Invasive Plants, Toxic Cyanobacteria & Fish and Wildlife Health

Susan B. Wilde and John C. Maerz

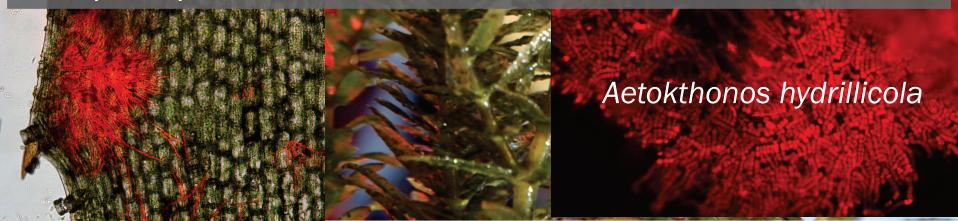
500 μm



Novel cyanobacterial species growing on invasive aquatic plants all 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





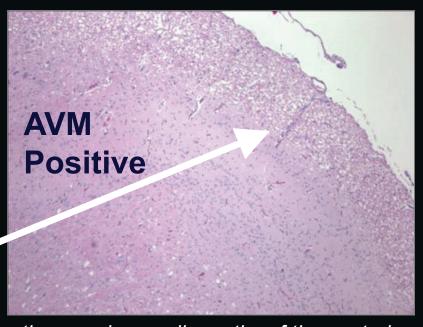
Diagnosis: Unique brain lesions



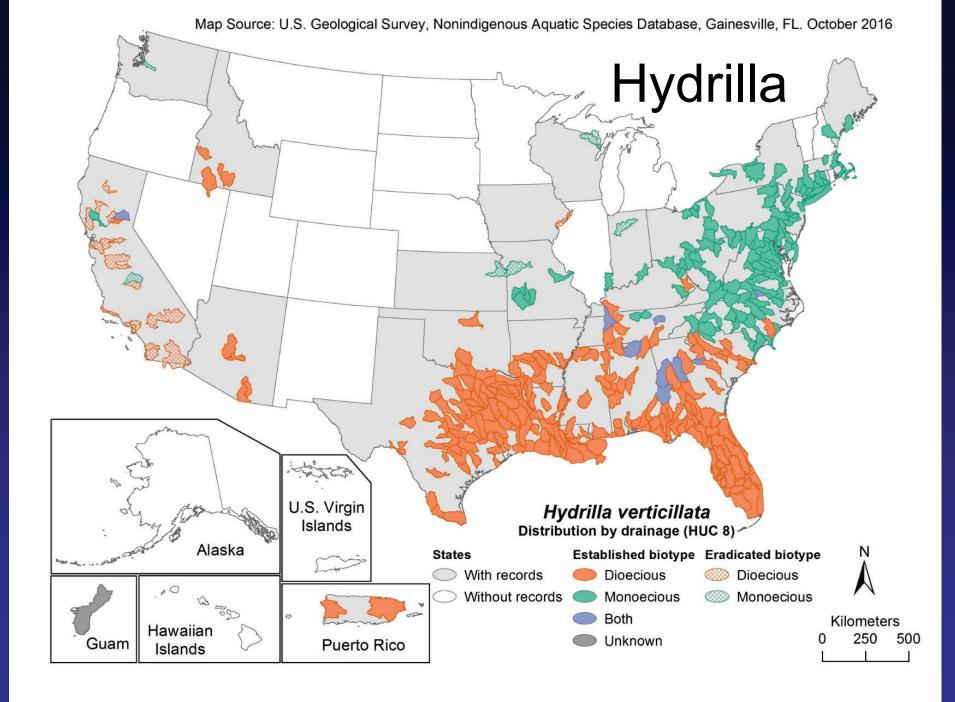
National Wildlife Health Center

Southeastern Cooperative Wildlife Disease Study

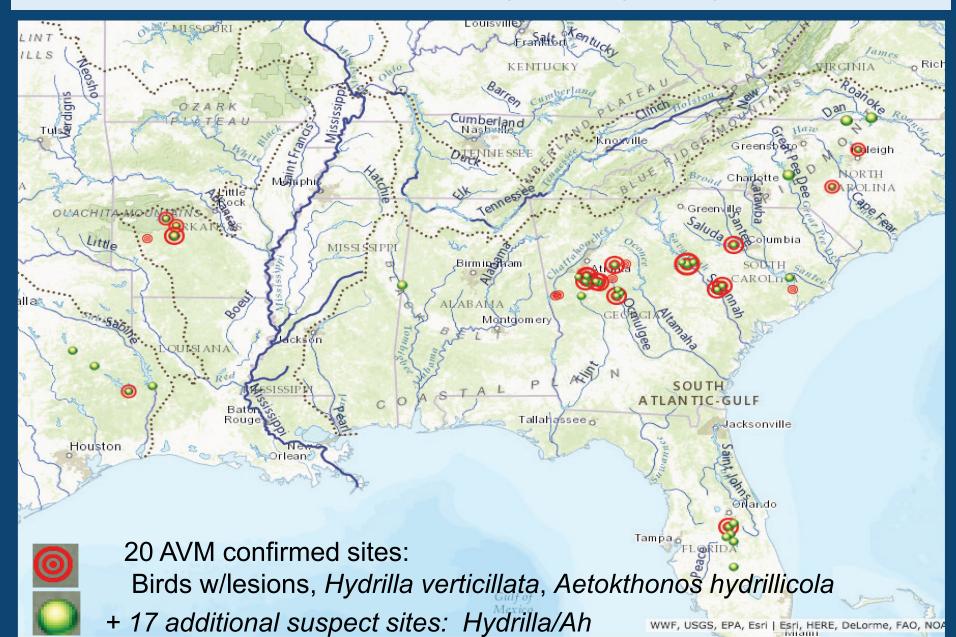
Open spaces in:
white matter of the central
nervous system, specifically an
intramyelinic edema



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



"Avian" Vacuolar Myelinopathy 2017



Expanding Taxa Fish Salamanders Frogs S **Mammals Turtles** Crocodiles, Alligators, Caimans Birds Lizards, Snakes





Journal of Aquatic Animal Health



ISSN: 0899-7659 (Print) 1548-8667 (Online) Journal homepage: http://www.tandfonline.com/loi/uahh20

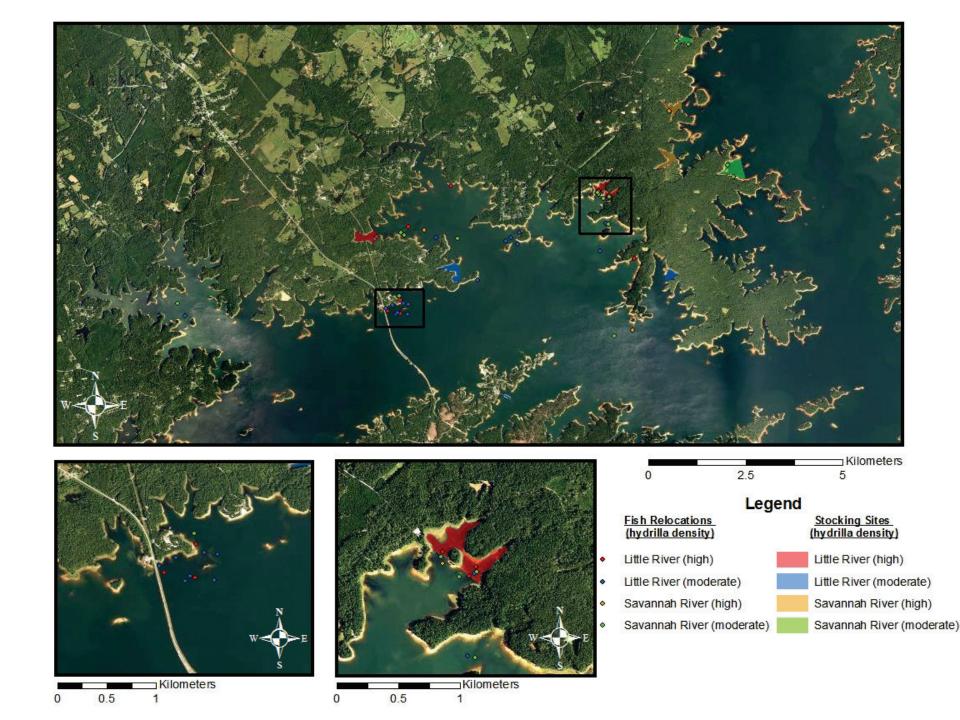
Triploid Grass Carp Susceptibility and Potential for Disease Transfer when used to Control Aquatic Vegetation in Reservoirs with Avian Vacuolar Myelinopathy

Rebecca S. Haynie , William W. Bowerman , Sarah K. Williams , John R. Morrison , John M. Grizzle , John M. Fischer & Susan B. Wilde

- First AVM study with grass carp (field and lab trials)
- No mortality or abnormal behavior found in treatment or control fish in laboratory study

Exposure type	n	Fish with lesions	
Lab Treatment	20	11	
Lab Control	20	0	
Field	23	7	

Chickens consuming grass carp did not develop AVM lesions.



Evaluating Behavioral and Physiological Responses of Triploid Grass Carp Consuming Hydrilla/Aetokthonos hydrillicola

Austin Haney & Paul Braun

Dr. Susan Wilde, Dr. Robert Bringolf, & Dr. Alvin Camus

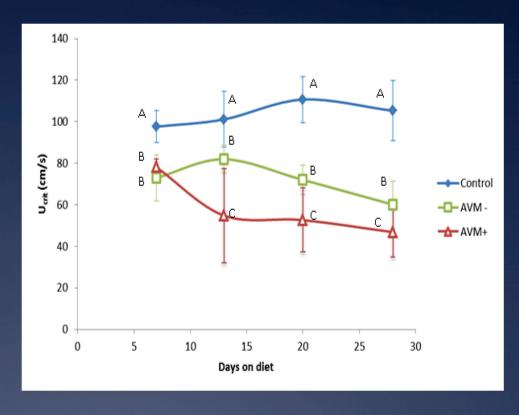






Swimming Performance Results

- AVM (+) significantly differ after 14 days
- 45% decline in swim
 performance of toxic group
 compared to control



Consistent histology, Vacuolar myelinopathy

Grass Carp VM Lesions



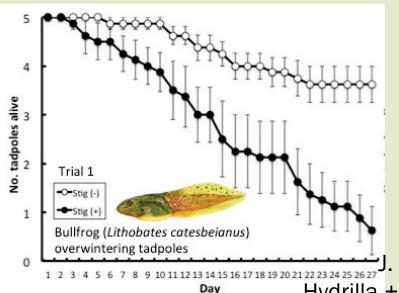


J. Strom Thurmond Hydrilla + Aetokthonos hydrillicola

Lake Wylie Hydrilla

Consistent dose response mortality

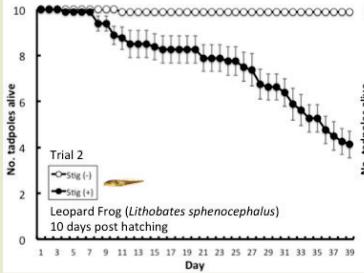


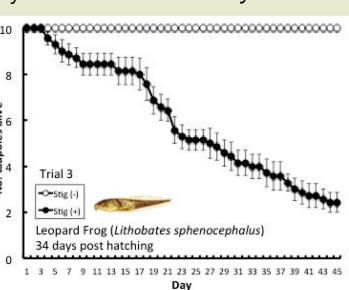


Lake Wylie Hydrilla

J. Strom Thurmond
Hydrilla + Aetokthonos hydrillicola

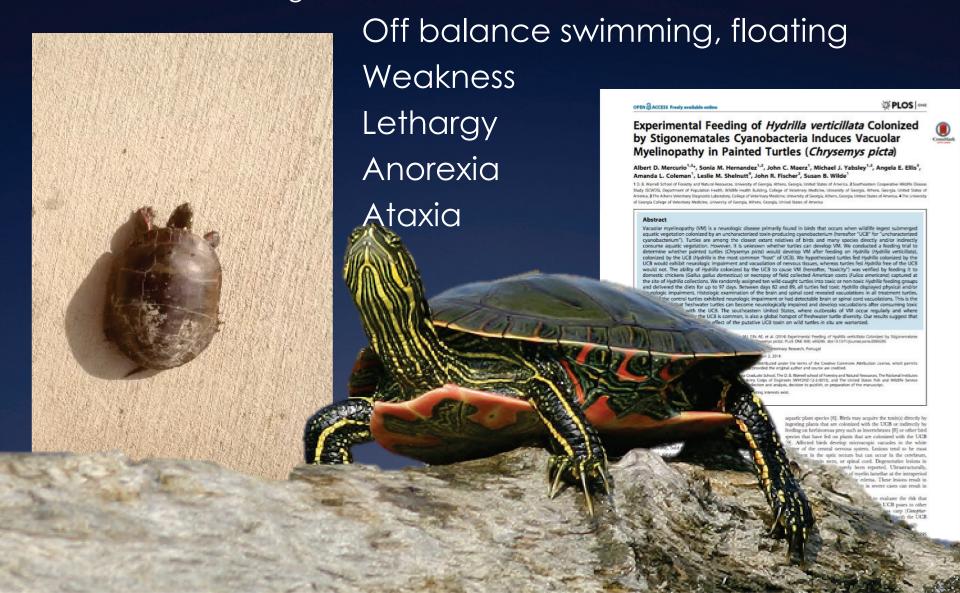






Consistent clinical impairment

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



Expanding submerged aquatic plant hosts





Florida Snail Kite Rostrhamus sociabilis

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









Potentially toxic cyanobacterial colonies grow on hydrilla and other aquatic plants



Apple snails readily consume hydrilla and other aquatic vegetation

Native and invasive apple snails are consumed by Snail Kites

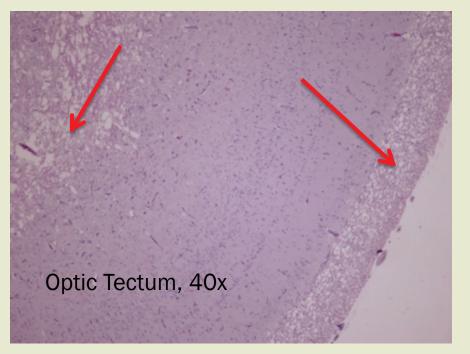
Apple Snail Feeding Trial

(snails fed hydrilla from Thurmond)

3/5 birds displayed clinical signs

Treatment

Severe lesions (5/5)

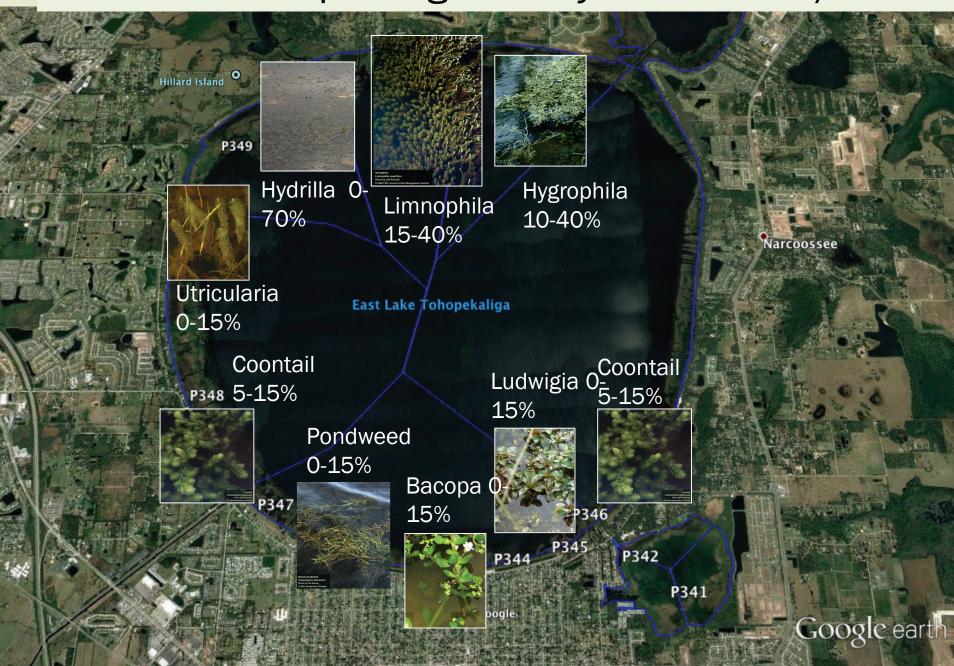


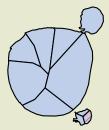
Control

Normal (5/5)



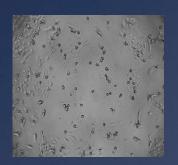
East Lake Tohopekaliga % A.hydrillicola 9/9 sites





Toxicity screening methods

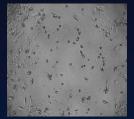
- * Aquatic vegetation collected from AVM and non-AVM locations
- * Screened for *A. hydrillicola* cyanobacterial colonies
- * Toxin extracted with methanol
- * Partitioned into chloroform
- * Bioassays
 - * Avian Chicken (*Gallus gallus*)
 - * In vitro C6 rat glioma cells
 - * Invertebrate Ceriodaphnia dubia







Comparing in vitro and invertebrate, and avian bioassays





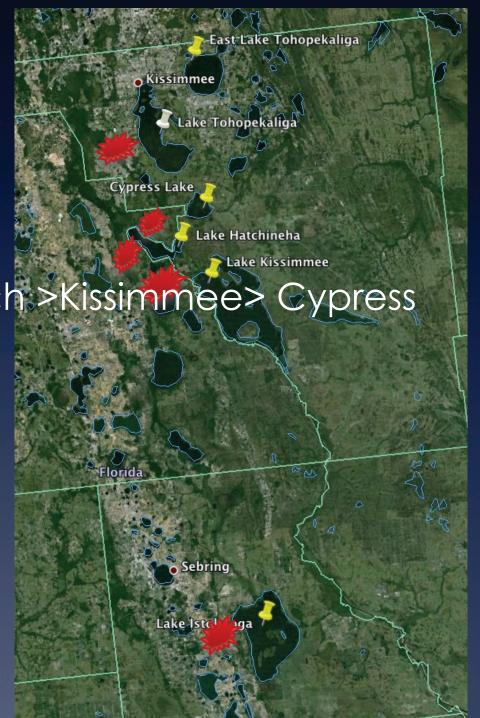


)	
	Cell line Bioassay	<i>C. dubia</i> bioassay	Avian bioassay
Hydrilla + <i>A. hydrillicola</i> JST Nov2015	Positive +++	Positive +++ Mortality +++ Reproduction	Positive +++
Hydrilla + <i>A. hydrillicola</i> JST Nov 2014	Positive +++	Positive ++ Mortality ++ Reproduction	Positive ++
Lake Wylie Hydrilla Control	Negative	Negative	Negative
Lake Seminole Hydrilla Control	Negative	Negative	Negative
A. hydrillicola cells removed from JST hydrilla	+++	+++	Insufficient material for testing
Apple snail tissues (P. insularum) fed JST hydrilla	++	++	+++
Chloroform fraction JST Hydrilla + A. hydrillicola	++	++	++

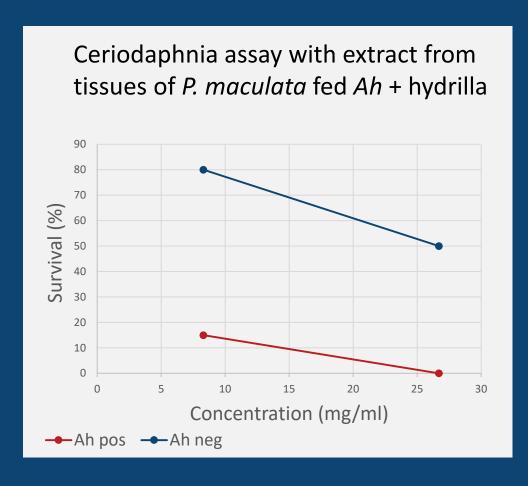
Relative toxicity

March 2016
Cell line bioassay
Toho > Istokpoga > Hatch > Kissimmee > Cypress

March 2017
Daphnia
Toho, East Lake Toho
& Istokpoga



In vivo bioassays show promise for AVM toxicity studies



Behavioral response of larval zebrafish (*D. rerio*) to *Ah* + hydrilla extract

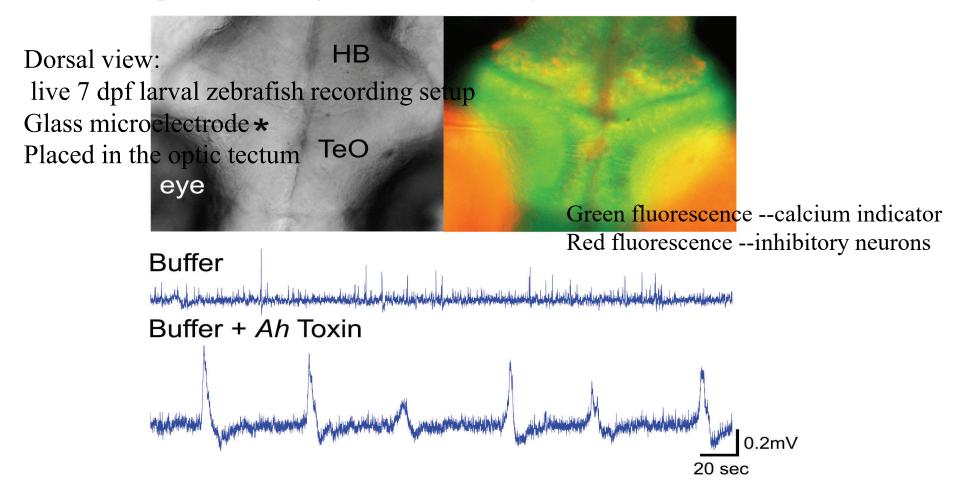
Ah neg - swimming movements



Ah pos – swimming movements



Ah-toxin exposure changes neural activity in the larval zebrafish brain.



Field recording from zebrafish larvae exposed:

Top: (normal) Buffer

Bottom: (seizure-like activity) *Ah*-positive *Hydrilla* extract containing active toxin

Amphibian and Reptile Vulnerability to *Hydrilla* Invasions and the Spread of *Aetokthonos hydrillicola*



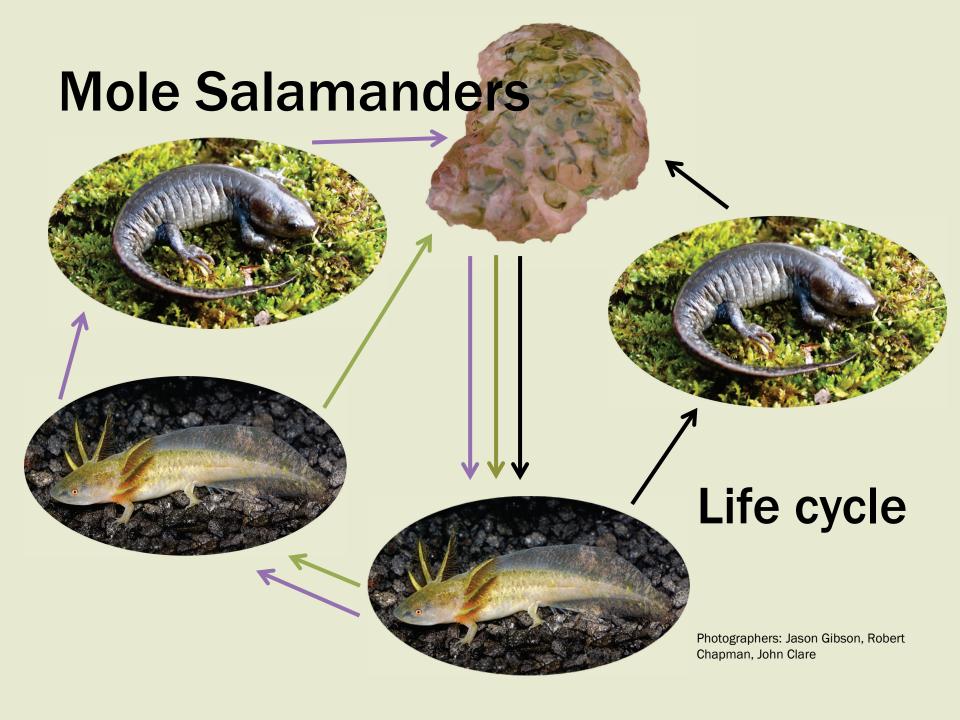
Trophic Transfer of Aetokthonos hydrillicola in Mole Salamanders (Ambystoma talpoideum)

Hunter Smith
John Maerz and Susan Wilde



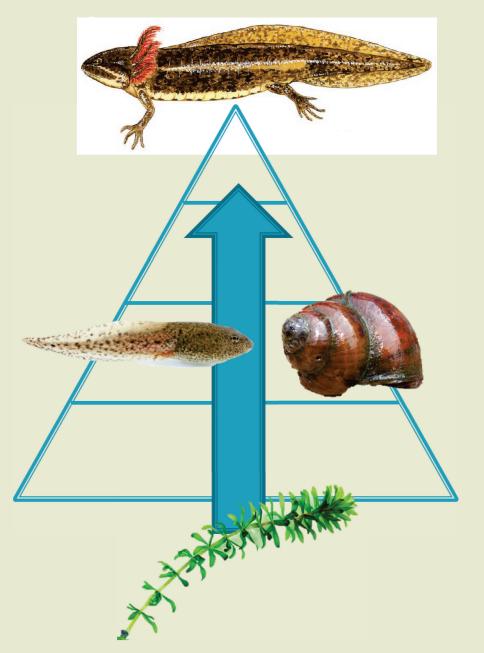






Objectives

 Determine whether paedomorphic mole salamanders are affected by Ah by consumption of an Ah+ gut-loaded intermediate food source.

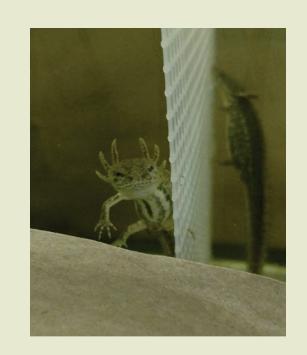


Gut-loading Prey

- Gut-loaded both snails and tadpoles
- Prey were fed hydrilla that had either Ah+ or were Ah-
- Every prey fed on hydrilla for at least 24 hours
- 15g of hydrilla were added everyday

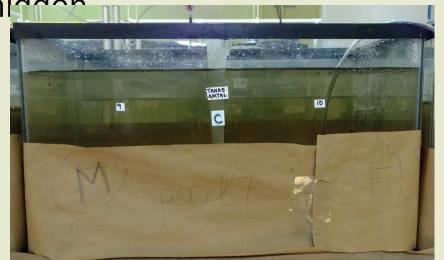
Monitoring

- Before feeding we noted:
 - Number of prey items left in tank:
 alive or dead



Behavior: listing, active, hidden

 If they were listing, we stimulated with touch to notate response



Neurologic Tests

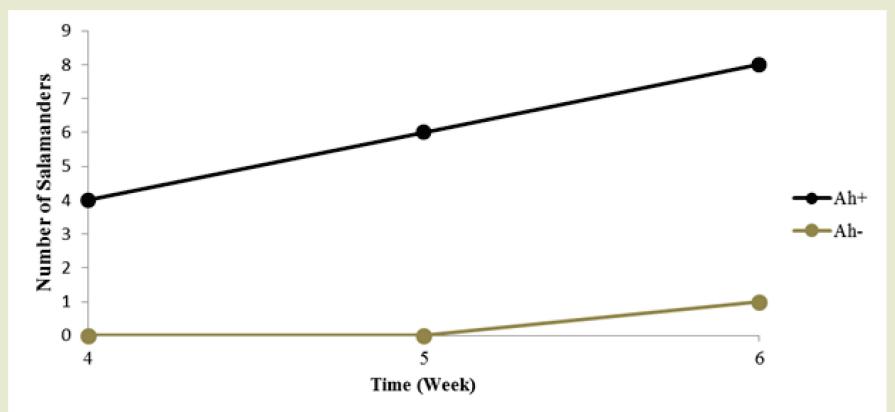
- Daily Stimulation Responses
 - Touch with a straw to gauge response type (if listing):
 - delayed swim, immediate swim, aggressive, none





- Righting Response Test
 - Flip over and time how long it took to correct

Righting Response Results



Graph 2. The cumulative salamanders that experienced a not normal righting response time.

Righting Response Results





Other signs noticed:

- Seizures
- Total paralysis
- Partial paralysis
- Head twitching
- Muscle atrophy

Other Neurologic Signs Noticed







- Listing
- Tail curling
- Inability to balance
- Inability to hold head up
- Curving body in one direction and apparent inability to straighten out



EVALUATING SENSITIVITY OF NORTHERN AND BANDED WATERSNAKES TO AETOKTHONOS HYDRILLICOLA EXPOSURE THROUGH EXPERIMENTAL TROPHIC TRANSFER STUDY



Audrey Vaughn

John Maerz, Susan Wilde, and Heather Fenton

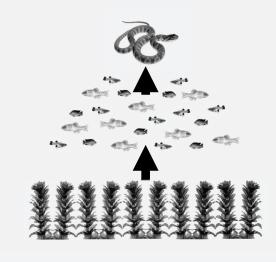
INTRODUCTION:

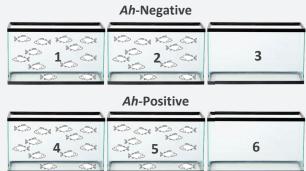
NORTHERN & BANDED WATERSNAKES

- Explanation for use in study
 - Likelihood of exposure to Ah
 - Habitat
 - Diet
 - Likelihood of susceptibility
 - Trophic status
 - Bioaccumulation
 - Other reasons
 - Lab setting









METHODS:

FEEDING EXPERIMENT

- Control vs. treatment groups
- Prey species
 - Gut-loading
 - Hydrilla preparation process
 - Rotation-based schedule
- Feeding snakes

BEHAVIORAL RESPONSES AH+ VS AH-

- Reduced Appetite
- Slow Righting response
- Reduced Tongue-flicking
 - Latency in response to alcohol cue





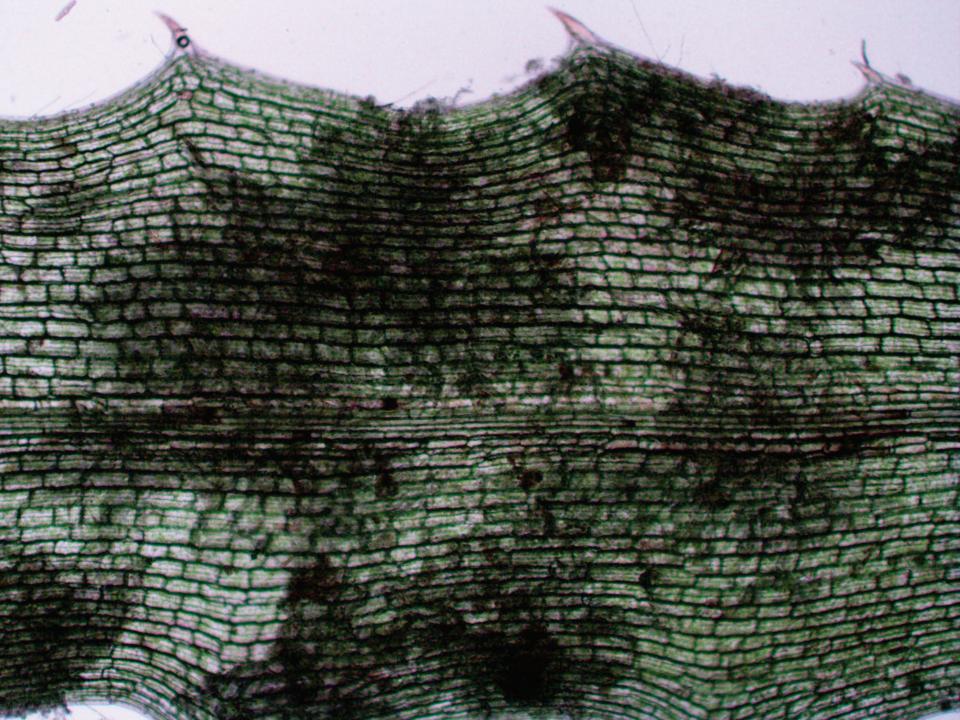
We know:

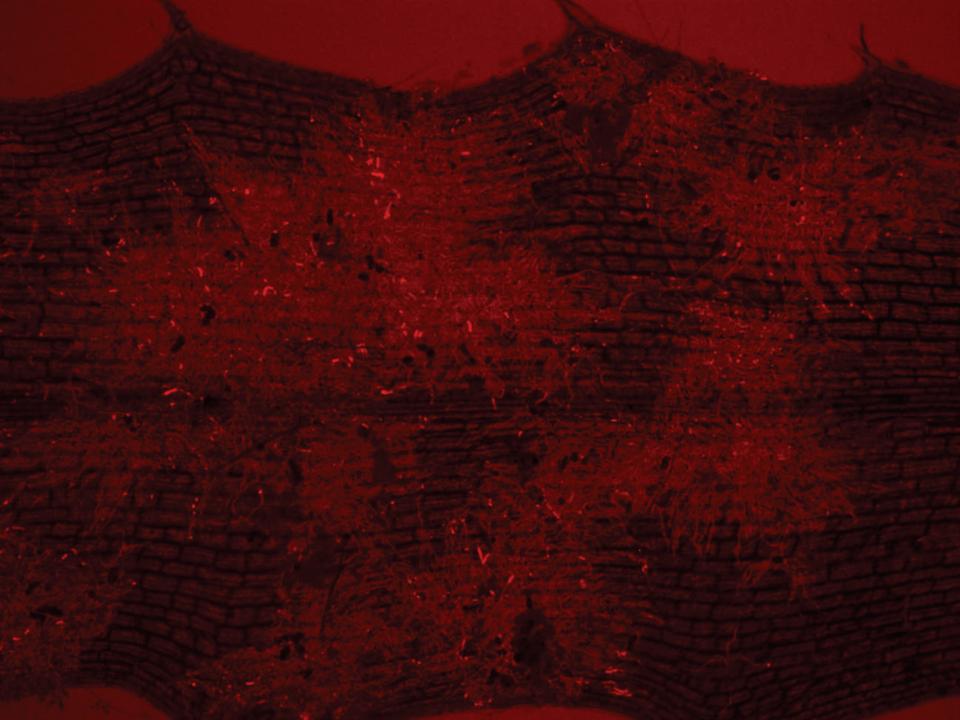
- 1. Newly described cyanobacteria, *Aetokthonos hydrillicola* (*Ah*), cyanobacteria forms colonies *Hydrilla verticillata*, an expanding invasive in the United States.
- 2. Consumption of impaired/dead waterbirds eating Hydrilla/Ah is a threat to Bald eagles
- 3. Food web toxin transfer causes impairment/mortality freshwater turtles, tadpoles, snakes, and fish.

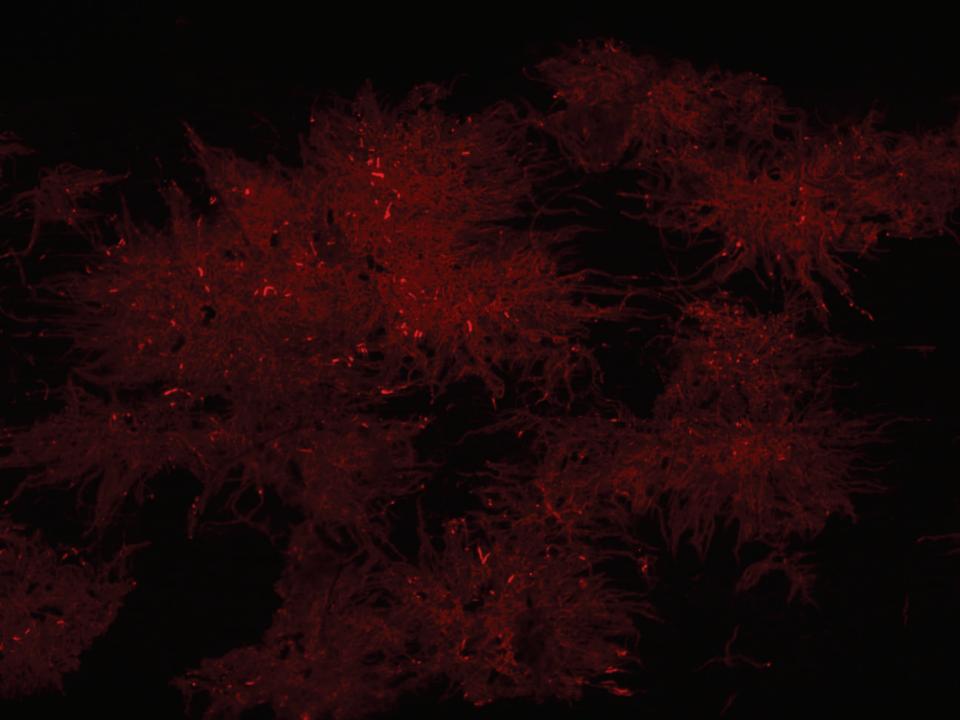
But to improve risk models to guide management, we need to know:

- 1. Toxin(s) produced by Ah cyanobacteria (polar & non-polar compounds)
- 2. Origin of Ah (monoecious vs. dioecious?)
- 3. Current geographical distribution (climate change shifts)
- 4. Environmental factors inducing toxin-production (geology, water quality, temperature?)









Acknowledgements





Wilde Lab UGA

Rebecca Haynie James Herrin **Shelley Dodd** Jamie Morgan Jenny Garrison **Brad Bartelme Brigette Haram Garon Brandon** Wallace Woods Tabby Phillips Wes Gerrin Melissa Martin



Gulf & South Atlantic Regional Panel On Aquatic Invasive Species



Telemetry study of AVM in bald eagles

