Eagle Killer, Living on Hydrilla: Investigating the Combined Risk of Invasive Submerged Plants & Toxic Epiphytic Cyanobacteria

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Overview

- Background on AVM
- Food Chain Transfer Experiments
- Expanding locations and taxa affected
- Toxin Investigations
- Management

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

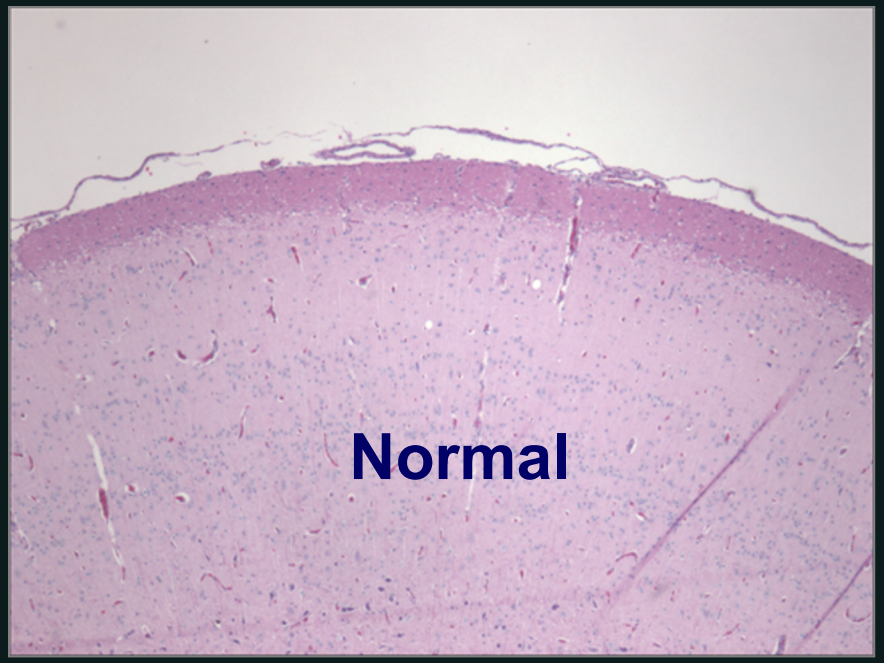
Neurological impairment

- Eagle with drooping wings

- Unresponsive coot

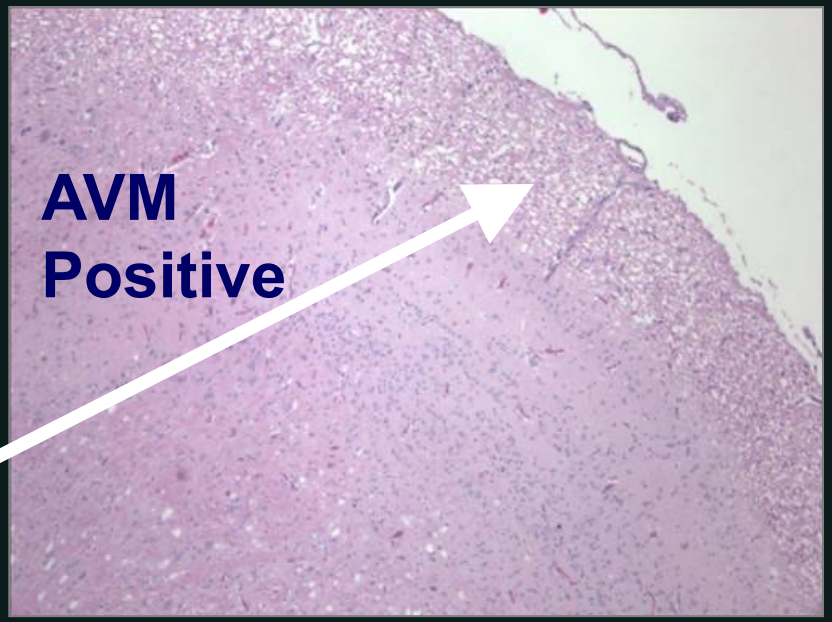
- Waterfowl and eagles wobble in flight
- Birds observed stumbling on land
- Coots may dive and then not right themselves
- Eagles may overshoot perches or fly into objects

Diagnosis: Unique brain lesions



National Wildlife Health Center

Southeastern Cooperative Wildlife Disease Study



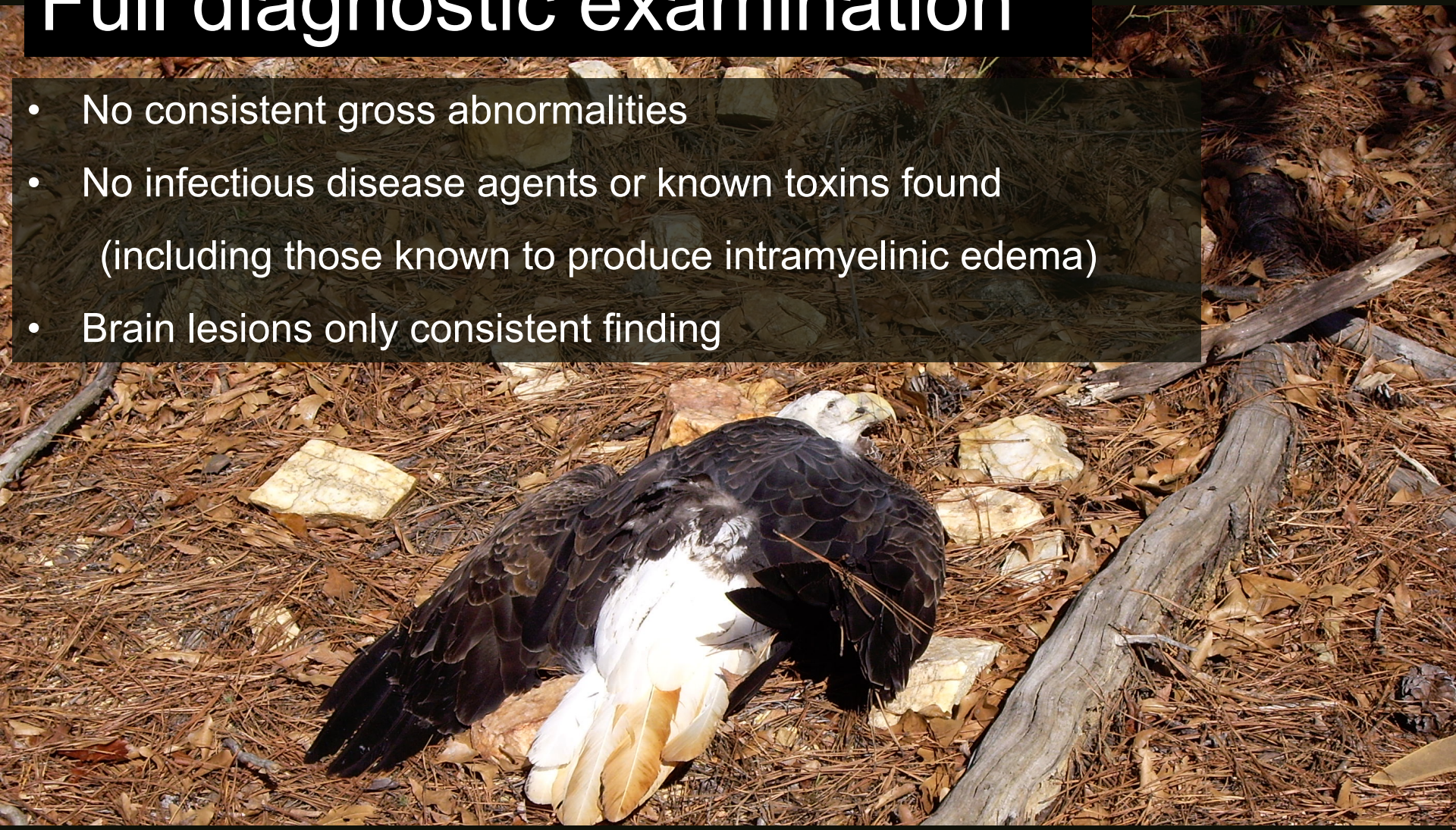
Open spaces in:
white matter of the central nervous system, specifically an intramyelinic edema

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 americana). Veterinary Pathology 35:479-487

Full diagnostic examination

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

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



Thomas, NJ, et al, 1998.

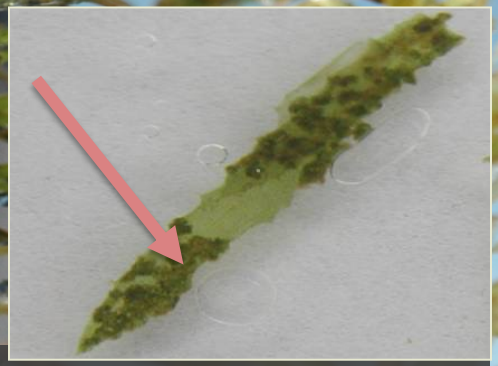
Dodder, NG, B Strandberg, T Augspurger, and RA Hites. 2003. Lipophilic organic compounds in lake sediment and American coot (Fulica americana) tissues, both affected and unaffected by avian vacuolar myelinopathy. Science Total Environment 311:81-89.

Reservoir surveys: 2001-present

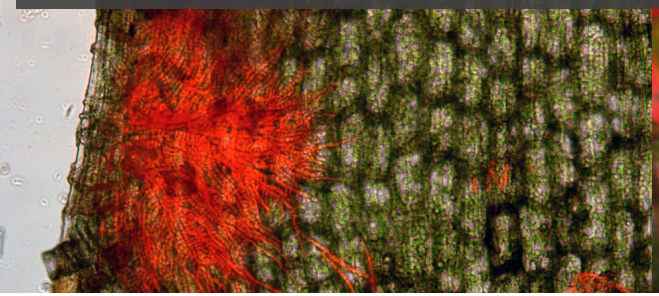
10 AVM Sites

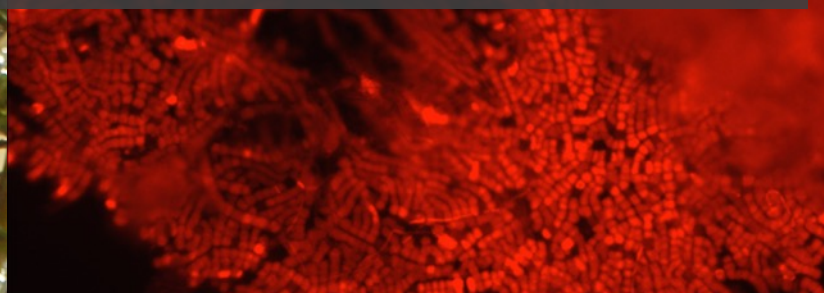
- Man-made ponds/reservoirs
- Nutrients low to moderate
- No harmful algal blooms in the water
- Dense non-native aquatic plants

Novel cyanobacterial species growing on invasive aquatic plants all AVM sites



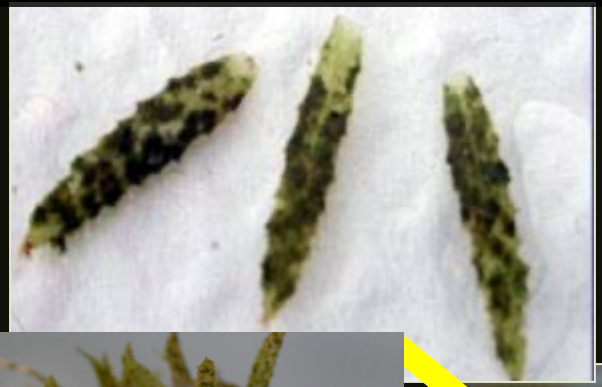
- Previously undescribed cyanobacterial species
- Cyanobacteria (or blue-green algae) are photosynthetic bacterial species that can produce liver and nerve toxins
- Grows as an epiphyte on hydrilla and other invasive exotic aquatic plants in all AVM sites



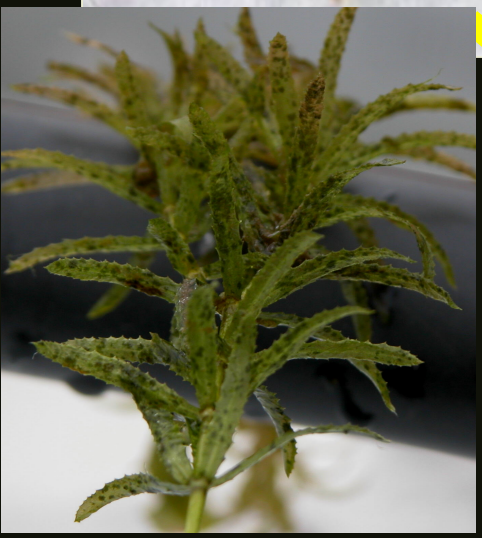


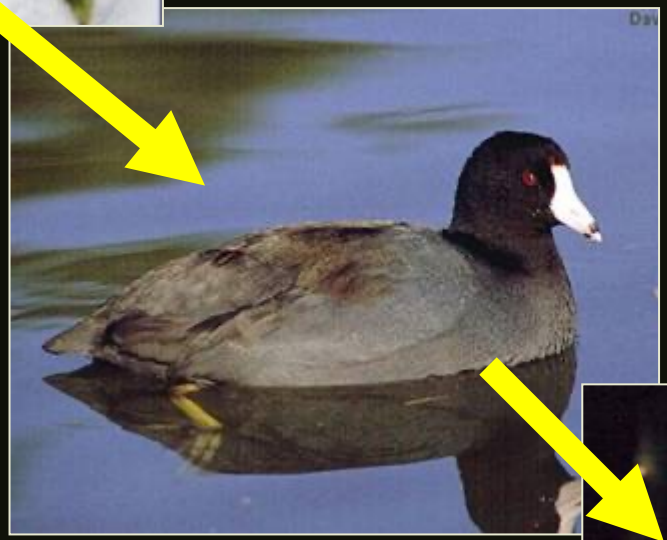
Wilde, S. B., T. M. Murphy, C. P. Hope, S. K. Habrun, J. Kempton, A. Birrenkott, F. Wiley, W. W. Bowerman, and A. J. Lewitus. 2005. Avian vacuolar myelinopathy (AVM) linked to exotic aquatic plants and a novel cyanobacterial species. Environmental Toxicology 20:348-353.

Food Chain Transfer Hypothesis



Potentially toxic cyanobacterial colonies on hydrilla and other aquatic plants in AVM sites

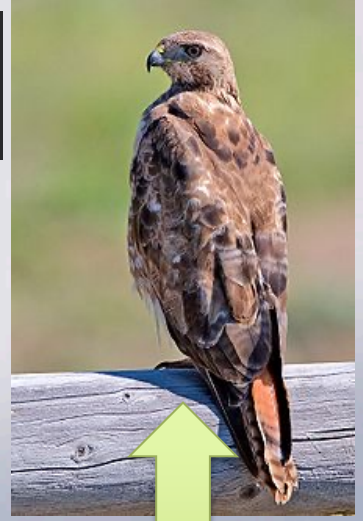




Aquatic plants and epiphytic algae are primary food source for coots

Sick waterfowl are consumed by Bald Eagles

Food Chain Transfer, Part I



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

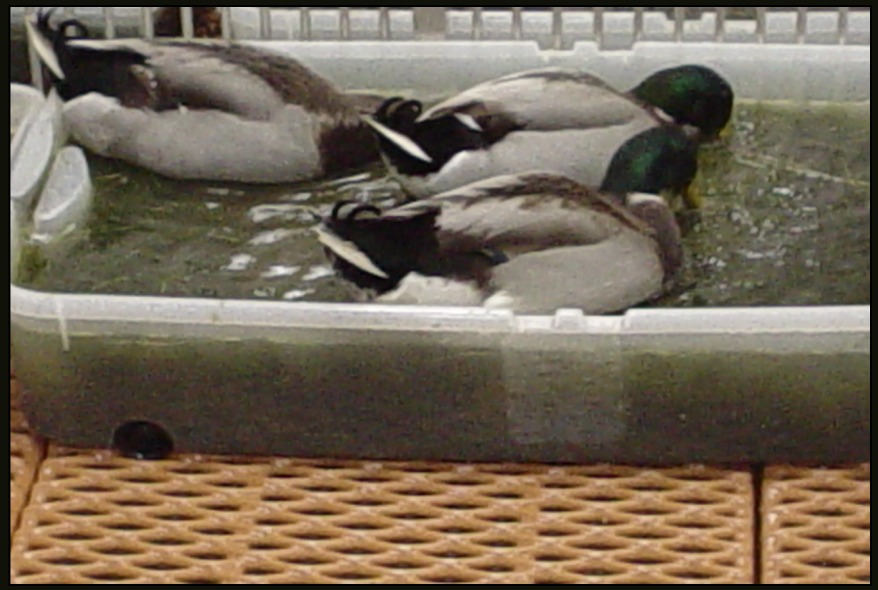
Fischer, J, LA Lewis-Weis, and CM Tate. 2003. Experimental vacuolar myelinopathy in red-tailed hawks. Journal of Wildlife Diseases 39:400-406.

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

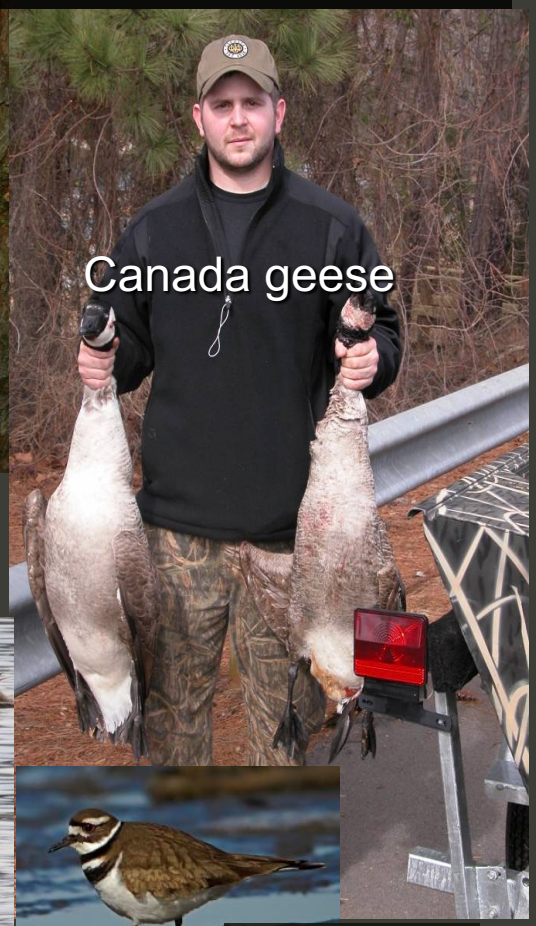


Birrenkott, AH, SB Wilde, JJ Hains, JR Fischer, TM Murphy, CP Hope, PG Parnell, and WW Bowerman. 2004. Establishing a food-chain linkage between aquatic plant material and Avian Vacuolar Myelinopathy in mallard ducks (Anas platyrhynchos). Journal of Wildlife Diseases 40:435-492

Sentinel Mallard: Untreated Reservoir Hydrilla/*Ah*

Bird species with AVM brain lesions

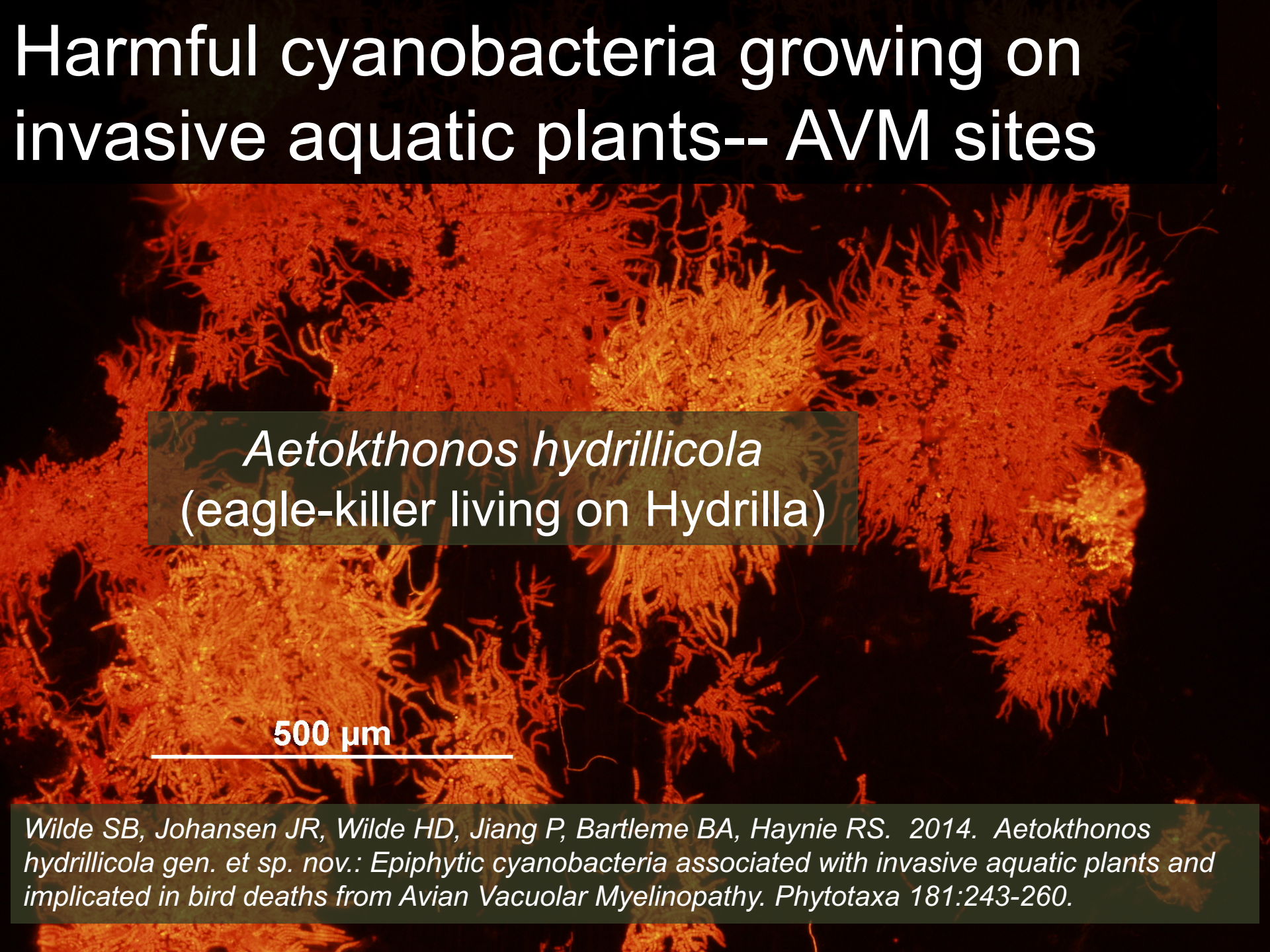
Mallards, Ring-necked ducks
Buffleheads, American wigeon



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

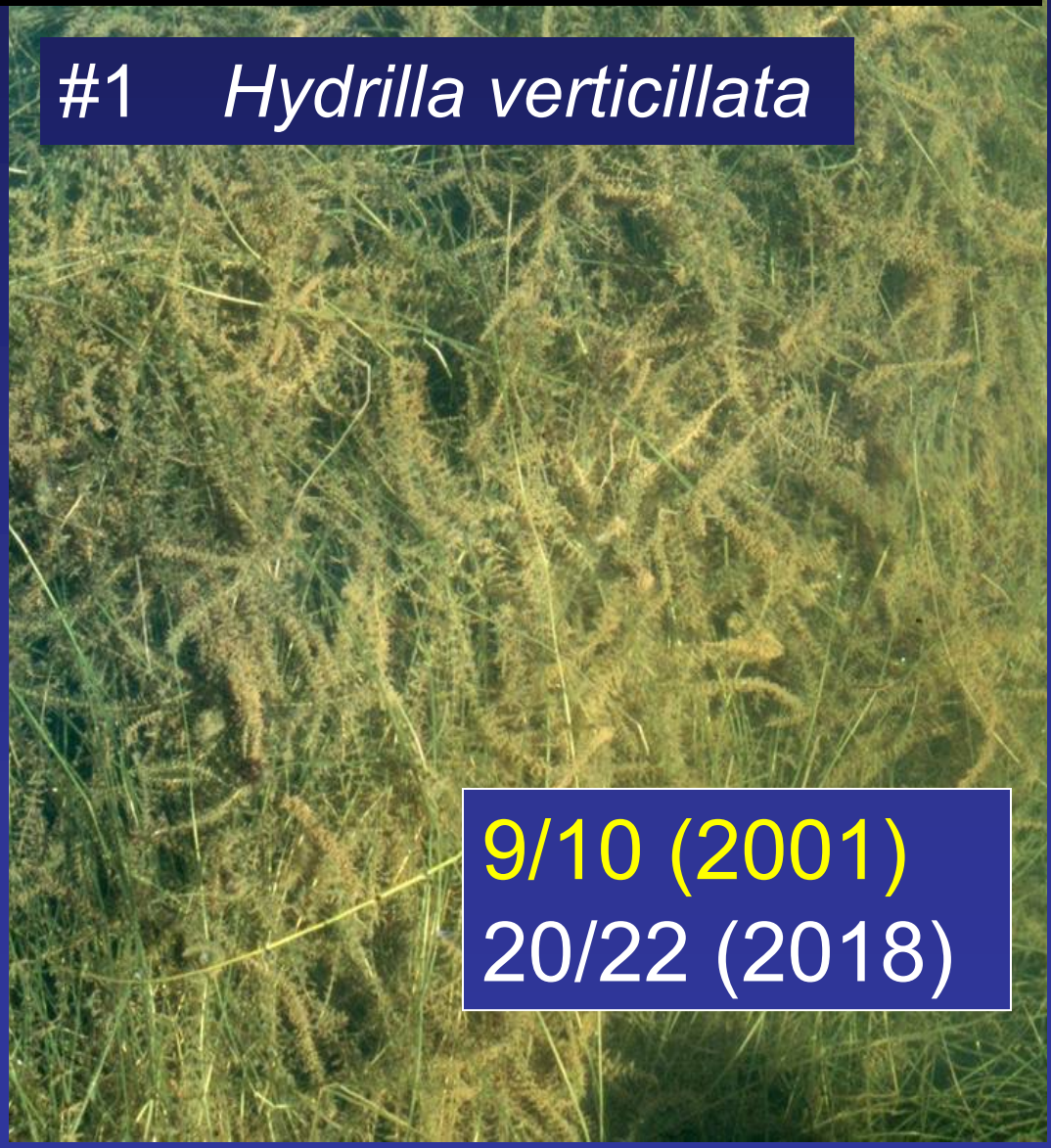
Harmful cyanobacteria growing on invasive aquatic plants-- AVM sites



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.

Submerged non-native aquatic plants

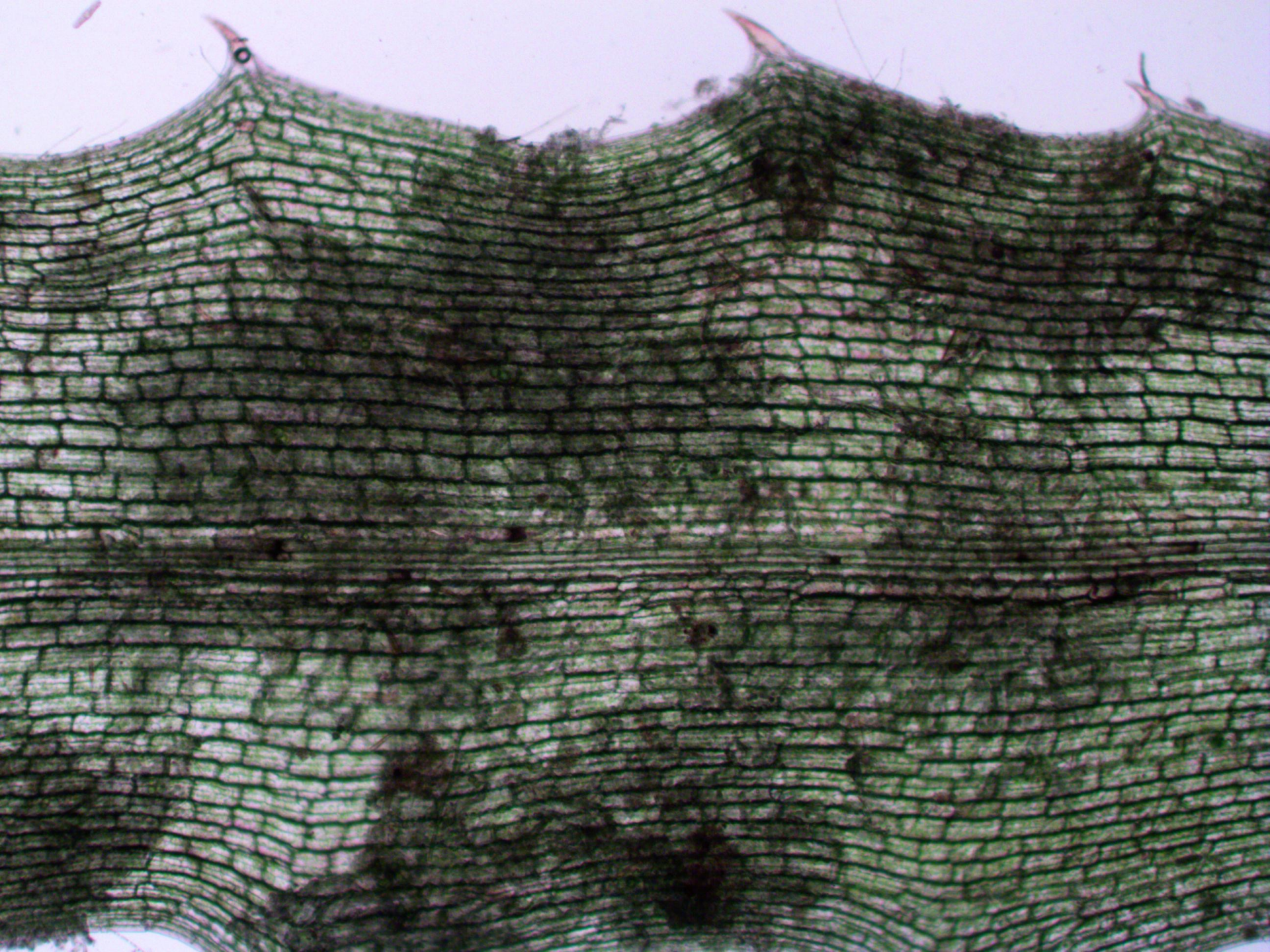
1994	DeGray Lake, AR
1996	Quachita, AR
1998	Woodlake, NC
1998	Thurmond, SC/GA
1998	Lake Juliette, GA
1998	Par Pond, SC
1998	L Lake, SC
1999	Lake Murray, SC
1999	Sam Rayburn, TX
2003	Davis Pond, SC
2003	Emerald Lake, GA
2005	Lake Horton, GA
2005	Smith Reservoir, GA
2005	Coachmans Trail, NC
2007	Lake Varner, GA
2010	Upper Towaliga, GA
2011	Longbranch, GA
2012	Lake Tohopekaliga, FL
2013	Lake Istokpoga, FL
2016	Kerr Reservoir, NC/VA
2018	Lake Tussehaw, GA
2020	Phillpott Reservoir, VA

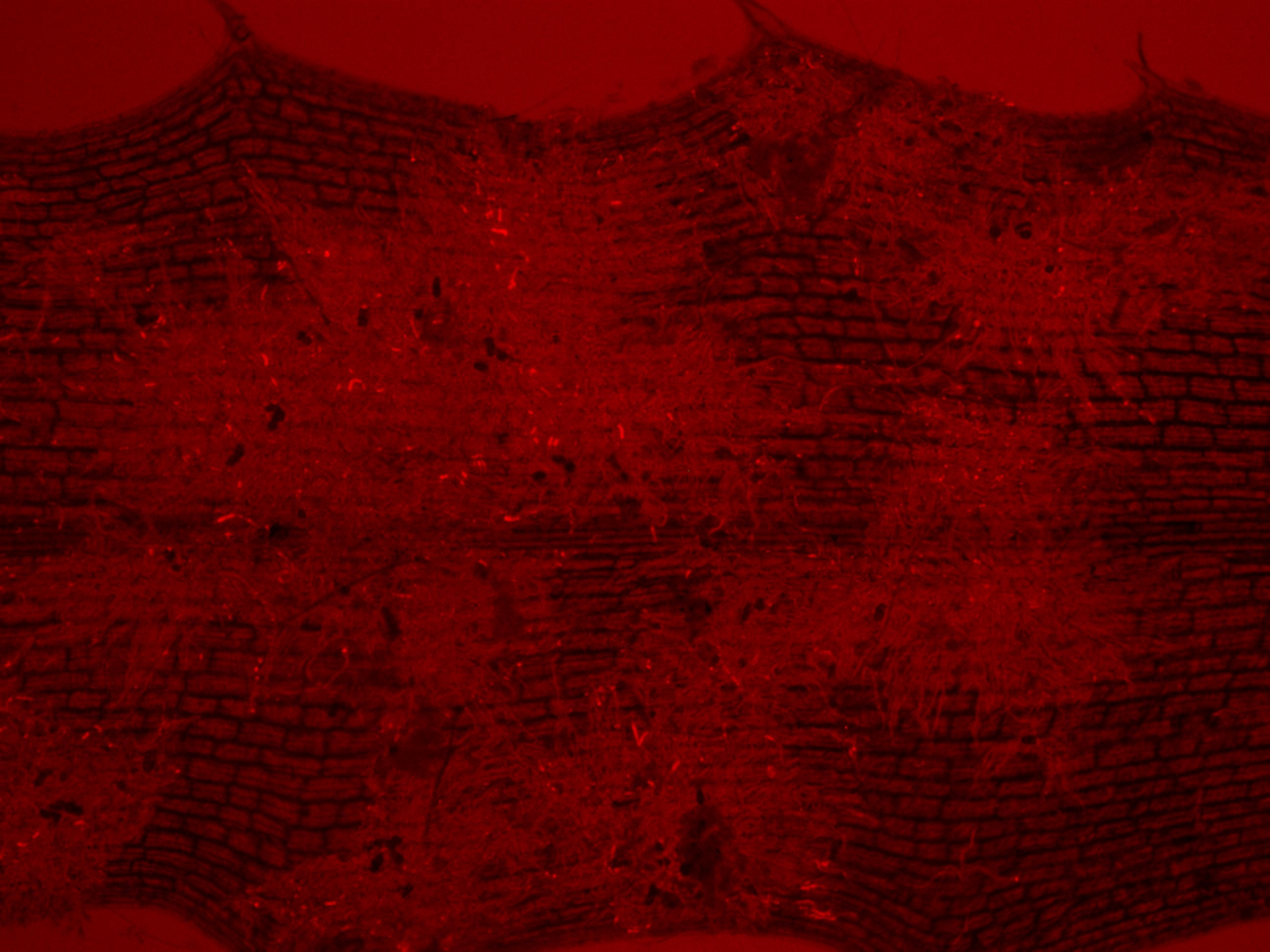


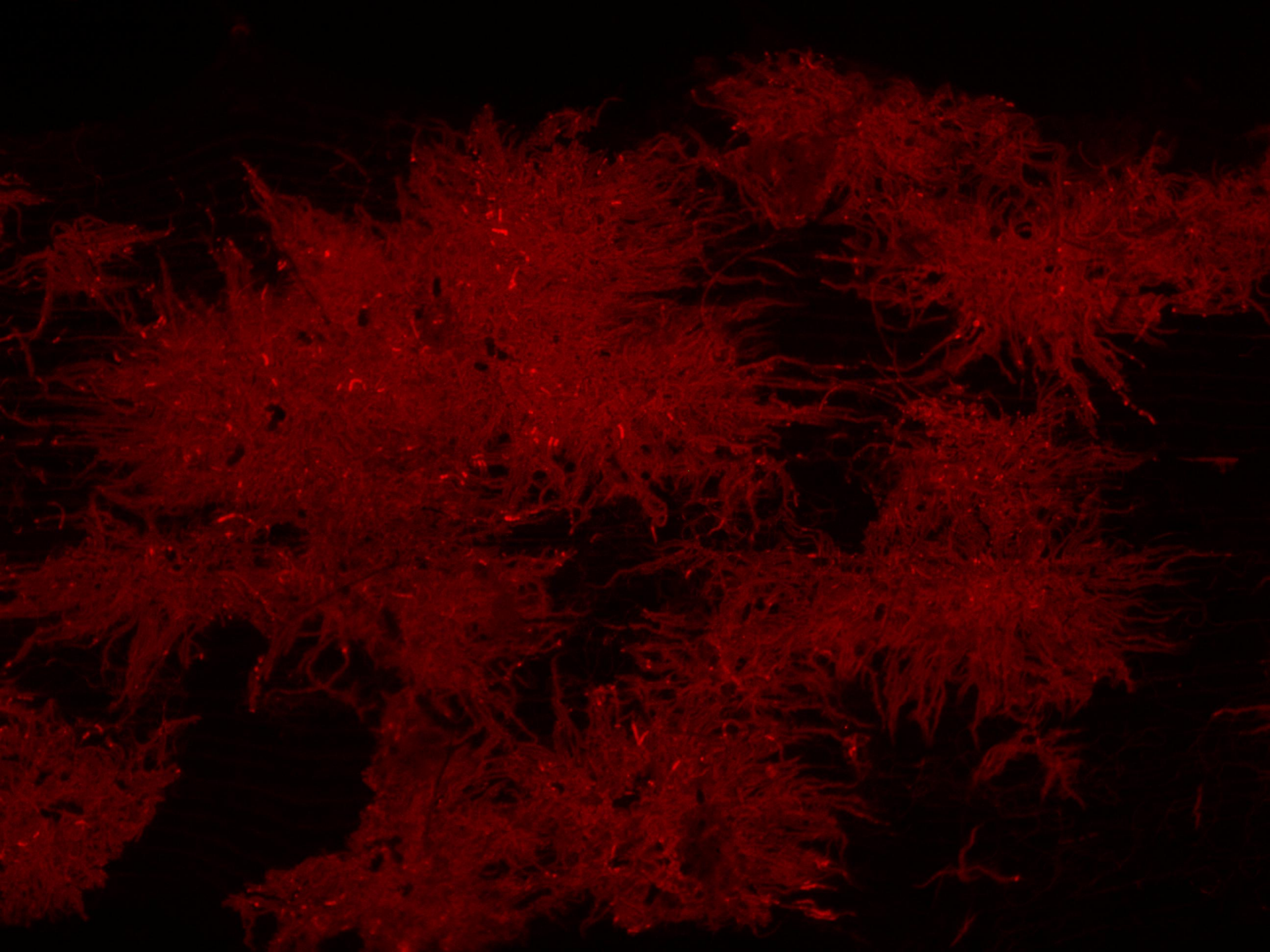
1997 Hamilton, AR
1998 SRS- L Lake, SC
1998 SRS-Par Pond, SC
1998 Lake Juliette, GA

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

EXPANDING LOCATIONS AND TAXA AFFECTED

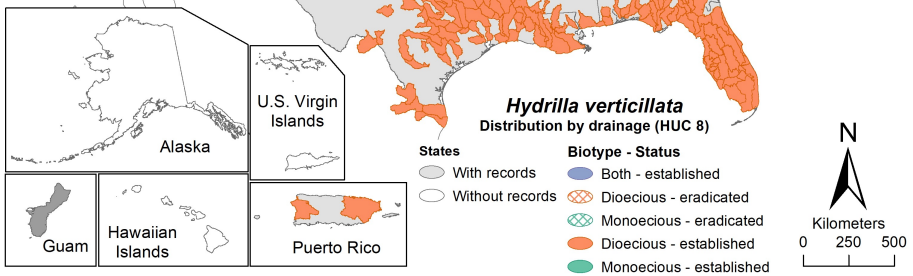






EXPANDING LOCATIONS AND TAXA AFFECTED

Hydrilla distribution



Hydrilla verticillata
Distribution by drainage (HUC 8)

States
- With records
- Without records

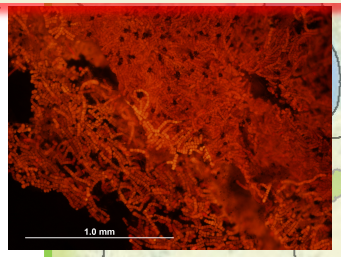
Biotype - Status
- Both - established
- Dioecious - eradicated
- Monoecious - eradicated
- Dioecious - established
- Monoecious - established
- Unknown - established

N

Kilometers
0 250 500

USGS, Nonindigenous Aquatic Species Database (nas.er.usgs.gov). February 2020

A. hydrillicola Distribution



N

Kilometers
0 125 250

- Vacuolar Myelinopathy
- *A. hydrillicola* confirmed
- *A. hydrillicola* not observed
- Not sampled

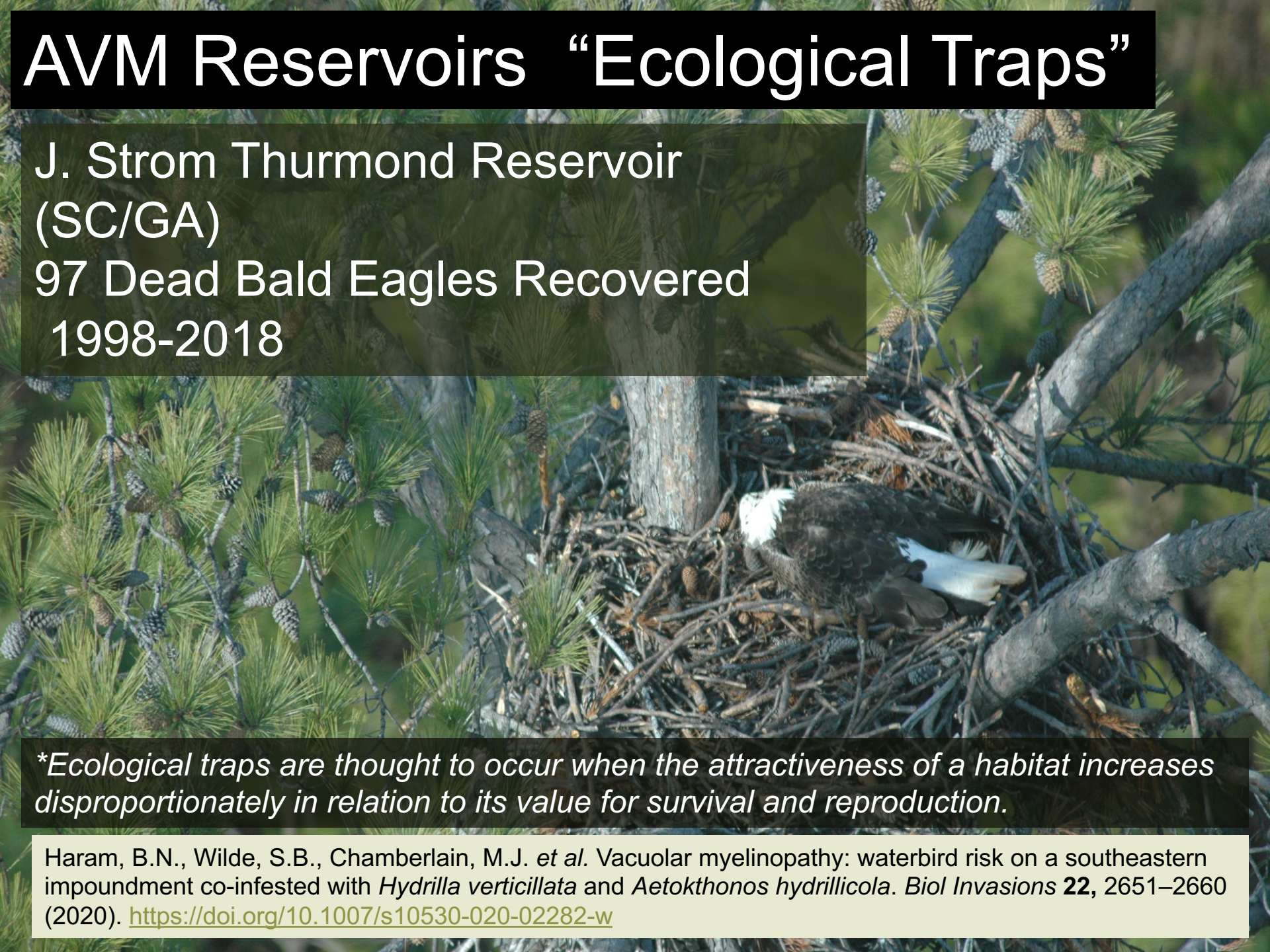
September 2019

Leslie J. Mehrhoff, Univ. of Connecticut, Bugwood.org

Robert Vidéki, Doronicum Kft., Bugwood.org

AVM Reservoirs "Ecological Traps"

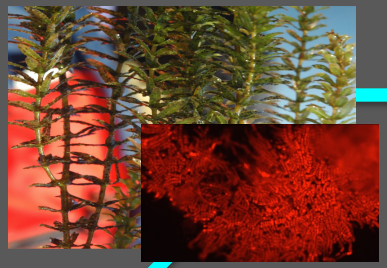
J. Strom Thurmond Reservoir (SC/GA)
97 Dead Bald Eagles Recovered
1998-2018



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

Haram, B.N., Wilde, S.B., Chamberlain, M.J. *et al.* Vacuolar myelinopathy: waterbird risk on a southeastern impoundment co-infested with *Hydrilla verticillata* and *Aetokthonos hydrillicola*. *Biol Invasions* **22,** 2651–2660 (2020). https://doi.org/10.1007/s10530-020-02282-w

Expanding Food Chain



Fish

Salamanders

Frogs

Mammals

Turtles

Alligators

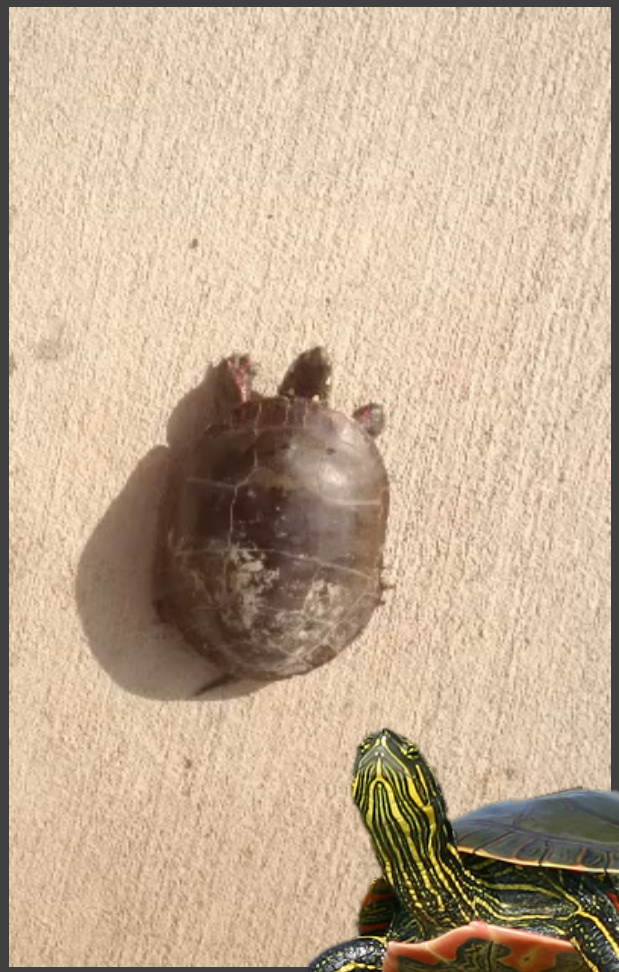
Birds

Snakes

Diagram provided by
Dr. John C. Maerz

Reptiles- Painted Turtles

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



Off balance swimming, floating

Weakness

Lethargy

Anorexia

Ataxia

OPEN ACCESS Freely available online

PLOS ONE

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[1,3], Angela E. Ellis[3], Amanda L. Coleman[1], Leslie M. Shelnutt[4], John R. Fischer[2], Susan B. Wilde[1]

1 D. B. Warnell School of Forestry and Natural Resources, University of Georgia, Athens, Georgia, United States of America, 2 Southeastern Cooperative Wildlife Disease Study (SCWDS), Department of Population Health, Wildlife Health Building, College of Veterinary Medicine, University of Georgia, Athens, Georgia, United States of America, 3 The Athens Veterinary Diagnostic Laboratory, College of Veterinary Medicine, University of Georgia, Athens, Georgia, United States of America, 4 The University of Georgia College of Veterinary Medicine, University of Georgia, Athens, Georgia, United States of America

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 89, 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. None of the control turtles exhibited neurologic impairment or had detectable brain or spinal cord vacuolations. This is the first evidence that freshwater turtles can become neurologically impaired and develop vacuolations after consuming toxic *Hydrilla* colonized with the UCB. The southeastern United States, where outbreaks of VM occur regularly and where vegetation colonized by the UCB is common, is also a global hotspot of freshwater turtle diversity. Our results suggest that further investigations into the effect of the putative UCB toxin on wild turtles *in situ* are warranted.

Citation: Mercurio AD, Hernandez SM, Maerz JC, Yabsley MJ, Ellis AE, et al. (2014) Experimental Feeding of *Hydrilla verticillata* Colonized by Stigonematales Cyanobacteria Induces Vacuolar Myelinopathy in Painted Turtles (*Chrysemys picta*). PLoS ONE 9(4): e93295. doi:10.1371/journal.pone.0093295

Editor: Mónica V. Cunha, INIAV, I.P.- National Institute of Agriculture and Veterinary Research, Portugal

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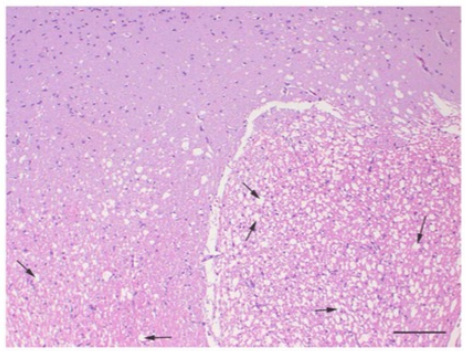


Figure 1. Histopathological slide of the optic tectum of a painted turtle fed toxic *Hydrilla* material. Painted turtle (*Chrysemys picta*), brain: Numerous clear vacuoles (black arrows) representing myelin degeneration and dilation of axonal sheaths are present in the white matter of a turtle treated with toxic hydrilla. H&E, 100X. Scale bar is 100 μm.
doi:10.1371/journal.pone.0093295.g001

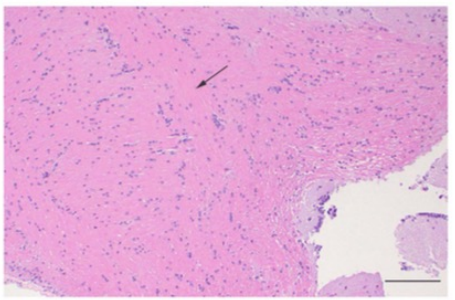
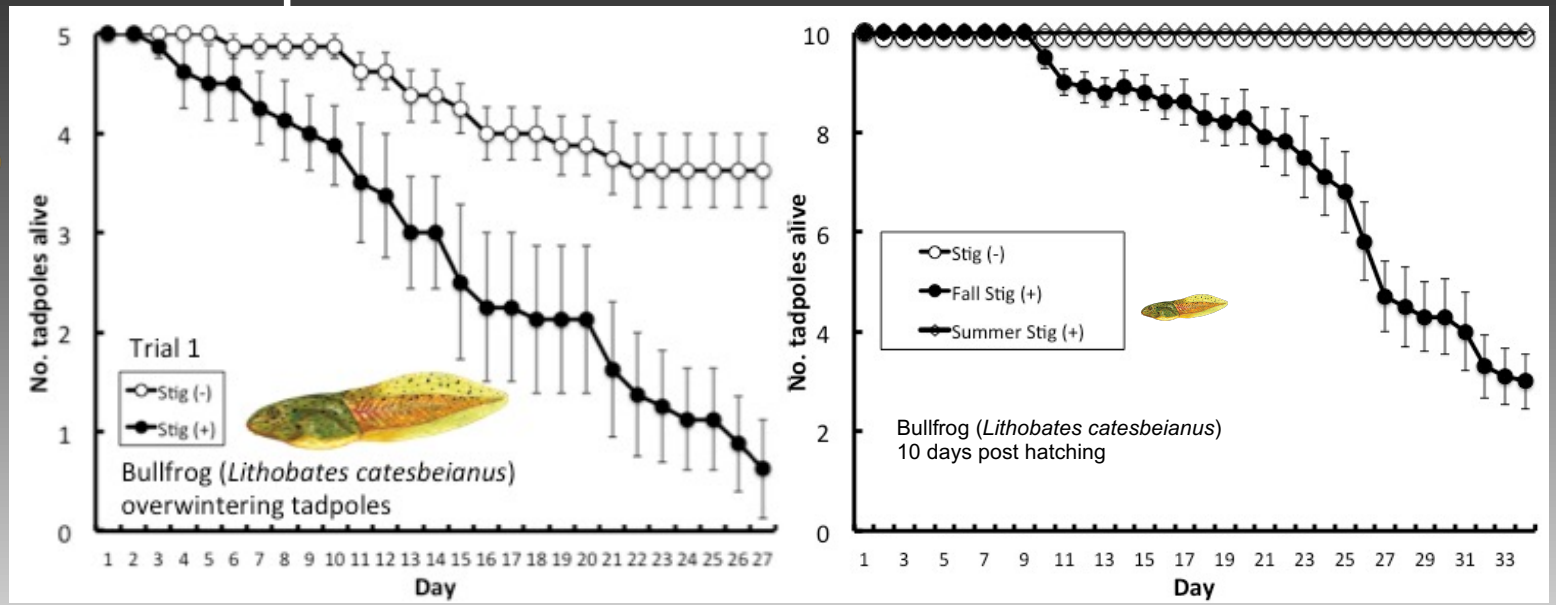


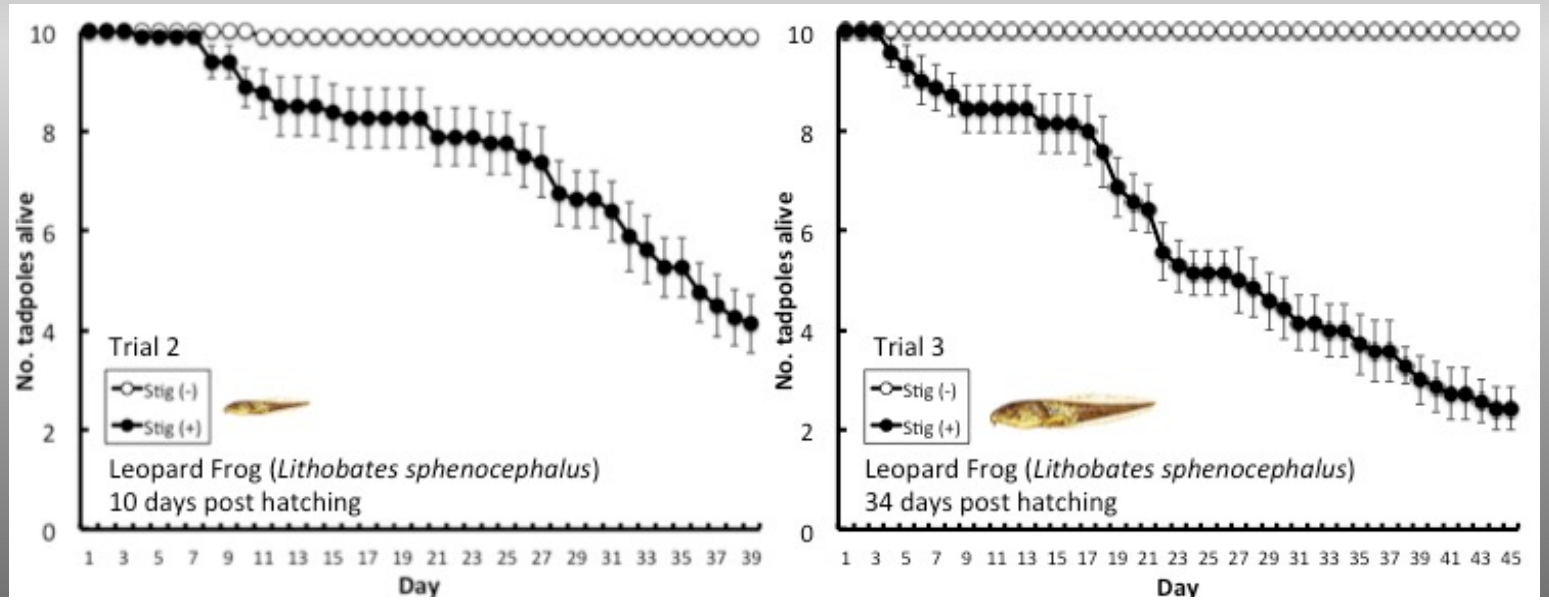
Figure 3. Histopathological slide of the optic tectum of a normal turtle. Painted turtle (*Chrysemys picta*), brain: white matter, indicated by black arrows, appears normal with no evidence of vacuolation or myelin degeneration. H&E, 100X. Scale bar is 100 μm.
doi:10.1371/journal.pone.0093295.g003

correlations could be identified. While some variation in distribution and severity was present among the cerebellar lesions, this did not appear to correlate with any differences in the clinical signs. These findings are similar to those described in birds with VM

Amphibians- tadpoles

Impairment, mortality, VM lesions





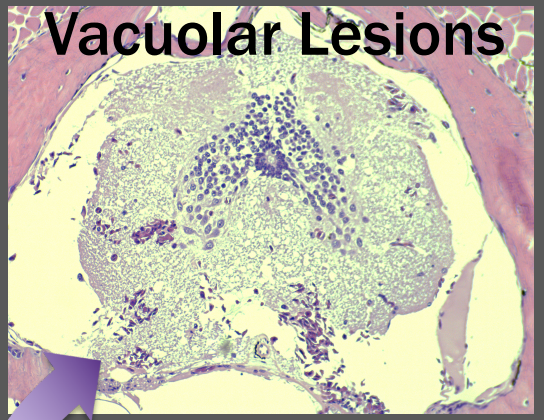
Maerz, J.C., Wilde, S.B., Terrell, V.K. *et al.* Seasonal and plant specific vulnerability of amphibian tadpoles to the invasion of a novel cyanobacteria. *Biol Invasions* **21,** 821–831 (2019).

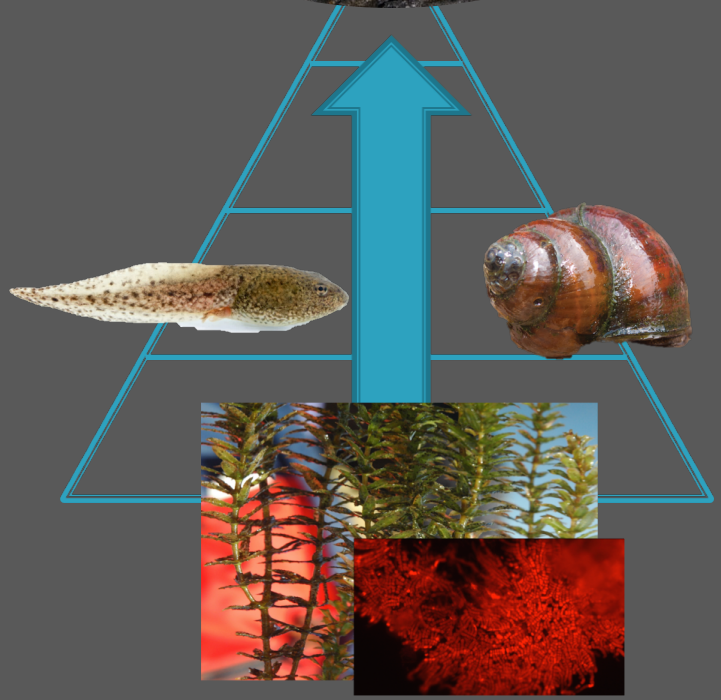
Amphibians- Salamanders

Consistent Clinical Impairment

- Seizures
- Paralysis
- Head twitching
- Muscle atrophy
- Inability to Right themselves

Mole Salamanders (*Ambystoma talpoideum*)





Reptiles: Water snakes (*Nerodia spp*)

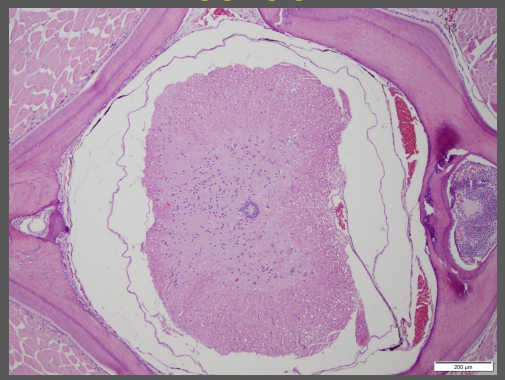
Herbivorous fish

Tilapia

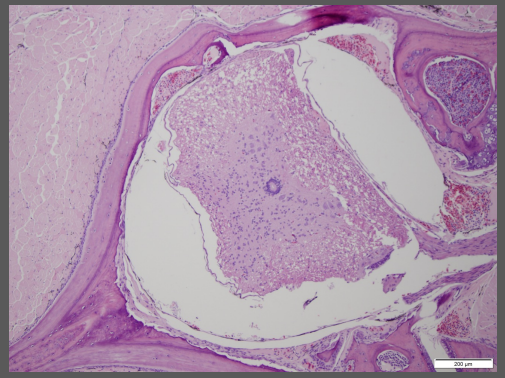
Fathead minnows

Water snakes

Control



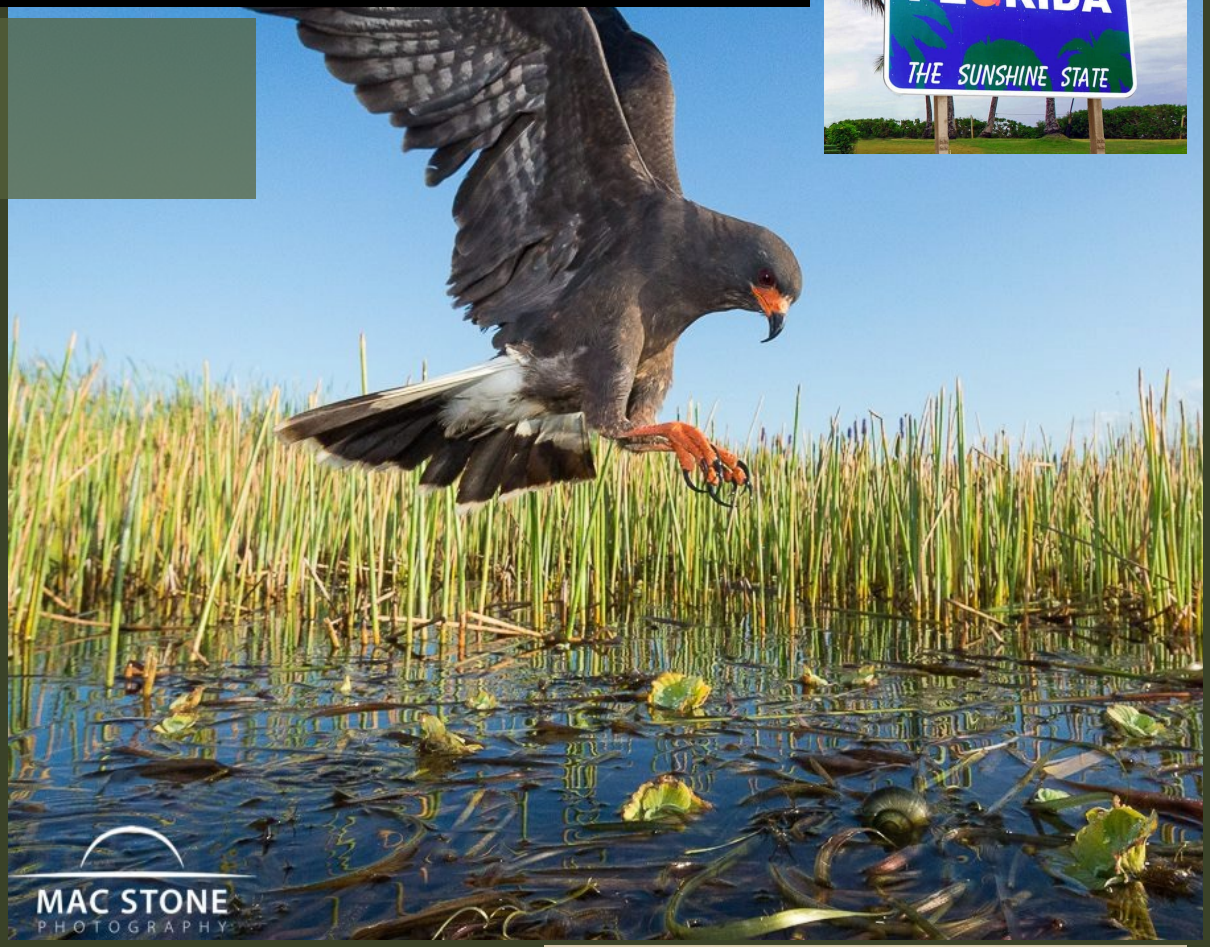
VM lesions in Spinal cord



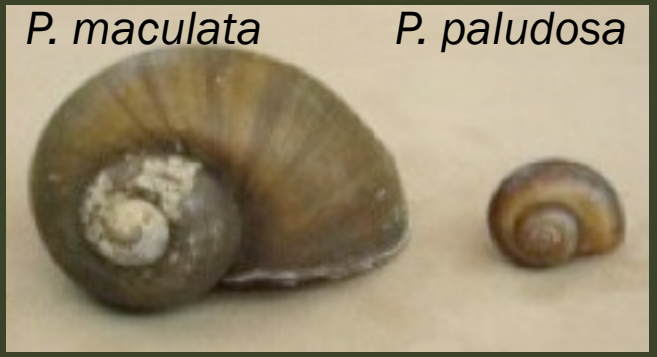
Expanding risk to endangered species

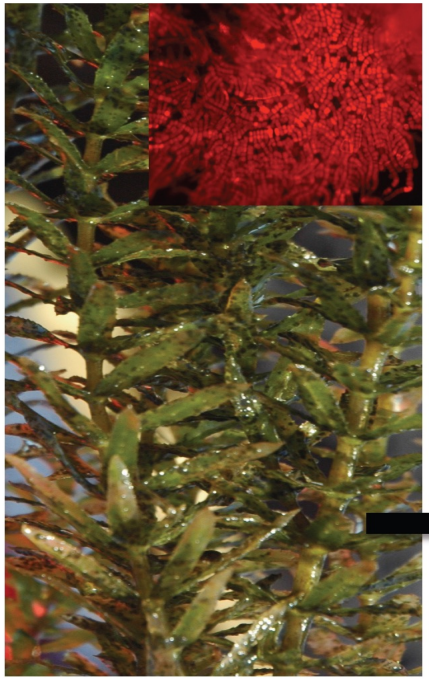
Florida Snail Kite
Rostrhamus sociabilis

Apple snails readily consume hydrilla and other aquatic vegetation

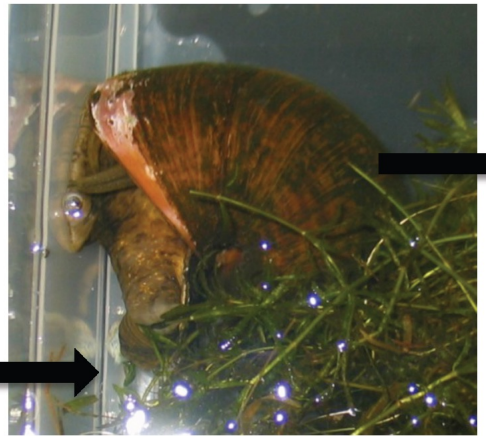


- Snail kites endangered in Florida
- Apple snails >99% of snail kite diet
- Kites forced to switch to exotic snail





A. hydrillicola growing on hydrilla leaflets produces biotoxin



Exotic apple snails feed on hydrilla and accumulate biotoxin

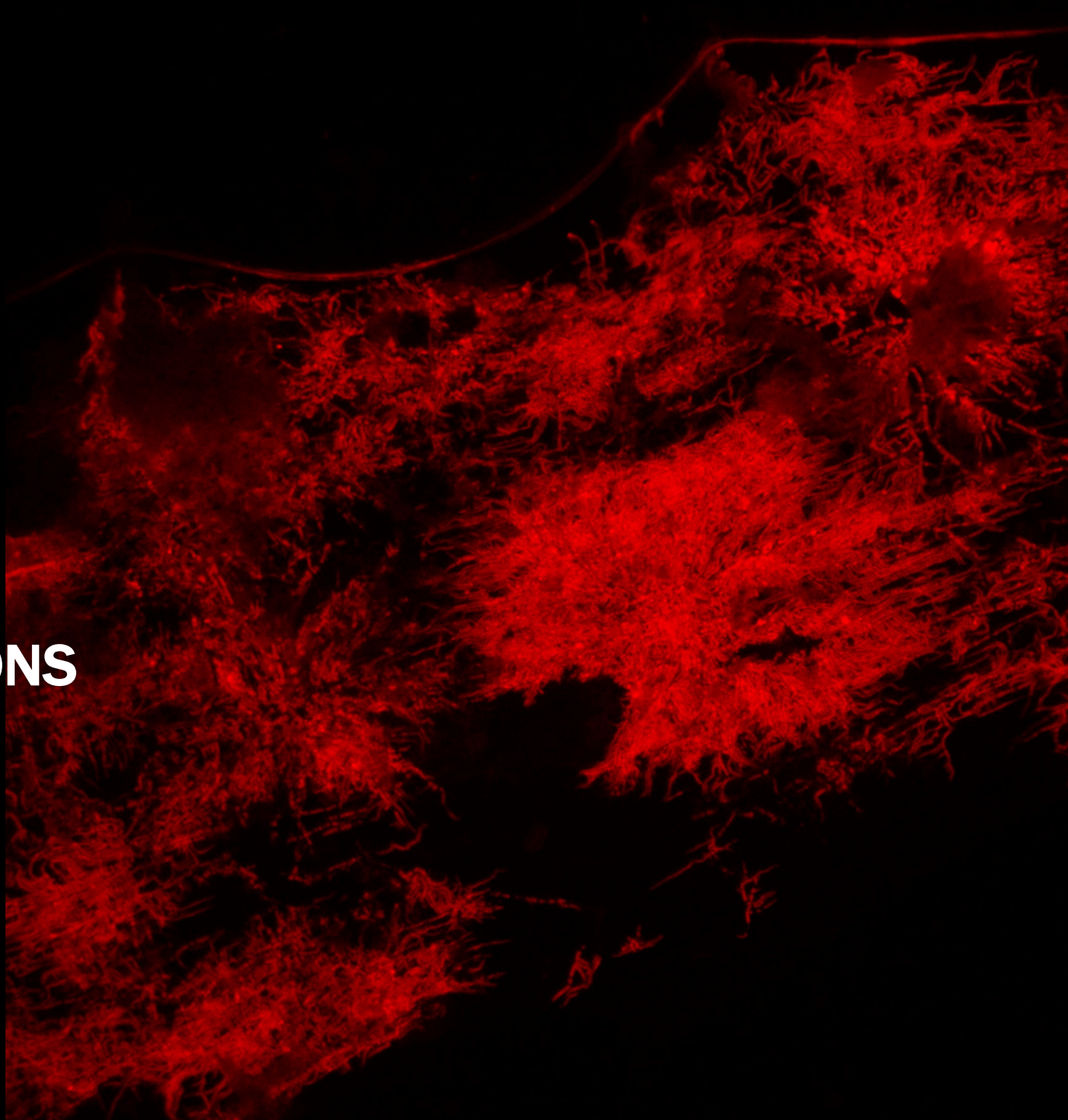
Hard to recover sick or deceased snail kites

Snail kites feed on exotic snails and ingest biotoxin

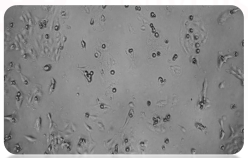
- Experiments showed VM toxin can be transferred through an invertebrate

Dodd, SR, RS Haynie, SM Williams, and SB. Wilde (2016). 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.

TOXIN INVESTIGATIONS

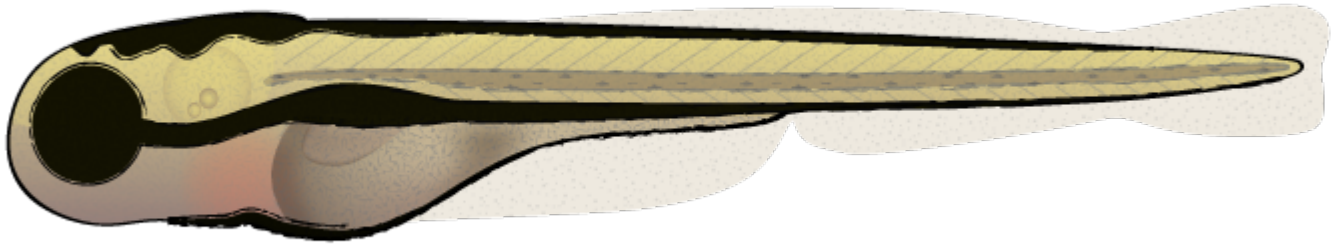


Bioassays for investigating VM Toxin

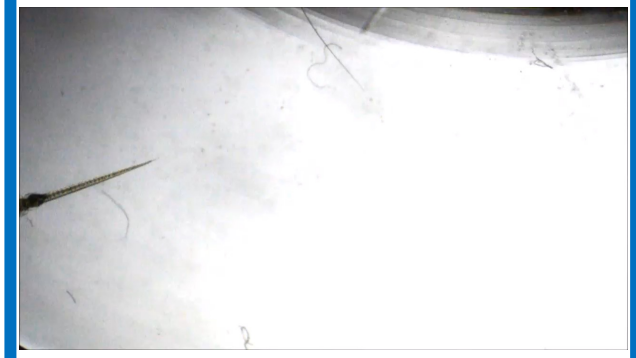



	Vertebrate **Avian Bioassay**	*Tissue Culture* **Cell line Bioassay**	*Invertebrate* ***C. dubia*** **bioassay**	*Vertebrate* **Larval Zebrafish Bioassay**
Positive VM Toxin				
Hydrilla + *A. hydrillicola* **J. S. Thurmond Nov 2015**	Positive +++	Positive +++	Positive +++	Positive +++
Hydrilla + *A. hydrillicola* **Lake Toho FL Feb 2010**	Positive +	Positive +	Positive +	Positive +
Negative VM Toxin				
Hydrilla Control **Lake Wylie, SC**	Negative	Negative	Negative	Negative
Hydrilla Control **Lake Seminole, FL**	Negative	Negative	Negative	Negative
Hydrilla + *A. hydrillicola* **J. S. Thurmond Aug**	Negative	Positive +	Negative	Negative
Hydrilla Control **Lake Oliver, GA**	--	--	--	Negative
Hydrilla Control **Walter F. George Reservoir**	--	--	--	Negative

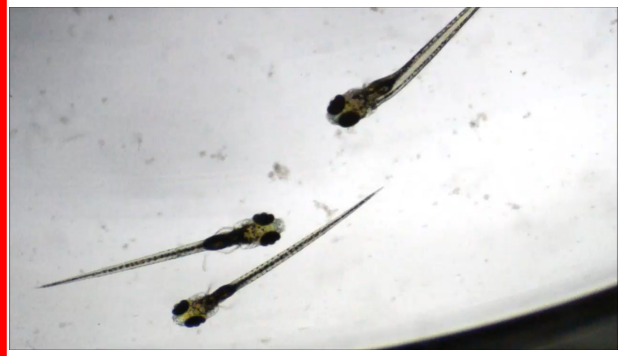
Zebrafish behavior after 24 hr exposure (7 days old)

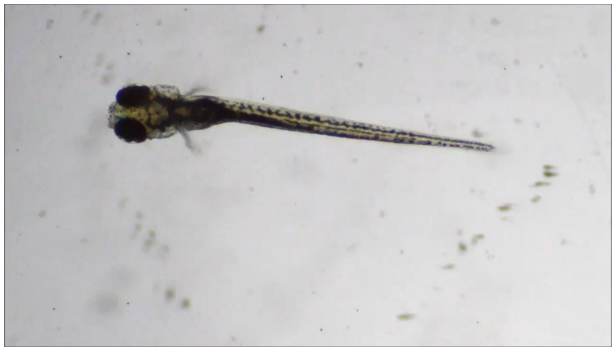


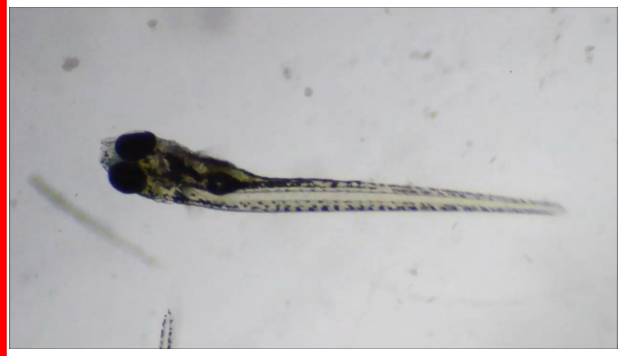
Solvent Control



VM toxin Exposed



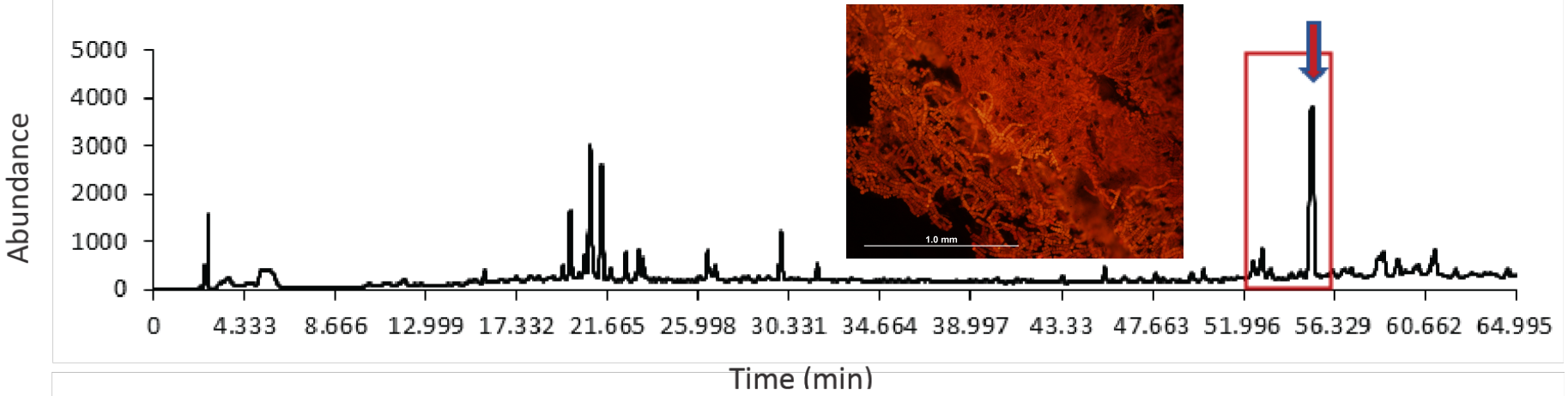




Aetokthonotoxin: a novel brominated neurotoxin discovered with AVM positive hydrilla

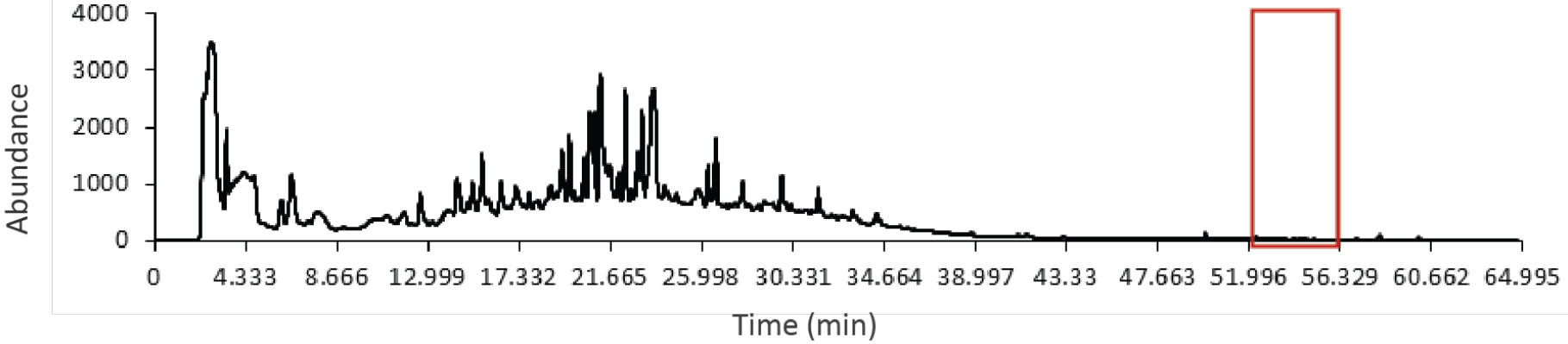
A

Hydrilla with *Aetokthonos hydrillicola* and Aetokthonotoxin (AETX)



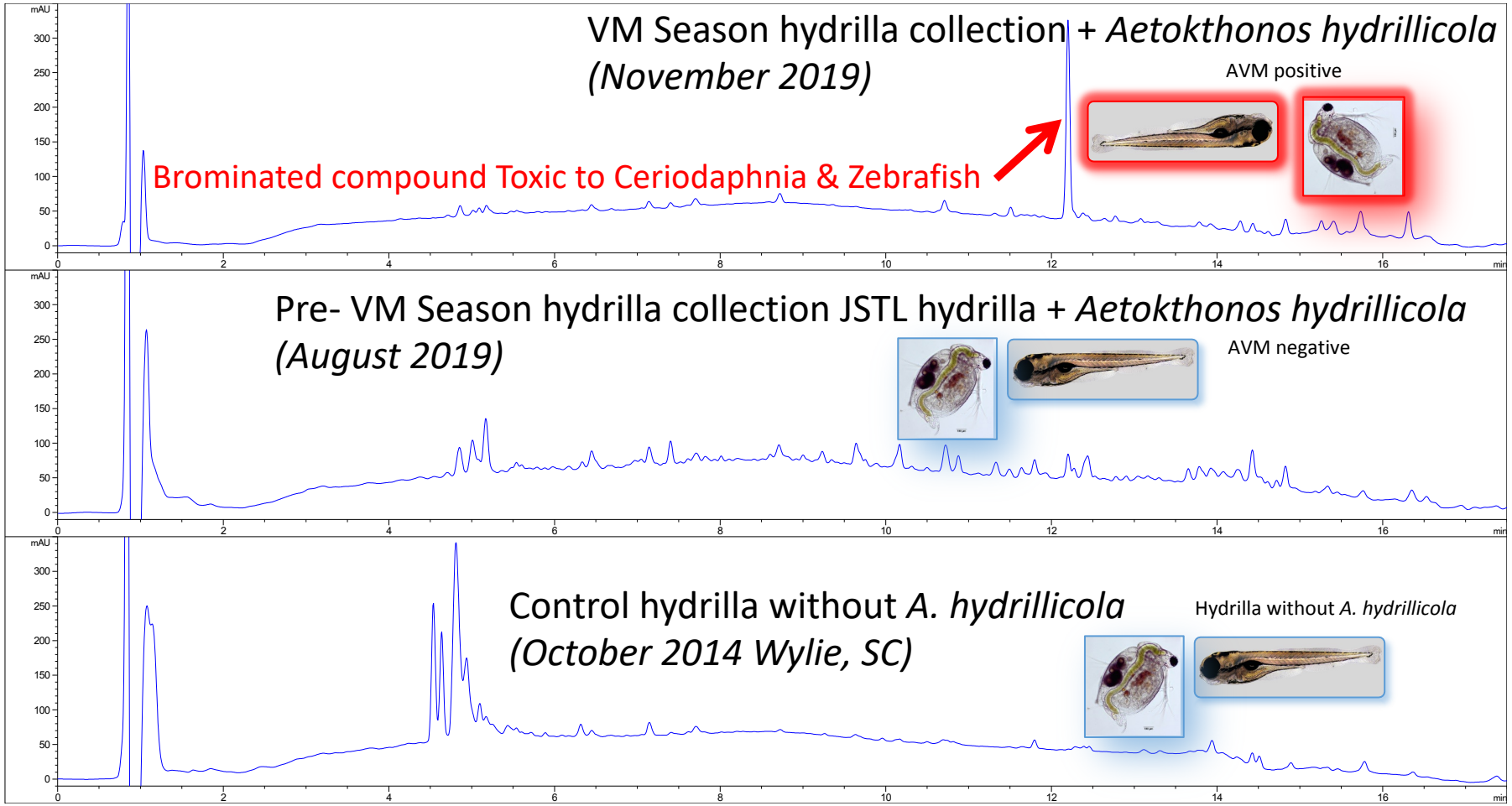
B

Hydrilla without *A. hydrillicola*



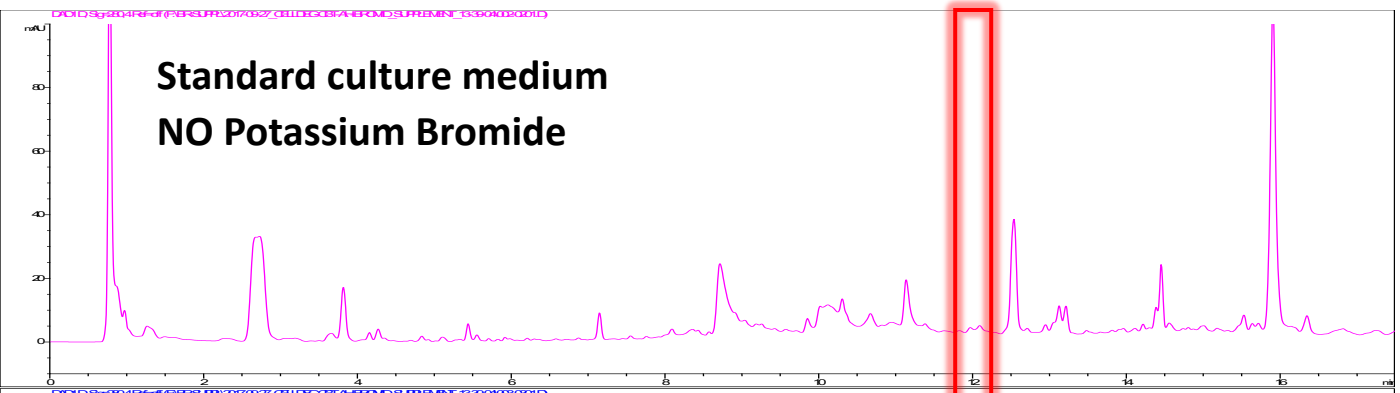
HPLC chromatograms (UV detection at 210 nm) methanol extracts

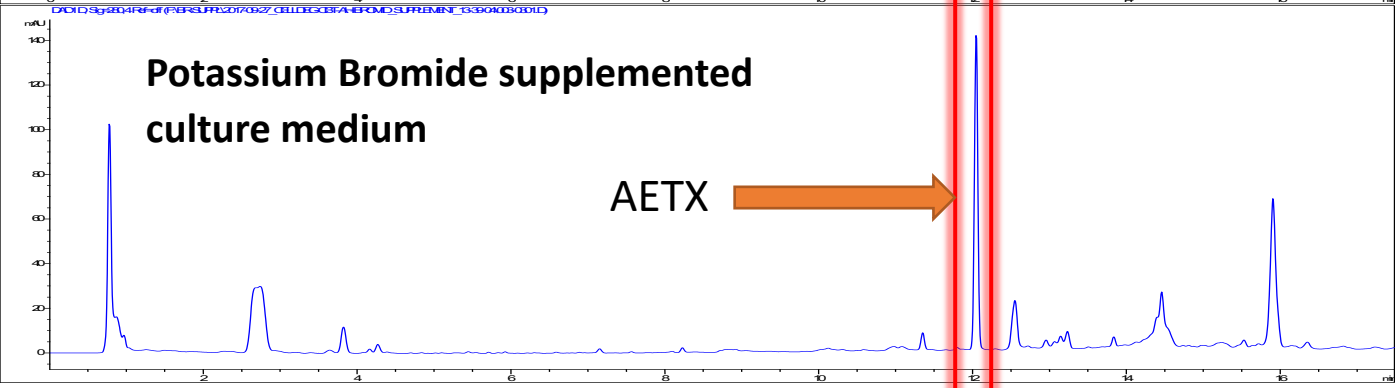
↗ unique brominated toxin shown in the top panel

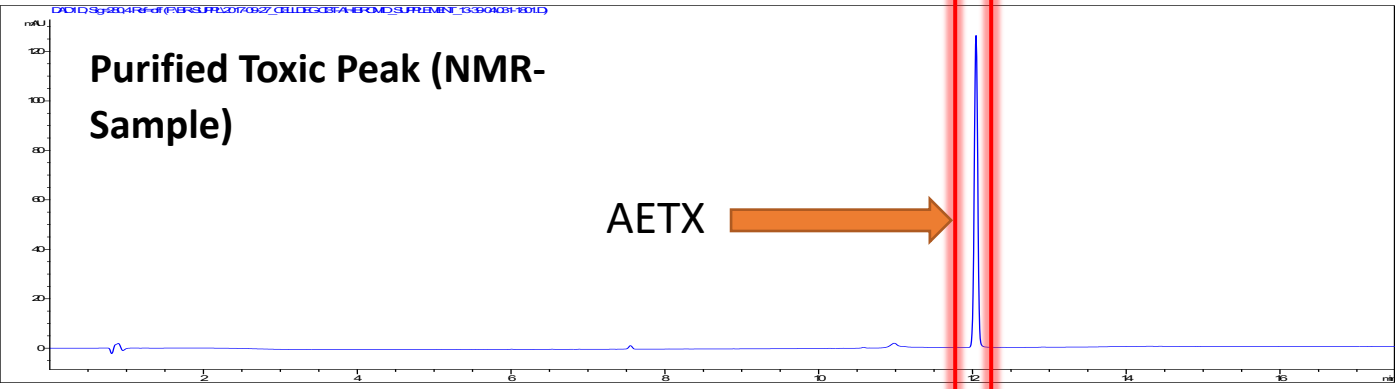


Aetokthonotoxin Production in Laboratory

Novel compound produced in cultured *A. hydrillicola* adding bromide to culture medium







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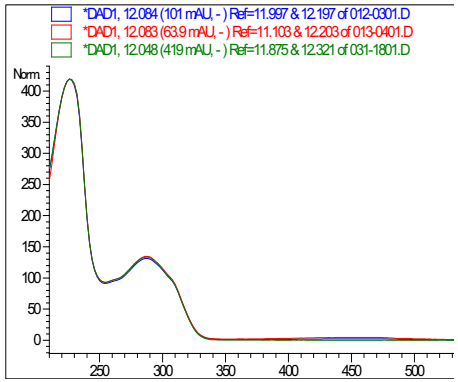
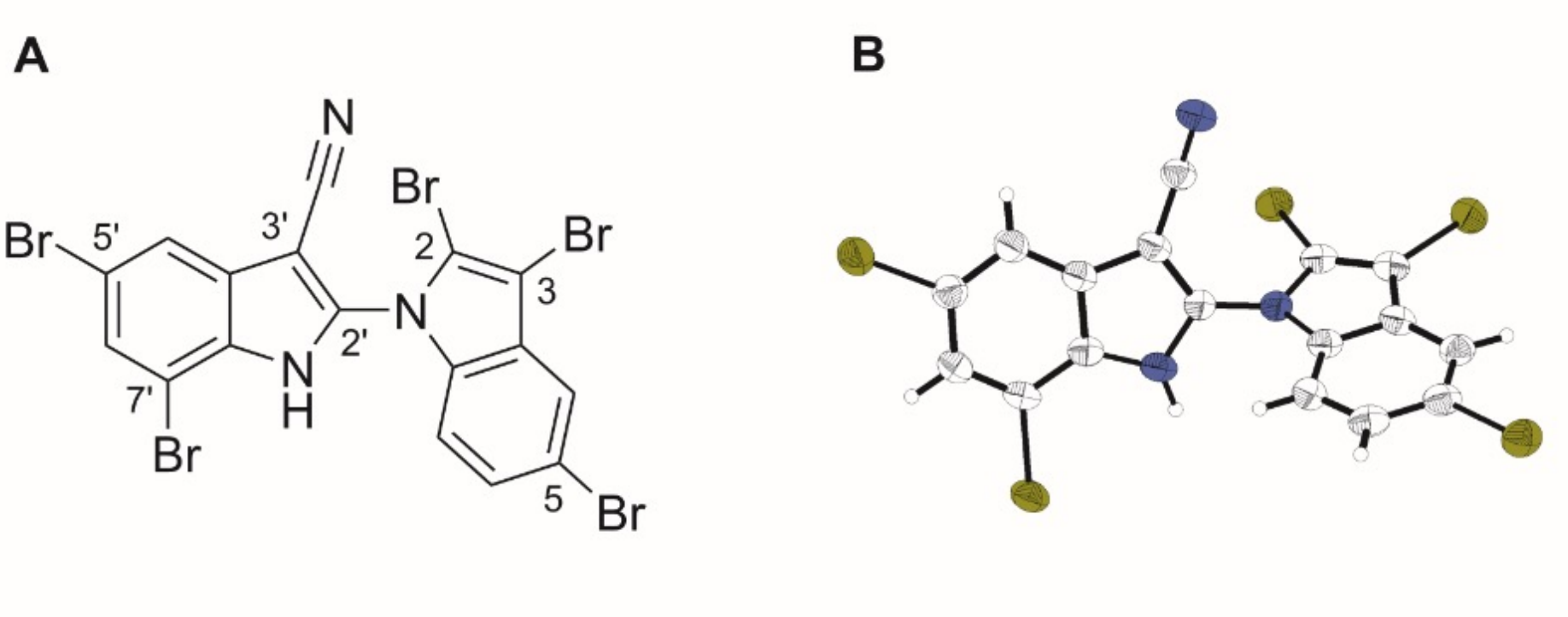
Dr. Timo Niedermeyer
PhD student
Steffen Breinlinger

UV-spectra overlay of the peaks at RT 12,2 min and isolated toxic peak



(A) Planar structure and (B) X-ray crystallography structure of aetokthonotoxin (AETX)



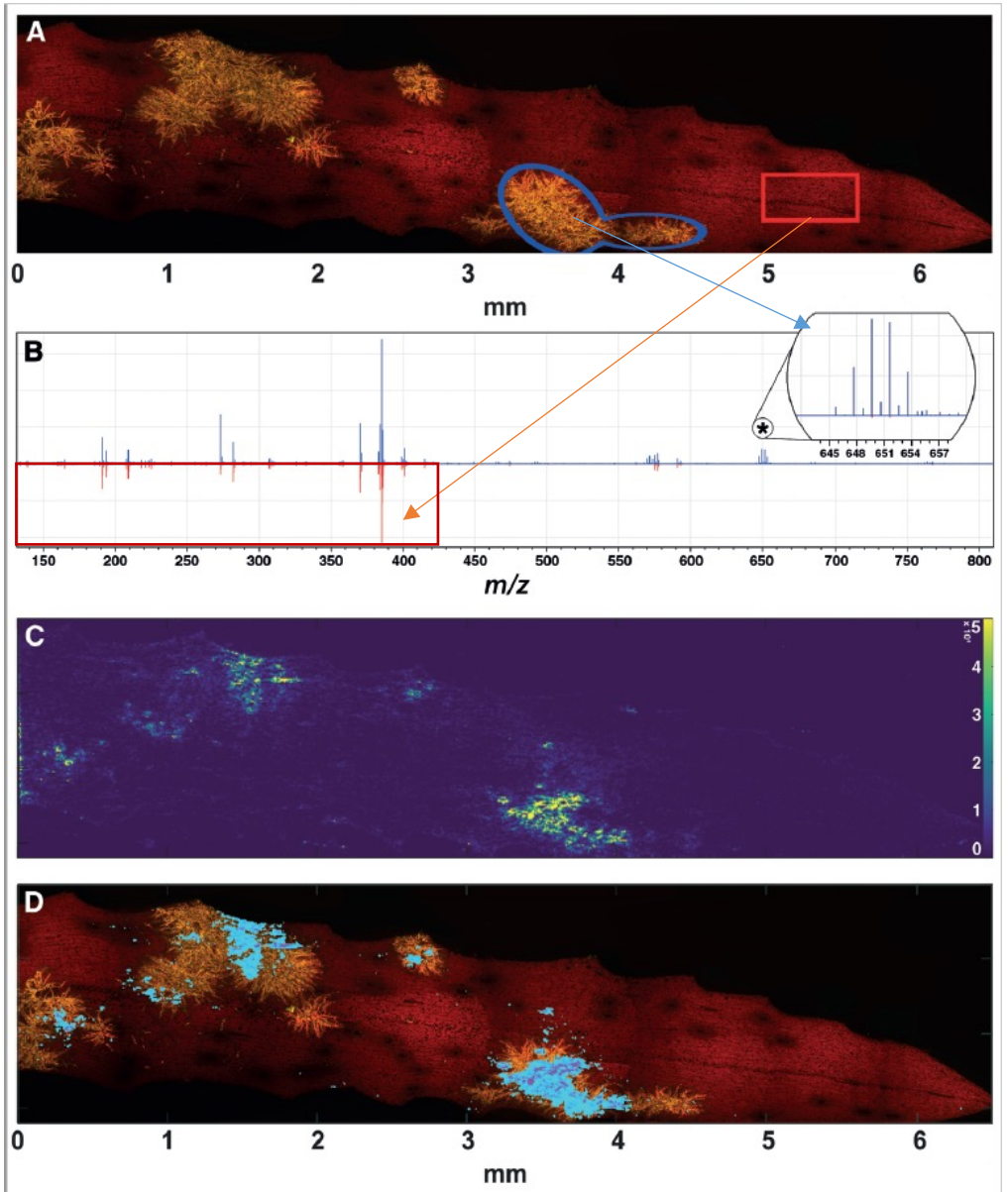
AP-MALDI-MSI of *A. hydrillicola* colonies growing on *H. verticillata* reveals a cyanobacterium-specific metabolite

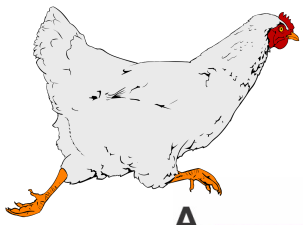
(**A).** *A. hydrillicola* colonies on *H. verticillata* leaf.

(**B**) Blue outline region -- pentabrominated metabolite associated with the cyanobacterial colony.

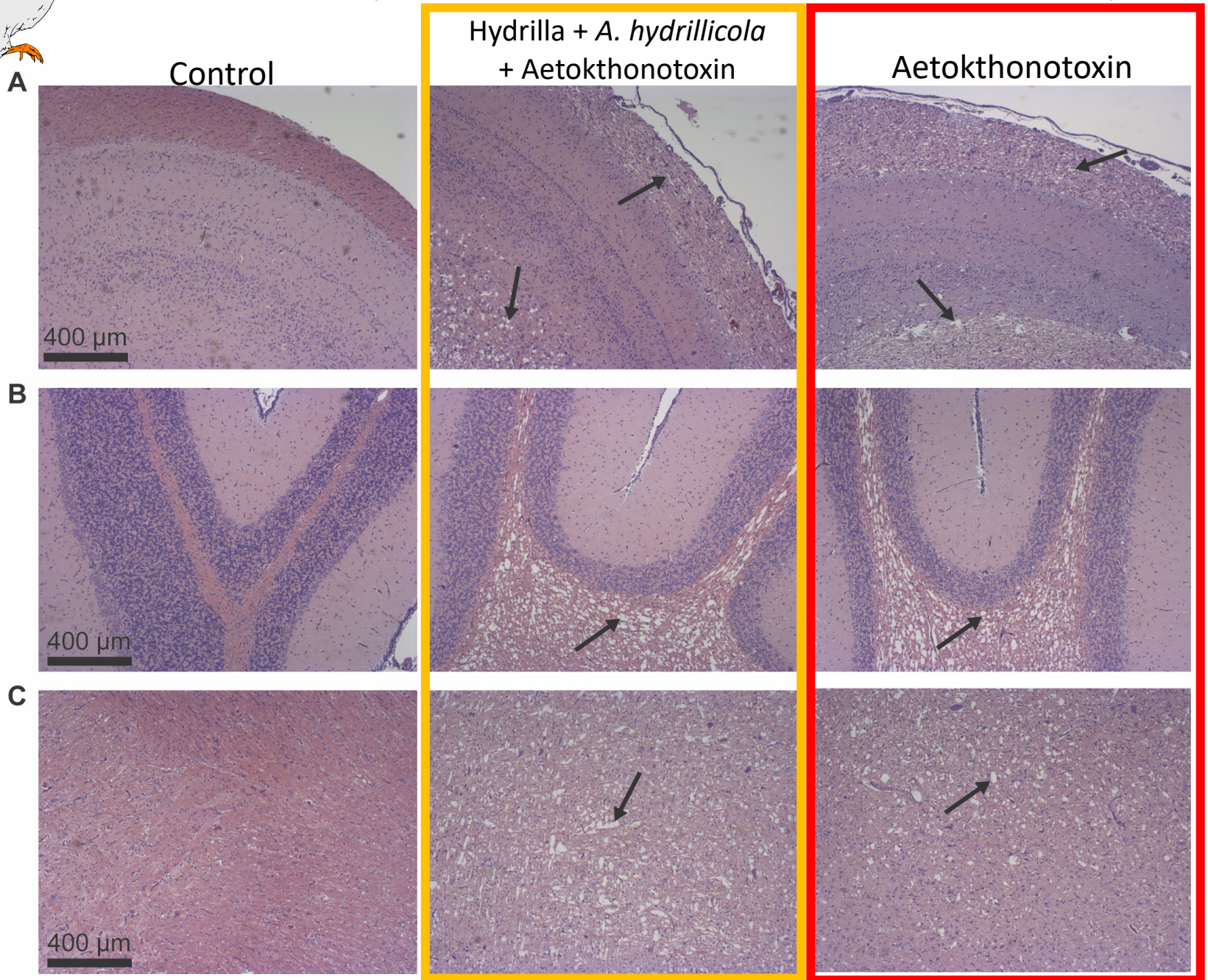
(**C**) AP-MALDI image showing the spatial distribution of AETX

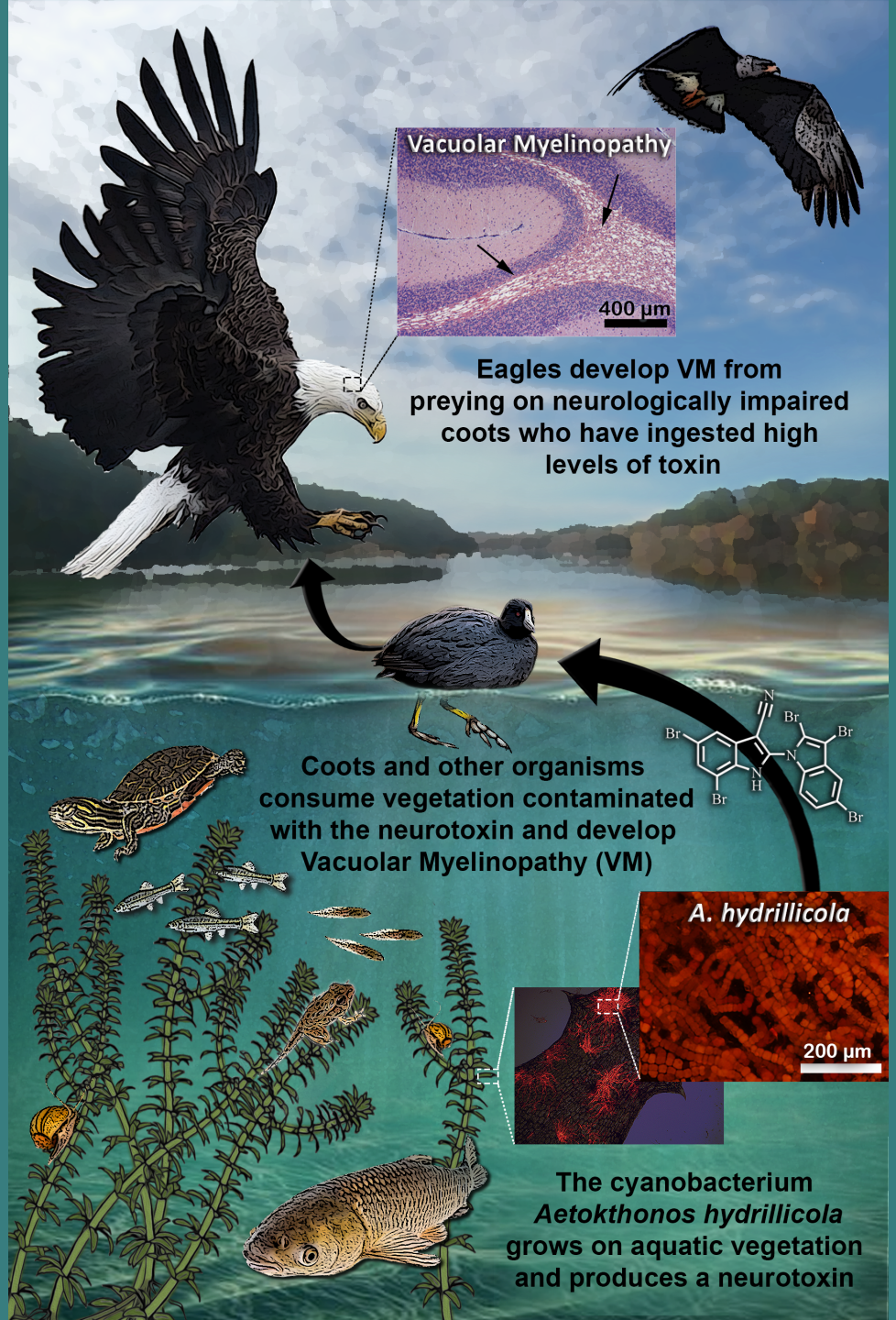
(**D**) Overlay of micrograph and *m/z* feature 649.6382 ± 2 ppm.





Chickens exposed to Aetokthonotoxin develop VM

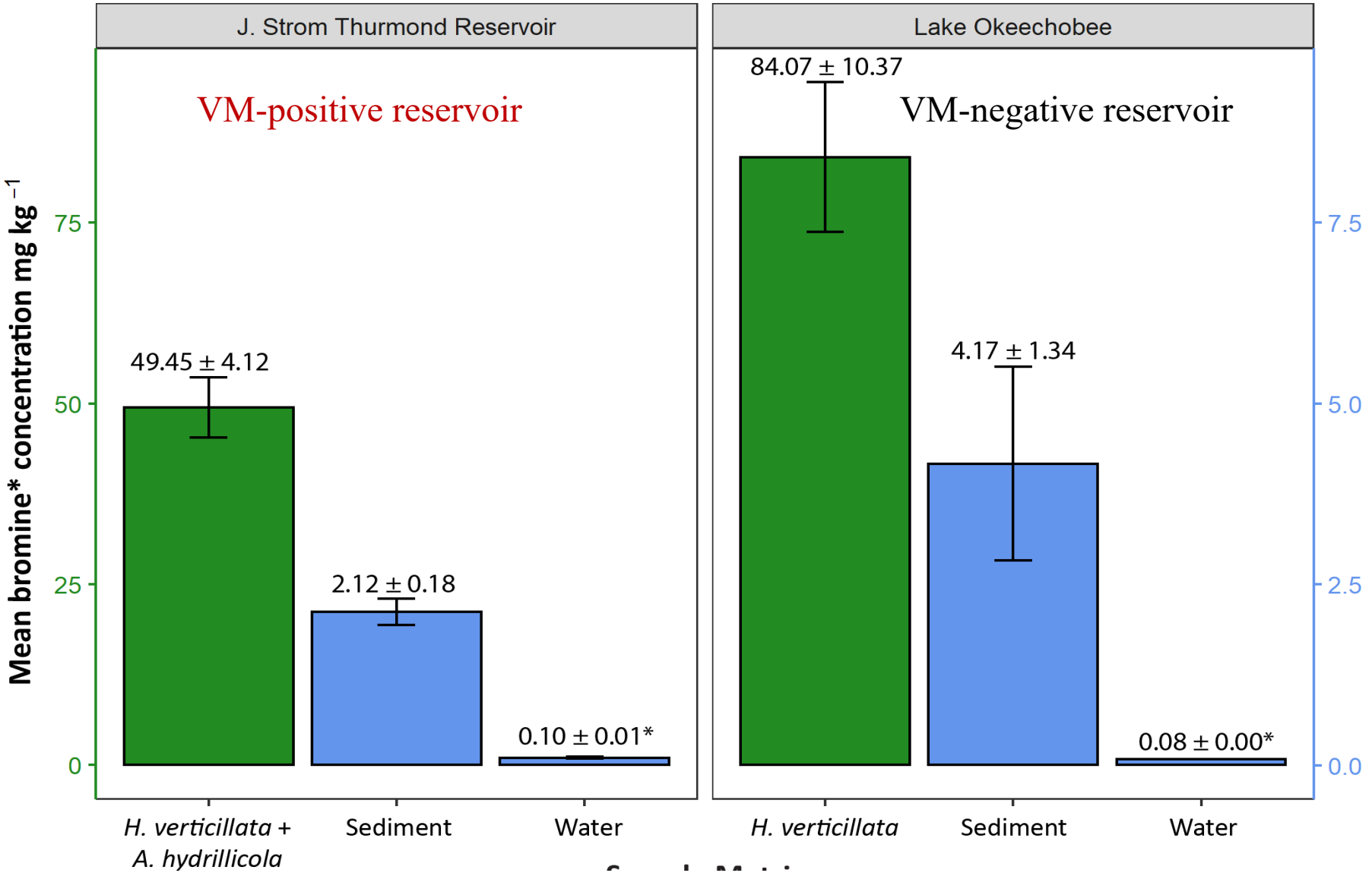




- Aetokthonotoxin is a neurotoxic, lipophilic compound, not water soluble.
- Most cyanotoxins are water soluble.
  - Microcystins, anatoxin, saxitoxin, domoic acid, brevetoxin
- Concern about bioaccumulation of toxin in tissues.

Breinlinger S, T Phillips, B Haram, J Mareš, JA Martínez Yerena, P Hrouzek, R Sobotka, WM Henderson, P Schmieder, SM Williams, JD Lauderdale, HD Wilde, W Gerrin, A Kust, C Wagner, B Geier, M Liebeke, H Enke, THJ Niedermeyer, SB Wilde. (submitted 10/29/20). Hunting down the eagle killer: A novel cyanobacterial neurotoxin causes Vacuolar Myelinopathy. Science.

Source of Bromide
hydrilla >sediment>water



Wes Gerrin

Aetokthonotoxin in wild coot tissue

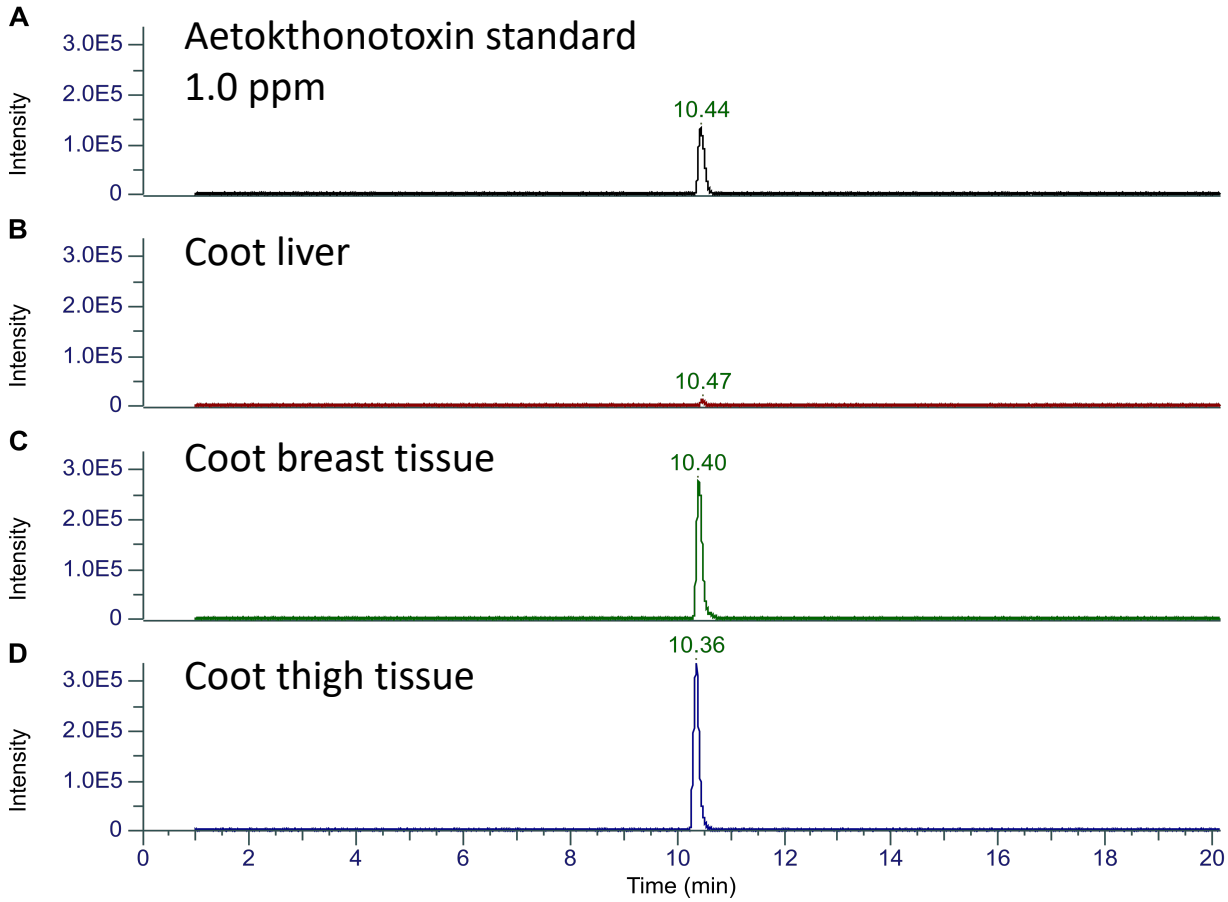


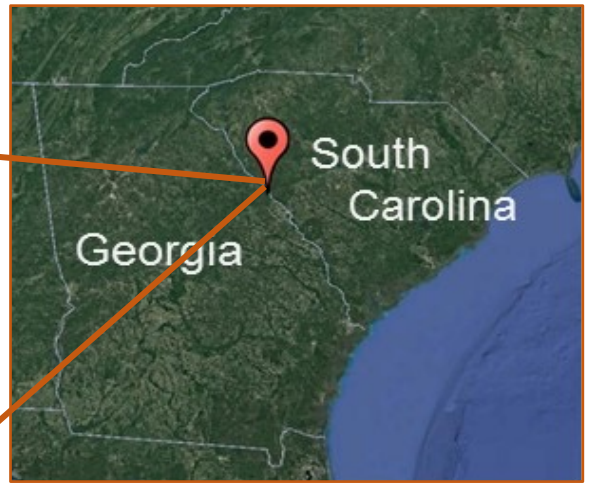
Fig. S5. Tissue screening for aetokthonotoxin (AETX) of wild American Coots (*Fulica americana)* succumbed to Vacuolar Myelinopathy (VM). A targeted mass spectrometry analysis (HPLC-SRM-MS) was carried out to selectively screen the tissues for AETX presence. Chromatograms show the SRM traces of most intense fragments (m/z 570, m/z 491) of the AETX parent ion. (**A**) AETX standard (t_R 10.44 min). (**B**) Liver tissue extract. (**C**) Breast tissue extract. (**D**) Thigh tissue extract.

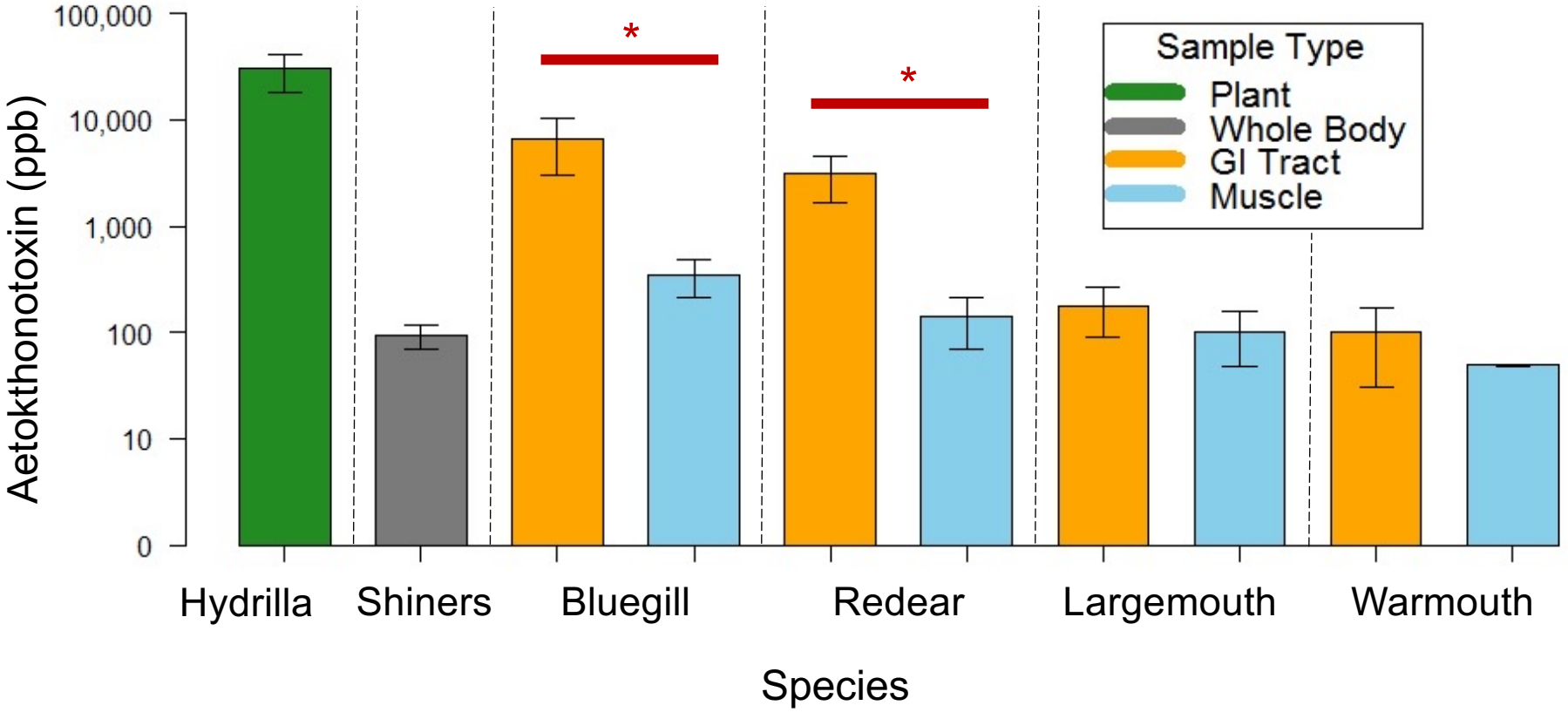
Breinlinger S, T Phillips, B Haram, J Mareš, JA Martínez Yerena, P Hrouzek, R Sobotka, WM Henderson, P Schmieder, SM Williams, JD Lauderdale, HD Wilde, W Gerrin, A Kust, C Wagner, B Geier, M Liebeke, H Enke, THJ Niedermeyer, SB Wilde. (submitted 10/29/20). Hunting down the eagle killer: A novel cyanobacterial neurotoxin causes Vacuolar Myelinopathy. Science.

Aetokthonotoxin in wild fish tissue

The University of Georgia
WARNELL
SCHOOL OF FORESTRY & NATURAL RESOURCES

MS Thesis
Alex Pelletier

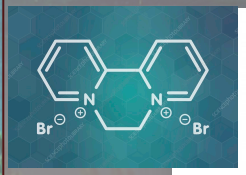




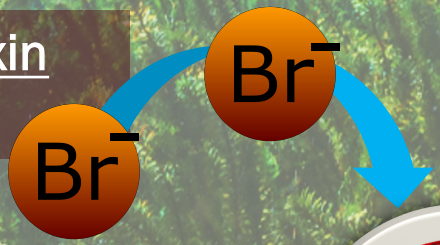
Management Solutions

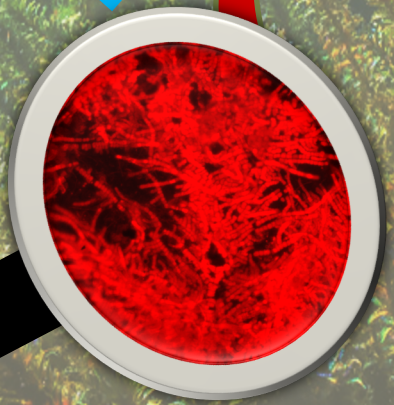
Biological

Chemical

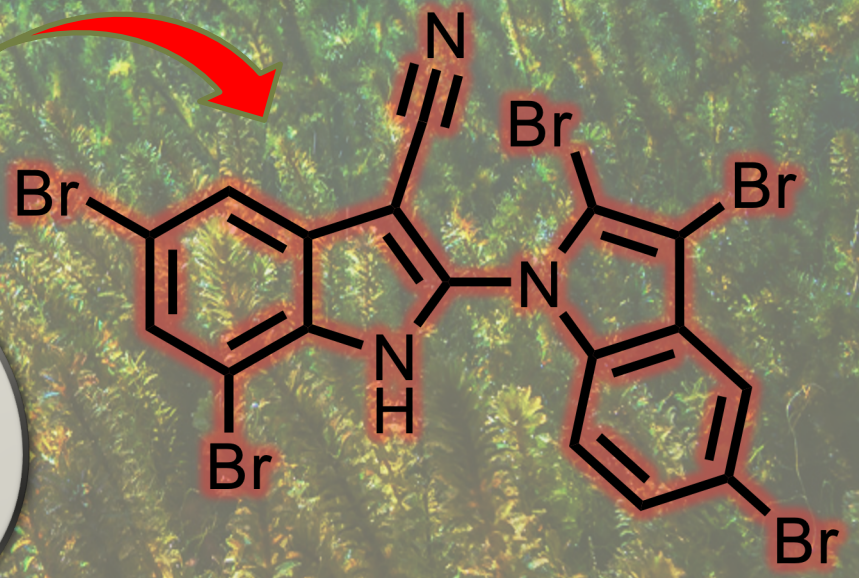


Needed for toxin production:





Aetokthonos hydrillicola



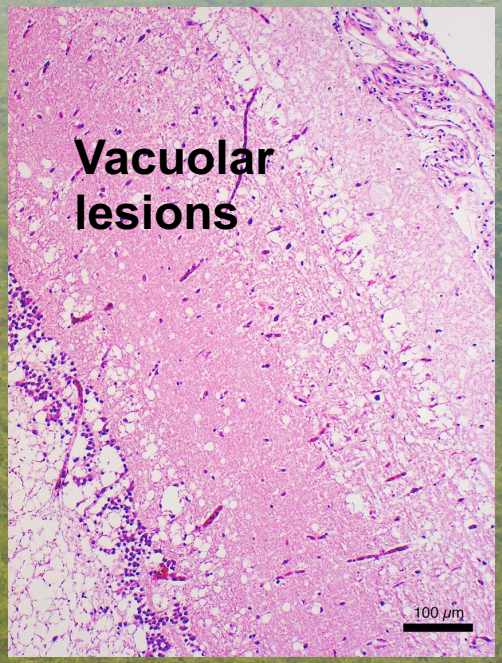
Aetokthonotoxin (AETX)

Management solutions

Triploid Sterile Grass Carp

10-12” Fish, Field & Lab Trials

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



Management Solutions: Herbicide + Grass Carp

- All sentinel mallards with access to *hydrilla/Ah*-- VM +
- Mallards in hydrilla free coves, no impairment, no VM lesions

Acknowledgements

The University of Georgia
WARNELL
SCHOOL OF FORESTRY & NATURAL RESOURCES

Wilde Lab UGA

Susan Wilde, Michael Netherland, John Maerz, Sonia Hernandez, Jim Lauderdale, Dayton Wilde, Al Camus, Jeffrey Johansen, Timo Niedermeyer, Robert Bringolf, Susan Williams, Matthew Henderson, John Washington, Dean Jones, Vanessa Kinney, Brigette Haram, Steffen Breinlinger, Tabby Phillips, Melissa Martin, Wes Gerrin, Mandy Howard, Alex Pelletier, Rebecca Haynie, James Herrin, Shelley Dodd, Jamie Morgan, Jenny Garrison, Brad Bartelme, Brigette Haram, Garon Brandon, Wallace Woods

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Franklin College of Arts and Sciences
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DNR

GULF & SOUTH ATLANTIC REGIONAL PANEL ON AQUATIC INVASIVE SPECIES

Gulf & South Atlantic Regional Panel On Aquatic Invasive Species

GULF STATES MARINE FISHERIES COMMISSION

Continuing Research

- AETX is lipid soluble, and we have demonstrated of trophic transfer-- potential for **bioaccumulation**
- **Multiple taxa are susceptible** to AETX (birds, fish, amphibians, reptiles, and invertebrates)
- **Expanded monitoring** of AETX in aquatic environments and animal tissues to determine environmental risk
- **Increase public awareness.** People can help us detect invasive species, harmful cyanobacteria and protect water resources
- Critical need for research on **mammalian susceptibility** and human health risks from consumption of fish and waterbirds from VM reservoirs


AETX
Aetokthonos hydrillicola