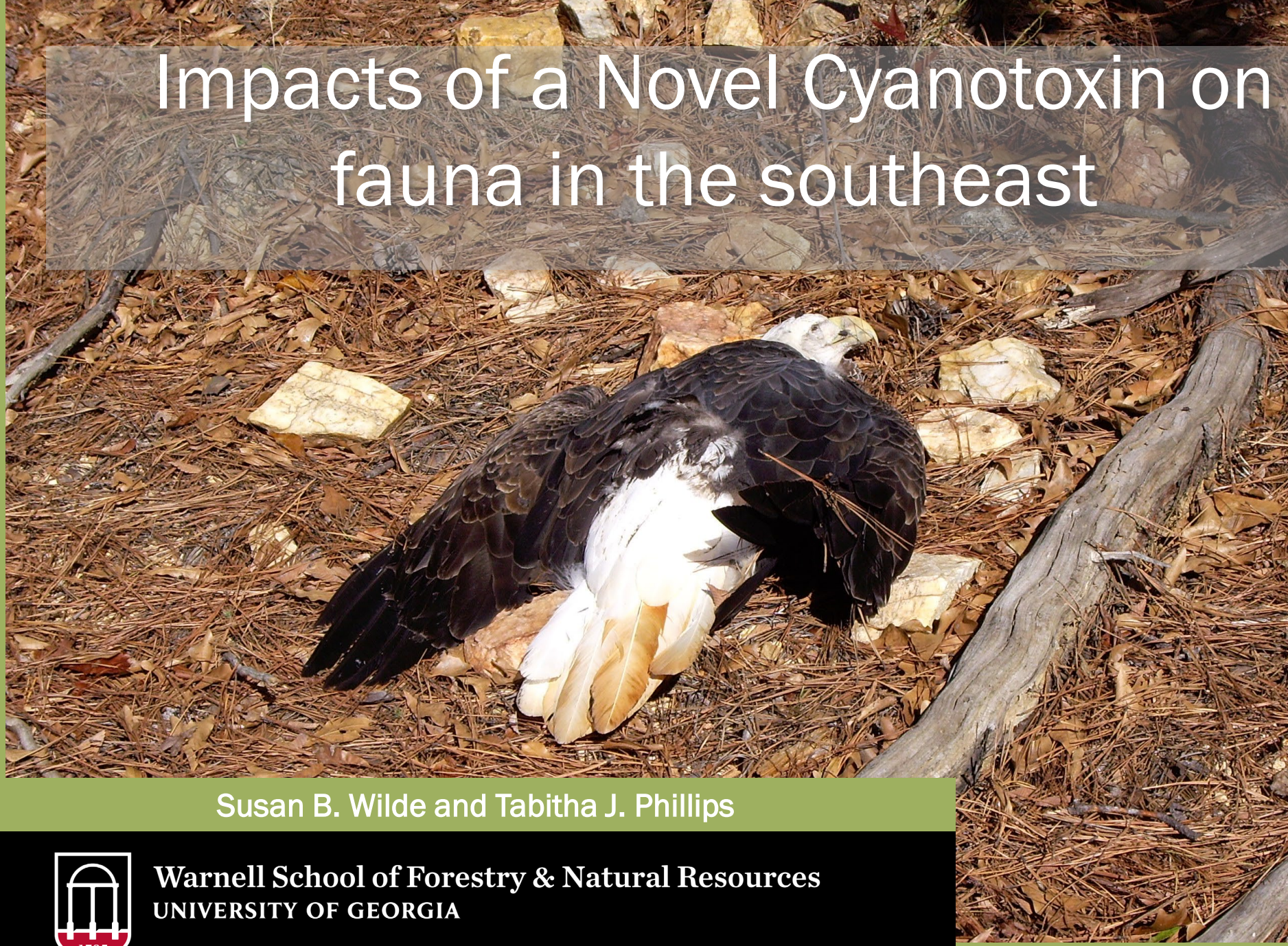
Impacts of a Novel Cyanotoxin on fauna in the southeast



Susan B. Wilde and Tabitha J. Phillips

Warnell School of Forestry & Natural Resources
UNIVERSITY OF GEORGIA

Avian Vacuolar Myelinopathy (AVM)

1994-2022 Past, Present, Future

1. Reservoir monitoring
2. Field studies
3. Food chain transfer trials
4. Expanding locations
5. Toxin discovery
6. More taxa affected-- VM
7. Managing invasive SAV --reservoirs
8. Human health concerns

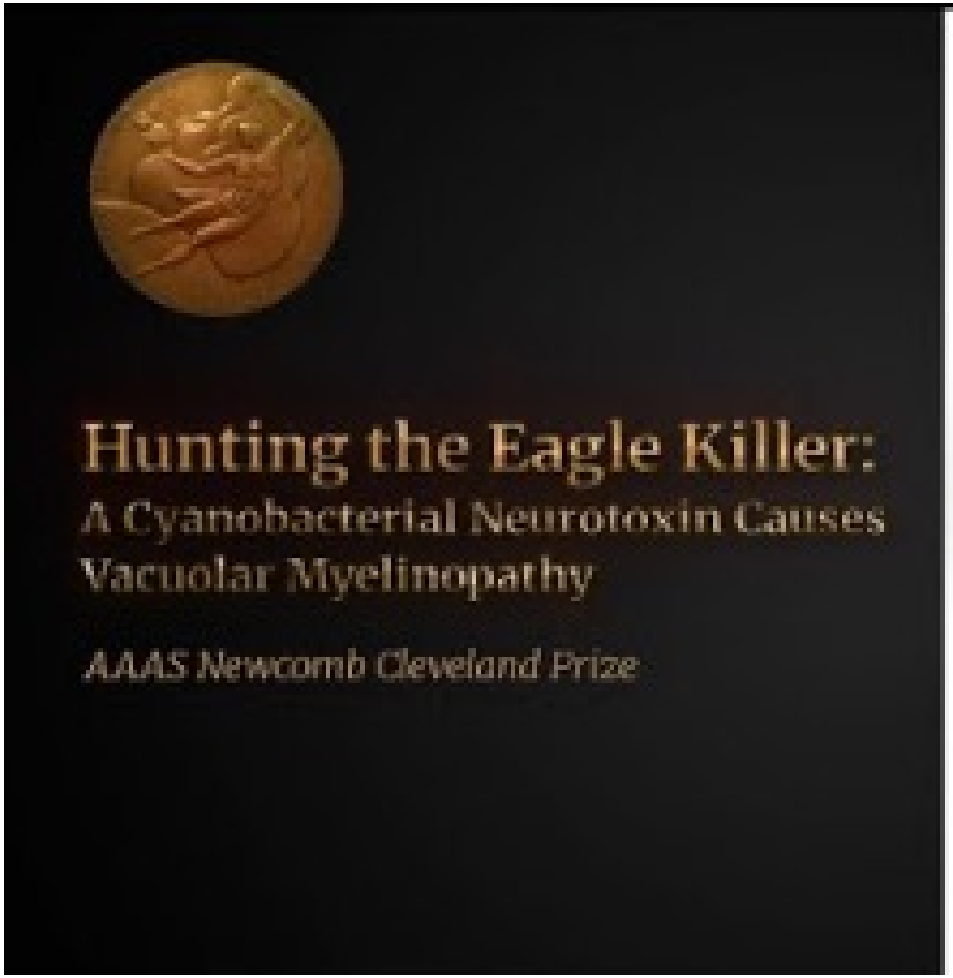
Science

$15
26 MARCH 2021
sciencemag.org

AAAS

Steffen Breinlinger and Tabitha J. Phillips
Timo Neidermeyer and Susan B. Wilde

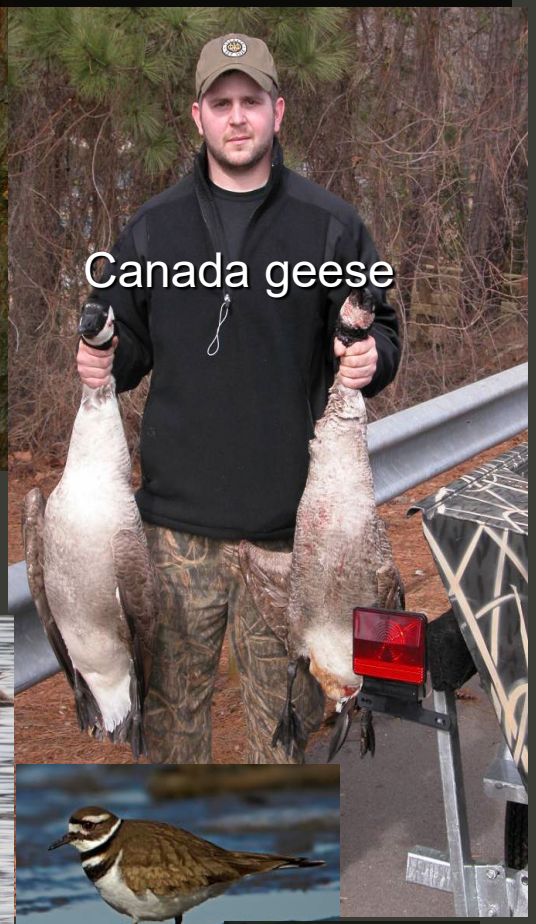
DEADLY CASCADE



https://www.youtube.com/watch?v=E-bEOU7-tYQ&t=2s

Bird species with AVM brain lesions

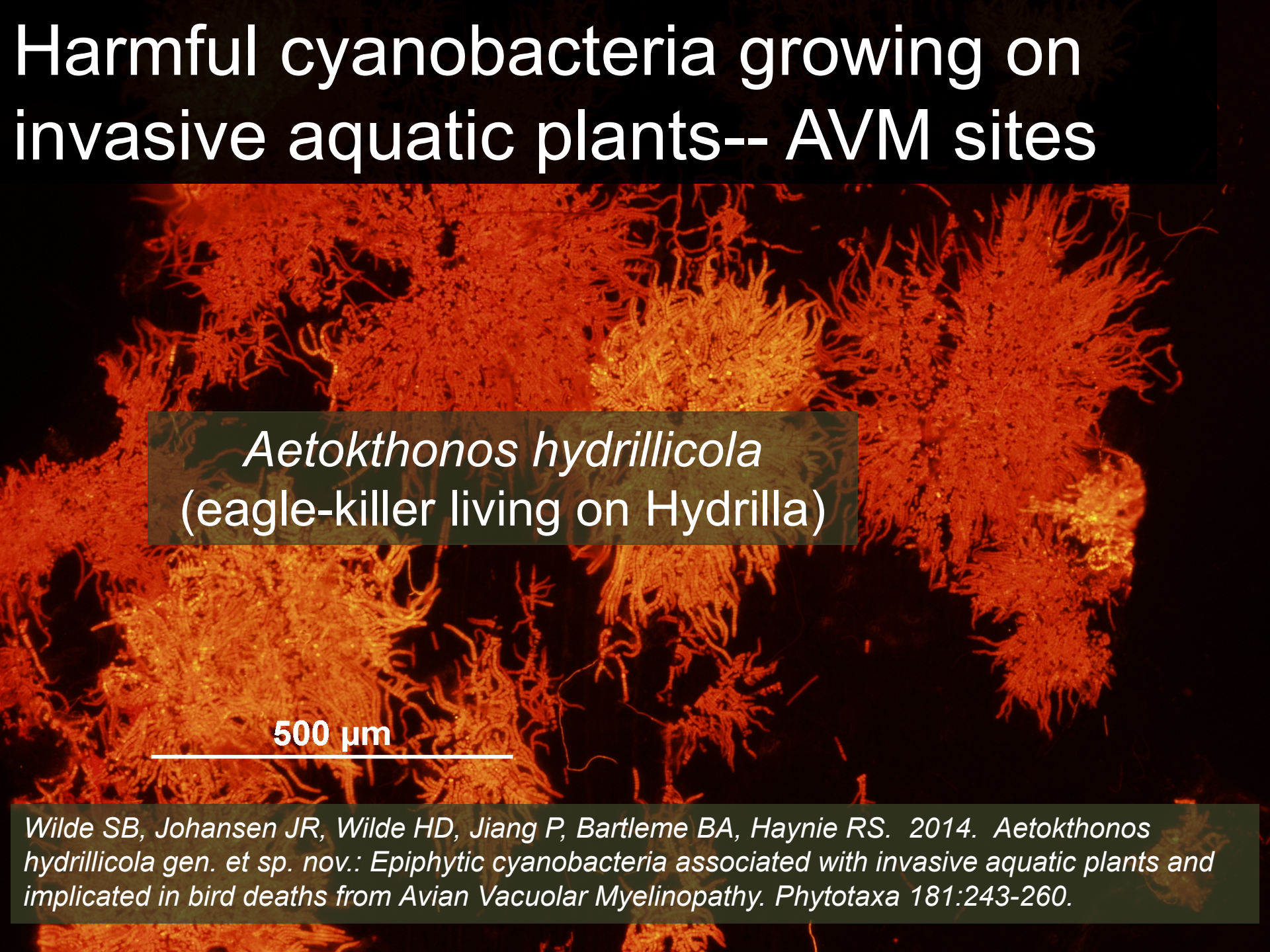
Mallards, Ring-necked ducks
Buffleheads, American wigeon



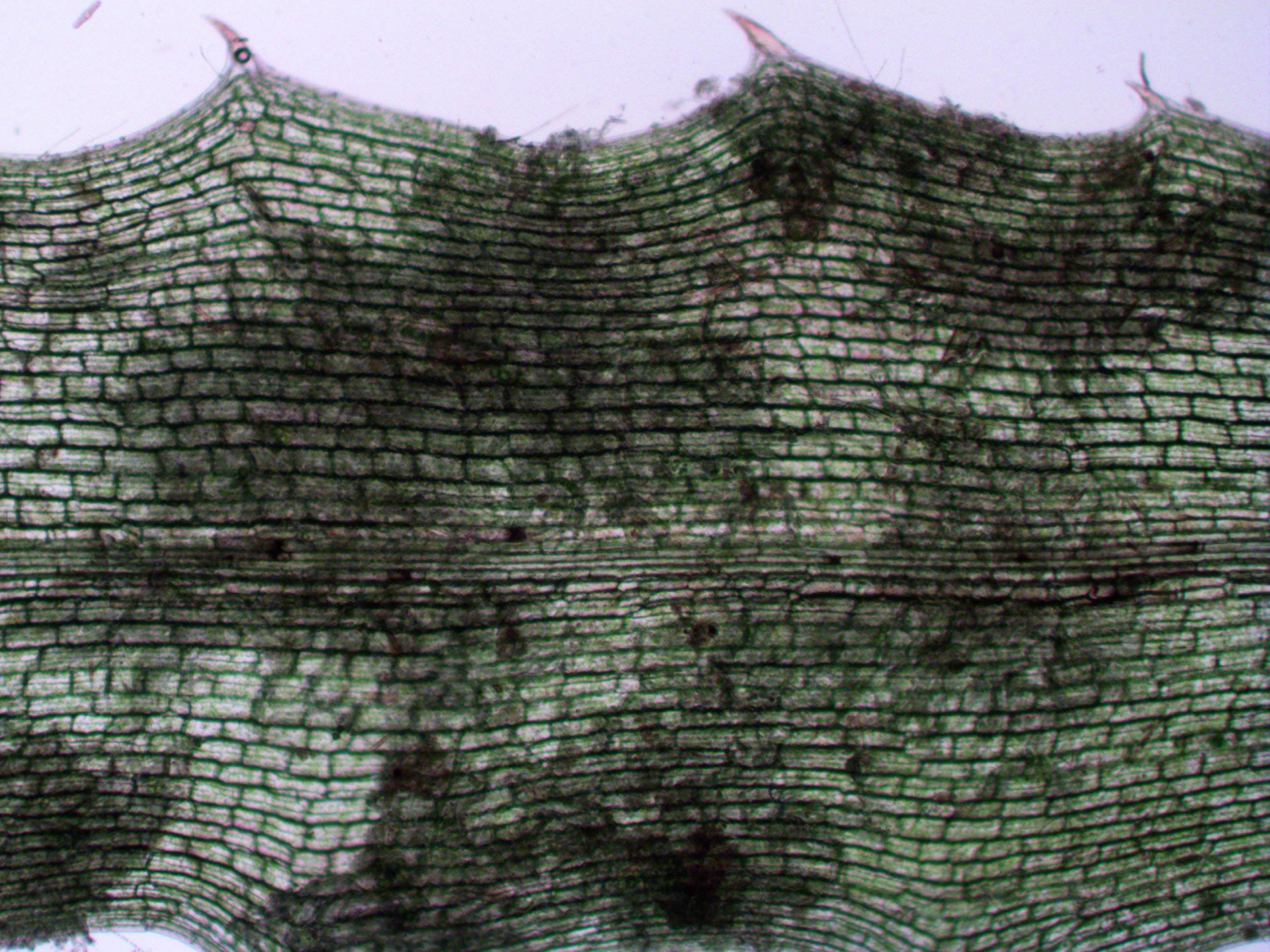
Killdeer

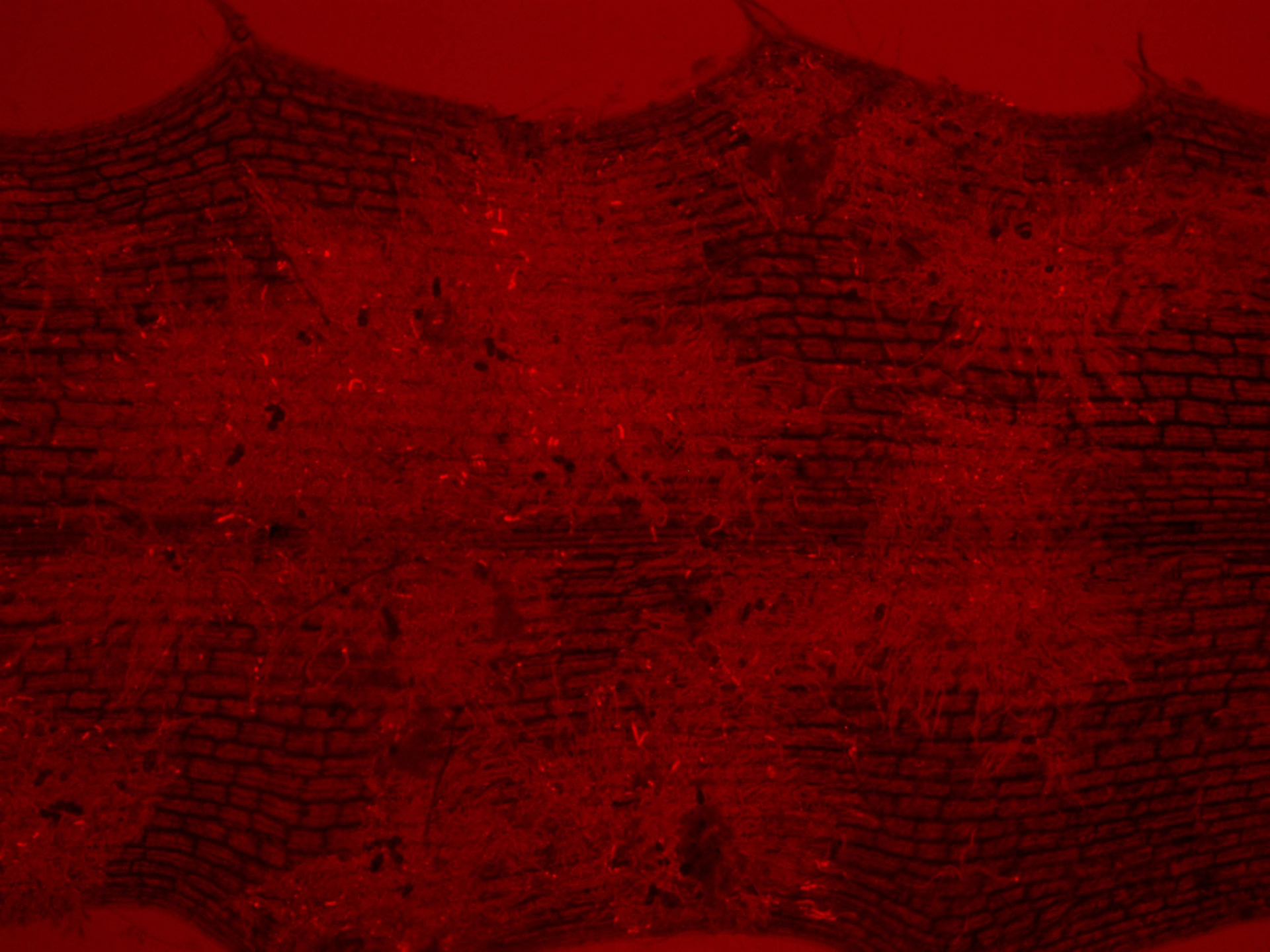
Augspurger, T, JR Fischer, NJ Thomas, L Sileo, RE Brannian, KJG Miller, and TE Rocke. 2003. Vacuolar myelinopathy in waterfowl from a North Carolina impoundment. JWD 39:412-417.
Fischer, J, LA Lewis-Weis, CM Tate, JK Gaydos, RW Gerhold, RH Poppenga. 2006. Avian vacuolar myelinopathy outbreaks at a southeastern reservoir. JWD 42:501-510

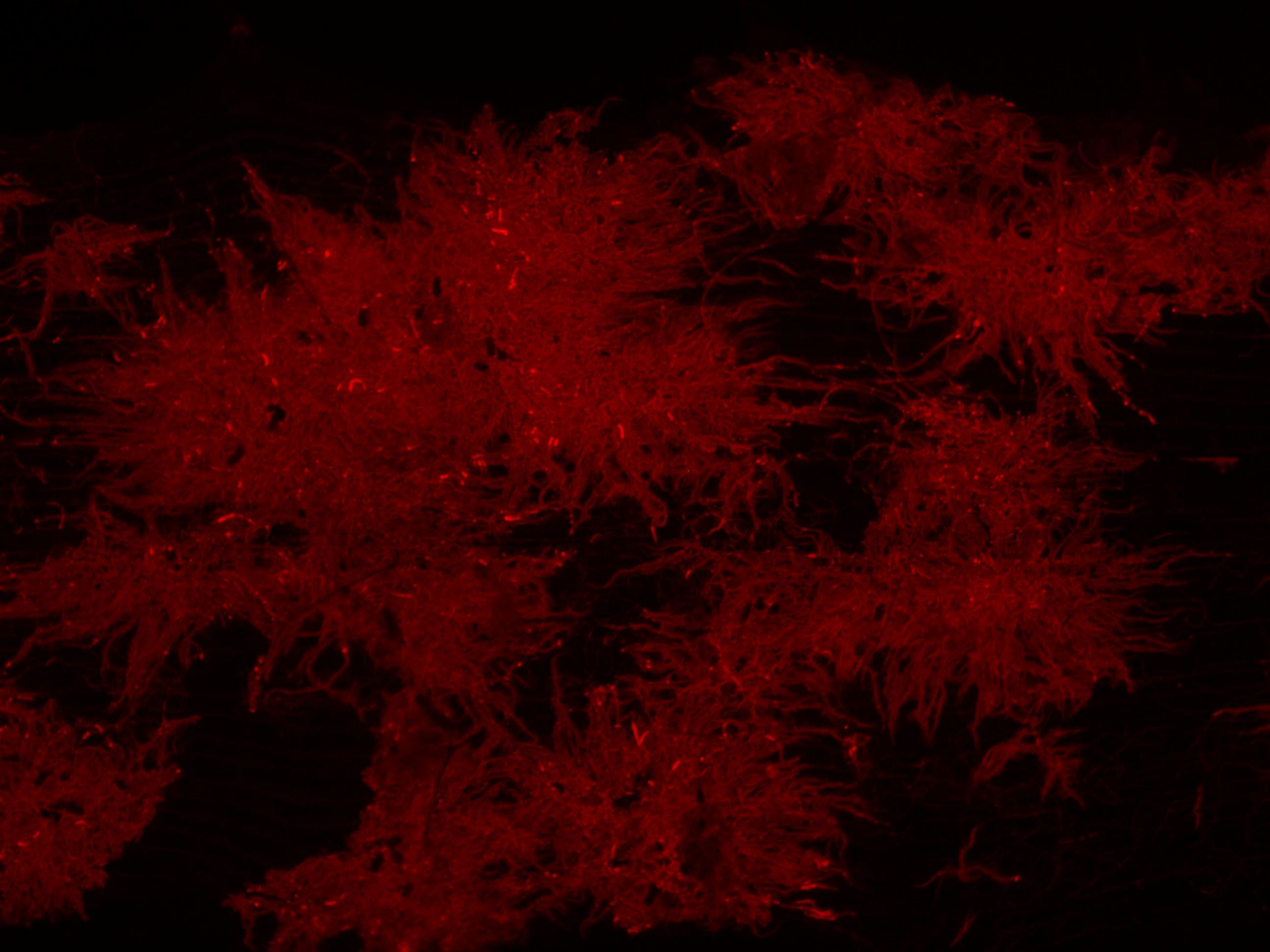
Harmful cyanobacteria growing on invasive aquatic plants-- AVM sites



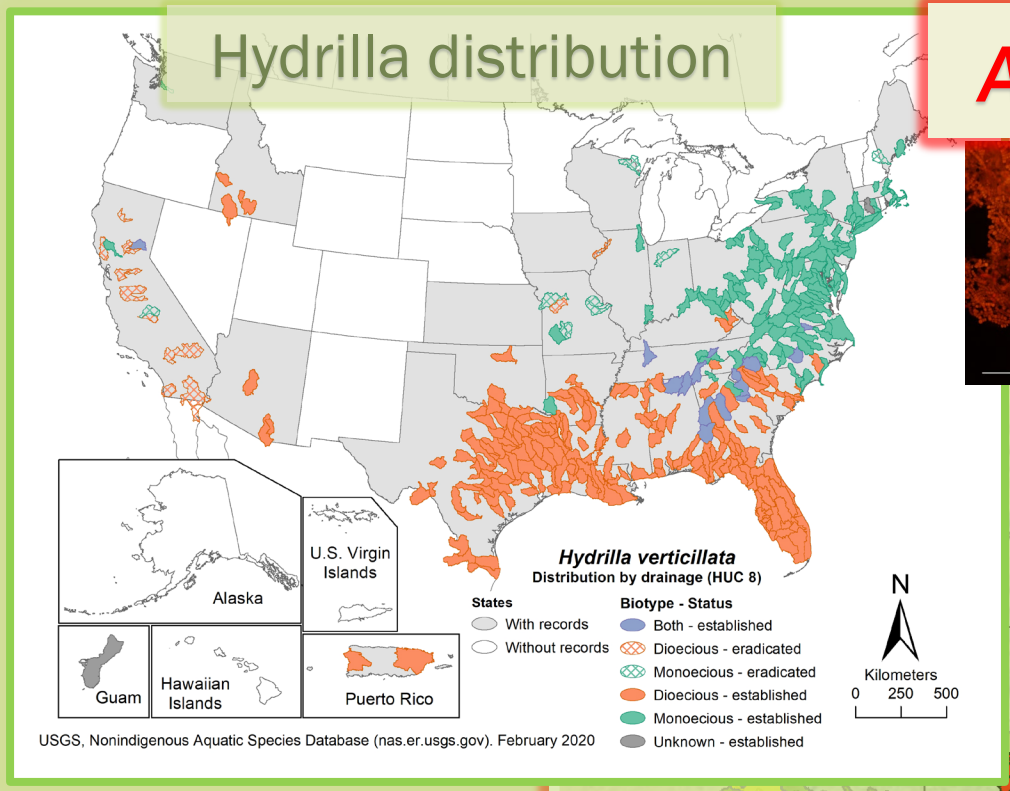
Wilde SB, Johansen JR, Wilde HD, Jiang P, Bartleme BA, Haynie RS. 2014. Aetokthonos hydrillicola gen. et sp. nov.: Epiphytic cyanobacteria associated with invasive aquatic plants and implicated in bird deaths from Avian Vacuolar Myelinopathy. Phytotaxa 181:243-260.



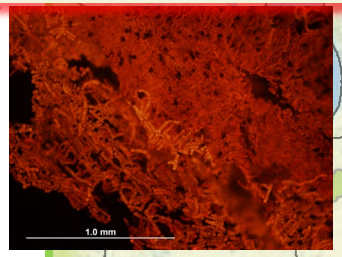




Hydrilla distribution



A. hydrillicola Distribution



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

Robert Vidéki, Doronicum Kft., Bugwood.org

N

Kilometers

0 125 250

Vacuolar Myelinopathy

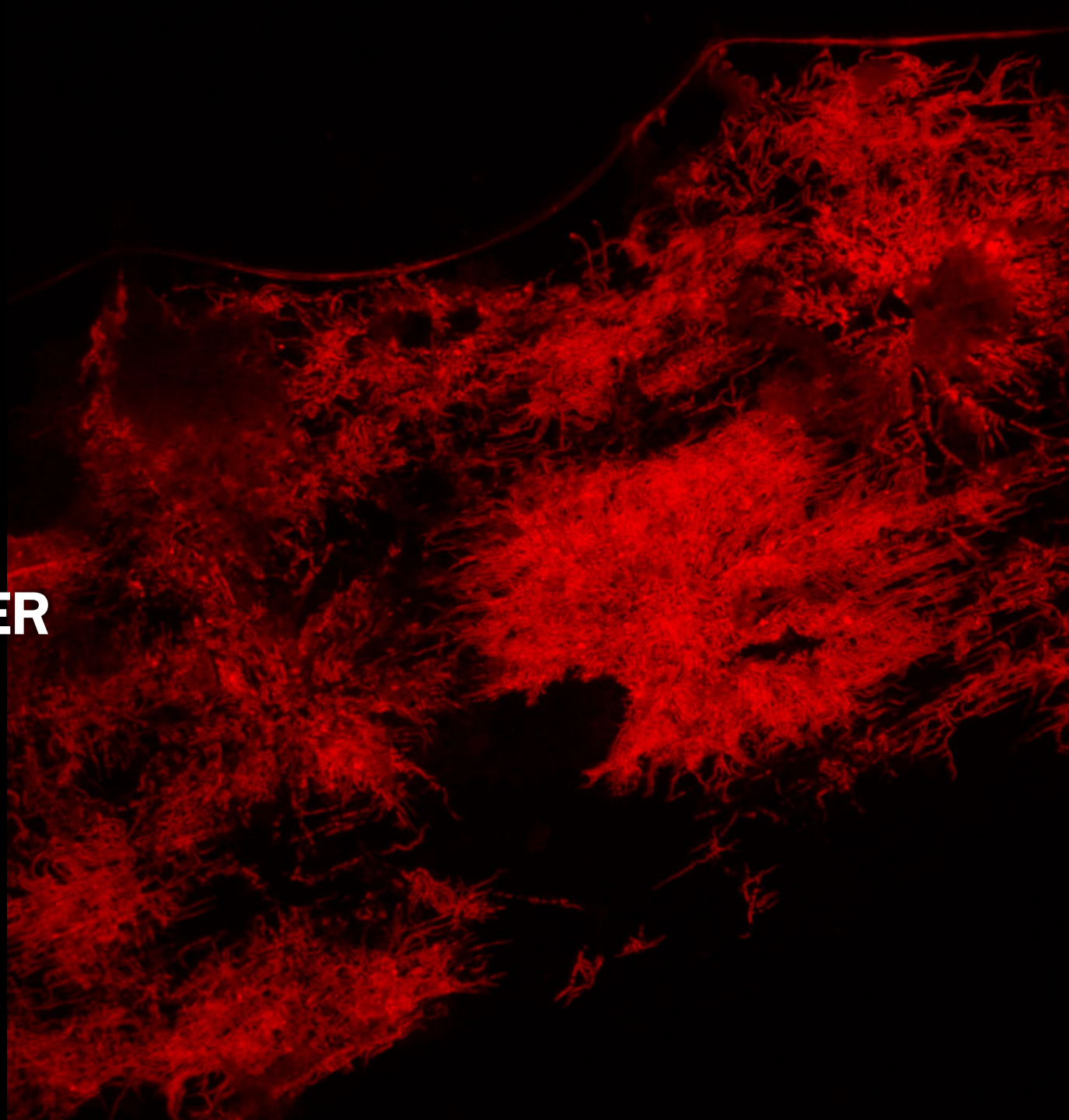
A. hydrillicola confirmed

A. hydrillicola not observed

Not sampled

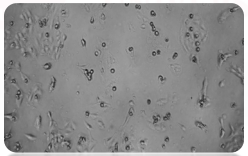
September 2019

EAGLE KILLER TOXIN



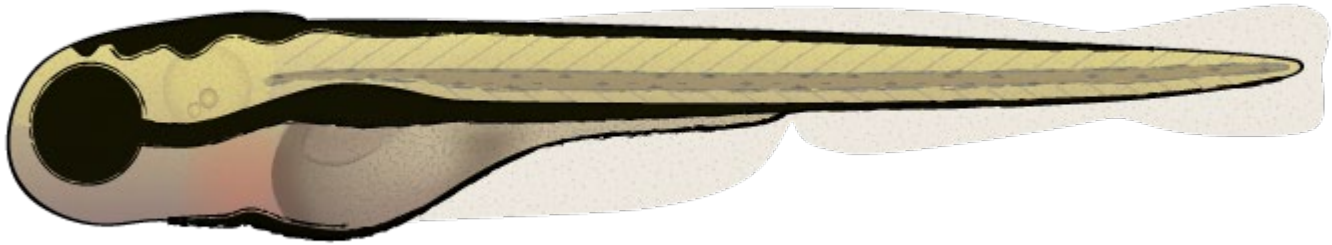
Sentinel Mallard: Untreated Reservoir Hydrilla/*Ah*

Bioassays for investigating VM Toxin

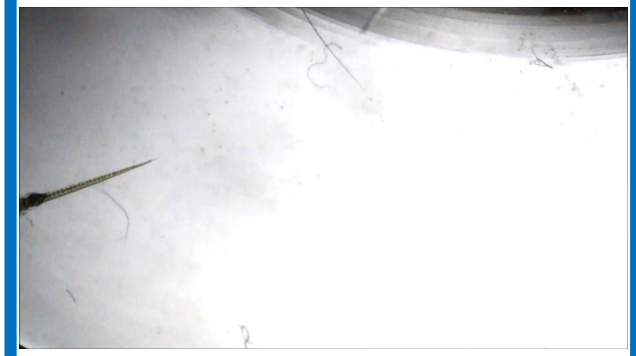


	Vertebrate **Avian Bioassay**	*Tissue Culture* **Cell line Bioassay**	*Invertebrate* ***C. dubia*** **bioassay**	*Vertebrate* **Larval Zebrafish Bioassay**
Positive VM Toxin				
Hydrilla + *A. hydrillicola* **J. S. Thurmond Nov 2015**	Positive +++	Positive +++	Positive +++	Positive +++
Hydrilla + *A. 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

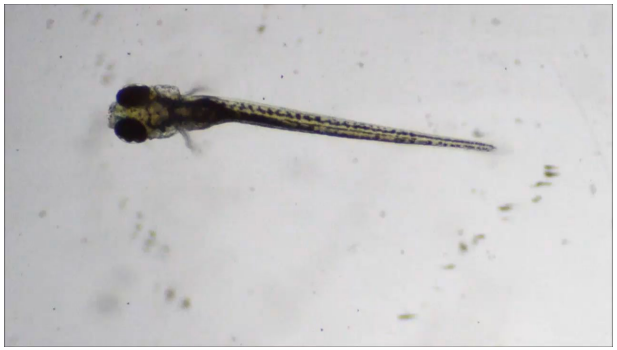
Zebrafish behavior after 24 hr exposure (7 days old)

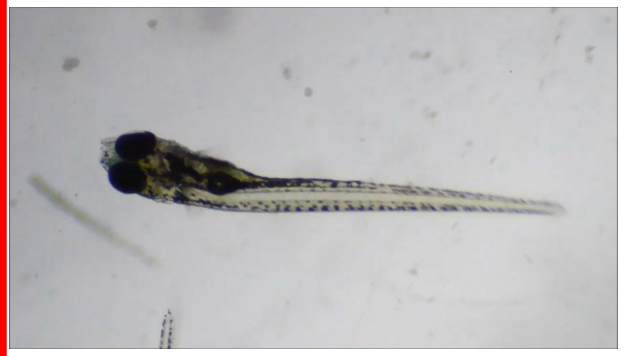


Solvent Control



VM toxin Exposed





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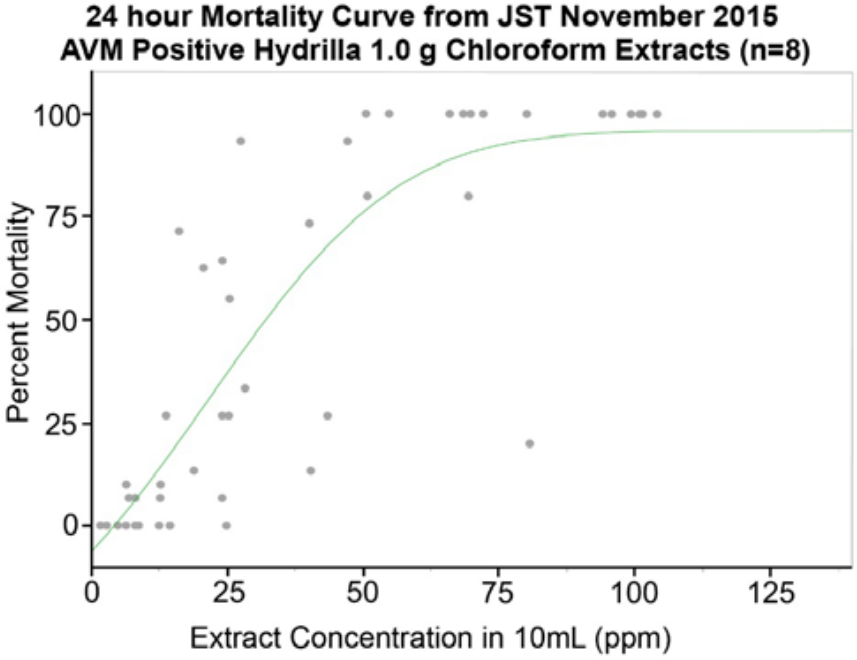
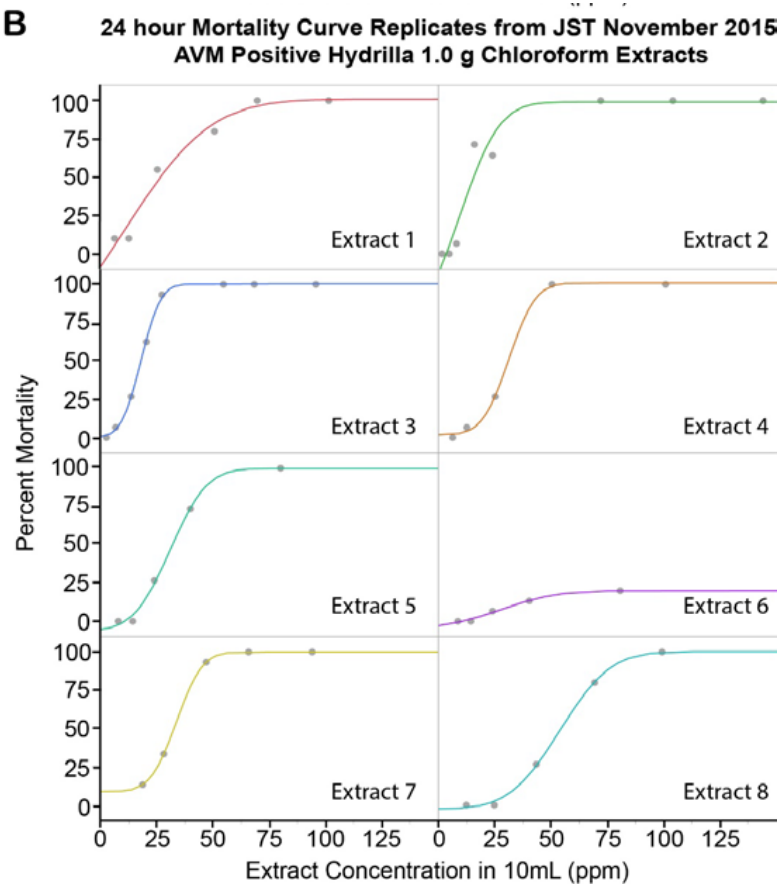
% Mortality Zebrafish Hydrilla/*A. hydrillicola* extracts

A

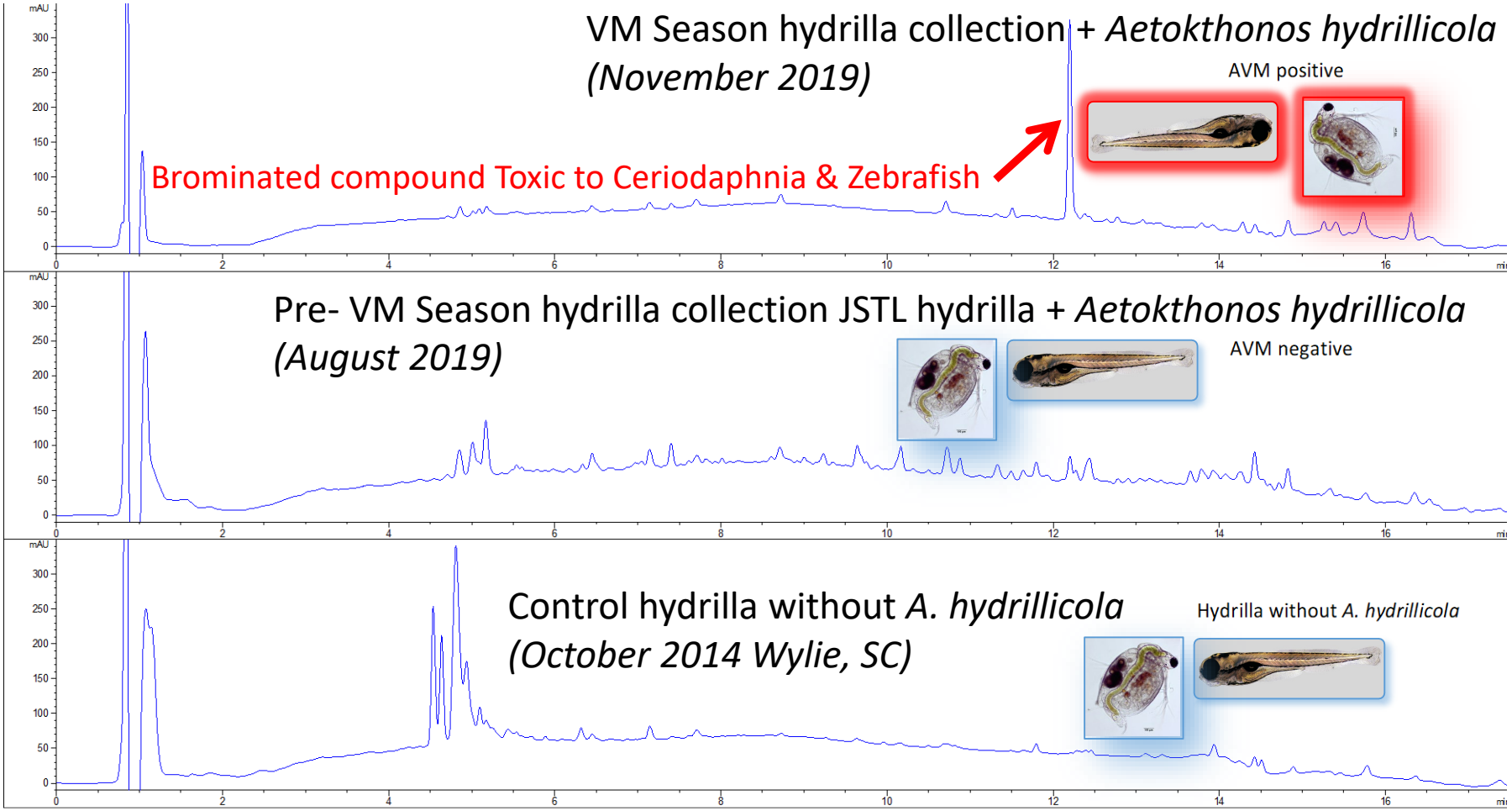


B



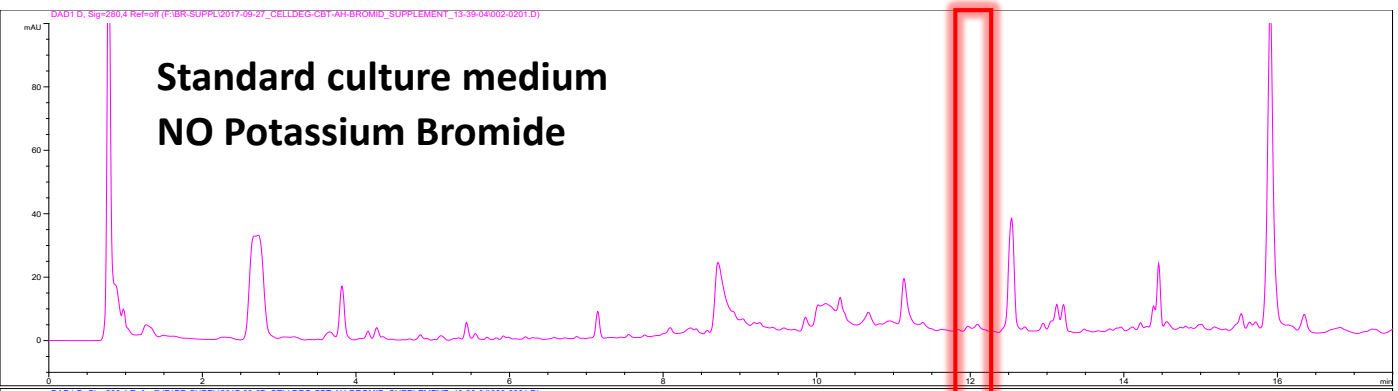
HPLC chromatograms (UV detection at 210 nm) methanol extracts

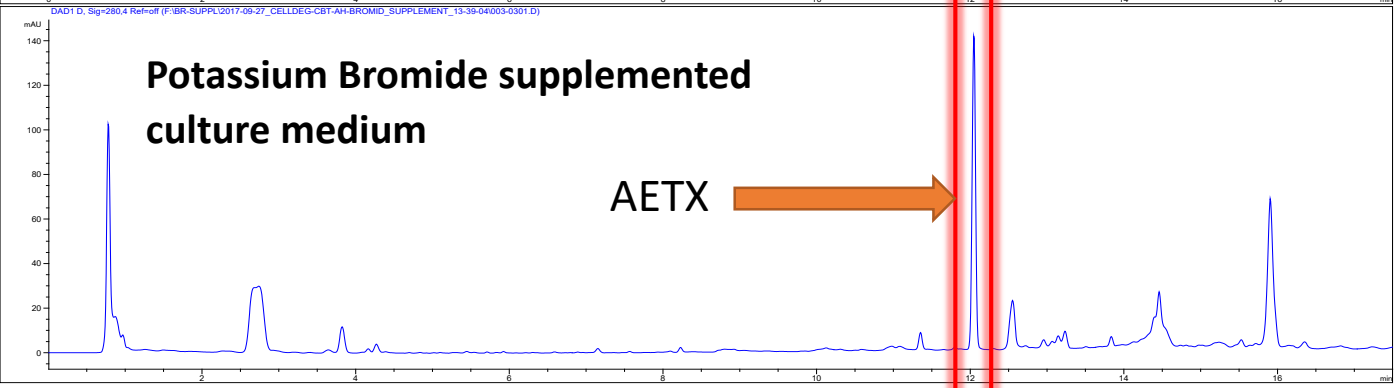
↗ unique brominated toxin shown in the top panel

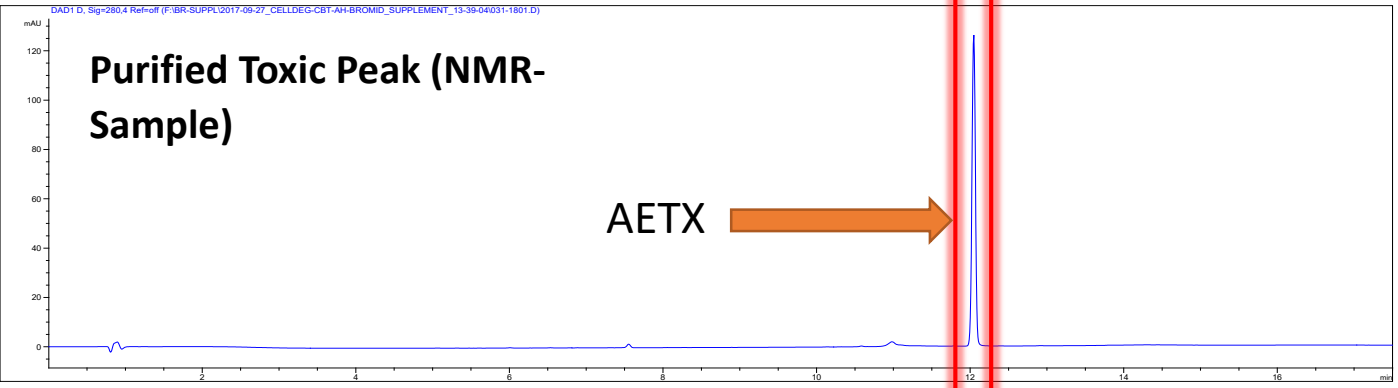


Aetokthonotoxin Production in Laboratory

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



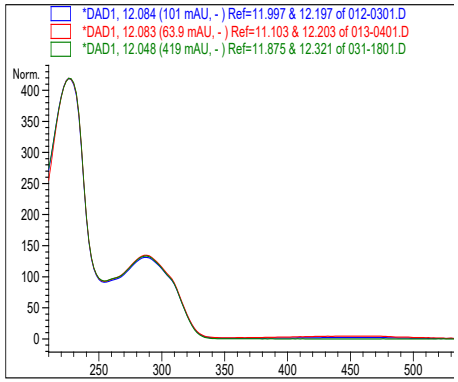




MARTIN-LUTHER
UNIVERSITÄT
HALLE-WITTENBERG

Dr. Timo Niedermeyer
PhD student
Steffen Breinlinger

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



Aetokthonotoxin in wild coot tissue

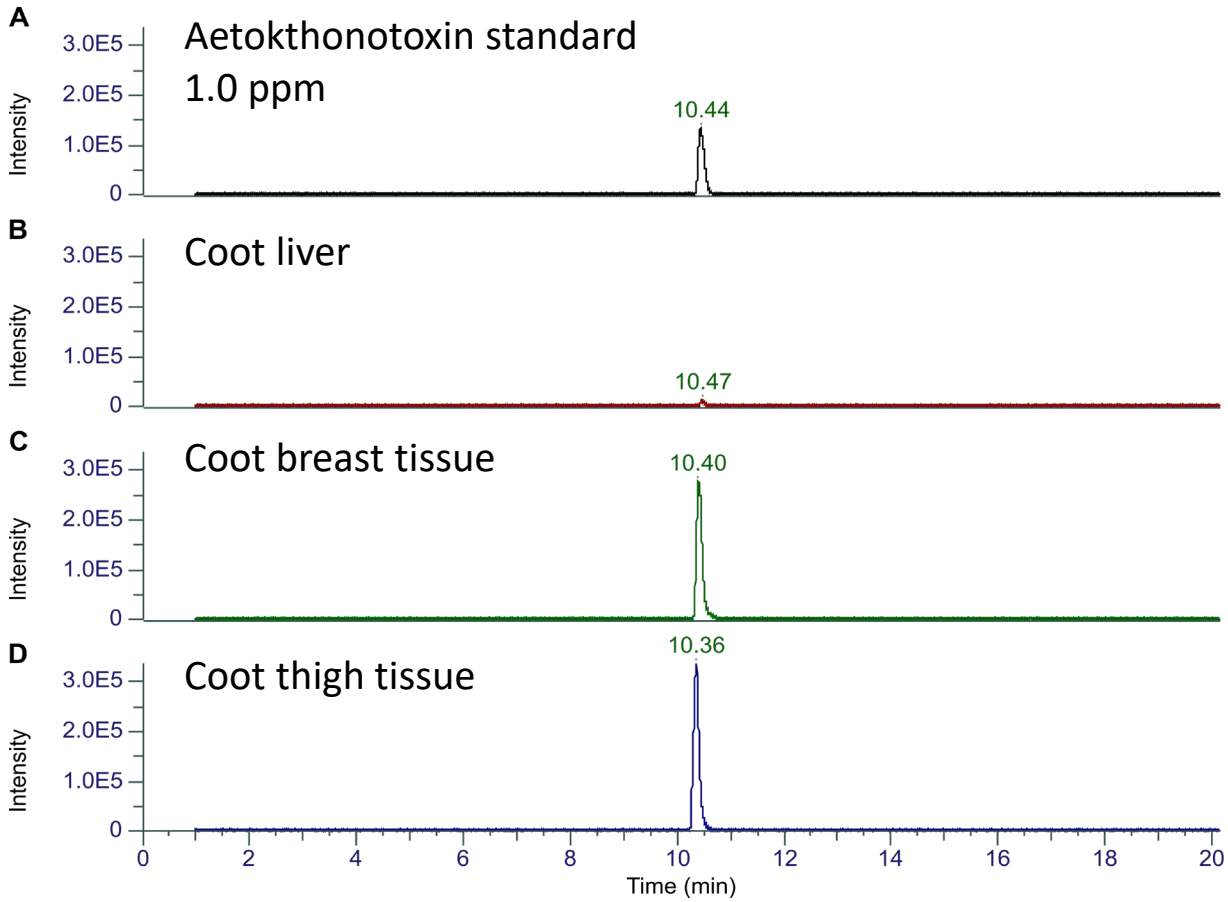
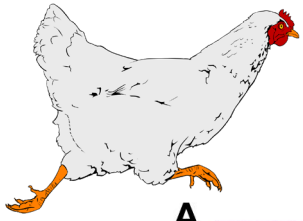
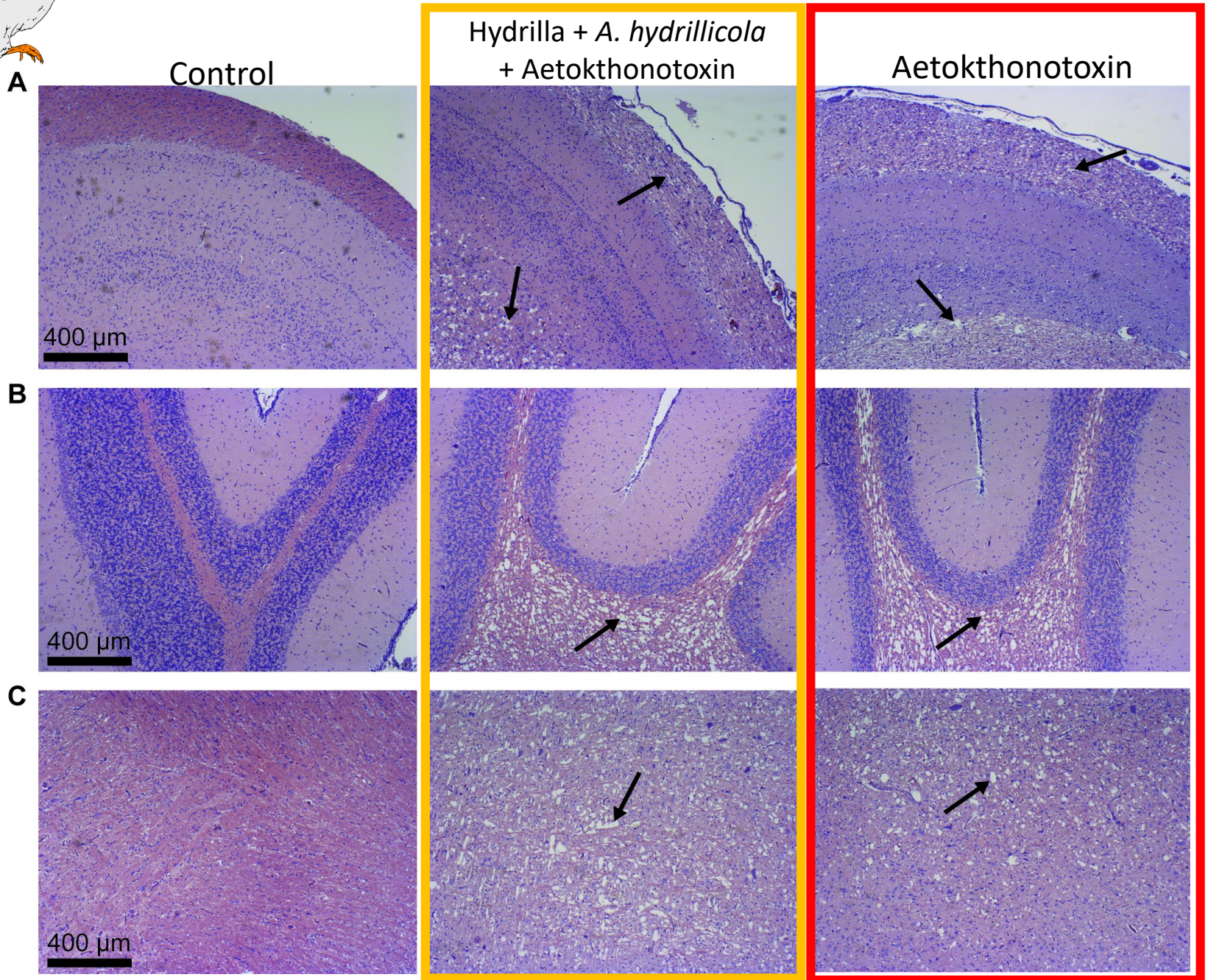


Fig. S5. Tissue screening for aetokthonotoxin (AETX) of wild American Coots (*Fulica americana)* succumbed to Vacuolar Myelinopathy (VM). A targeted mass spectrometry analysis (HPLC-SRM-MS) was carried out to selectively screen the tissues for AETX presence. Chromatograms show the SRM traces of most 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.

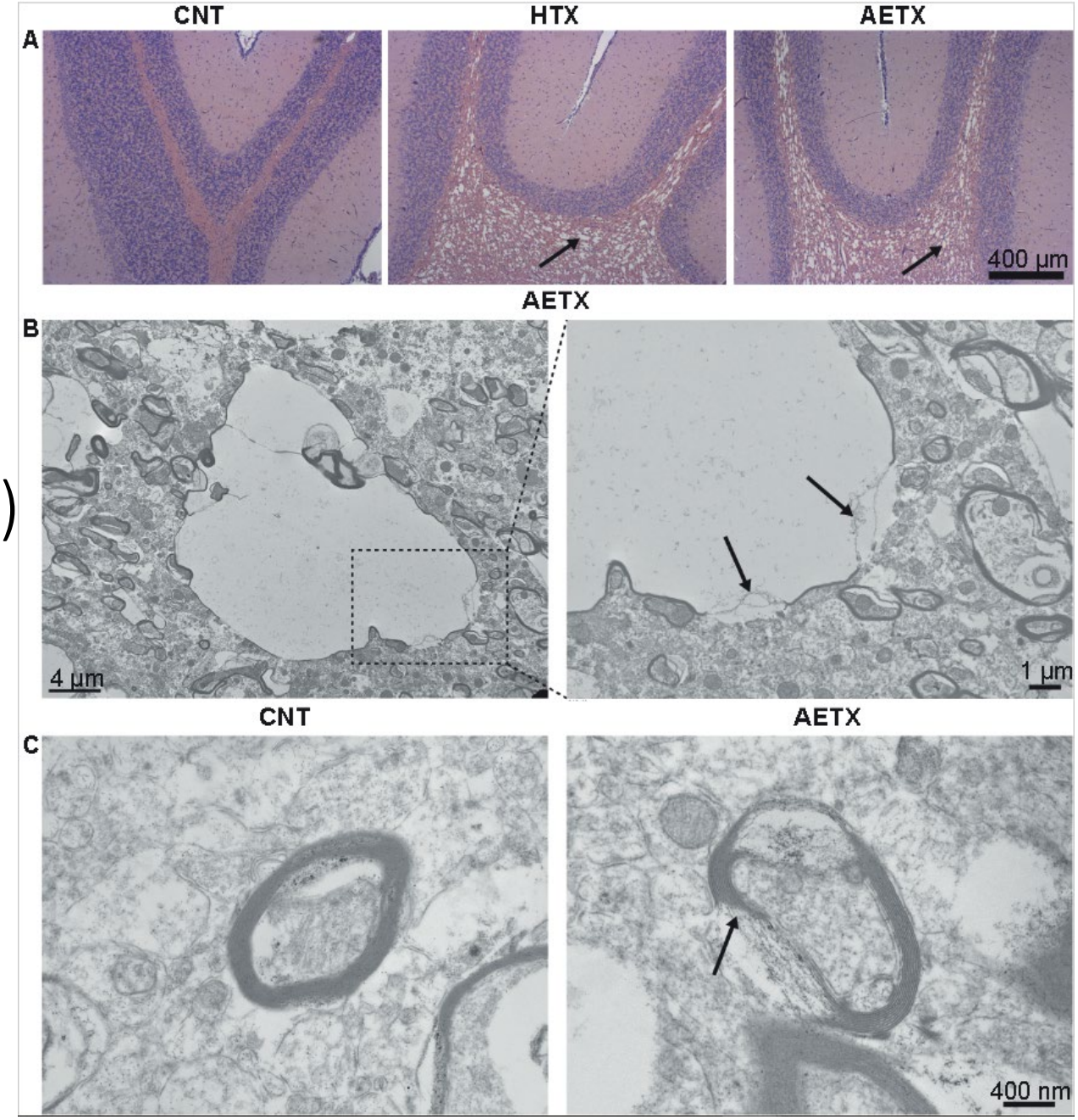
Breinlinger S, T Phillips, B Haram, J Mareš, JA Martínez Yerena, P Hrouzek, R Sobotka, WM Henderson, P Schmieder, SM Williams, JD Lauderdale, HD Wilde, W Gerrin, A Kust, C Wagner, B Geier, M Liebeke, H Enke, THJ Niedermeyer, SB Wilde. (submitted 10/29/20). Hunting down the eagle killer: A novel cyanobacterial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



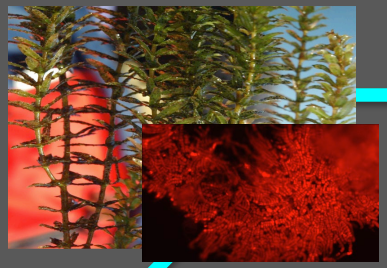
Chickens exposed to Aetokthonotoxin develop VM



Transmission Electron Microscopy(TEM) confirmed intramyelenic edema from AETX exposure



Expanding Food Chain



Fish

Salamanders

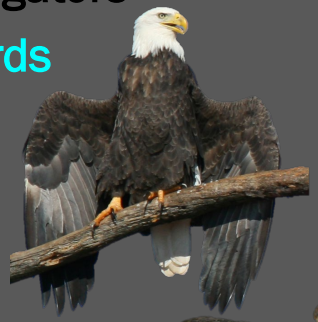
Frogs

Mammals

Turtles

Alligators

Birds



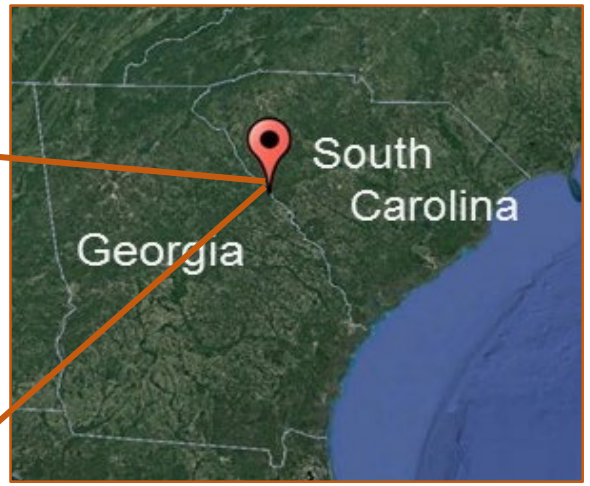
Snakes

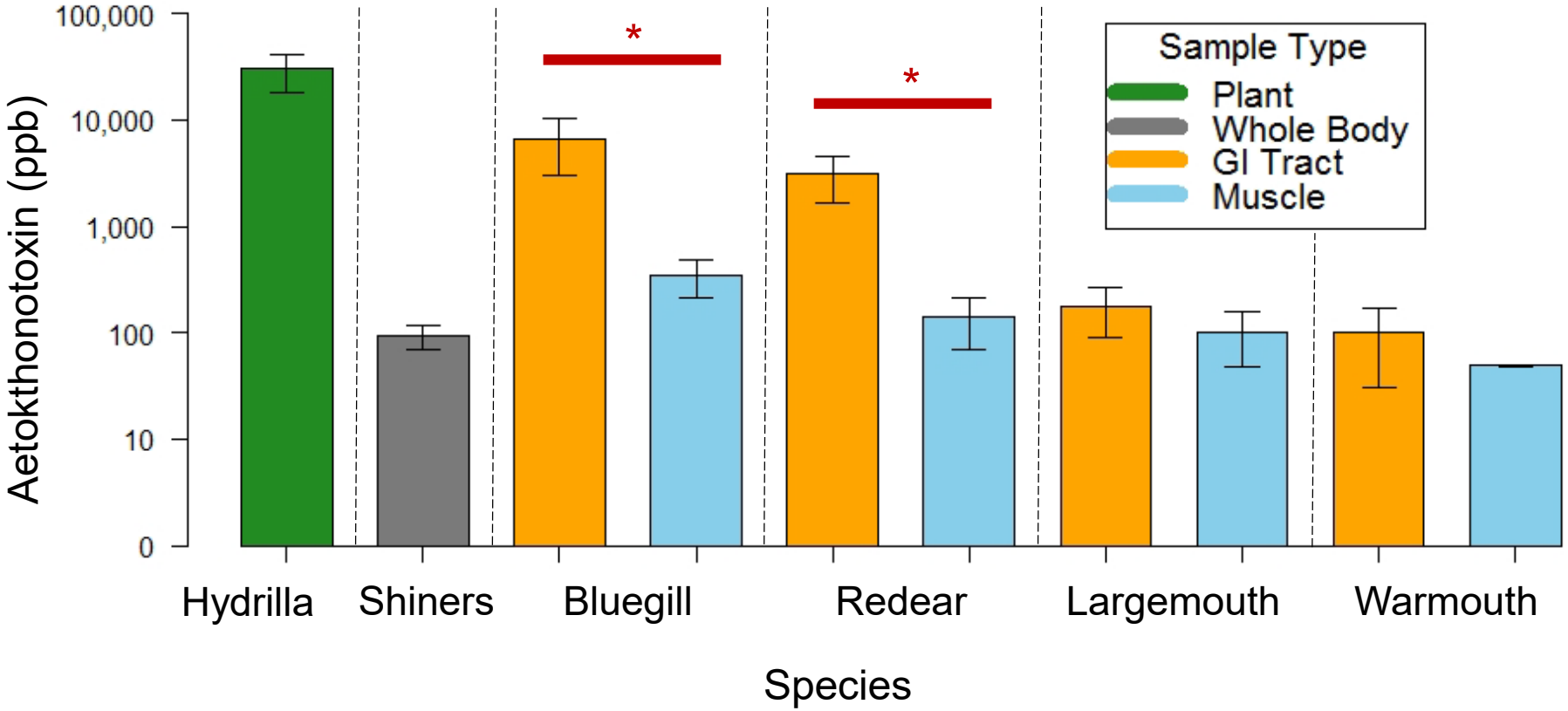
Diagram provided by
Dr. John C. Maerz

Aetokthonotoxin in wild fish tissue

The University of Georgia
WARNELL
SCHOOL OF FORESTRY & NATURAL RESOURCES

MS Thesis
Alex Pelletier

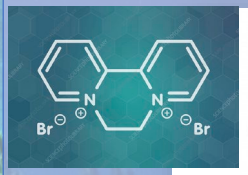


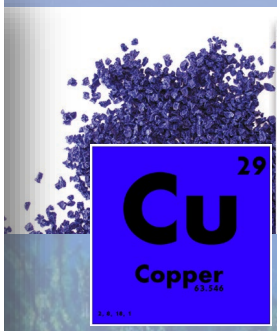


Management Solutions

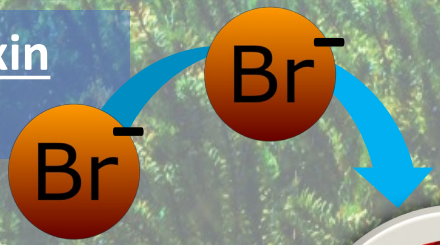
Biological

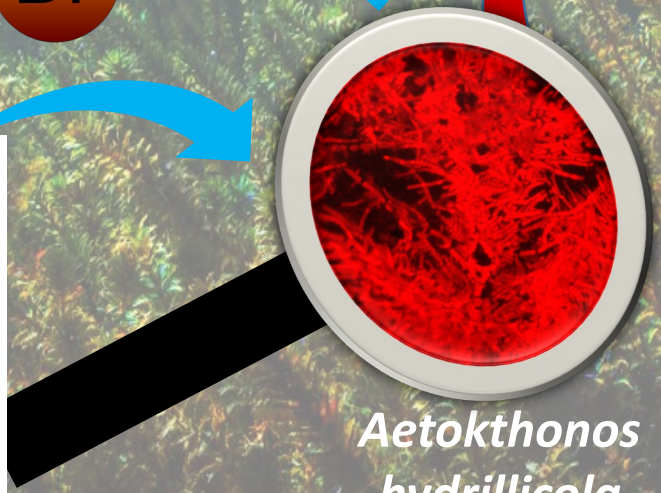
Chemical



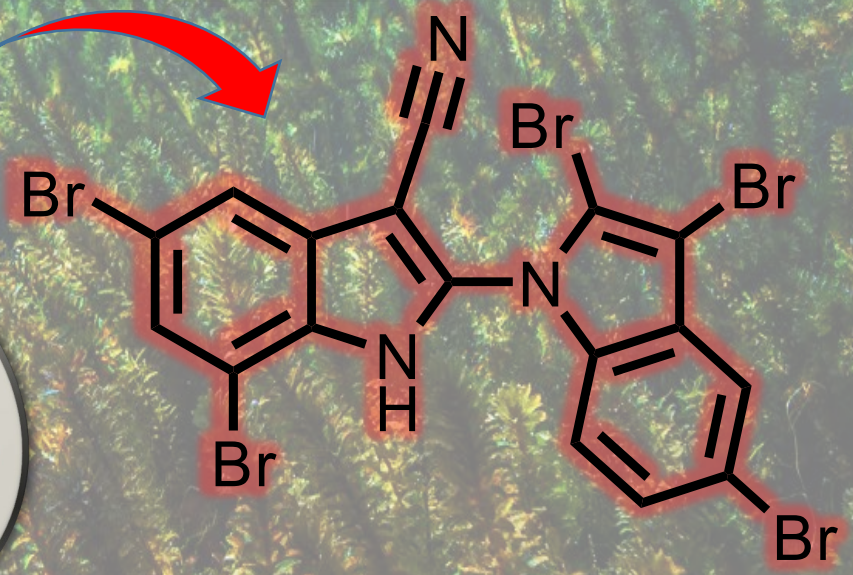


Needed for toxin production:



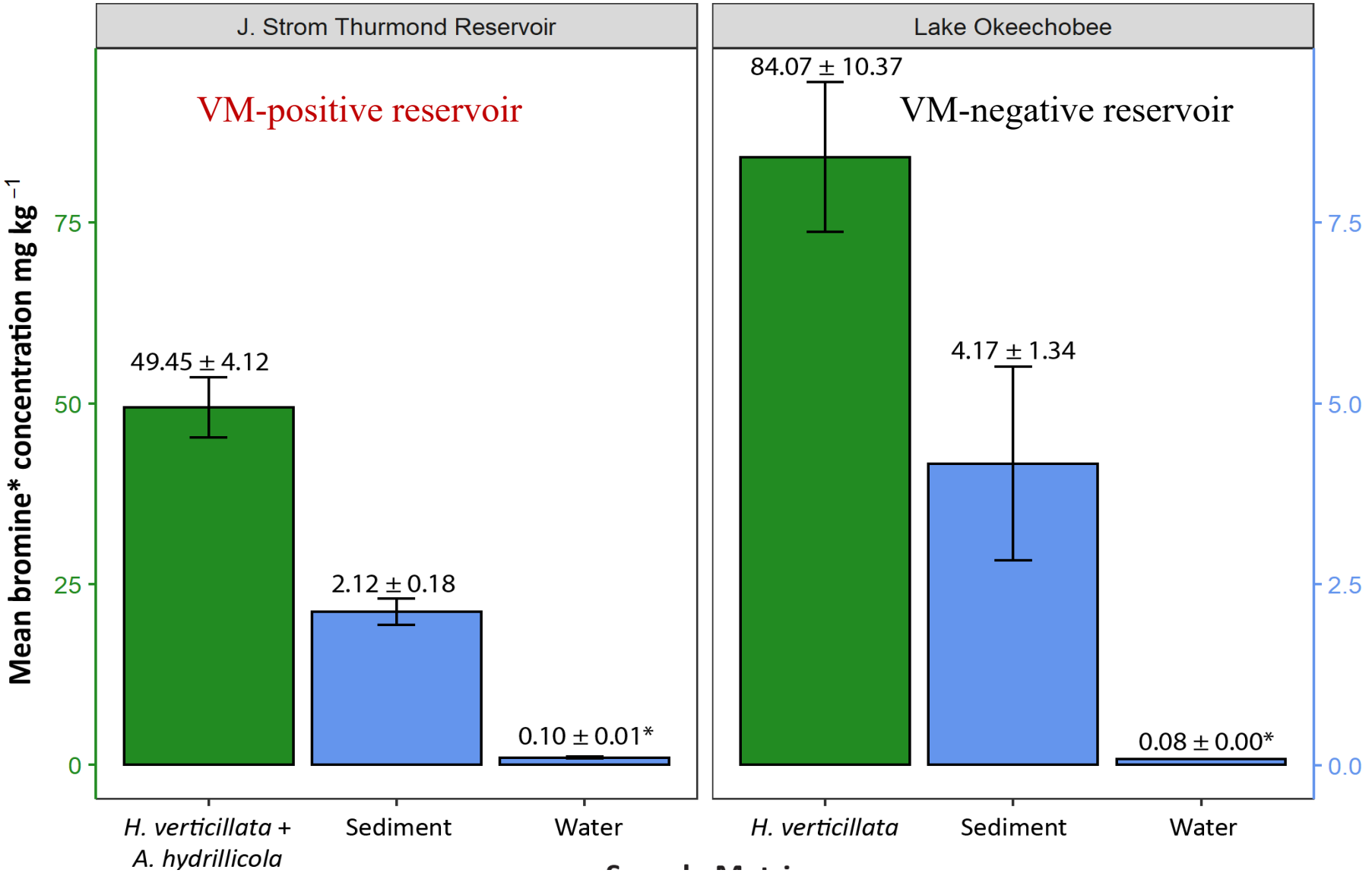


Aetokthonos hydrillicola



Aetokthonotoxin (AETX)

Source of Bromide
hydrilla >sediment>water



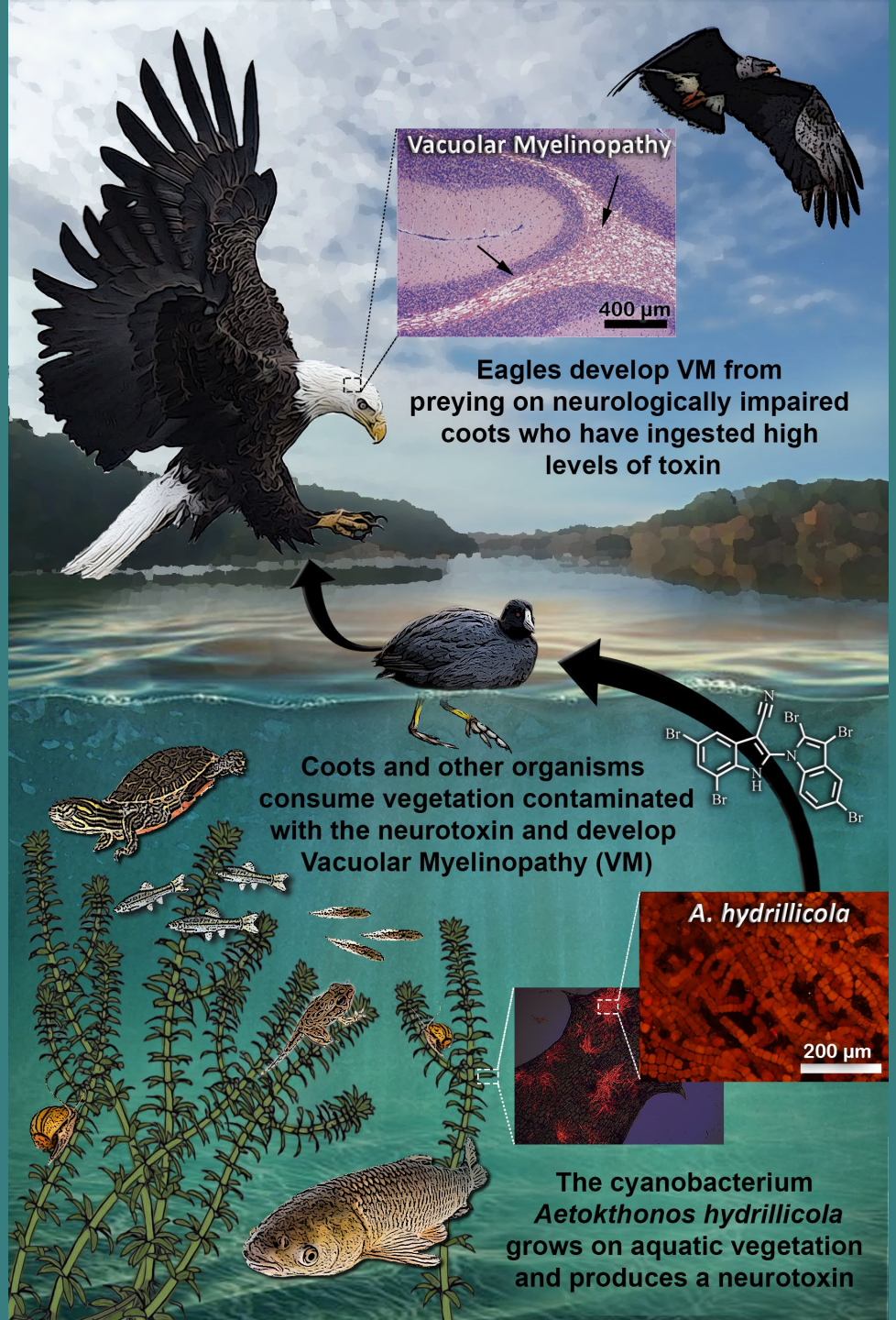
Wes Gerrin

Management solutions

Triploid Sterile Grass Carp

10-12” Fish, Field & Lab Trials

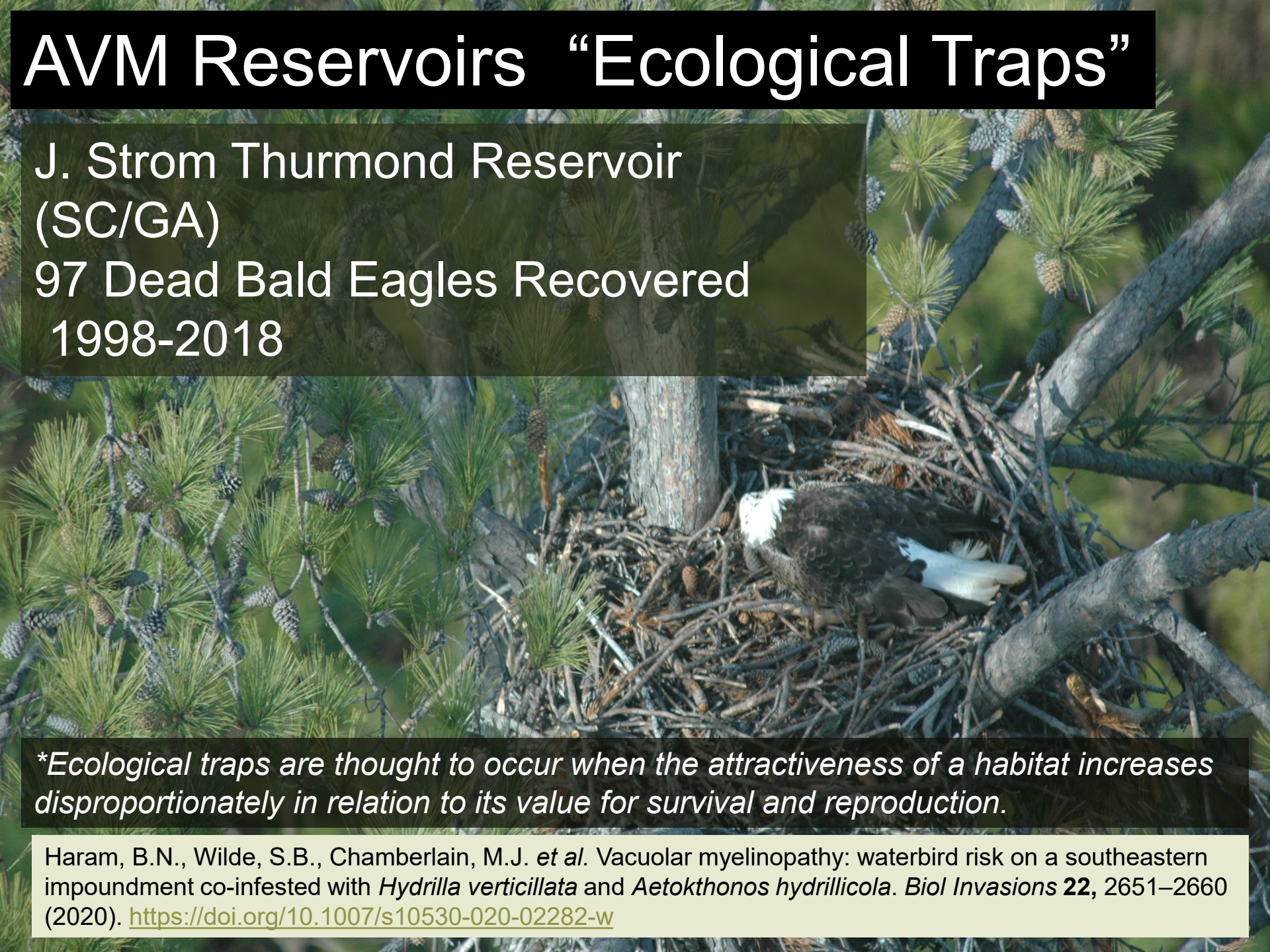
- Effective control of submerged aquatic plants
- Develop vacuolar lesions, but survive
- Did not induce lesions in birds



- Aetokthonotoxin (AETX) is a neurotoxic, lipophilic compound, not water soluble.
- Trophic transfer confirmed
- Accumulation of AETX in wild game and fish.

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

More questions…

Are game birds at risk?

Are aquatic mammals at risk?

Are fish and waterfowl living in these reservoirs safe to consume?

Are hunters and anglers at risk?

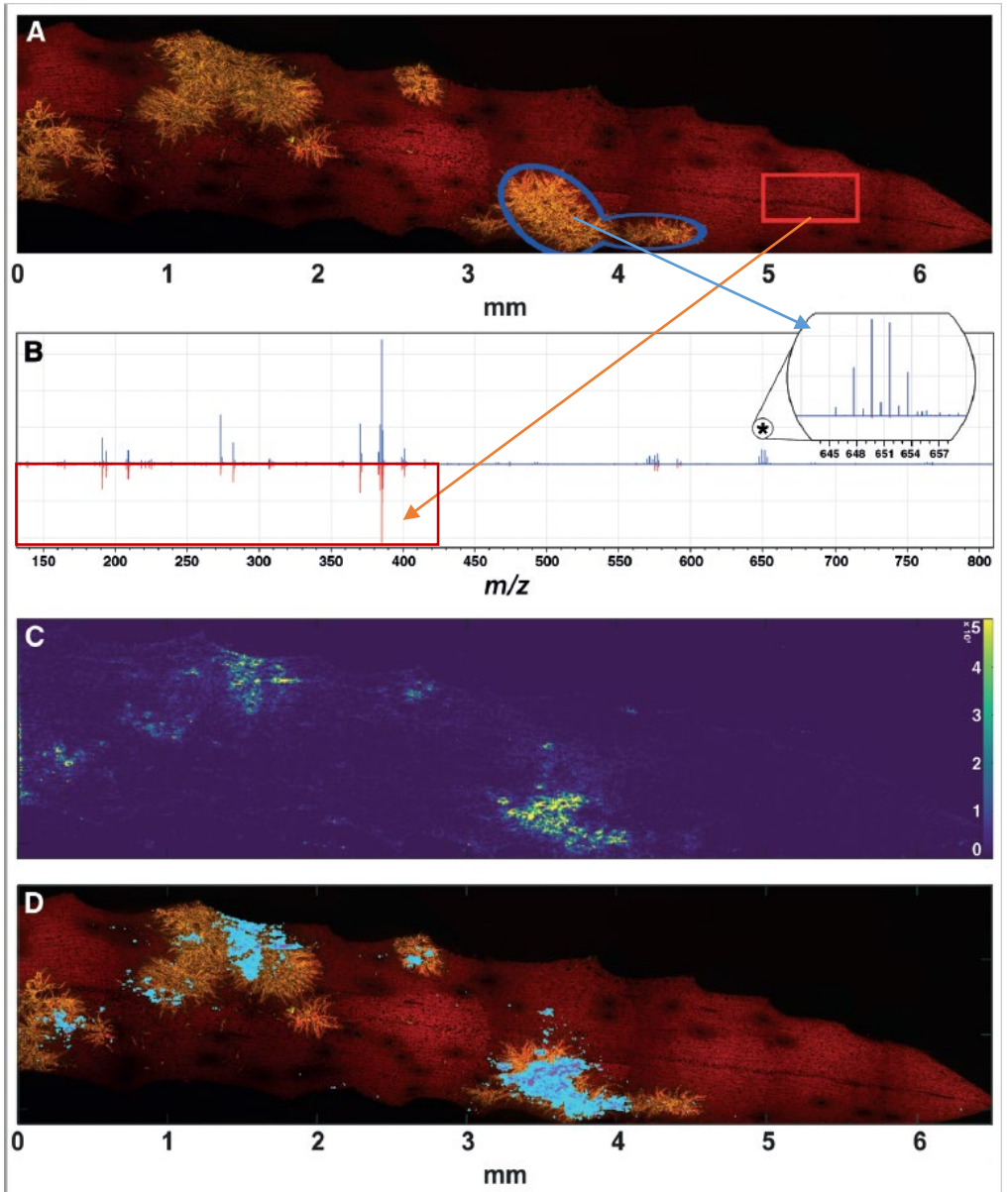
AP-MALDI-MSI of *A. hydrillicola* colonies growing on *H. verticillata* reveals a cyanobacterium-specific metabolite

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

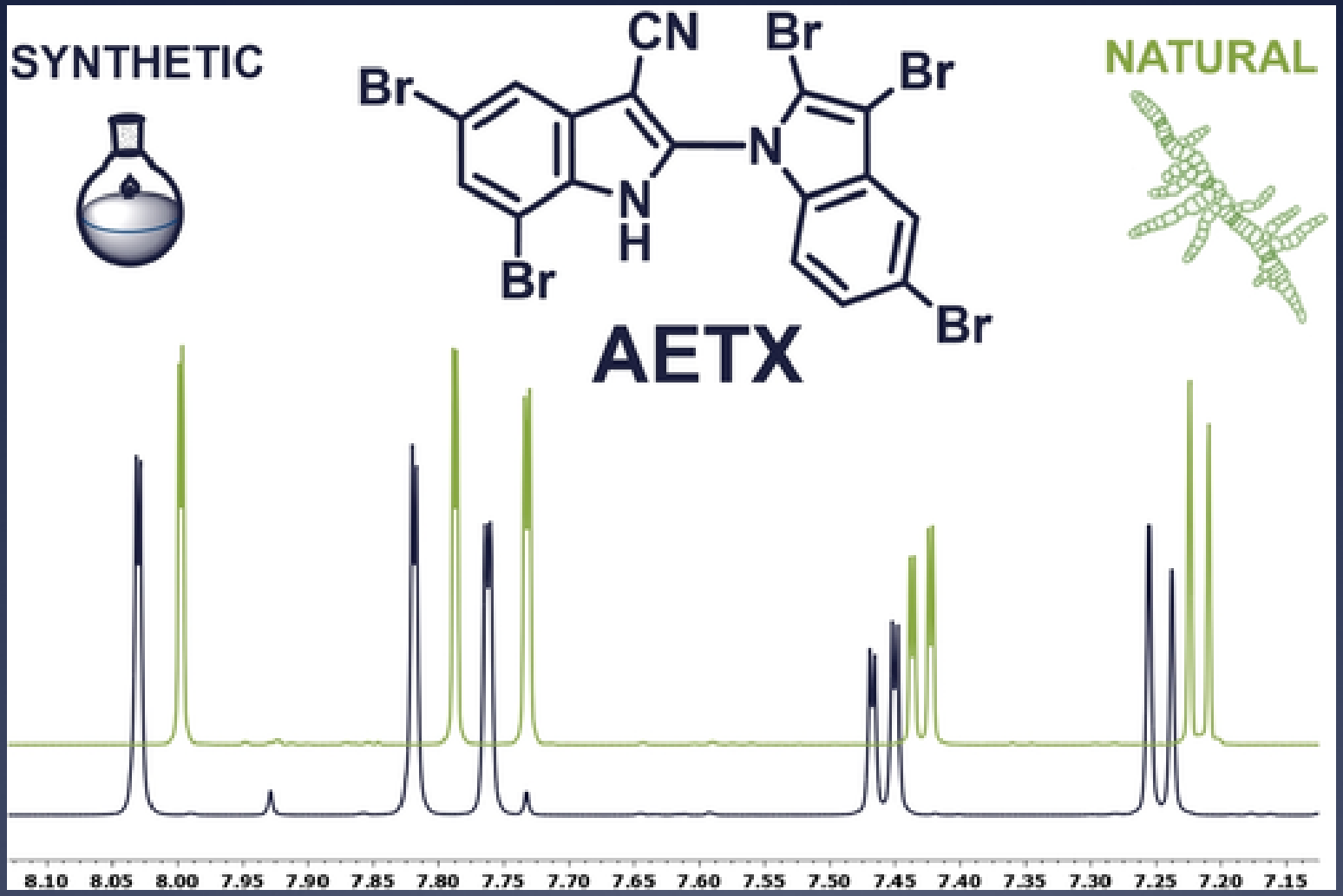
(**B**) Blue outline region -- pentabrominated metabolite associated with the cyanobacterial colony.

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

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



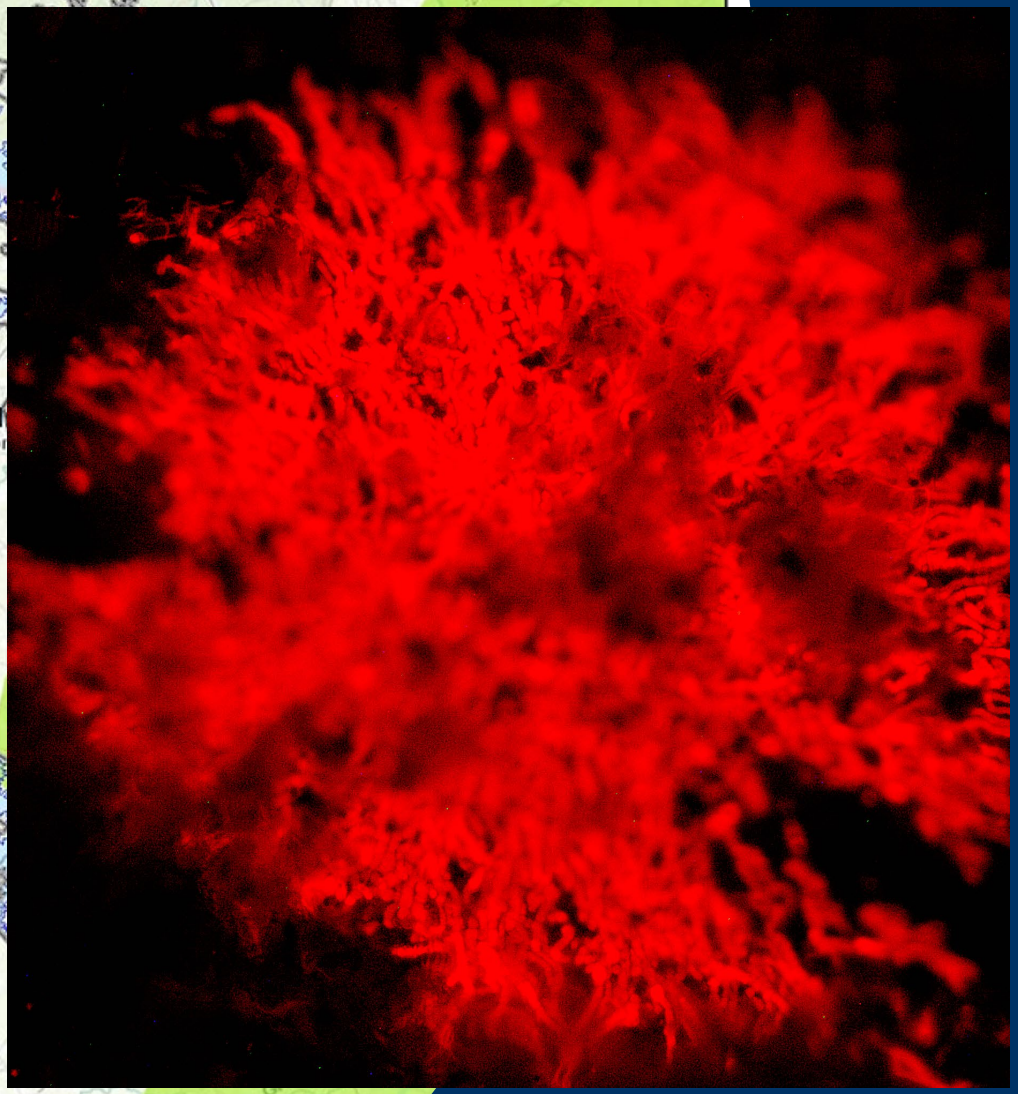
Total Synthesis of Aetokthonotoxin, the Cyanobacterial Neurotoxin Causing Vacuolar Myelinopathy (2021).



Heterocyclic chemistry:
Synthesizing the eagle killer will enable future research.
Total synthesis of this natural product was achieved in only five steps.

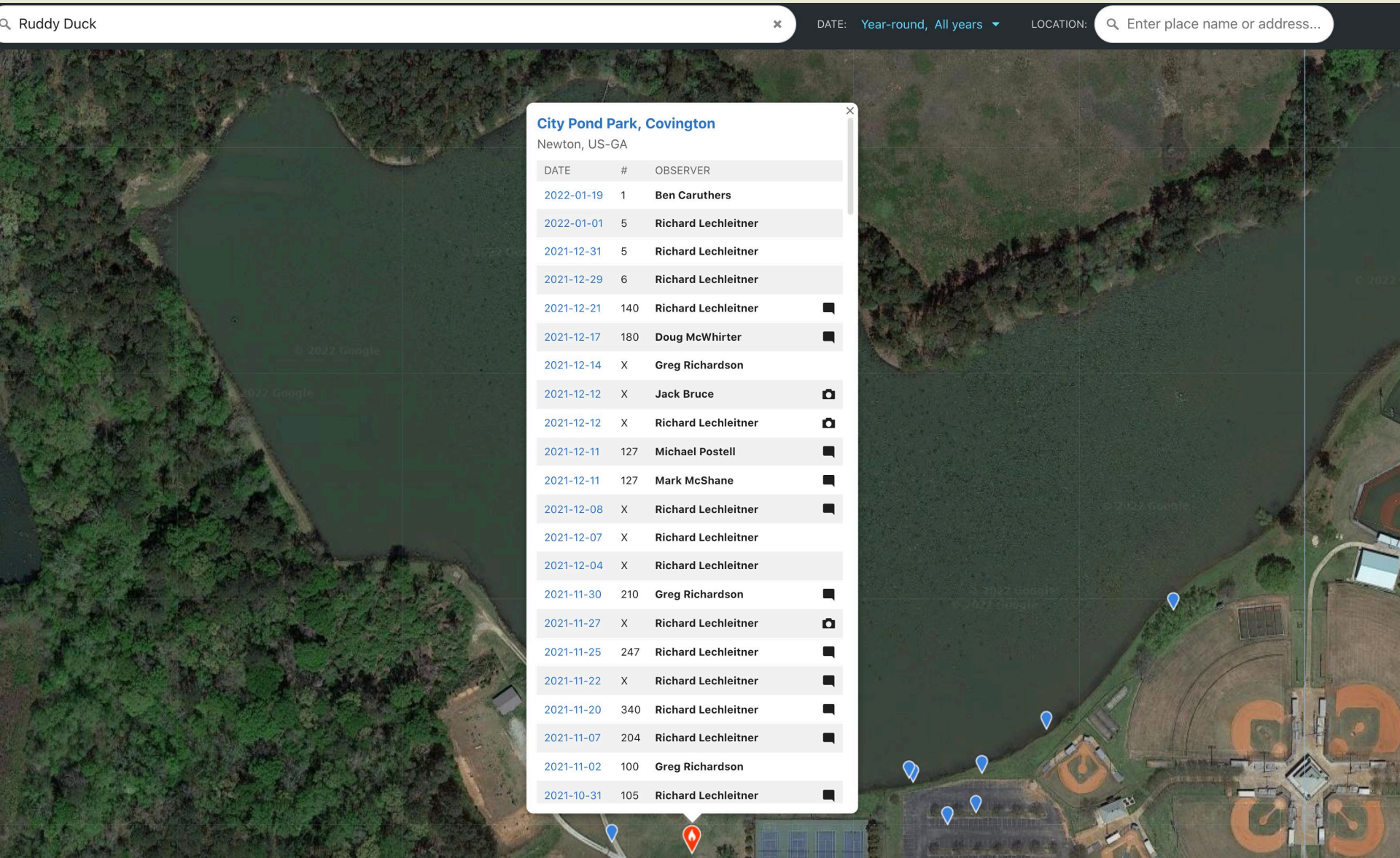
Additional substrates that *Aetokthonos* likes to grow on

- Pine needles
  - Especially Loblolly pine
- Water willow
  - Native, cultivated at JST, planted for fish & wildlife habitat



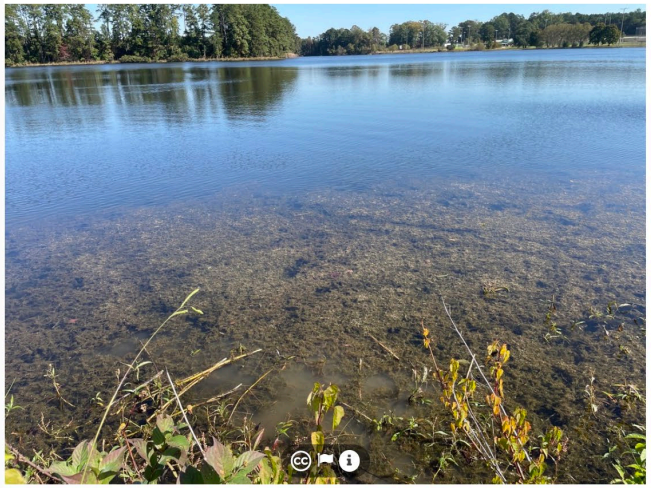
Legend
Historic Longleaf Pine Range
JST Property Boundary
Lincolnton Rd
Thomson Hwy
890 ft
Rocky Creek
COLUMBIA
Sources: Esri, HERE, Garmin
FAO, NPS, NRCAN, GeoBa

Ebird Covington Pond



Hydrilla (*Hydrilla verticillata*) Research Grade

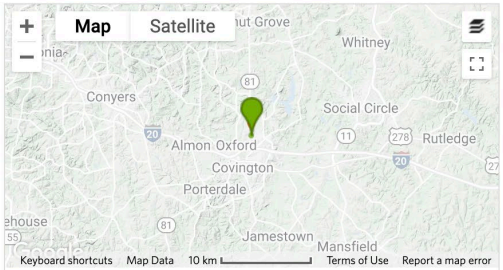
Follow



susanbwilde
10 observations

Observed:
Oct 22, 2021 · 12:47 PM EDT

Submitted:
Feb 23, 2022 · 1:27 PM EST



Covington, GA, USA Details

Activity

susanbwilde suggested an ID — Improving 4d

Hydrilla
Hydrilla verticillata

mjwarman suggested an ID 4d

Community Taxon

What's this?

Hydrilla (*Hydrilla verticillata*)
Cumulative IDs: 3 of 3

0 **2/3rds** 3

Agree About

Acknowledgements

The University of Georgia WARNELL SCHOOL OF FORESTRY & NATURAL RESOURCES

Wilde Lab UGA

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Gulf States Marine Fisheries Commission