refine cell line bioassay screening tool for toxic material

Rebecca S. Haynie, Tom Murphy, Charlotte Hope, Sarah Williams,
Faith Wiley, Bill Bowerman and Susan B. Wilde
South Carolina Department of Natural Resources
University of South Carolina
Clemson Institute of Environmental Toxicology

Where Are They Going?

Atlantic fly-way midwinter coot numbers decreased from 869,124 in 1988 to 96,297 in 2004

Photo credit Steven O. Muskie

Is AVM contributing significantly to the apparent decline?

Collections during winter months confirmed 50-95% of the coot populations residing in J. Strom Thurmond Reservoir show characteristic AVM lesions (*Southeastern Cooperative Wildlife Disease Study*)

Proposed Study Outline

- J. Strom Thurmond Reservoir: Neck band 200 coots upon arrival in respective treatment, control areas
- Half of each group will be wing-clipped and have contrasting neck band color

- Throughout season:
  - Conduct carcasses recovery, analyze for AVM
  - Validate cyanobacterium is present on vegetation using genetic probe
  - Validate cyanobacterium is producing toxin with mallard feeding trial and cell line bioassay

What portion of symptomatic coots die during the season?

How does AVM effect future migration success?

Successful Extraction of the Toxin Responsible for Avian Vacuolar Myelinopathy (AVM)

Faith Wiley, Michael Twiner,
Fran Van Dolah, Susan Wilde,
William Bowerman, Tod Leighfield

Avian Bioassay

Hydrilla collected weekly during trial; ½ frozen, ½ fresh (kept at room temp)

18 adult male mallards

6 Treatment JSTL

- **3 Frozen Hydrilla**
- **3 Fresh Hydrilla**

All AVM POSITIVE

6 Treatment Davis Pond

- **3 Frozen Hydrilla**
- **3 Fresh Hydrilla**

4/6 AVM POSITIVE

6 Control L Marion

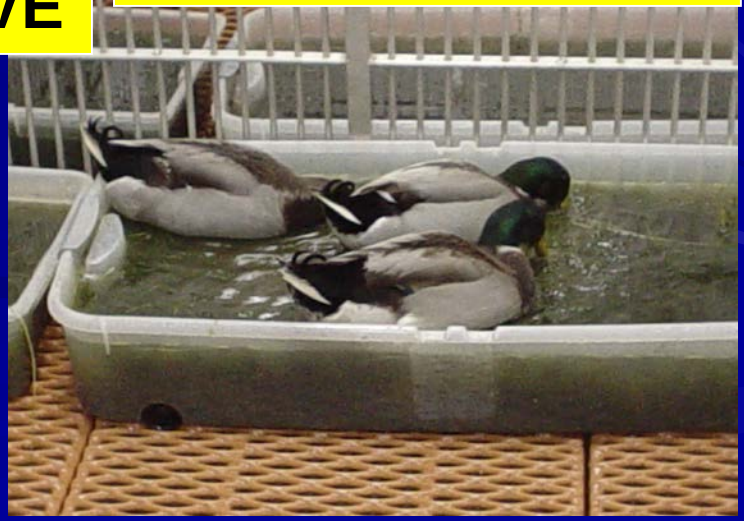
- **3 Frozen Hydrilla**
- **3 Fresh Hydrilla**

All AVM NEGATIVE

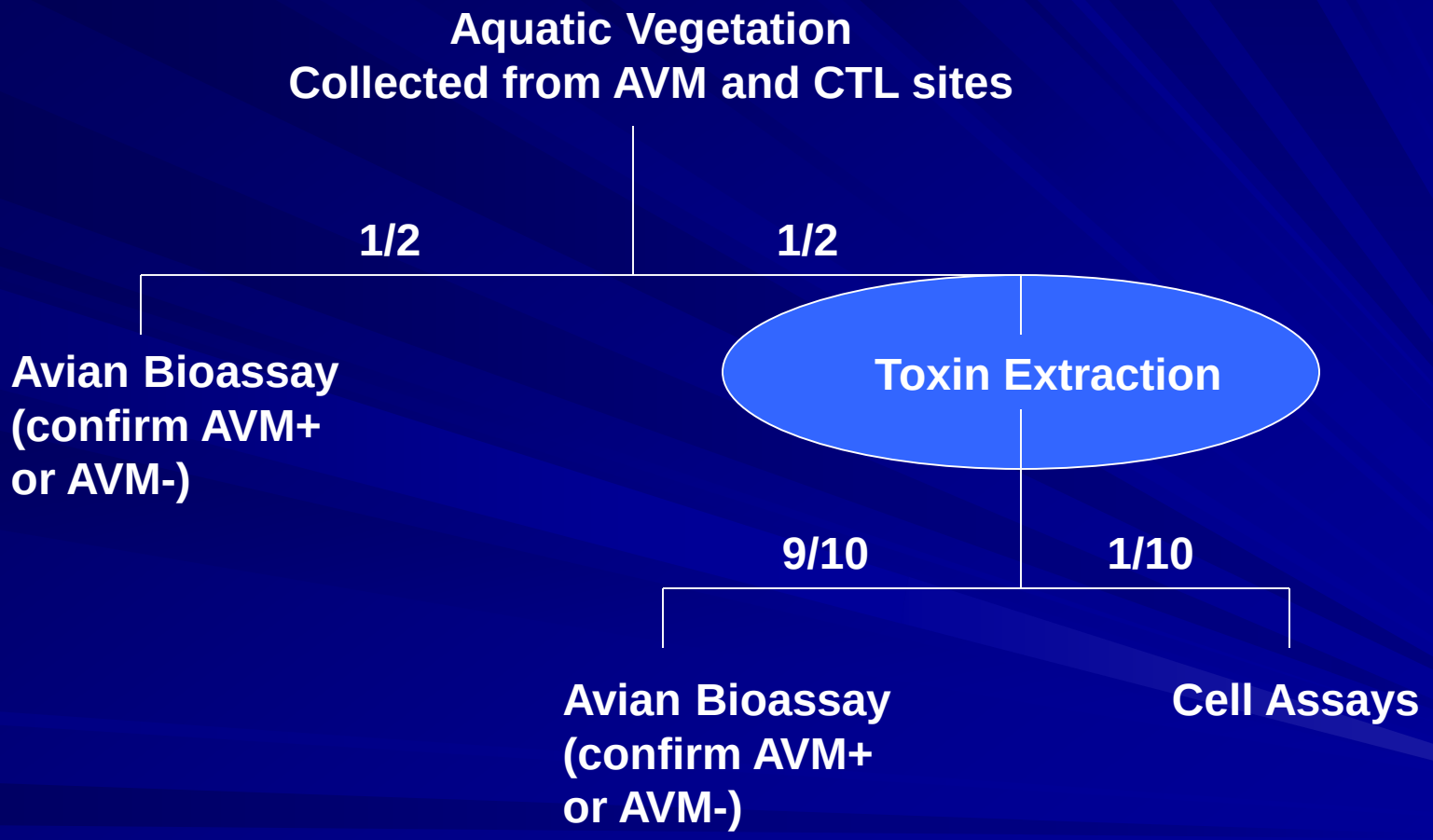
4 week trial (19 Nov – 17 Dec)

All birds sacrificed at end of 4 weeks

**2 birds developed signs of neurological impairment



Study Design



Hydrilla Extraction

Lyophilize, grind to coarse powder

Extract using a series of non-polar to polar solvents

Filter and Concentrate

Non-Polar

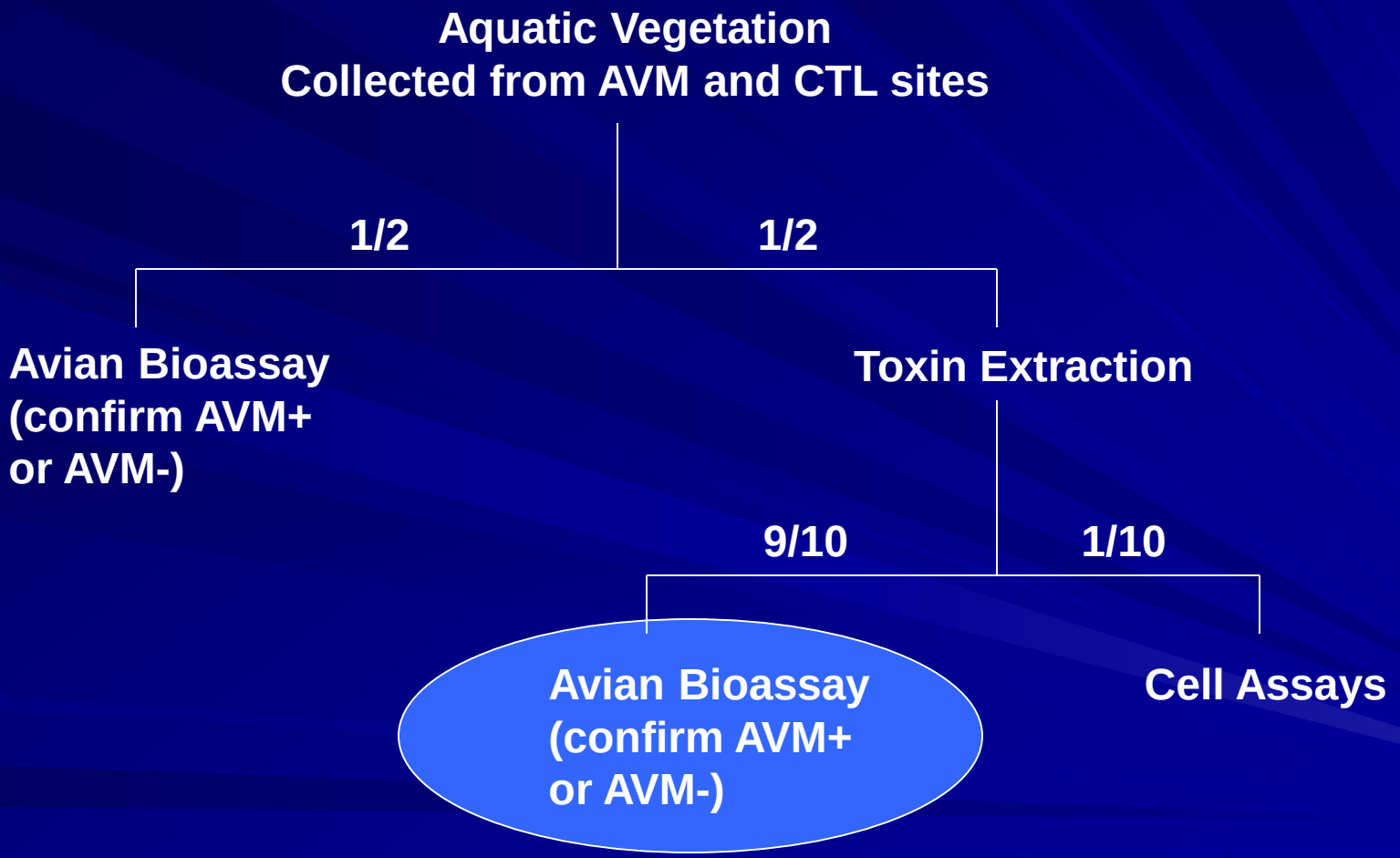
Polar

Hexane

Acetone

Methanol

Study Design



Avian Bioassay

Experimental Groups
(3 birds per group)

AVM (+)	AVM(-)
Hexane	Hexane
Acetone	Acetone
Methanol	Methanol

Extracts exchanged to non-toxic carriers

Mallards dosed by oral gavage
3x/week for 4 weeks

Avian Bioassay - Results

No clinical signs of disease

Regurgitation in methanol groups

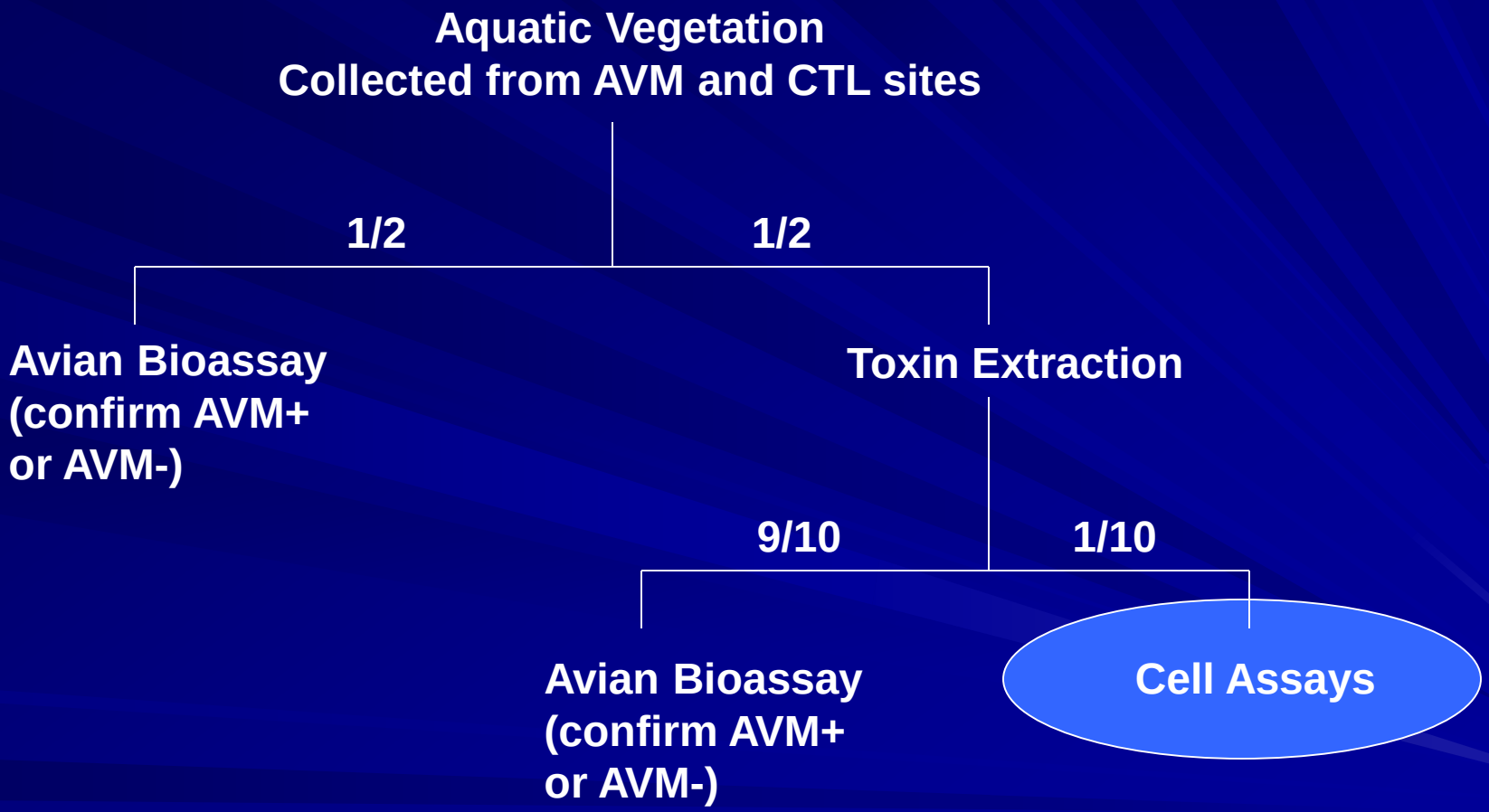
One bird in AVM methanol group broke wing on Day 14, euthanized

Brain analysis results:

AVM Hexane – no apparent lesions
AVM Acetone – 1 with possible mild lesions, 2 no apparent lesions
AVM Methanol – all 3 with definite, moderately severe lesions

Control Hexane – no apparent lesions
Control Acetone – no apparent lesions
Control Methanol – no apparent lesions

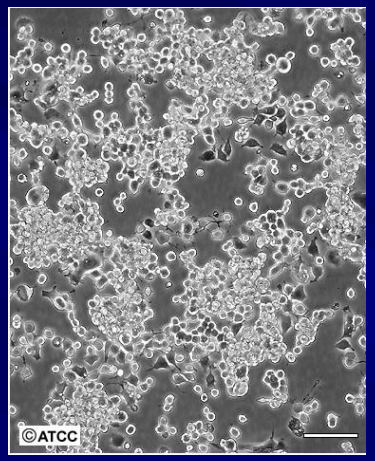
Study Design



Cell Bioassays

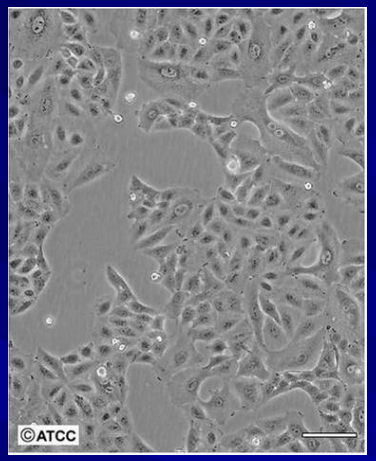
Conduct cytotoxicity tests on established cell lines

Neuro2a



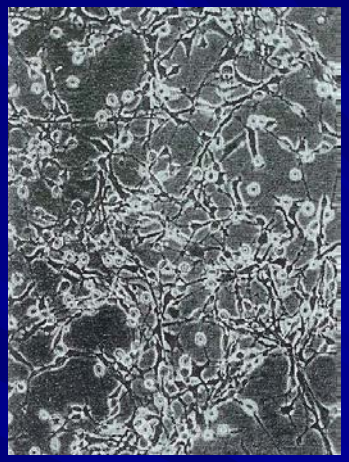
mouse
neuronal

A549



human
lung epithelial

QNR/D



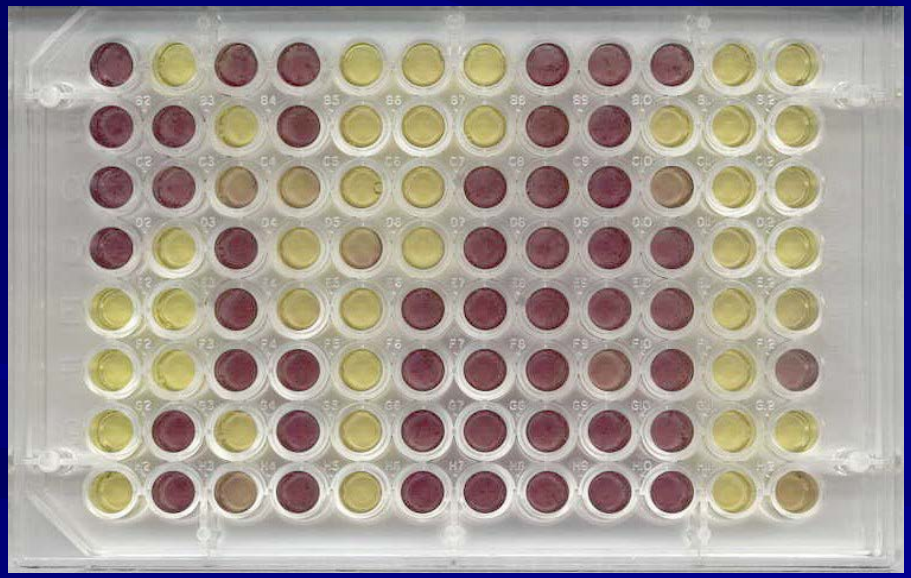
quail
neuroretinal

showed possible cytotoxic response to extracts in preliminary study (NOAA 2002-2003)

only avian neuronal line

Cell Bioassays

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

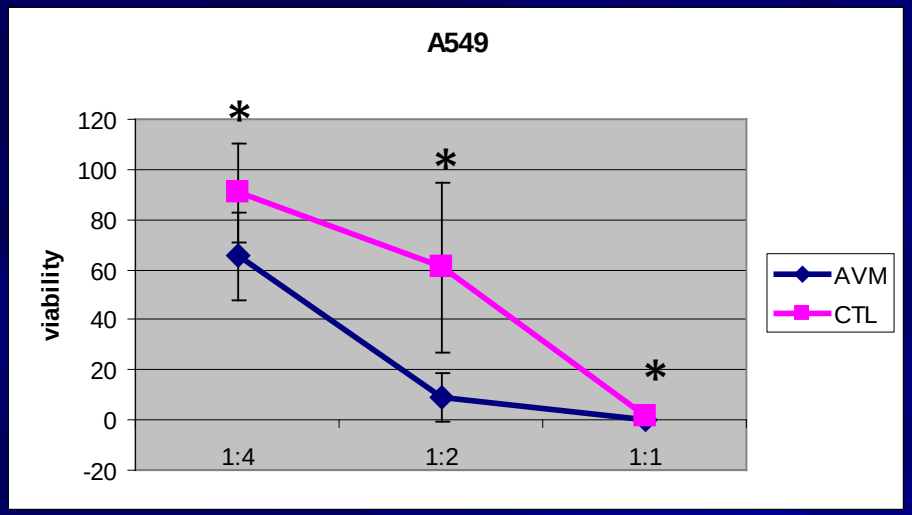


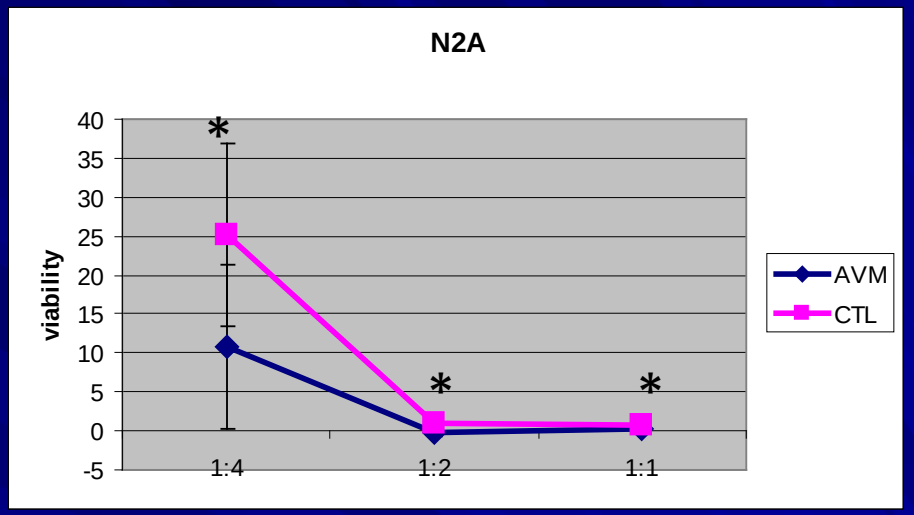
Live cells will take up the MTT dye and precipitate purple formazan crystals in mitochondria

Purple → live cells, no toxicity

Yellow → dead cells, toxic

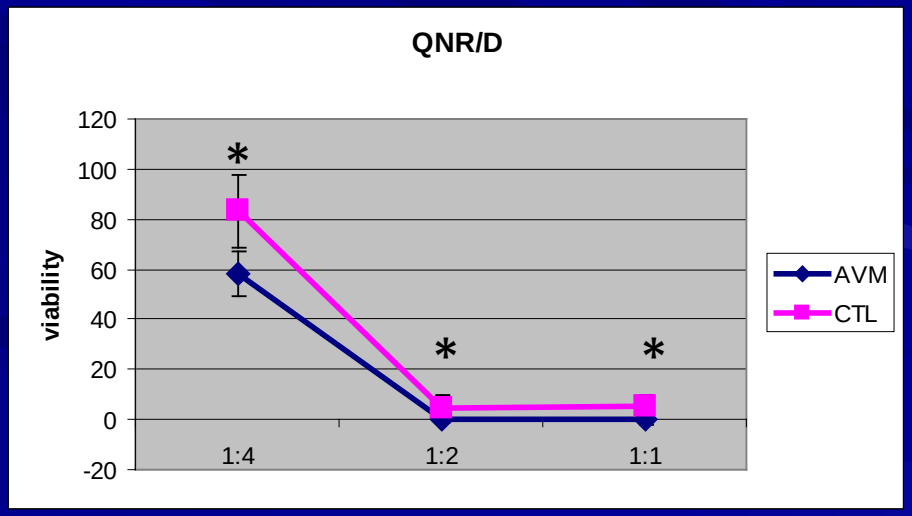
Cytotoxicity Results
Methanol Fraction
Bin 1 (JSTL/AVM 11-18-03; L Marion/Control 11-17-03)





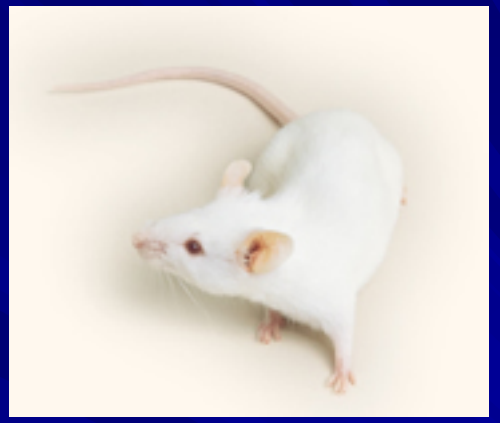
***significant difference between AVM (+) and AVM (-)**

1:1 = 10 mg hydrilla (dry weight)
1:2 = 5 mg hydrilla
1:4 = 2.5 mg hydrilla



Current Research

- Methanol Extract Fractionation
  - Avian Bioassay
  - Cell Bioassay
- Mammalian Susceptibility
  - Tested mice using same material from mallard assay
  - Suspect lesions, artifact?
  - Repeat experiment with new fixative



Acknowledgements

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