Stalking an expanding disease; new locations and species at risk from

Avian Vacuolar Myelinopathy (AVM)

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THE UGA® COLLEGE OF VETERINARY MEDICINE
Poultry Diagnostic & Research Center

Avian Vacuolar Myelinopathy (AVM)

- The beginning: 1st disease locations
- Disoriented birds and brain lesions
- No disease agents or toxins found
- Food chain transfer
- Bird species found with AVM lesions

- Invasive aquatic plants/cyanobacterial monitoring
- Field sentinel trials
- Laboratory toxin trials
- Expanding locations
- Expanding taxa
- Management solutions
- Remaining questions
- Future directions

Back to the beginning

1994/95 DeGray Lake, AR

29 bald eagle mortalities

1996/97 DeGray, Ouachita, Hamilton, AR

26 eagle mortalities, disease confirmed in American coots

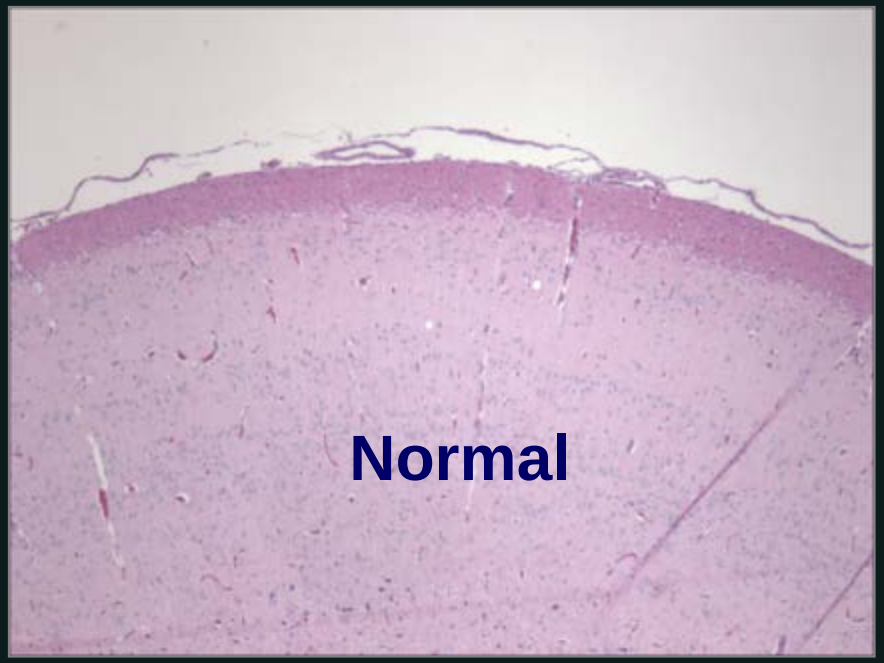
"Avian Vacuolar Myelinopathy (AVM) is the most significant unknown cause of eagle mortality in the history of the United States"

Photo by Tom Murphy; SCDNR

Neurological impairment

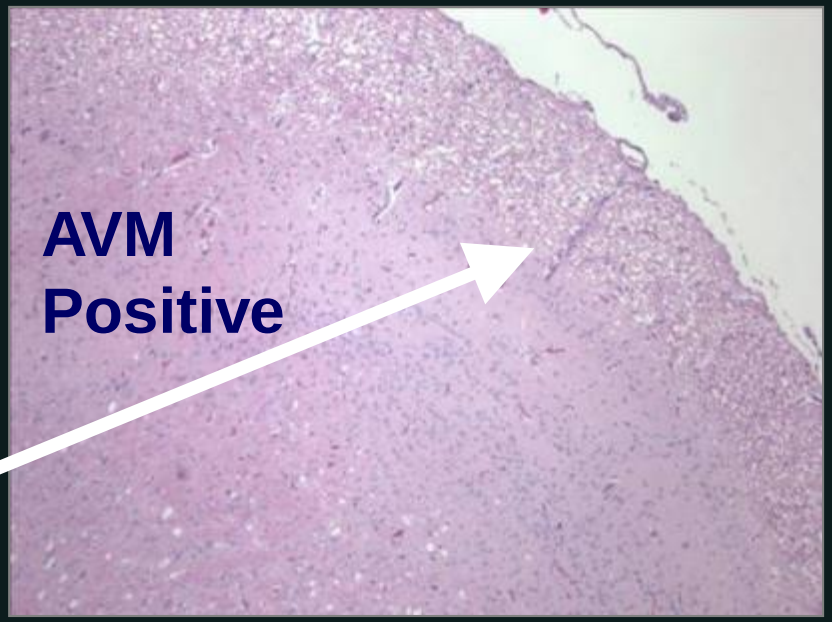
- Eagles may overshoot perches or fly into objects

Diagnosis: Unique brain lesions



National Wildlife Health Center

Southeastern Cooperative Wildlife Disease Study



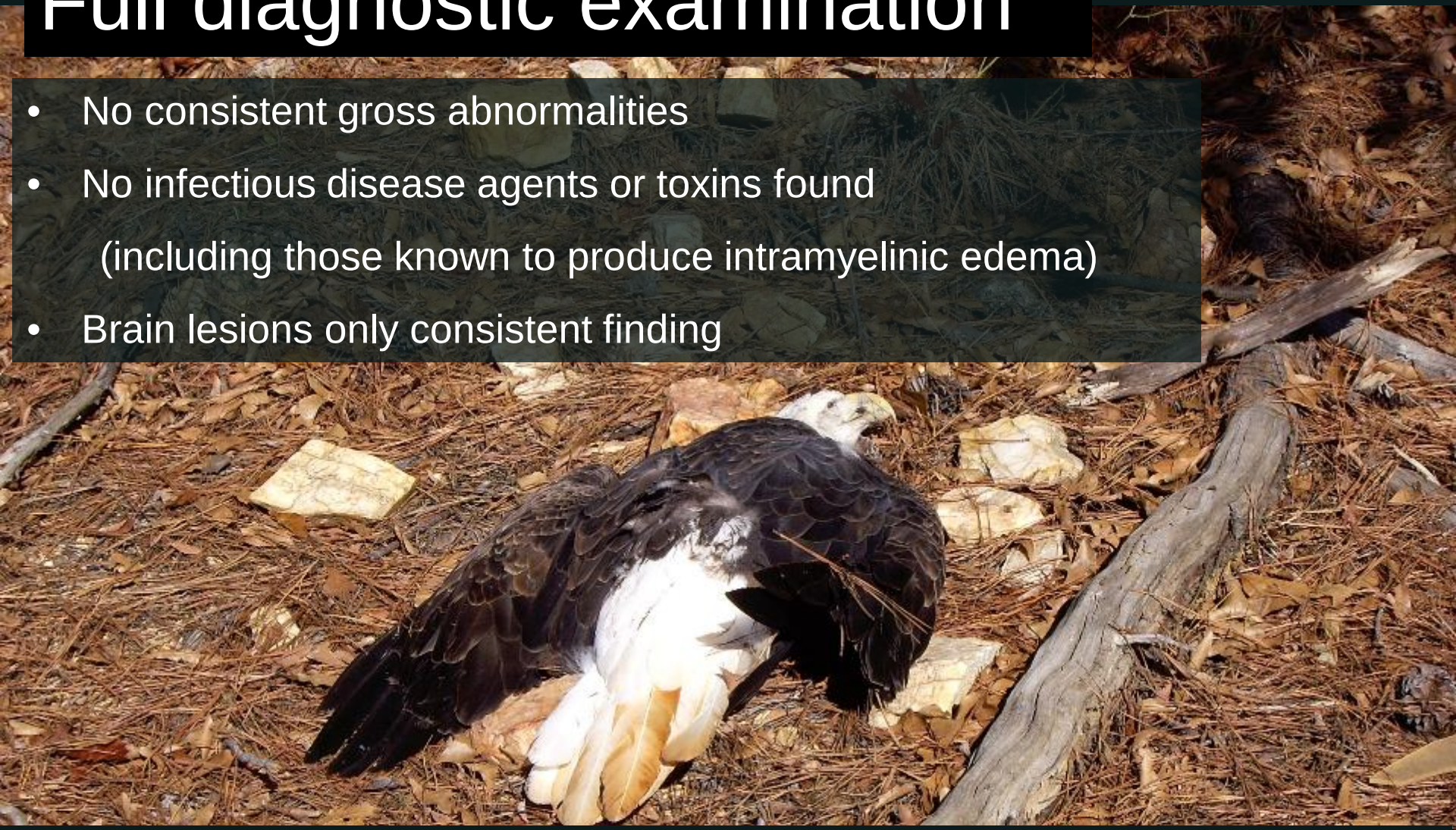
Open spaces in:
white matter of the central nervous system, specifically an intramyelinic edema

Thomas, NJ, CU Meteyer, and L Sileo, 1998. Epizootic vacuolar myelinopathy of the central nervous system of bald eagles (Haliaeetus leucocephalus) and American coots (Fulica American). Veterinary Pathology 35:479-487

Full diagnostic examination

- No consistent gross abnormalities
- No infectious disease agents or toxins found

  (including those known to produce intramyelinic edema)
- Brain lesions only consistent finding



Thomas, NJ, et al, 1998.

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 Total Environment 311:81-89.

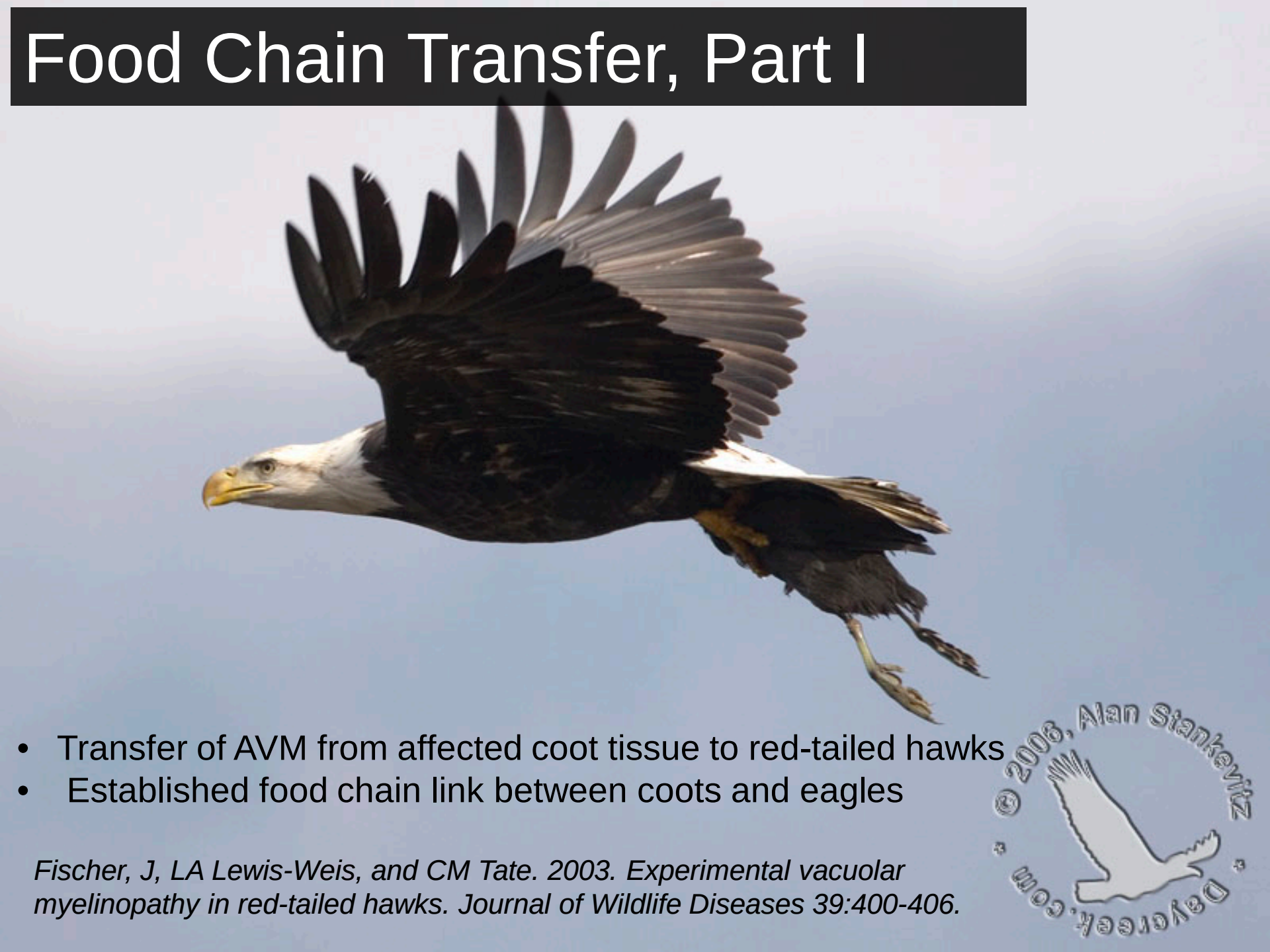
AVM Reservoirs 2000

- AVM is site-specific
- Rapid onset (5 days)
- Seasonal occurrence (late fall-winter)

Fischer, JR, LA Lewis, T. Augspurger, TE Rocke 2002. Avian Vacuolar Myelinopathy: A Newly Recognized Fatal Neurological Disease of Eagles, Waterfowl and Other Birds. Trans. Am. Wild. Res. Conf. 67: 51–61.

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.

Food Chain Transfer, Part I

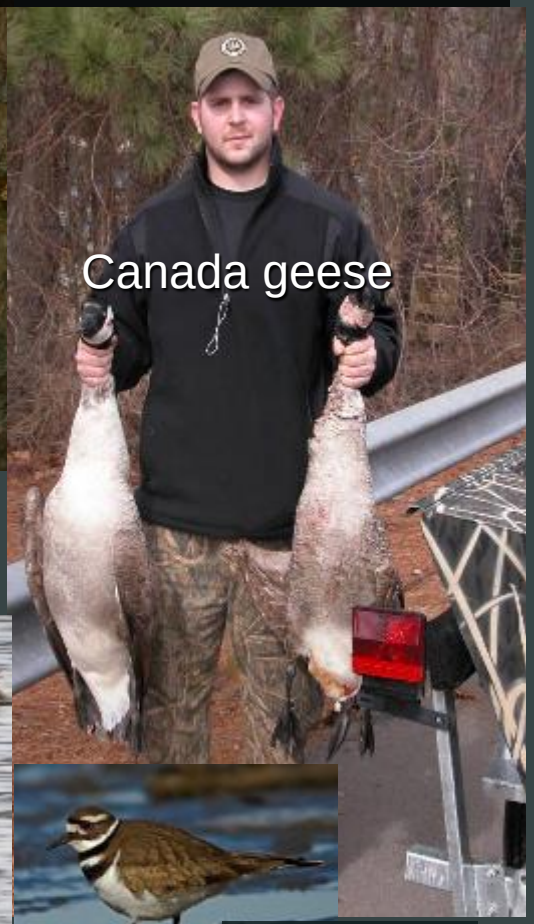


- Transfer of AVM from affected coot tissue to red-tailed hawks
- Established food chain link between coots and eagles

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

Bird species with AVM brain lesions

Mallards, Ring-necked ducks
Buffleheads, American wigeon



Killdeer

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. JWD 39:412-417.

Fischer, J, LA Lewis-Weis, CM Tate, JK Gaydos, RW Gerhold, RH Poppenga. 2006. Avian vacuolar myelinopathy outbreaks at a southeastern reservoir. JWD 42:501-510

Reservoir surveys: 2001-present

All AVM Sites

- Man-made ponds/reservoirs
- Nutrients low to moderate
- No harmful algal blooms in the water
- Dense non-native aquatic plants

Submerged non-native aquatic plants

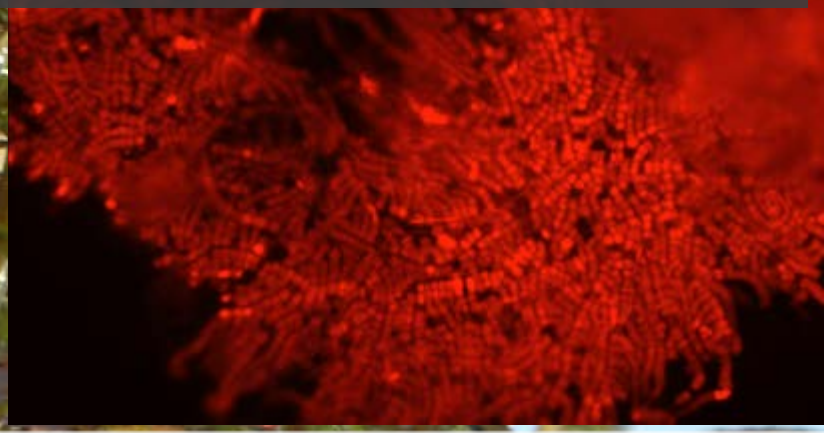
1994	DeGray Lake, AR
1996	Quachita, AR
1998	Woodlake, NC
1998	Thurmond, SC/GA
1998	Lake Juliette, GA
1998	Par Pond, SC
1998	L Lake, SC
1999	Lake Murray, SC
1999	Sam Rayburn, TX
2003	Davis Pond, SC
2003	Emerald Lake, GA
2005	Lake Horton, GA
2005	Smith Reservoir, GA
2005	Coachmans Trail, NC
2007	Lake Varner, GA
2010	Upper Towaliga, GA
2011	Longbranch, GA
2012	Lake Tohopekaliga, FL

1997 Hamilton, AR
1998 SRS- L Lake, SC
1998 SRS-Par Pond, SC
1998 Lake Juliette, GA

1994 DeGray Lake, AR
1996 Lake Ouachita, AR
1998 Lake Juliette, GA

Harmful cyanobacteria growing on invasive aquatic plants-- AVM sites

- Previously undescribed cyanobacterial species
- Cyanobacteria (or blue-green algae) are photosynthetic bacterial species that can produce liver and nerve toxins
- Grows as an **epiphyte on hydrilla** and other invasive exotic aquatic plants in all AVM sites



Food Chain Transfer, Part II

Laboratory feeding trial, when mallards were fed:

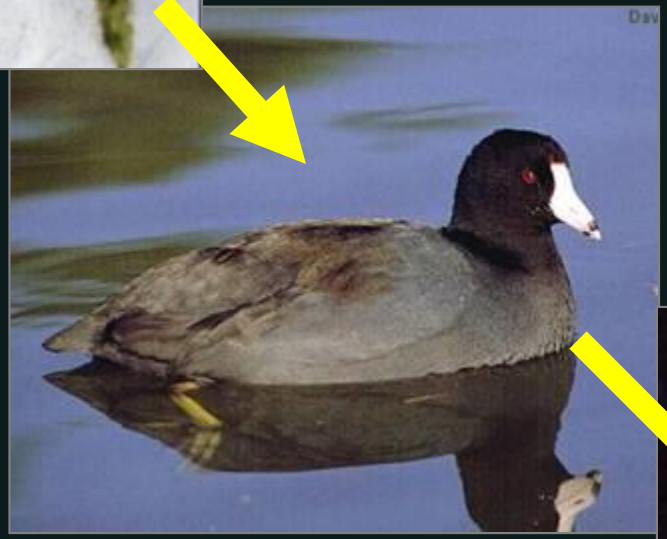
+ Hydrilla w/novel cyanobacteria (AVM site)-- AVM lesions

- Hydrilla w/o novel cyanobacteria (control lake)– no lesions

Birrenkott, AH, SB Wilde, JJ Hains, JR Fischer, TM Murphy, CP Hope, PG Parnell, and WW Bowerman. 2004. Establishing a food-chain linkage between aquatic plant material and Avian Vacuolar Myelinopathy in mallard ducks (Anas platyrhynchos). Journal of Wildlife Diseases 40:435-492

Food Chain Transfer Hypothesis

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

Field Test: Food Chain Transfer Hypothesis

Mallard Sentinel Trial

- Hydrilla + uncharacterized cyanobacteria
- Released 20 farm raised mallards for 6 week trial
- All mallards developed AVM lesions
- 1st documentation of AVM in small pond

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

Extracting the AVM toxin

Lyophilize, grind to coarse powder

Extract using a series of non-polar to polar solvents

Filter and Concentrate

Non-Polar

Hexane

Acetone

Methanol

Polar

Laboratory Toxin Trial

- Experimental Groups
  - + AVM site (hydrilla/cyano) Hexane
  - + AVM site (hydrilla/cyano) Acetone
  - + AVM site (hydrilla/cyano) Methanol

  - - Control (hydrilla) Hexane
  - - Control (hydrilla) Acetone
  - - Control (hydrilla) Methanol

- Mallards dosed 3x/wk for 4 wks

+ AVM(hydrilla/cyano) Methanol – all had AVM lesions

Wiley FE, Twiner MJ, Leighfield TA, Wilde SB, Van Dolah FM, Fischer JR, Bowerman WW. 2009. An Extract of Hydrilla verticillata and Associated Epiphytes Induces Avian Vacuolar Myelinopathy in Laboratory Mallards. Environ Toxicol 24:362-368.

Cell line Toxin Assay

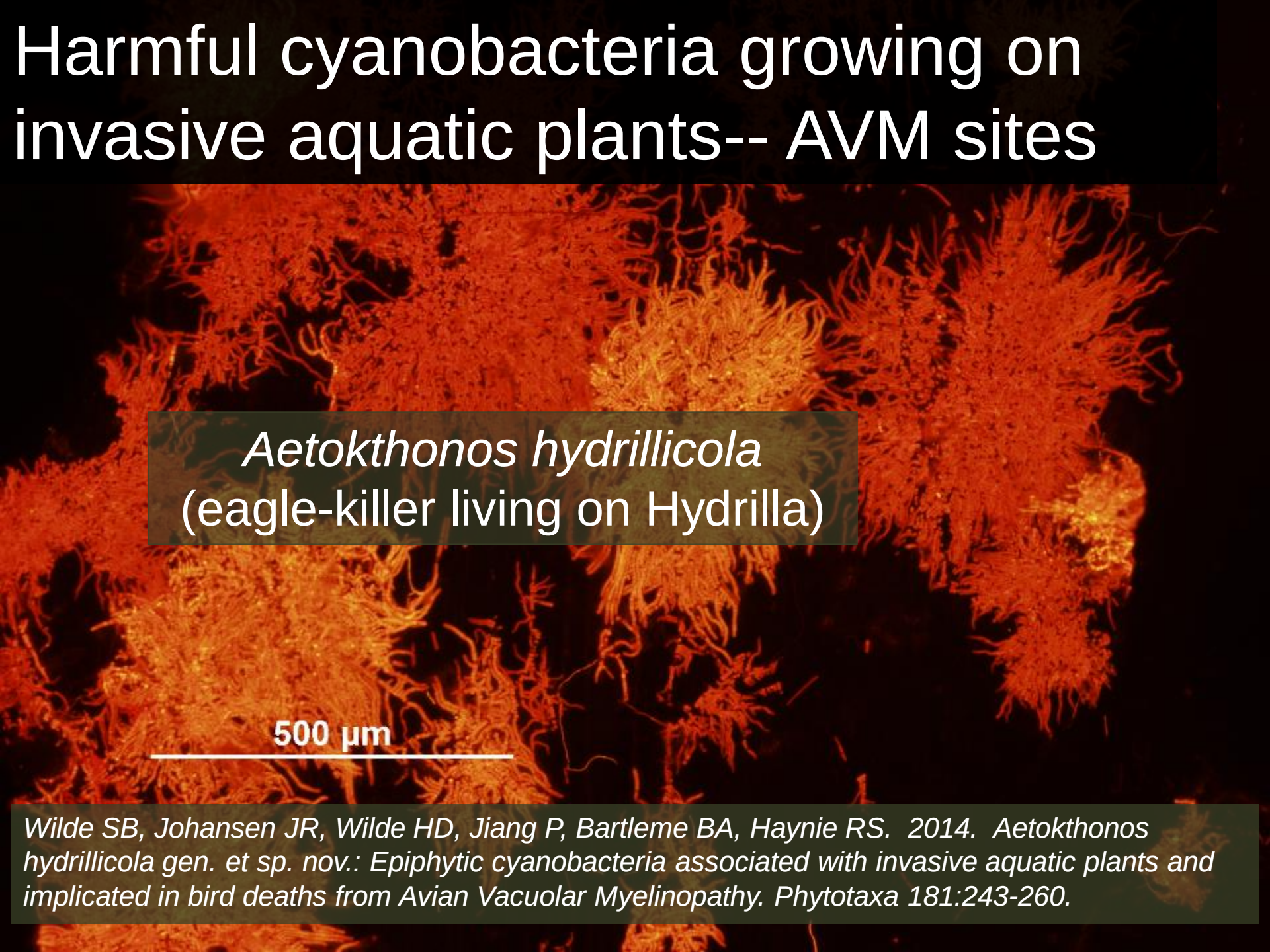
GOAL: Develop a method to detect the unknown toxin without testing on birds

- Consistent cell cycle arrest following 24hr exposure to methanol extracts from AVM positive sites (hydrilla/cyano)
- Healthy cell cultures following 24hr exposure to methanol extracts from control sites (hydrilla)

+AVM site (hydrilla/cyano) cells stop dividing

- Control (hydrilla) normal cells

Harmful cyanobacteria growing on invasive aquatic plants-- AVM sites



Wilde SB, Johansen JR, Wilde HD, Jiang P, Bartleme BA, Haynie RS. 2014. Aetokthonos hydrillicola gen. et sp. nov.: Epiphytic cyanobacteria associated with invasive aquatic plants and implicated in bird deaths from Avian Vacuolar Myelinopathy. Phytotaxa 181:243-260.

Expanding Locations: AVM Reservoirs 2015

30 sites (+5 FL)

Hydrilla &
Aetokthonos hydrillicola

166 eagles

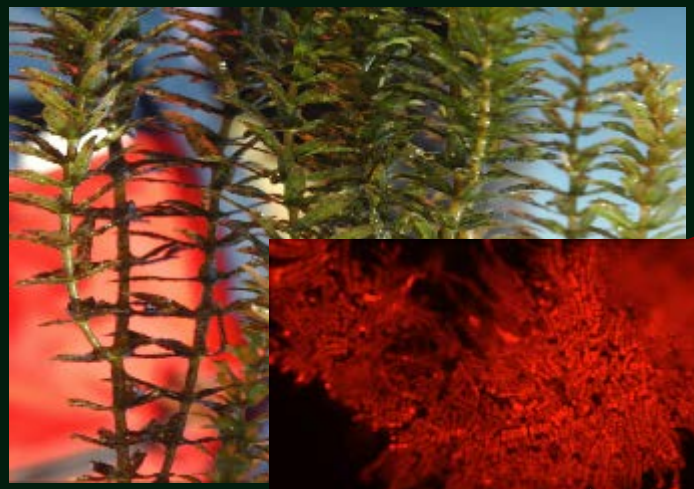
AVM confirmed birds

20 sites

AVM+, Hydrilla &
A. hydrillicola

Expanding Food Chain

More aquatic animals at risk?



Birrenkott 2004

Haynie 2013

Brandon current MS

Haram current PhD

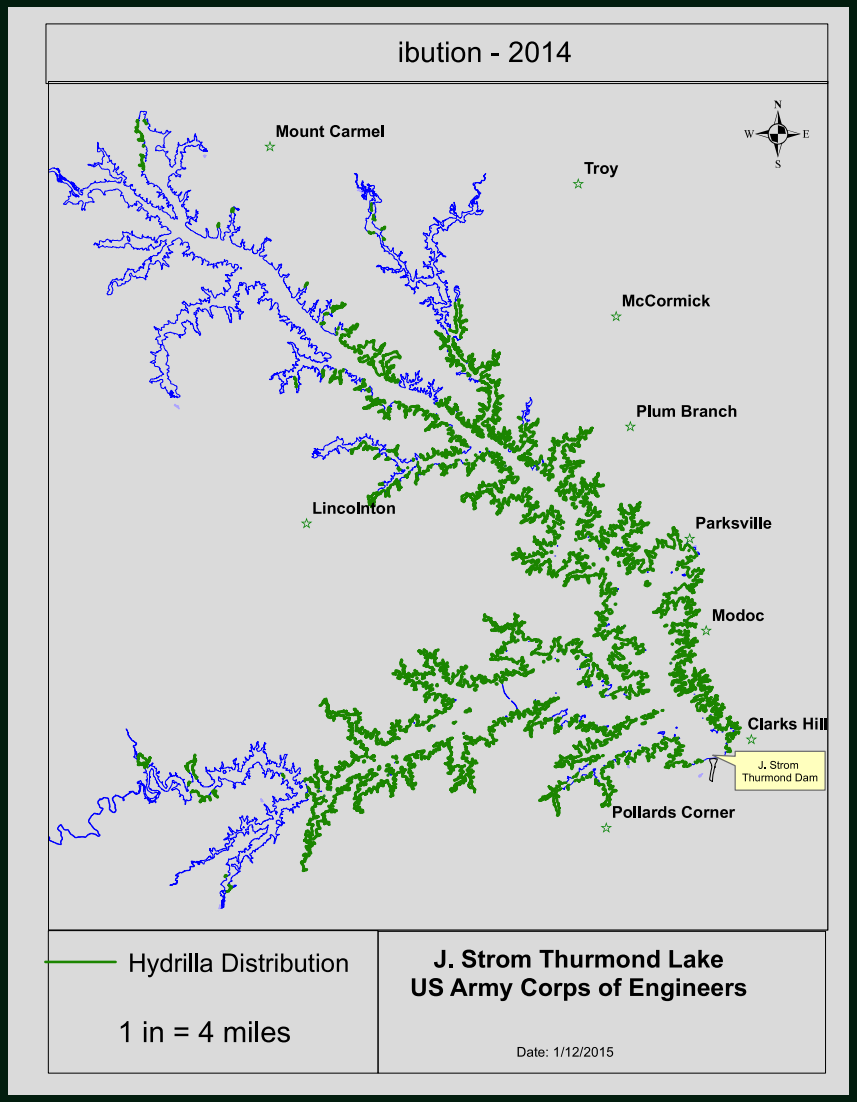
Dodd in press

Mercurio 2014

Maerz (in prep)

Expanding Food Chain

Lethargic beaver recovered from AVM positive site during late fall



J. Strom Thurmond Reservoir

Gut contents of neurologically impaired beaver

Aetokthonos hydrillicola

Hydrilla spine

Southeastern Cooperative Wildlife Disease Study pathologists documented severe vacuolar changes in the white matter of the brain on light microscopy, but were unable to confirm AVM lesions under electron microscopy

Expanding Food Chain

- Southeastern U.S. is a global hot spot of freshwater turtle diversity.
- Most species are omnivorous or herbivorous.
  - ~85% of adult diets are composed of aquatic plants and algae.
  - Hydrilla commonly reported as a dominant plant in turtle diets in SE US.

Expanding Food Chain

Laboratory feeding trial, 10 turtles were fed:

+ Hydrilla w/novel cyanobacteria (AVM site)
- Hydrilla w/o novel cyanobacteria (control lake)

Methods

- Humanely euthanized and performed a complete necropsy
- Organs in 10% formalin
- ½ brain stored in EM fixative
- Histology on major organs
- Electron microscopy on CNS

Experimental Feeding of *Hydrilla verticillata* Colonized by Stigonematales Cyanobacteria Induces Vacuolar Myelinopathy in Painted Turtles (*Chrysemys picta*)

Albert D. Mercurio[1,2]*, Sonia M. Hernandez[1,2], John C. Maerz[1], Michael J. Yabsley[1,2], Angela E. Ellis[3], Amanda L. Coleman[1], Leslie M. Shelnutt[4], John R. Fischer[4], Susan B. Wilde[1]

Abstract

Vacuolar myelinopathy (VM) is a neurologic disease primarily found in birds that occurs when wildlife ingest submerged aquatic vegetation colonized by an uncharacterized toxin-producing cyanobacterium (hereafter "UCB" for "Uncharacterized cyanobacterium"). Turtles are among the closest extant relatives of birds and many species directly and/or indirectly consume aquatic vegetation. However, it is unknown whether turtles can develop VM. We conducted a feeding trial to determine whether painted turtles (*Chrysemys picta*) would develop VM after feeding on *Hydrilla* (*Hydrilla verticillata*), colonized by the UCB (*Hydrilla* is the most common "host" of UCB). We hypothesized turtles fed *Hydrilla* colonized by the UCB would exhibit neurologic impairment and vacuolation of nervous tissues, whereas turtles fed *Hydrilla* free of the UCB would not. The ability of *Hydrilla* colonized by the UCB to cause VM (hereafter, "toxicity") was verified by feeding it to domestic chickens (*Gallus gallus domesticus*) or necropsy of field collected American coots (*Fulica americana*) captured at the site of *Hydrilla* collections. We randomly assigned ten wild-caught turtles into toxic or non-toxic *Hydrilla* feeding groups and delivered the diets for up to 97 days. Between days 82 and 85, all turtles fed toxic *Hydrilla* displayed physical and/or neurologic impairment. Histologic examination of the brain and spinal cord revealed vacuolations in all treatment turtles. [illegible] of the control turtles exhibited neurologic impairment or had detectable brain or spinal cord vacuolations. This is the [illegible] freshwater turtles can become neurologically impaired and develop vacuolations after consuming toxic [illegible] with the UCB. The southeastern United States, where outbreaks of VM occur regularly and where [illegible] the UCB is common, is also a global hotspot of freshwater turtle diversity. Our results suggest that [illegible] effect of the putative UCB toxin on wild turtles *in situ* are warranted.

Key Findings

Between days 80 and 90, all turtles fed *Aetokthonos* positive *Hydrilla* exhibited associated clinical signs of VM

- Weakness
- Lethargy
- Anorexia
- Floating abnormally
- Ataxia

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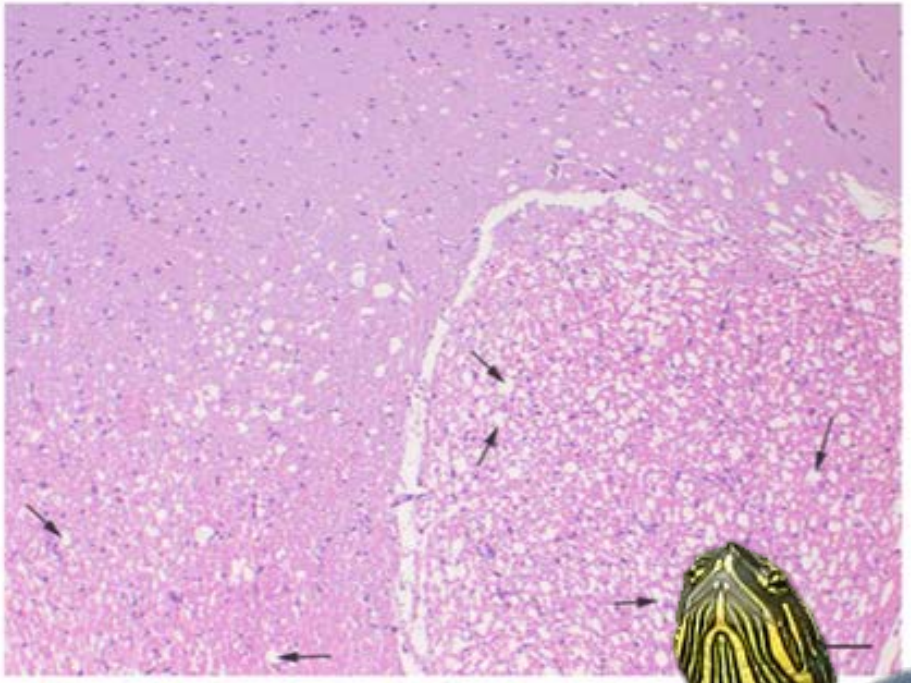
Key Findings



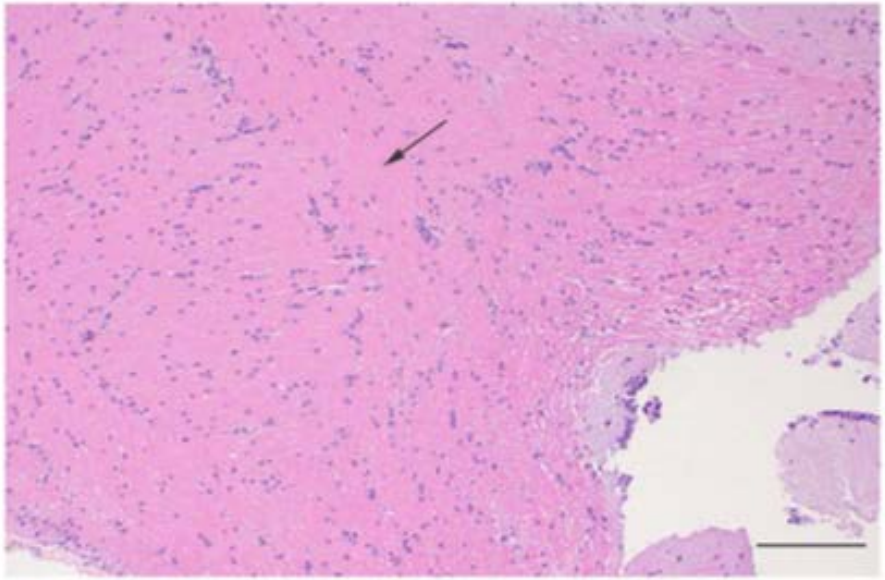


Figure 1. Histopathological slide of the optic … painted turtle fed toxic *Hydrilla* material. Painted tu… *picta*), brain: Numerous clear vacuoles (black arrow… myelin degeneration and dilation of axonal sheaths a… white matter of a turtle treated with toxic hydrilla. H&… is 100 μm.
doi:10.1371/journal.pone.0093295.g001

Figure 3. Histopathological slide of the optic tectum of a …ted turtle (*Chrysemys picta*), brain: white matter, … appears normal with no evidence of …ion. H&E, 100X. Scale bar is 100 μm.

…some variation in distribu-… cerebellar lesions, this did …es in the clinical signs. … birds with VM

Key Findings

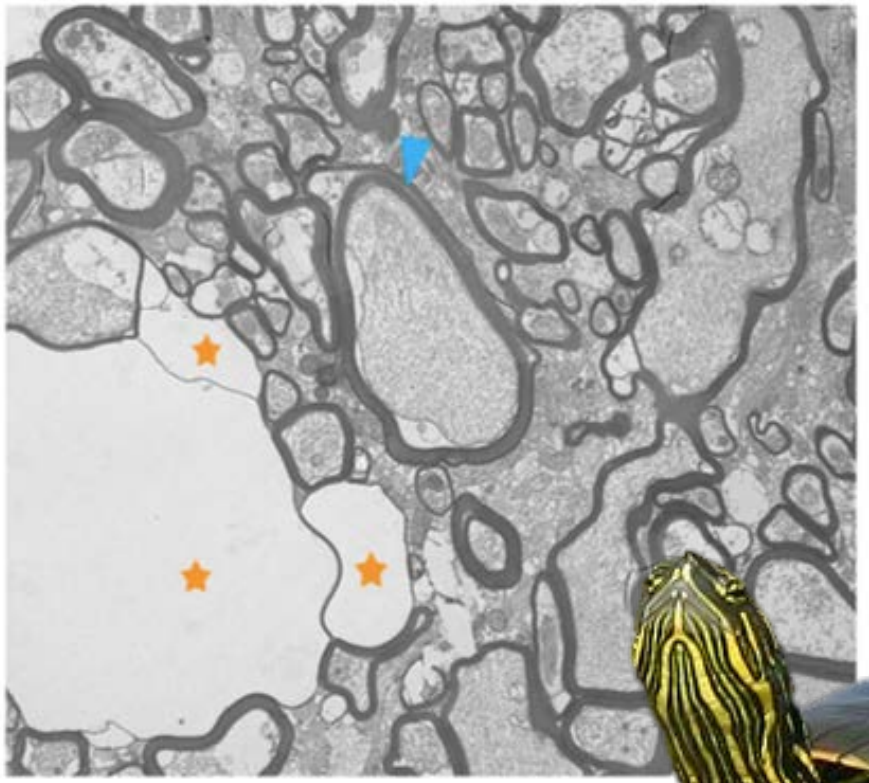


Figure 2. Electron Micrograph of central ner
painted turtle fed toxic *Hydrilla* material. El
painted turtle (*Chrysemys picta*), brain: Axon
degenerate and myelin sheaths are frequently
intramyelinic vacuoles (orange stars). In les
splitting can be seen to occur at the intraperi (blue

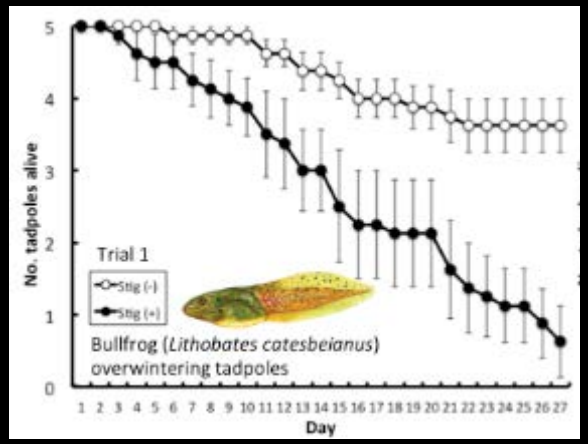
Southeastern U.S. has a rich diversity of amphibians.

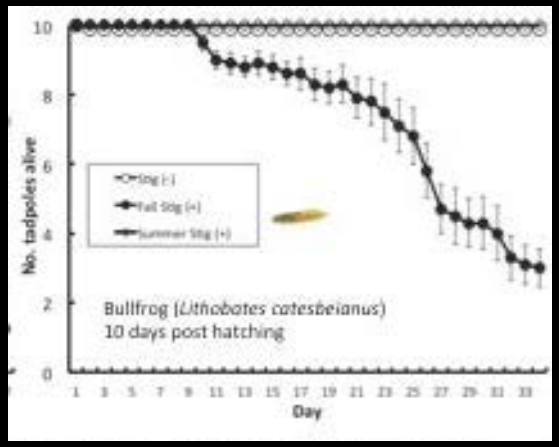
- Species breed in a range of water bodies including ponds and the littoral zones of lakes.
- Anuran [frogs and toads] have an aquatic tadpole stage that feeds on epiphytic biofilms on aquatic plants.

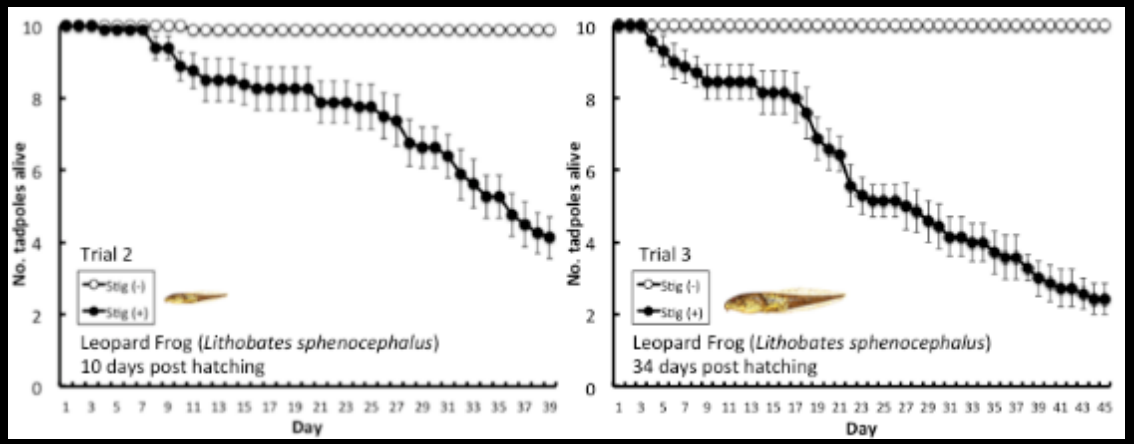
Methods

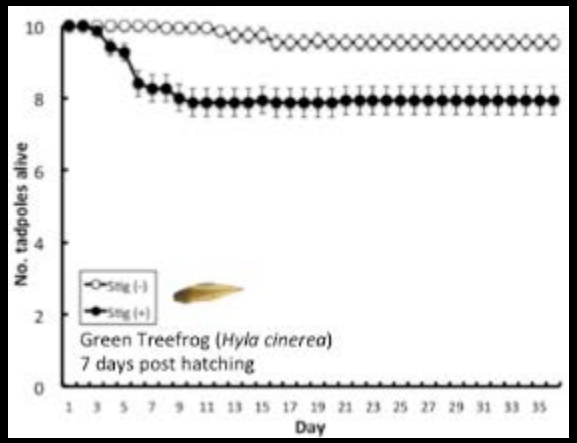
- 7-10 replicate aquaria containing 5-10 tadpoles [depending on size]
- Tested three species at various stages using fall collected *Aetokthonos* positive or negative *Hydrilla*
- Monitored survival and behavior daily

Results

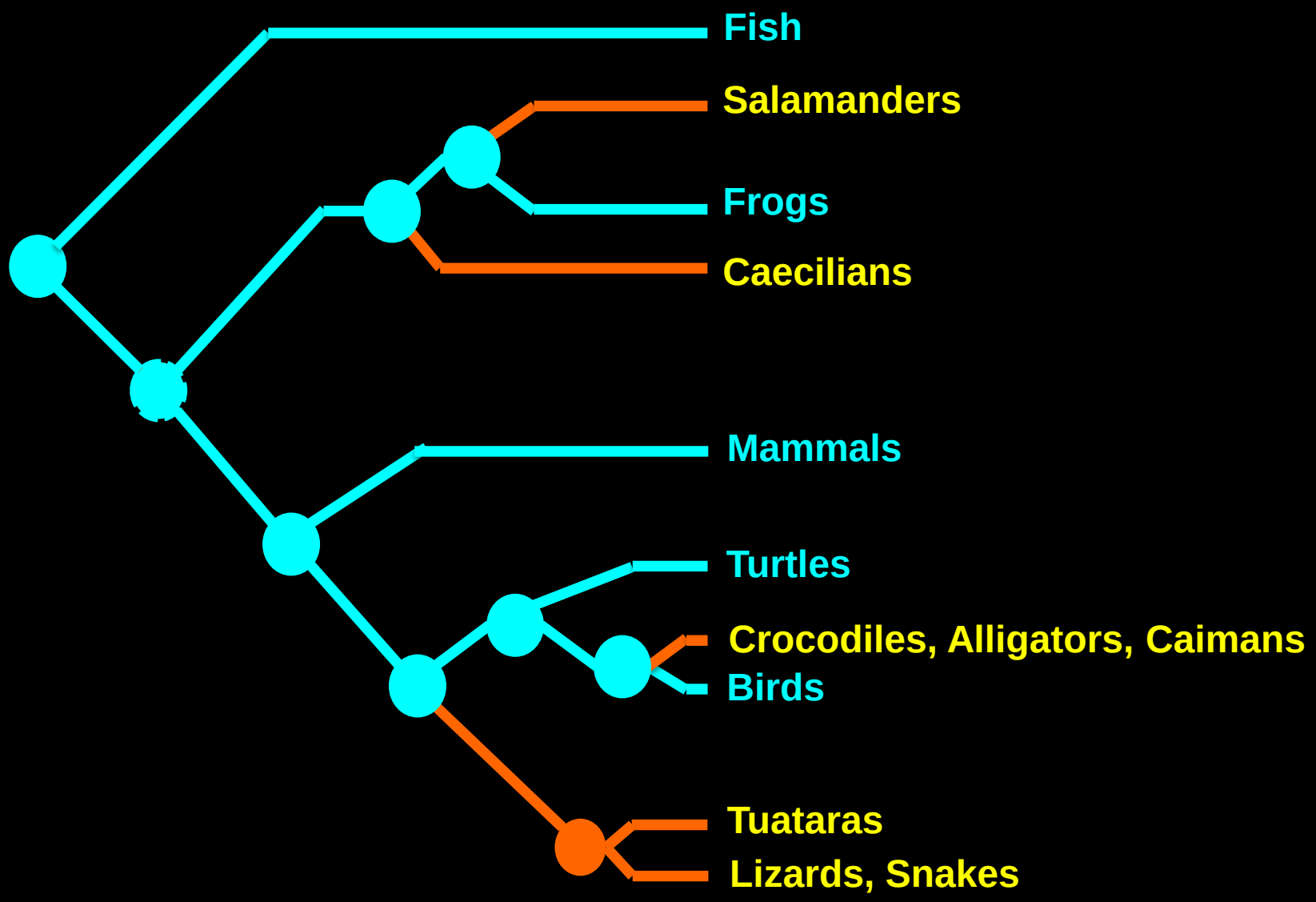

No. tadpoles alive
Day
Trial 1
Stig (-)
Stig (+)
Bullfrog (Lithobates catesbeianus)
overwintering tadpoles


No. tadpoles alive
Day
Bullfrog (Lithobates catesbeianus)
10 days post hatching

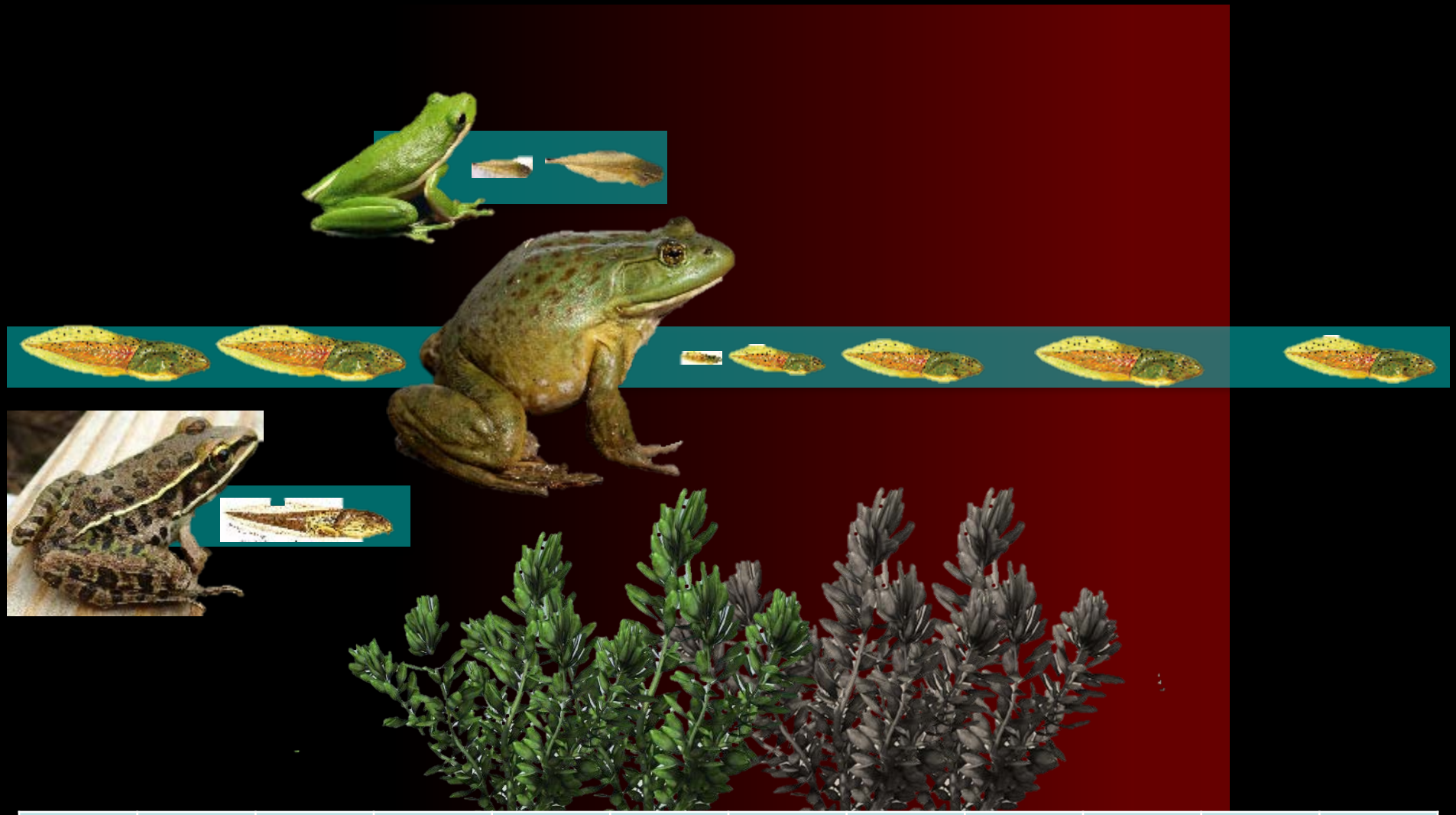

No. tadpoles alive
Day
Trial 2
Stig (-)
Stig (+)
Leopard Frog (Lithobates sphenocephalus)
10 days post hatching
Trial 3
Leopard Frog (Lithobates sphenocephalus)
34 days post hatching


No. tadpoles alive
Day
Stig (-)
Stig (+)
Green Treefrog (Hyla cinerea)
7 days post hatching

Taxa affected by ingesting *Aetokthonos* positive *Hydrilla*



Risk = Sensitivity + Exposure

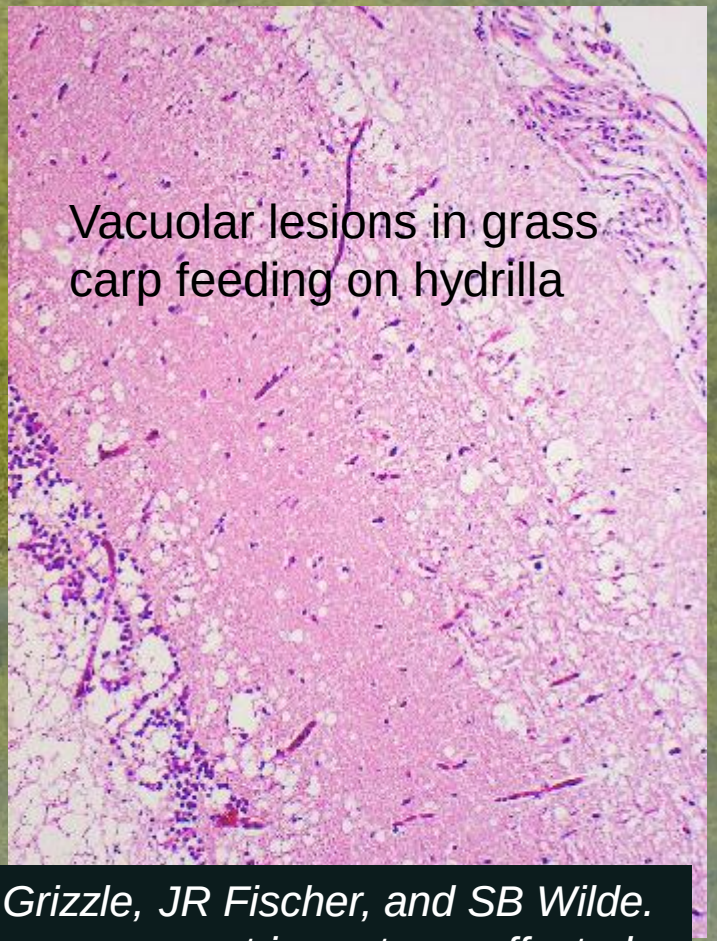


Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb

Management solutions

Triploid Chinese Grass Carp

- Effective control of submerged aquatic plants
- Develop vacuolar lesions, but survive
- Did not induce lesions in birds



Vacuolar lesions in grass carp feeding on hydrilla

Haynie, RH, WW Bowerman, SK Williams, JR Morrison, JR Grizzle, JR Fischer, and SB Wilde. 2013. Are triploid grass carp suitable for aquatic vegetation management in systems affected by Avian Vacuolar Myelinopathy? Journal of Aquatic Animal Health 25: 252–259.

Management solutions

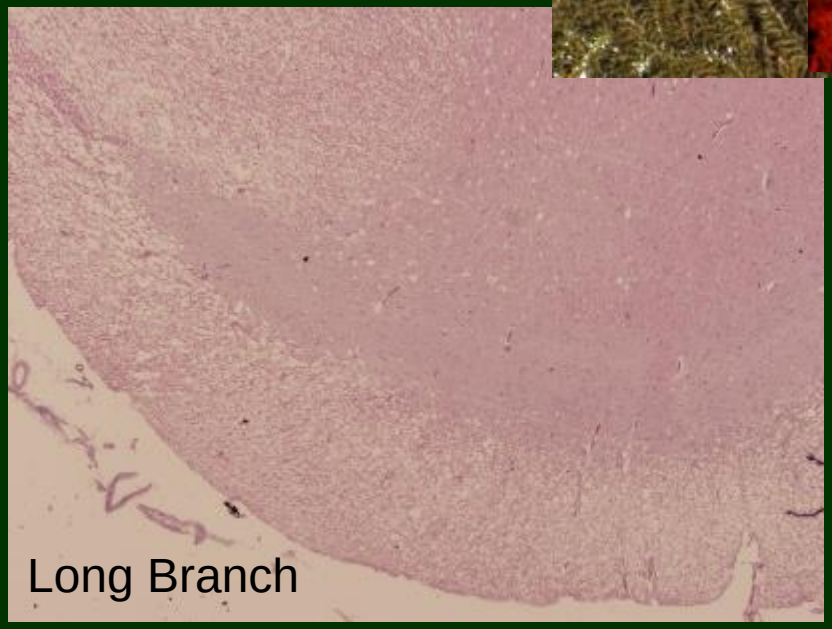
Towaliga

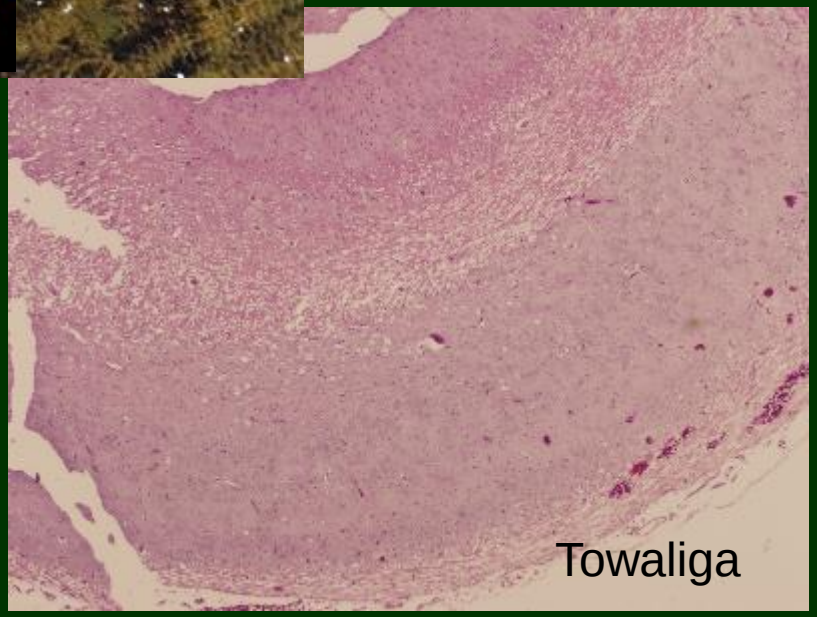
5000 10-12” triploid grass carp in April 2011
Additional 5000 April 2012

Long Branch

No management

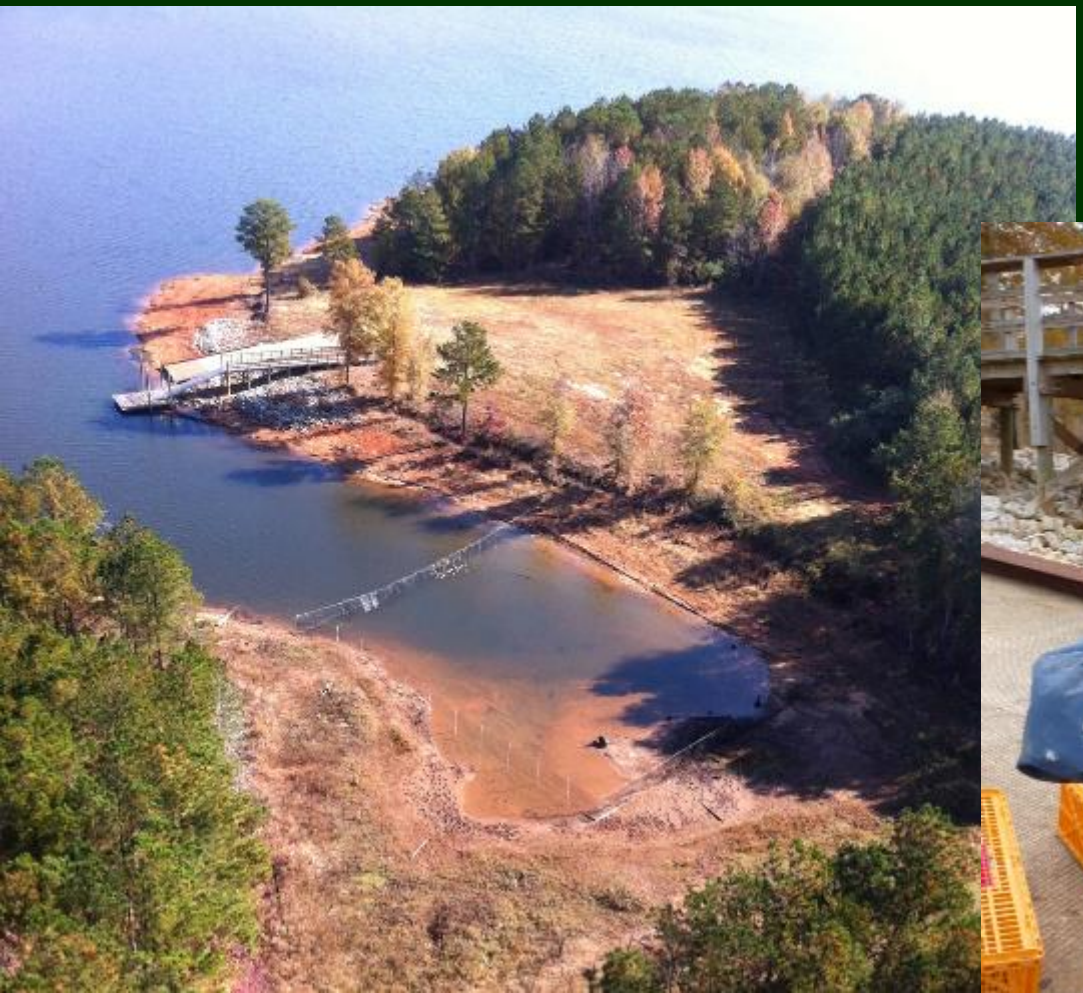
Sentinel Trial: Year 1





Sentinel Trial: Year 2

- All sentinel mallards with access to hydrilla-- AVM positive
- Mallards in hydrilla free zones-- AVM negative



Sentinel Mallard: Untreated Reservoir, Year 2

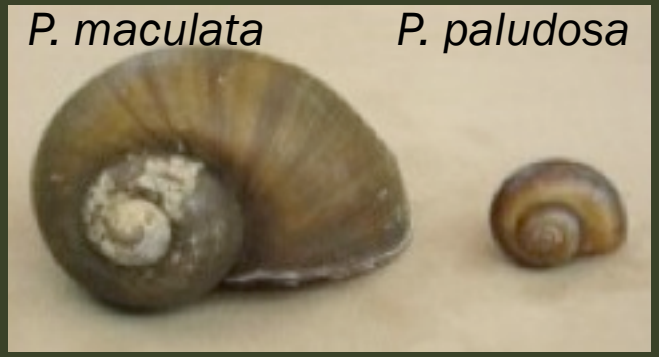
Expanding food chain & locations

Photo: Mac Stone

--Expanding risk

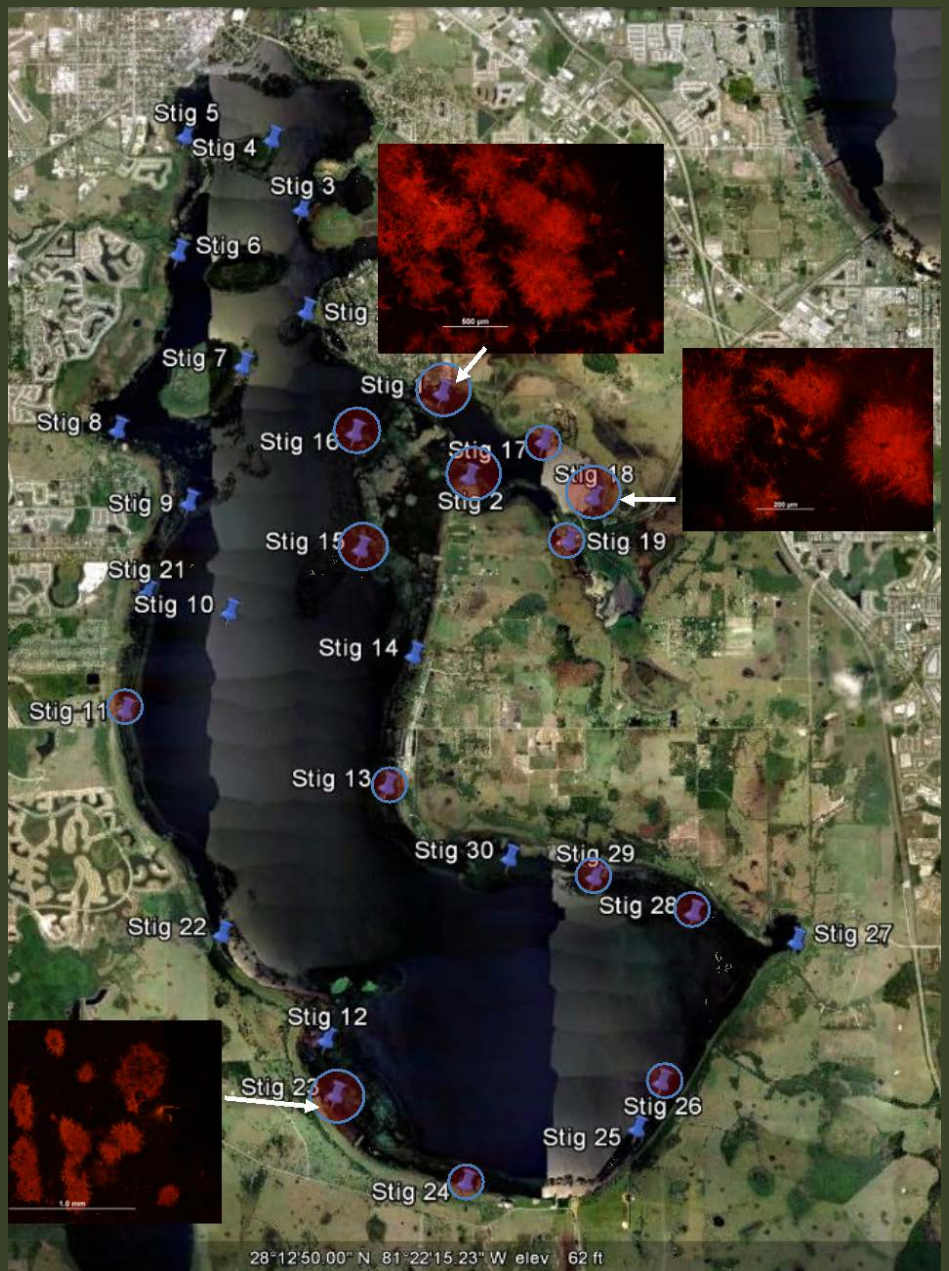
Florida Snail Kite
Rostrhamus sociabilis

- Snail kites endangered in Florida
- Apple snails >99% of snail kite diet
- Kites forced to switch to exotic snail



Expanding locations

- 1st found in Florida January 2012
- Lake Toho, critical snail kite nesting site
- *Aetokthonos hydrillicola* 15/30 sites



Expanding food chain

AVM+

Hydrilla + A.h.

1. ‘AVM +’ hydrilla material fed to apple snails

2. Apple snails fed to chickens

1. ‘AVM –’ hydrilla material fed to apple snails

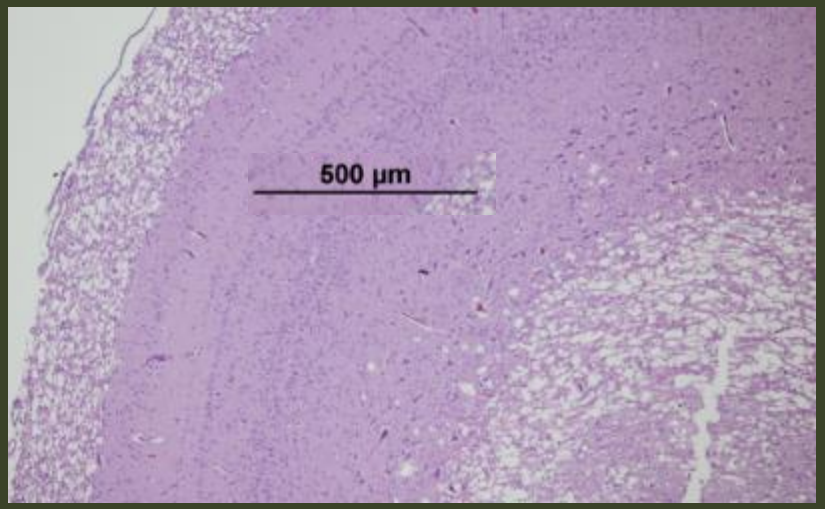
2. Apple snails fed to chickens

Control

Hydrilla, no A.h.

Expanding risk

- AVM lesions in **10/10** chickens that consumed hydrilla with *A. hydrillicola* from Lake Toho
- Severe AVM lesions in 5/5 chickens fed snails that had consumed hydrillla/*A. hydrillicola*
- AVM lesions in 5/10 coots collected from region in Toho with *A. hydrillicola*



A. hydrillicola growing on hydrilla leaflets produces biotoxin

Exotic apple snails feed on hydrilla and accumulate biotoxin

Snail kites feed on exotic snails and ingest biotoxin

- Hydrilla/*A. hydrillicola* is toxic in Lake Toho, FL
- Toxin can be transferred through an invertebrate

Dodd, SR, RS Haynie, SM Williams, and SB. Wilde (in press). Alternate food-chain transfer of the toxin linked to Avian Vacuolar Myelinopathy (AVM) and implications for endangered Florida snail kite, Rosthramus sociabilis. Journal of Wildlife Diseases.

Expanding locations

5 additional Florida Lakes *A. hydrillicola*

East Lake Tohopekaliga

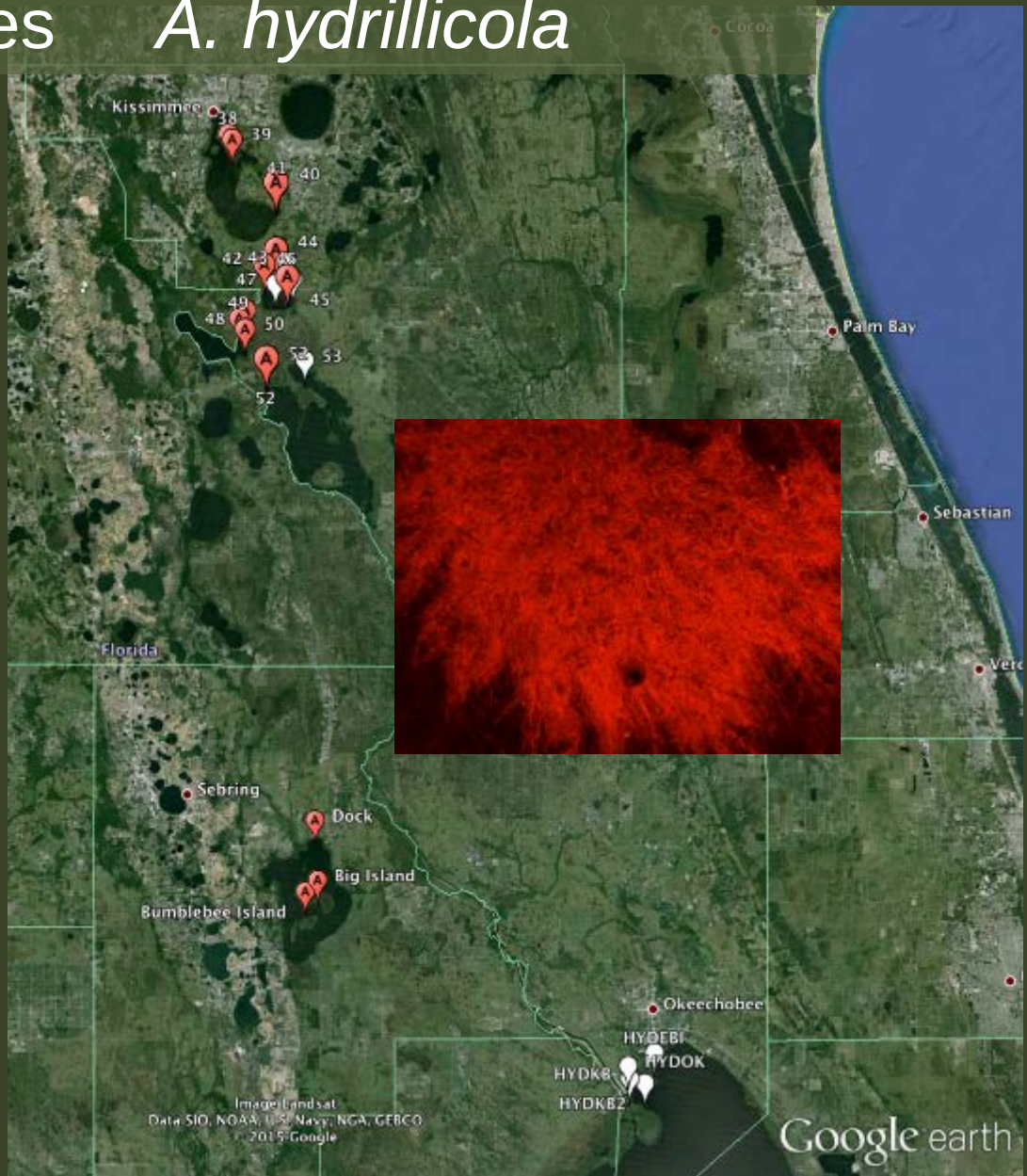
Tohopekaliga

Cypress

Kissimmee

Hatchineha

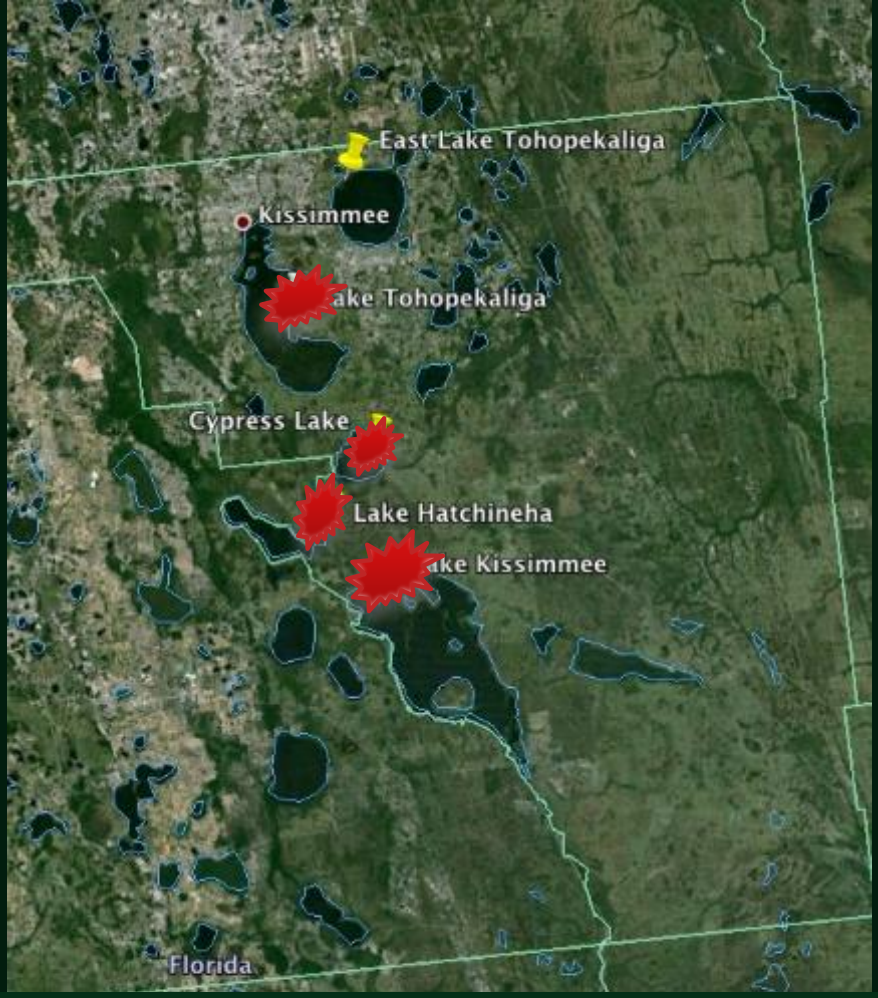
Istokpoga



Expanding Risk

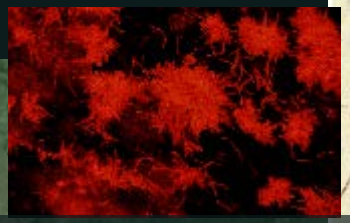
Relative toxicity
March 2015

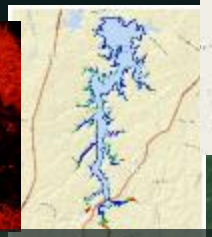
Cell line bioassay



Kissimmee> Istokpoga >Toho>Hatch> Cypress

Expanding locations



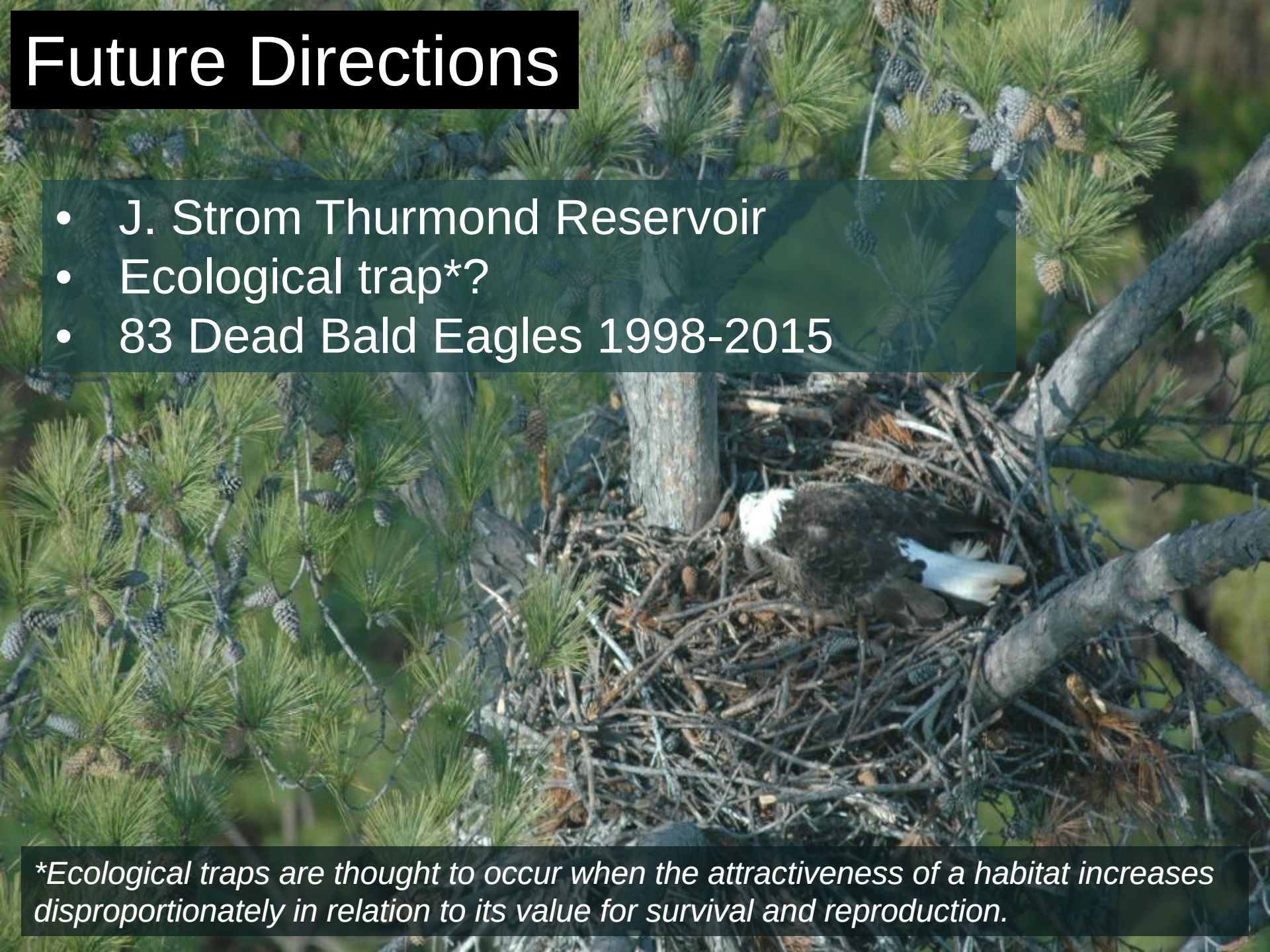


Mayo Lake, NC & Kerr Reservoir, NC/VA
September 10, 2015 *A. hydrillicola*

Coachmans Trail
Woodlake
Lake Murray
Davis Pond
Pollard Lake
J. Strom Thurmond
L Lake
Par Pond
Lake Walton
Lake Varner
Pond 6
Longbranch Reservoir
Pond 22
Pond 11a
Troupe County AVM eagle
Pond 11b
Still Branch Reservoir
Ouachita
Lake Hamilton
DeGray Lake
Lake Greeson
Caddo Lake
Crosslake
Lake Jacksonville
Lake Nacogdoches
Toledo Bend
Sam Rayburn Reservoir
East Lake Tohopekaliga
Cypress Lake
Lake Kissimmee
Lake Hatchineha
Lake Istokpoga

AVM sites (avian lesions, hydrilla + *A. hydrillicola*
Suspect sites (Hydrilla + *A. hydrillicola*)

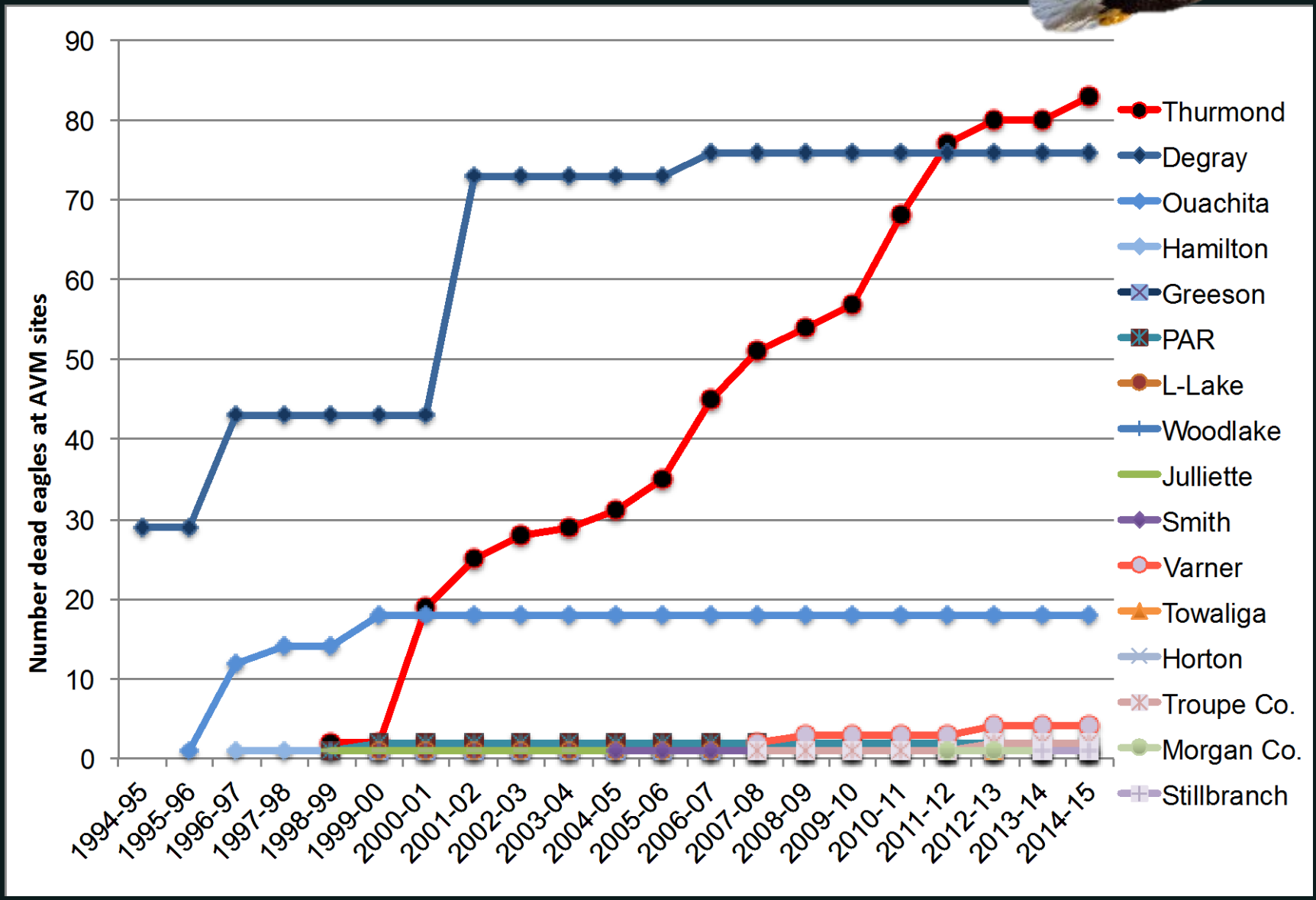
Future Directions



- J. Strom Thurmond Reservoir
- Ecological trap*?
- 83 Dead Bald Eagles 1998-2015

**Ecological traps are thought to occur when the attractiveness of a habitat increases disproportionately in relation to its value for survival and reproduction.*

Since 1998, eagles have been dying on J. Strom Thurmond Reservoir



Dense hydrilla, abundant coots, eagle nesting

Hydrilla

High coot densities

Eagle sightings

Eagle nests

Eagle mortalities

J. Strom Thurmond Reservoir

Brigette Haram, UGA

Eagle mortality & hydrilla data provided by Ken Boyd & Allen Dean, COE

Eagle nest data provided by Jim Ozier, GADNR

Stalking the surviving eagle nestlings

Expert assistance from:

Libby Mojica

The CENTER for CONSERVATION BIOLOGY

Craig Koopie

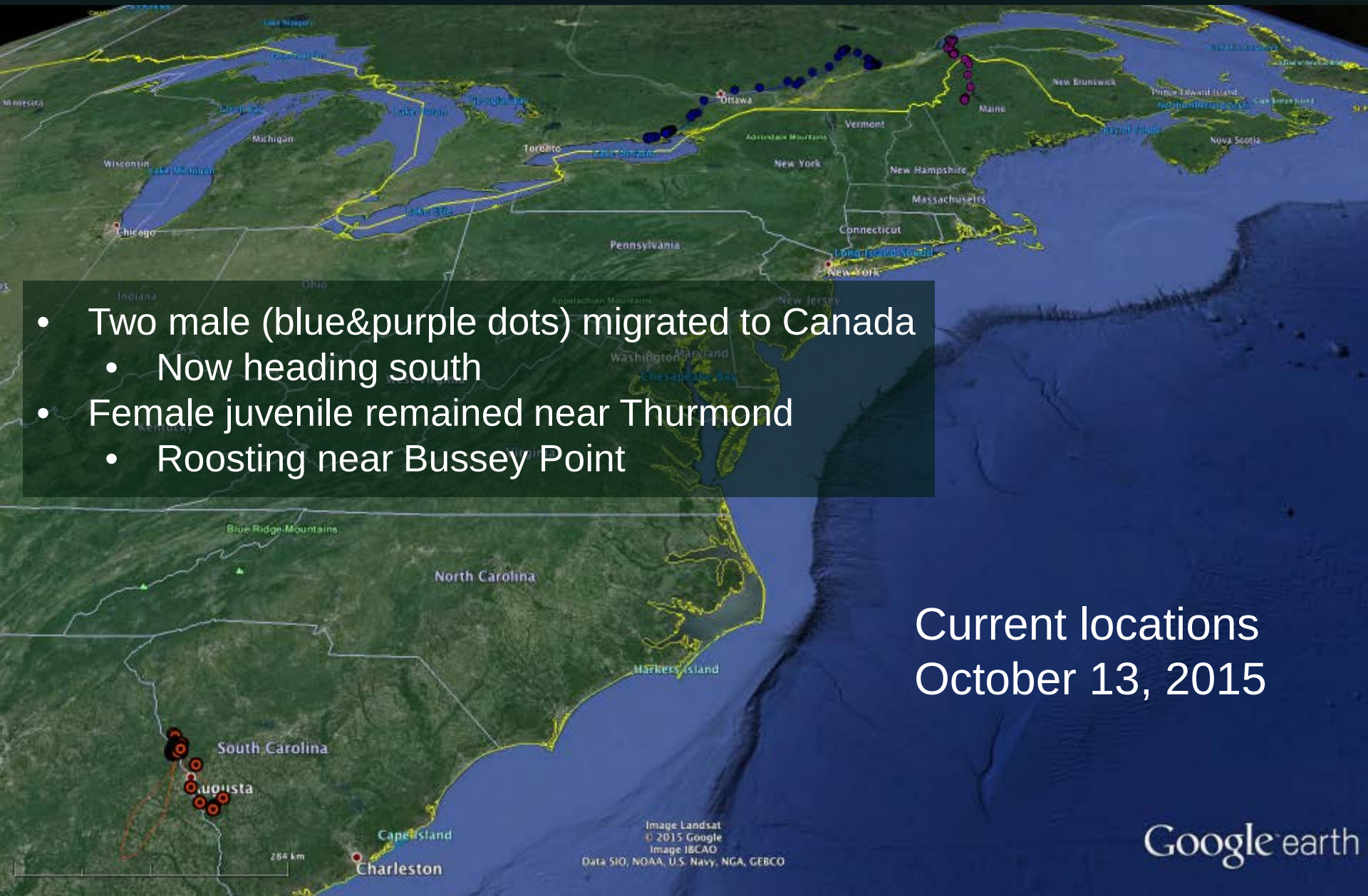
Thurmond nestling April 3, 2015

Brigette Haram photo

Satellite telemetry

- Solar powered PTT/GPS 70g units (Microwave Telemetry Inc)
- Transmit to Argos
- Programmed for the winter risk period (Nov-Jan) and sends a UHF signal during daylight hours

Stalking the surviving juvenile eagles



- Two male (blue&purple dots) migrated to Canada
  - Now heading south
- Female juvenile remained near Thurmond
  - Roosting near Bussey Point

Current locations
October 13, 2015

Management solutions

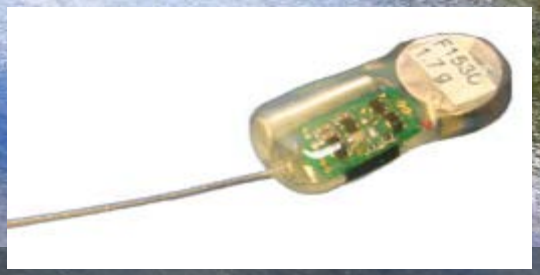
Combining chemical and biological control methods

Bussey Point region: dense hydrilla, sick waterfowl, and eagle mortality (1998-2015)

- 15 acres plots
- High density: ~10 acres hydrilla, grass carp, & grass carp/herbicide
- Low density ~5 acres hydrilla

Stalking the triploid grass carp

Radiotagged Grass Carp Stocking in J. Strom Thurmond



- 24 grass carp will be fitted with body implant radio transmitters to assess movement.
- Electroshocking boat used to collect a subset of stocked grass carp and other fish species for:
  - Health assessments (including vacuolar lesions)
  - Laboratory feeding trials (to test for risk of AVM toxin transmission)

Questions and future research

- Chemical structure of the VM neurotoxin?
  - Bioassay guided fractionation
    - Cell line bioassay, chicken trials
    - HPLC/MS peaks, NMR
- How does the VM toxin cause brain lesions?
  - Develop Zebrafish bioassay, mechanism of lesion formation, genetic changes induced by VM toxin
- Origin of *Aetokthonos hydrillicola?*
  - *Screen hydrilla from native range for A. hydrillicola*
  - *Determine heterogeneity in DNA sequences A. hydrillicola from all sites*

- What controls the distribution of *A. hydrillicola* and what triggers neurotoxin production?
  - Continue monitoring
    - AVM sites
    - AVM suspect sites
    - new hydrilla locations
  - Laboratory trials
    - *A. hydrillicola* cultures/sterile hydrilla
    - Model fall-winter reservoir environment
- Can native plants/*A. hydrillicola* cause VM?
  - Collect native plants from Thurmond & other sites where A. hydrillicola is growing on native
  - Test for toxicity, conduct feeding trials

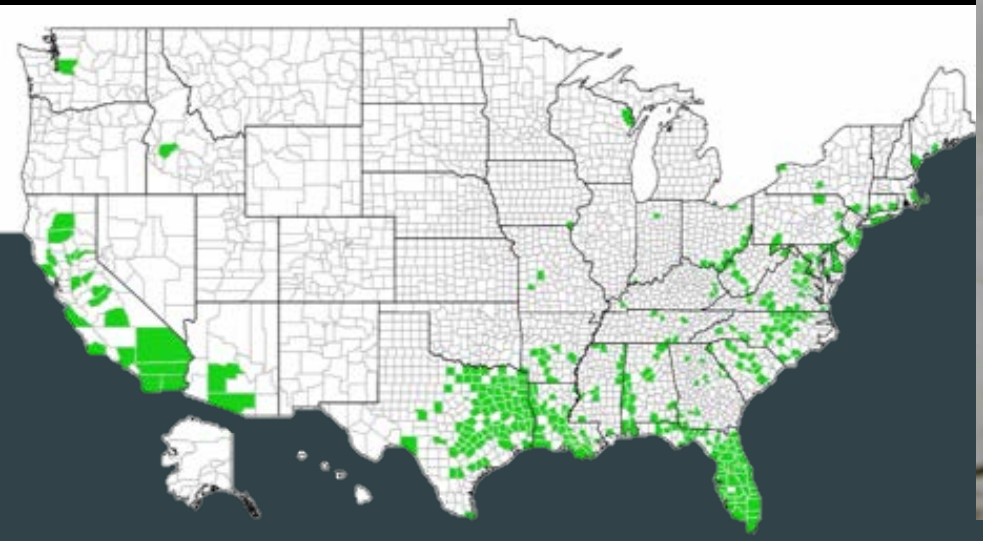
- Are there risks to human health?

  - Use Zebrafish model: they share 70% of our genome, used for toxin screening and human disease research
  - Mouse bioassay: standard technique for cyanotoxins
  - Test fish and waterfowl consumed by fishermen and hunters for VM toxin
  - Test meat from chickens used in feeding trials with AVM lesions for VM toxin*

*preliminary trial documented lesion formation in chickens consuming breast meat from AVM positive birds

Gulf & South Atlantic Regional Panel On Aquatic Invasive Species

Contact Information



- Please help expand AVM survey efforts
- Send hydrilla for screening during October-December
- Email swilde@uga.edu for collection and shipping information

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