Triple threat: Hydrilla and Aetokthonos + Bromide, More than an "eagle killer" toxin

Susan B. Wilde, Seth McWhorter, Matthew Henderson, Jeffrey Cullen, Cassidy Brown, Erika Klar, Ryann Heninger, Tobias Haymes, Katherine Callaghan, and Tabitha Hortenstine

Warnell School of Forestry & Natural Resources
UNIVERSITY OF GEORGIA

FLORIDA FISH AND WILDLIFE CONSERVATION COMMISSION

Vacuolar Myelinopathy (VM)

1994-2025 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. Aquatic mammals and Human health concerns

Science

$15
26 MARCH 2021
sciencemag.org

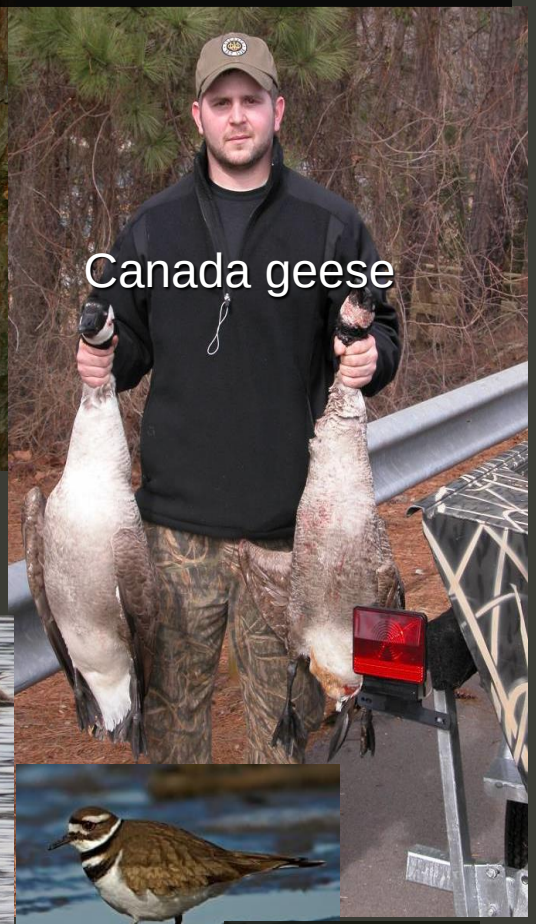
AAAS

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

DEADLY CASCADE

Bird species with AVM brain lesions

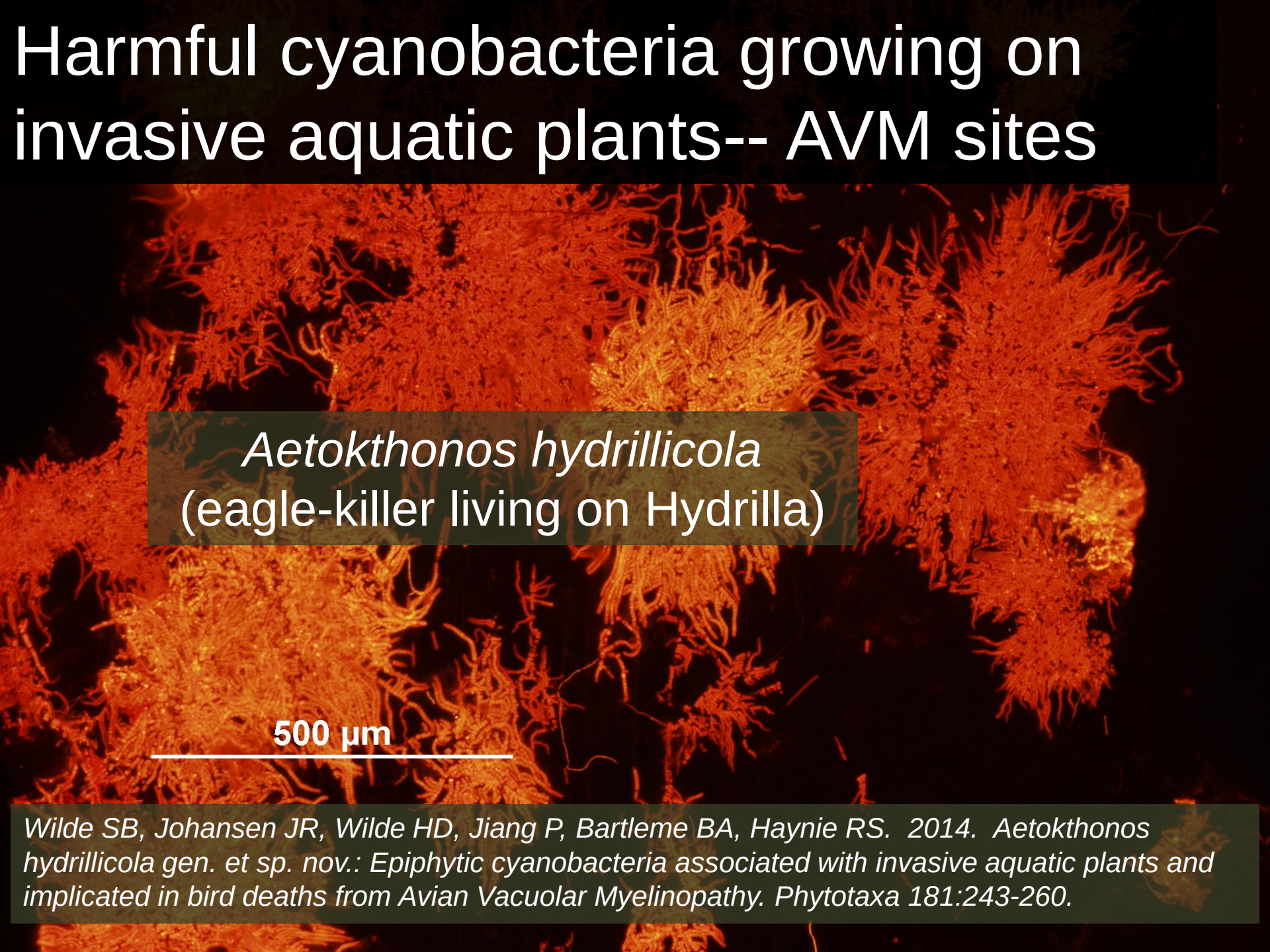
Mallards, Ring-necked ducks
Buffleheads, American wigeon



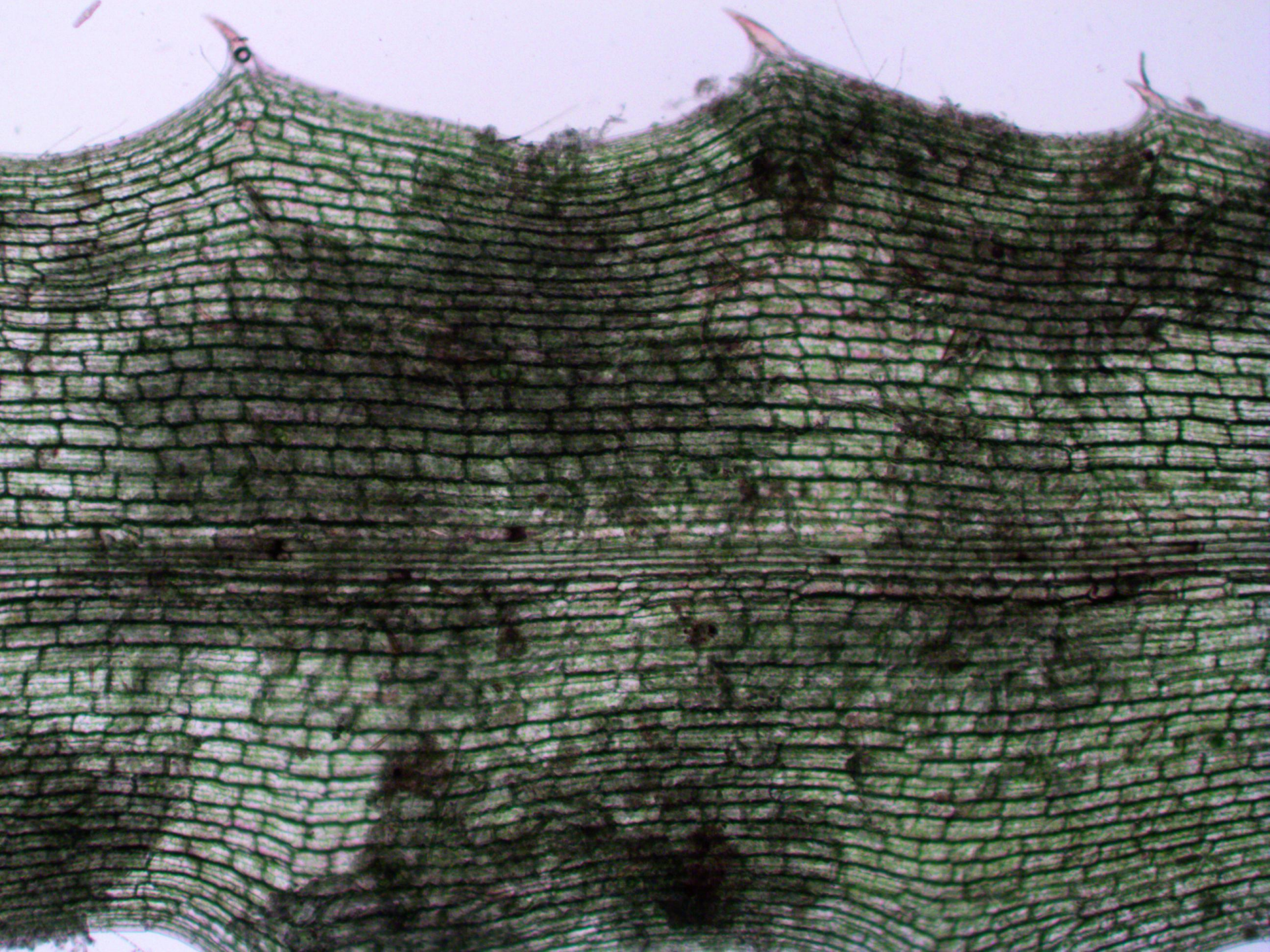
Killdeer

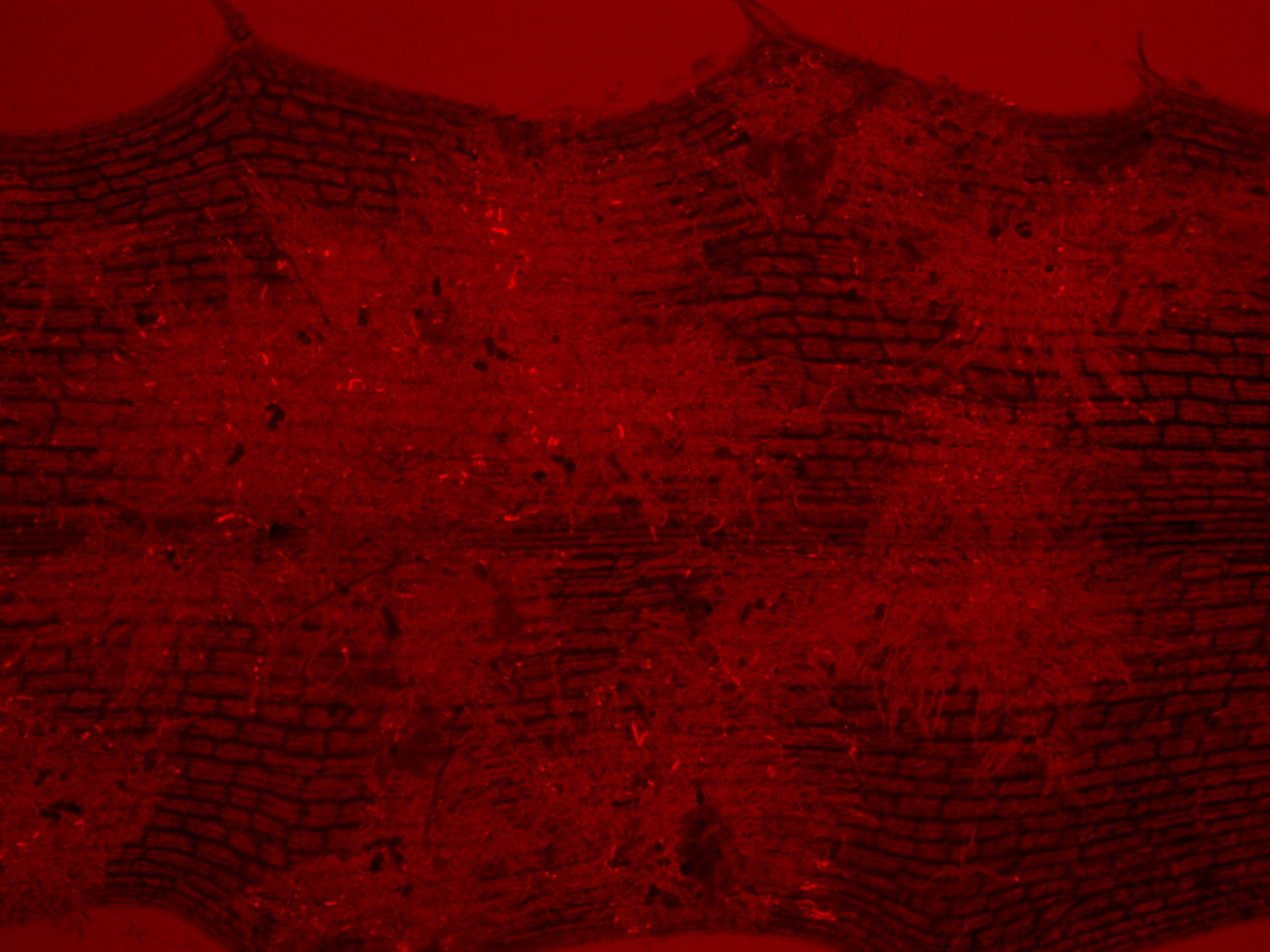
Augspurger, T, JR Fischer, NJ Thomas, L Sileo, RE Brannian, KJG Miller, and TE Rocke. 2003. Vacuolar myelinopathy in waterfowl from a North Carolina impoundment. JWD 39:412-417.
Fischer, J, LA Lewis-Weis, CM Tate, JK Gaydos, RW Gerhold, RH Poppenga. 2006. Avian vacuolar myelinopathy outbreaks at a southeastern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

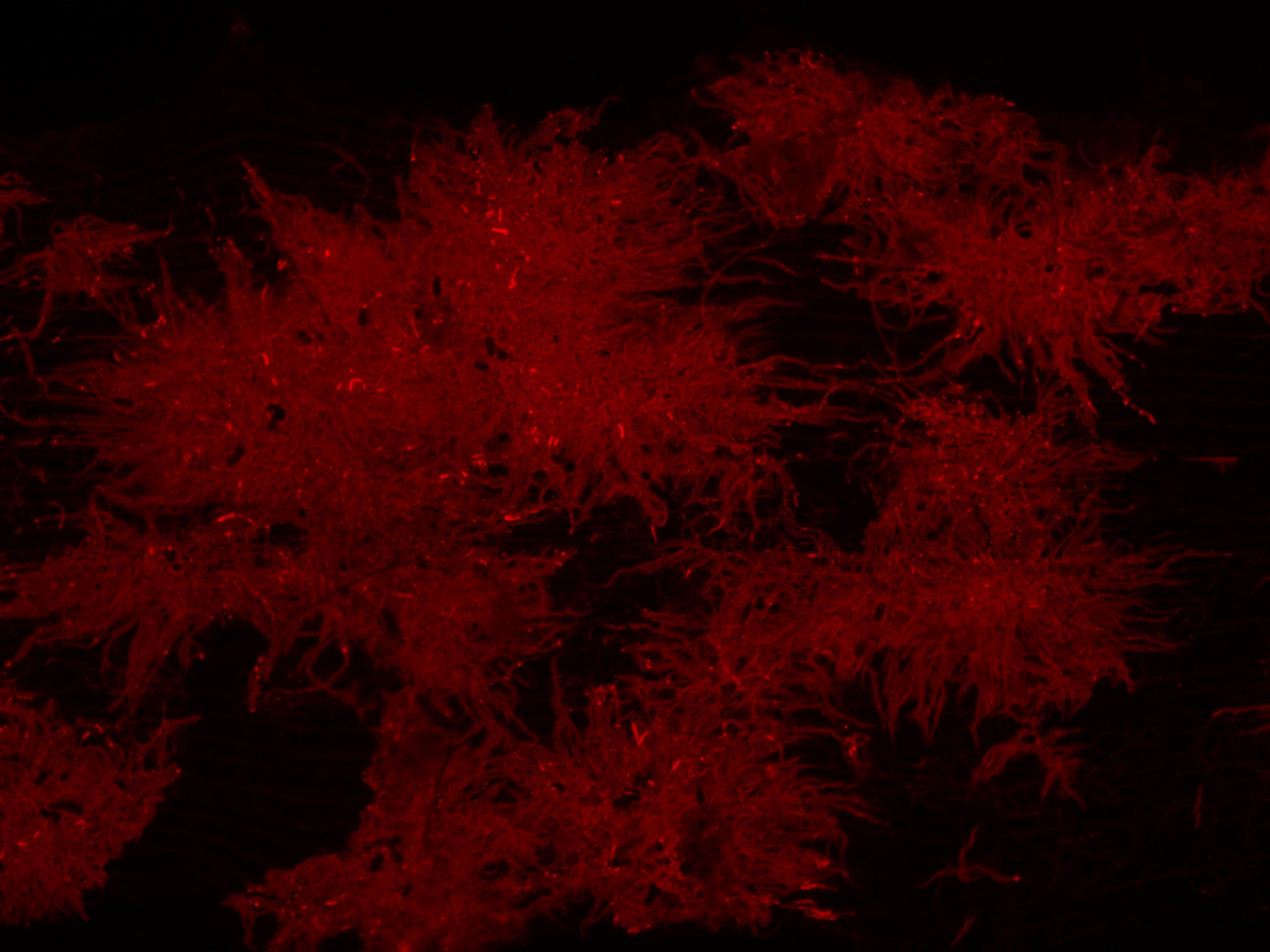
Harmful cyanobacteria growing on invasive aquatic plants-- AVM sites



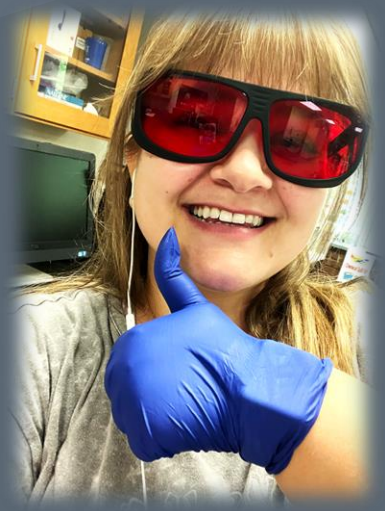
Wilde SB, Johansen JR, Wilde HD, Jiang P, Bartleme BA, Haynie RS. 2014. Aetokthonos hydrillicola gen. et sp. nov.: Epiphytic cyanobacteria associated with invasive aquatic plants and implicated in bird deaths from Avian Vacuolar Myelinopathy. Phytotaxa 181:243-260.







Visual Screening



NIGHTSEA Xite Fluorescent Flashlight system emitting green light with red barrier glasses

Amscope epifluorescent light microscope with filter to visualize phycocyanin pigments

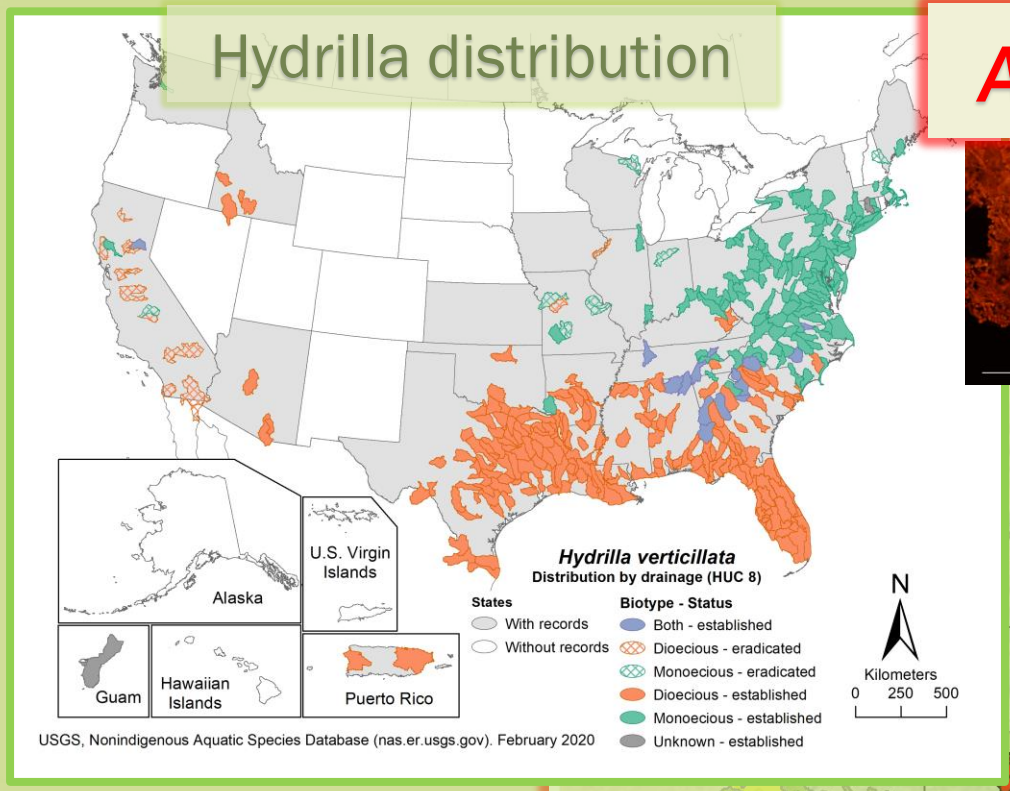
Expanding AETX Food Web

Br

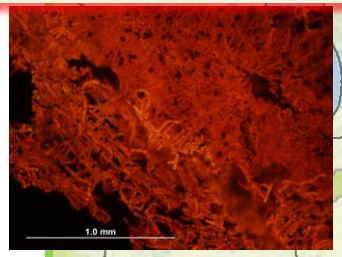
Snakes

Diagram provided by Dr. John C. Maerz

Hydrilla distribution



A. hydrillicola Distribution



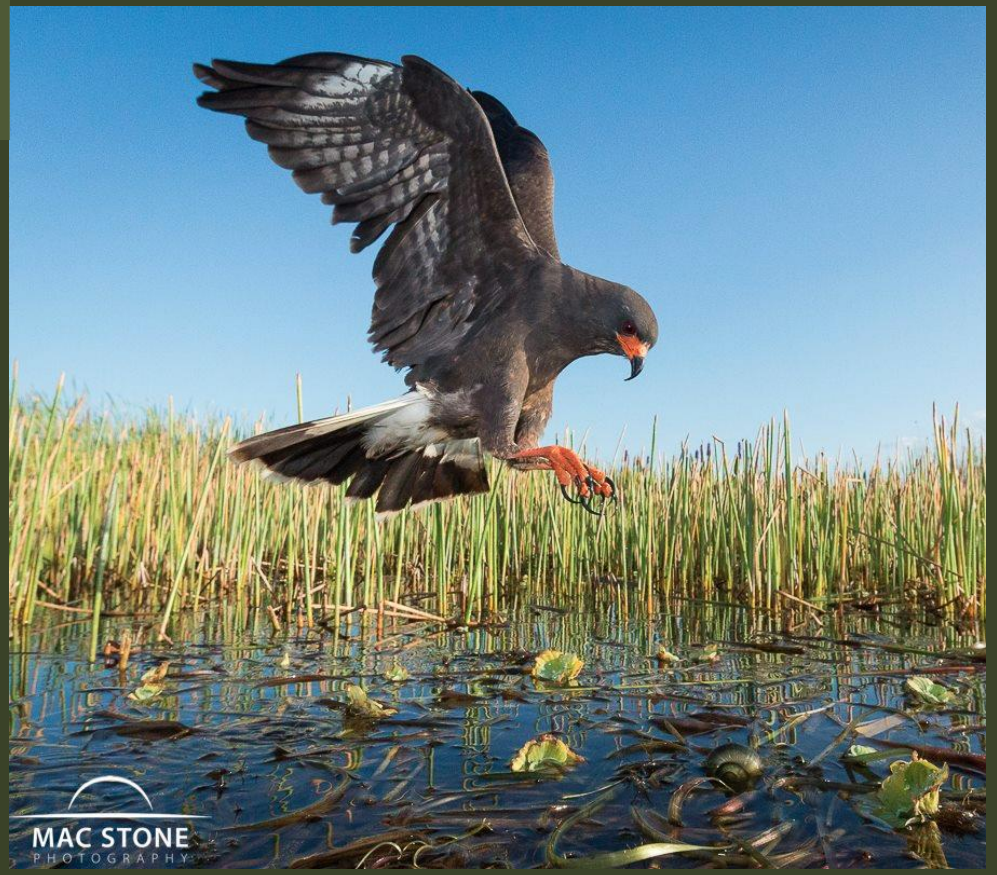
Leslie J. Mehrhoff, Univ. of Connecticut, Bugwood.org

Robert Vidéki, Doronicum Kft., Bugwood.org

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

Expanding food chain

AVM+

Hydrilla + A.h.

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

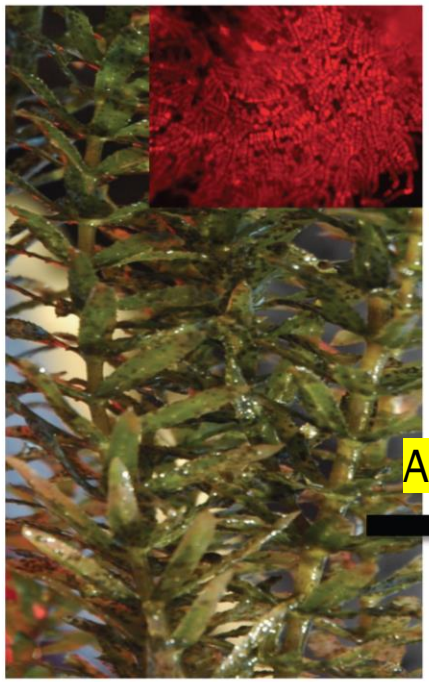
2. Apple snails fed to chickens

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

2. Apple snails fed to chickens

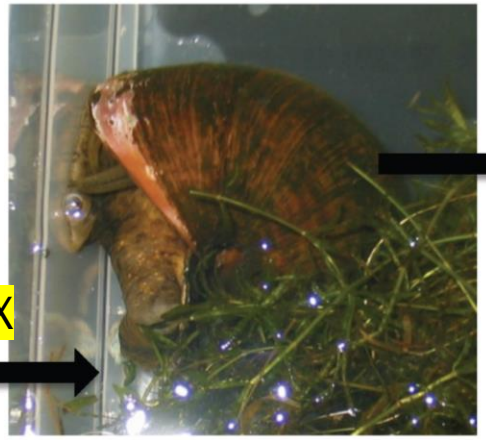
Control

Hydrilla, no A.h.



A. hydrillicola growing on hydrilla leaflets produces biotoxin

AETX



Exotic apple snails feed on hydrilla and accumulate biotoxin

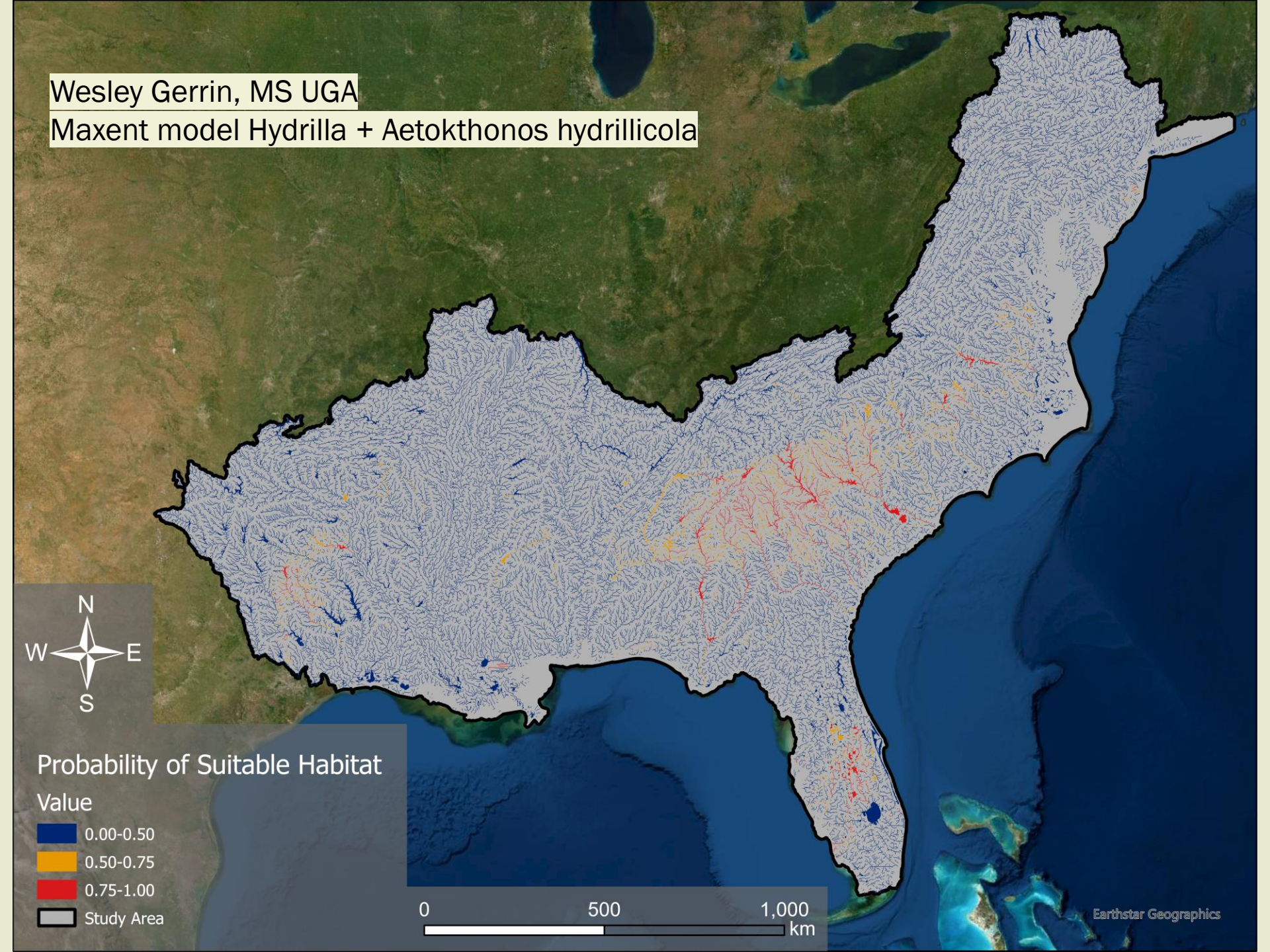
AETX

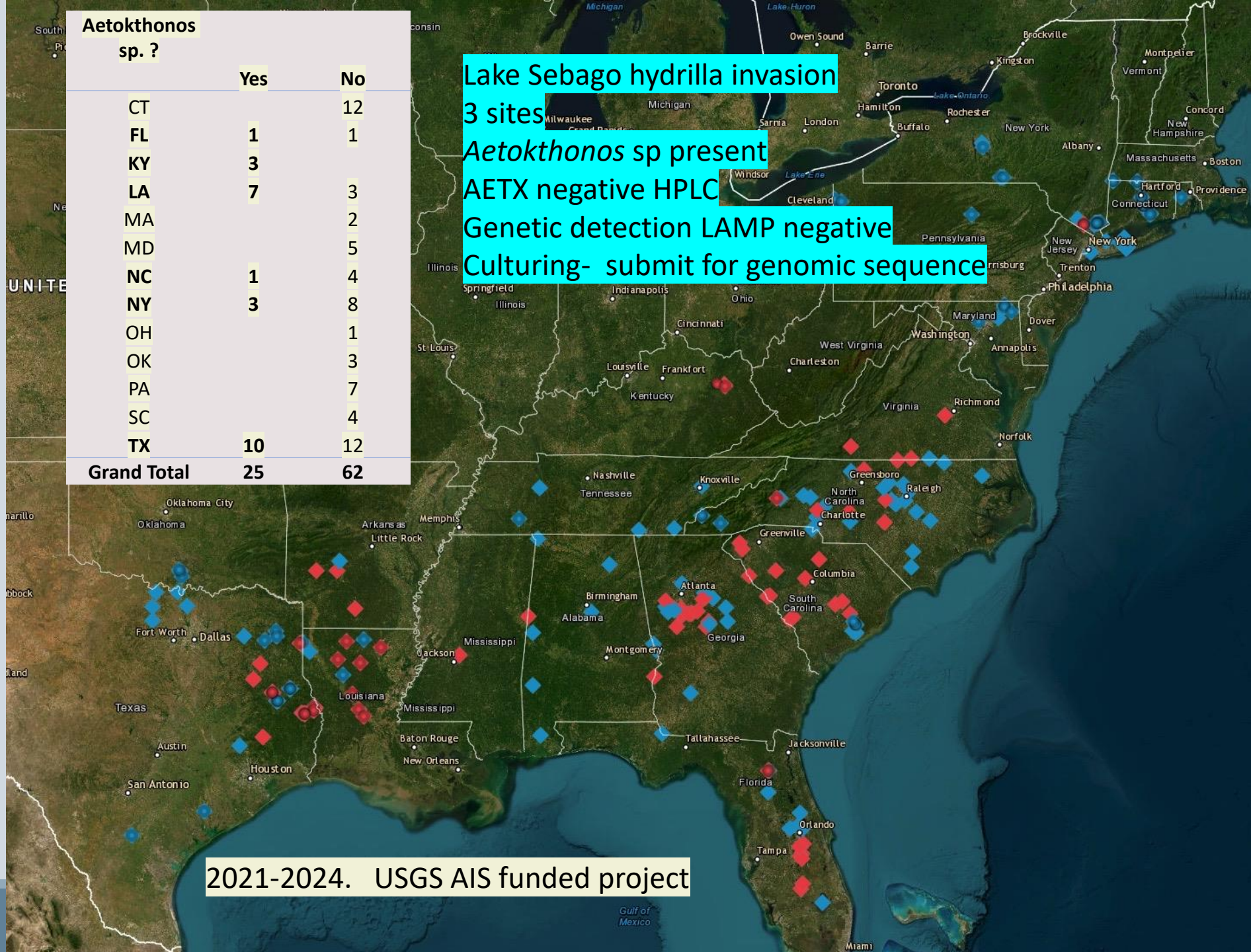
Snail kites feed on exotic snails and ingest biotoxin

Difficult to recover sick or deceased snail kites for histological diagnosis.

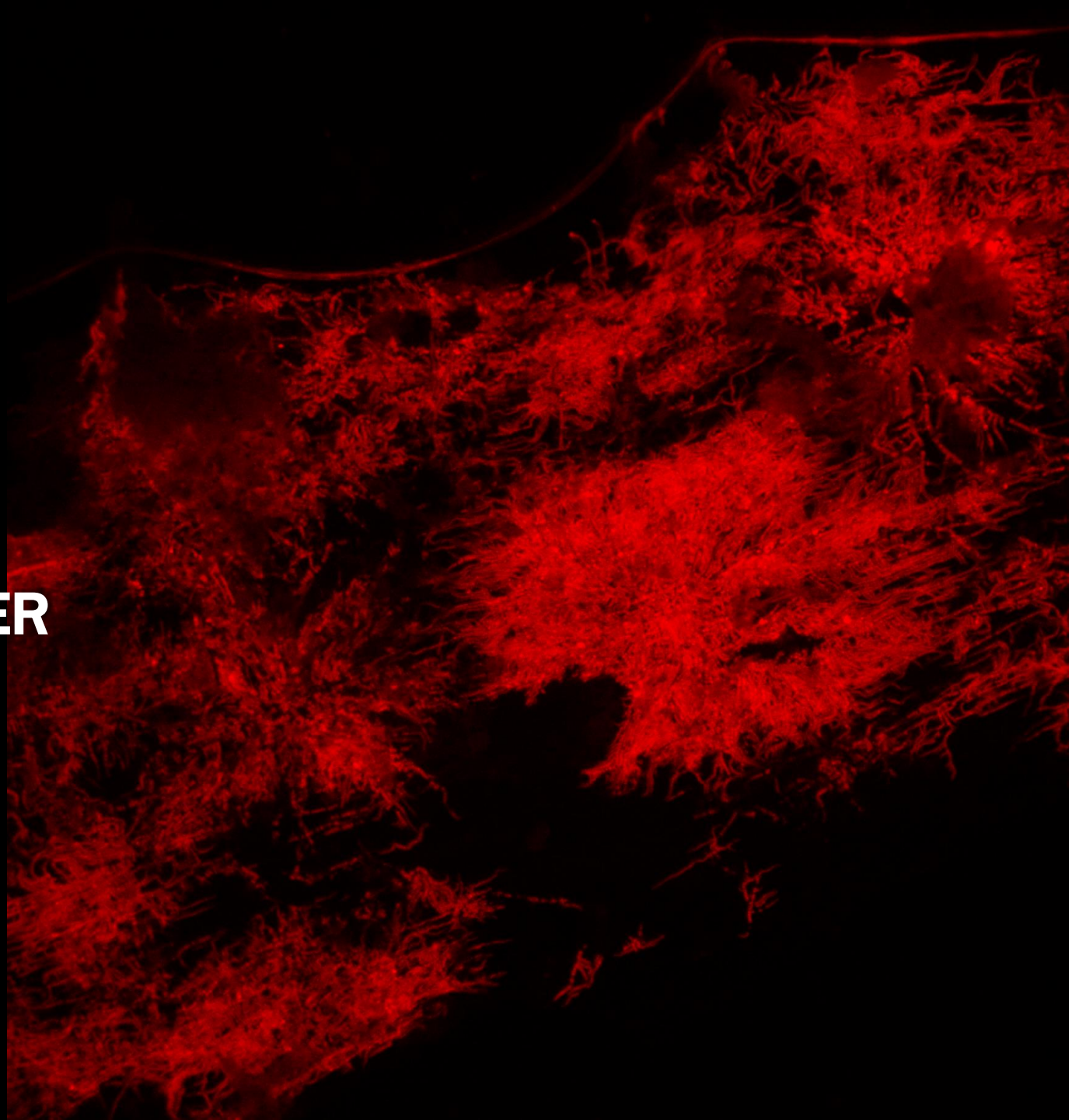
- Hydrilla/Ah laboratory trials confirm that VM toxin AETX can be transferred through an invertebrate

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.

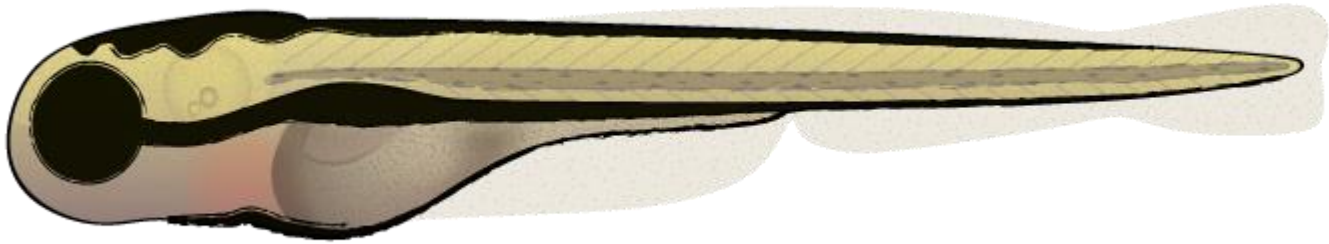

Wesley Gerrin, MS UGA
Maxent model Hydrilla + Aetokthonos hydrillicola
N
W
E
S
Probability of Suitable Habitat
Value
0.00-0.50
0.50-0.75
0.75-1.00
Study Area
0
500
1,000
km
Earthstar Geographics


Aetokthonos sp. ?
Yes
No
CT 12
FL 1 1
KY 3
LA 7 3
MA 2
MD 5
NC 1 4
NY 3 8
OH 1
OK 3
PA 7
SC 4
TX 10 12
Grand Total 25 62
Lake Sebago hydrilla invasion
3 sites
Aetokthonos sp present
AETX negative HPLC
Genetic detection LAMP negative
Culturing- submit for genomic sequence
2021-2024. USGS AIS funded project

EAGLE KILLER TOXIN

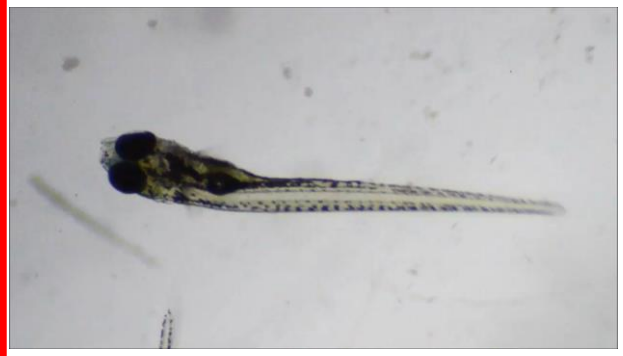


Zebrafish behavior after 24 hr exposure (7 days old)



Solvent Control

VM toxin Exposed



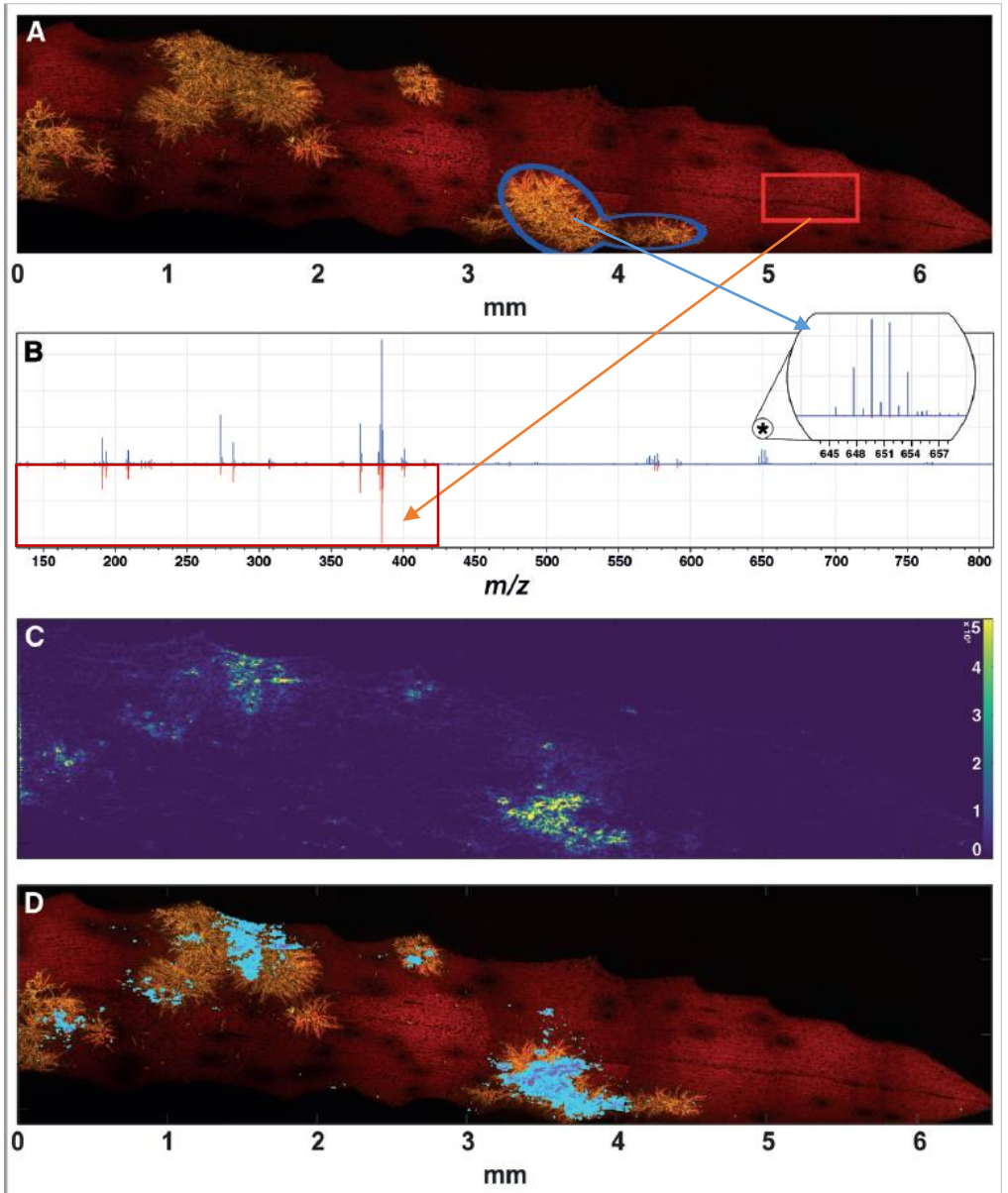
AP-MALDI-MSI of *A. hydrillicola* colonies growing on *H. verticillata* reveals a cyanobacterium-specific metabolite

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

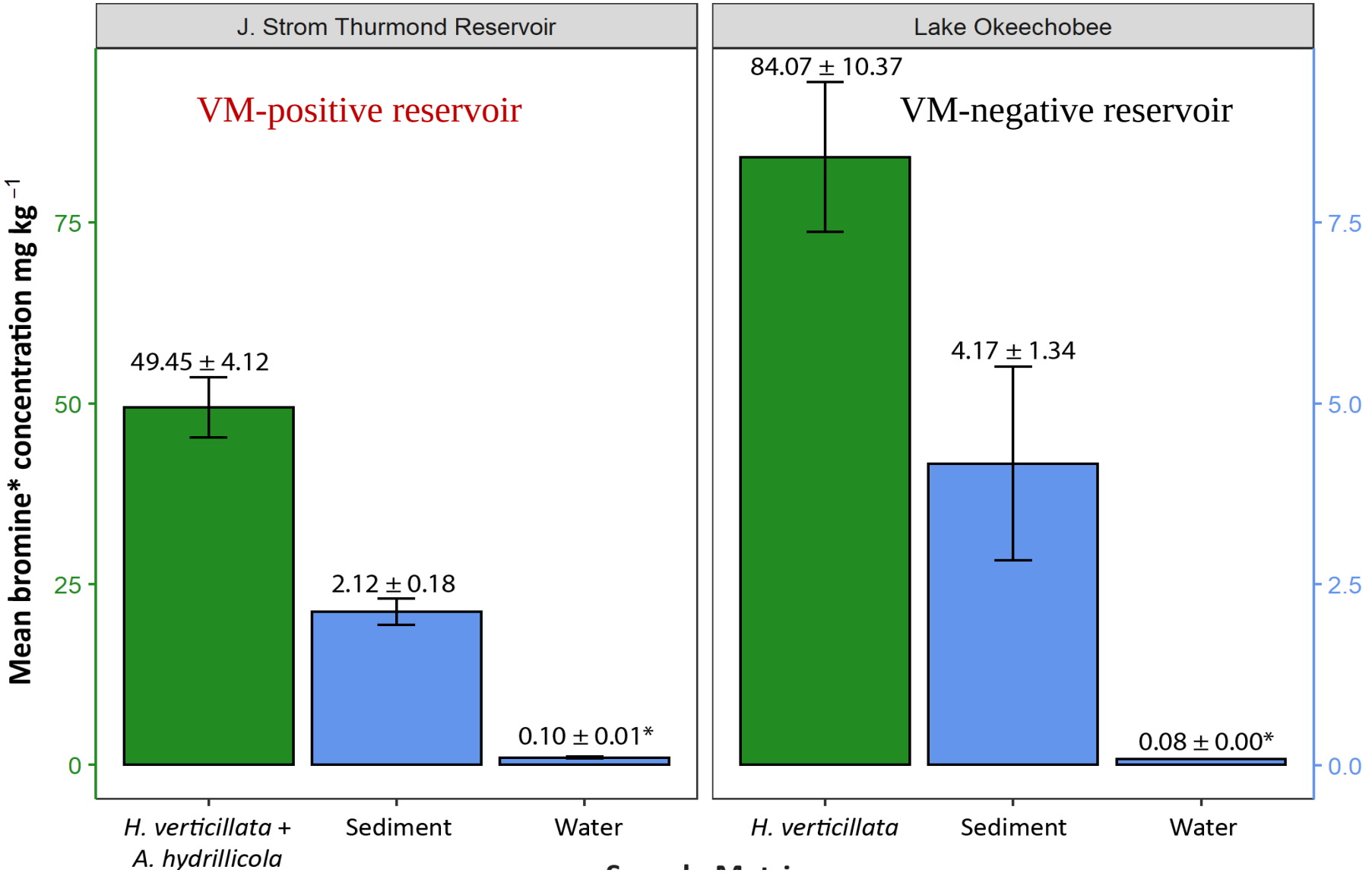
(**B**) Blue outline region -- pentabrominated metabolite associated with the cyanobacterial colony.

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

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

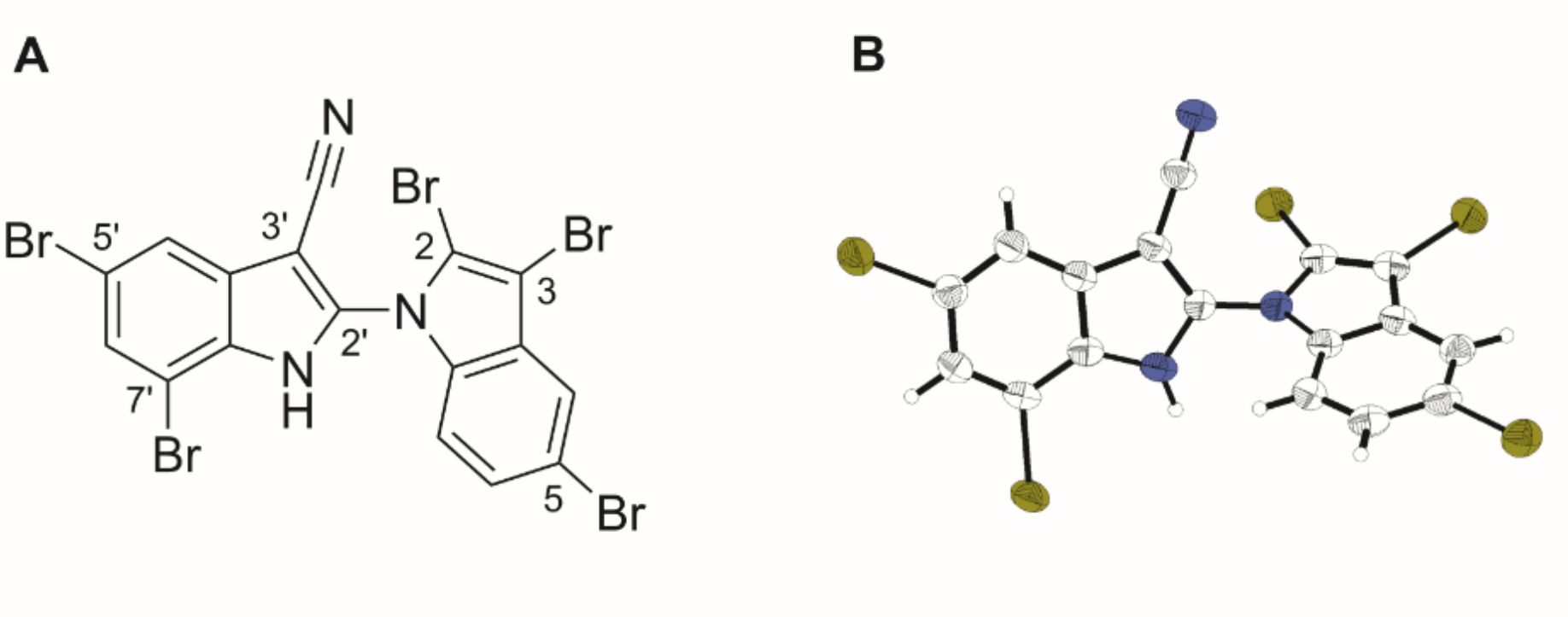


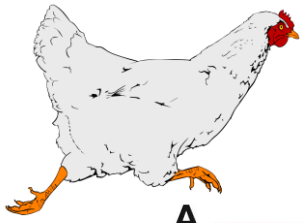
Source of Bromide
hydrilla >sediment>water



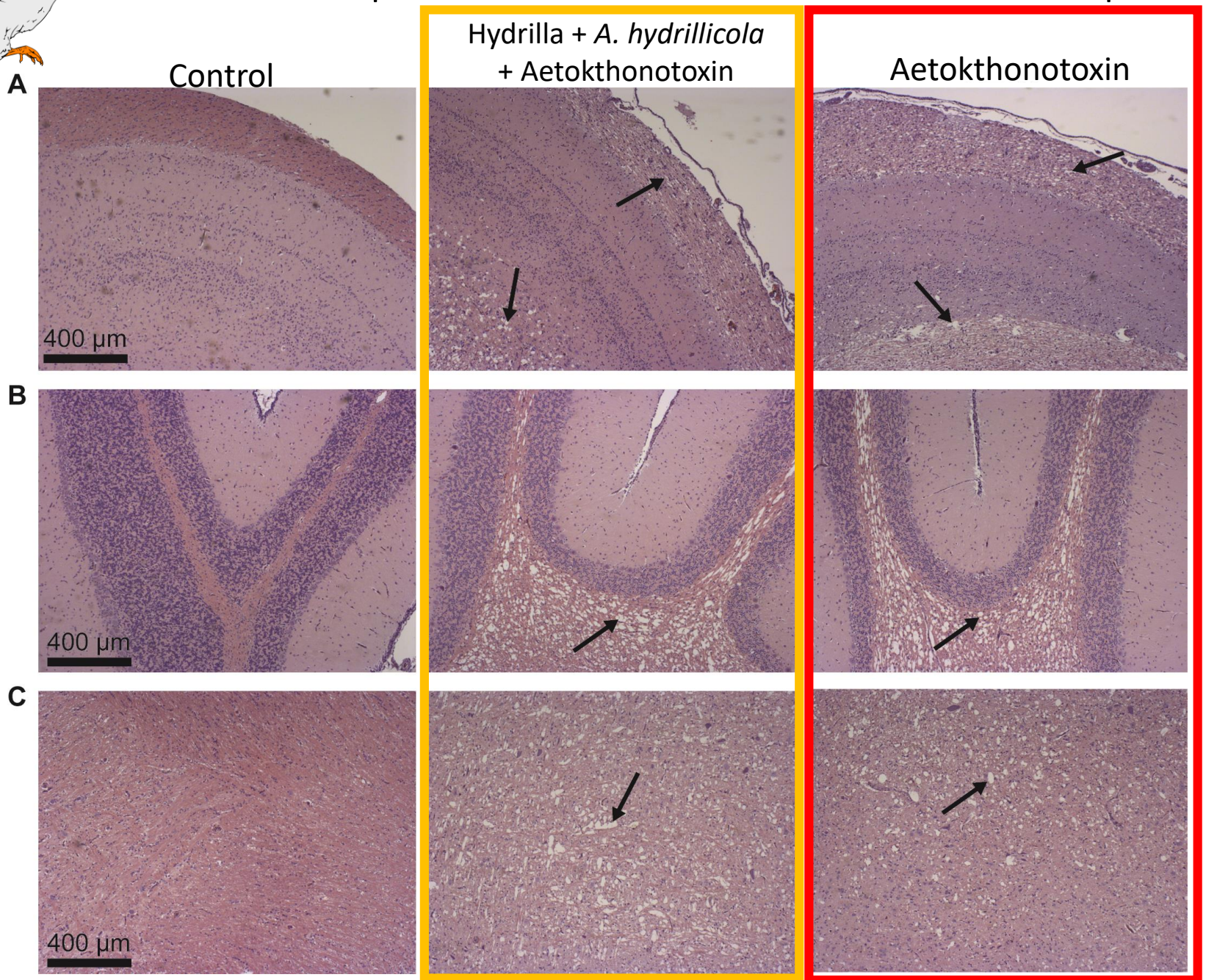
Wes Gerrin

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

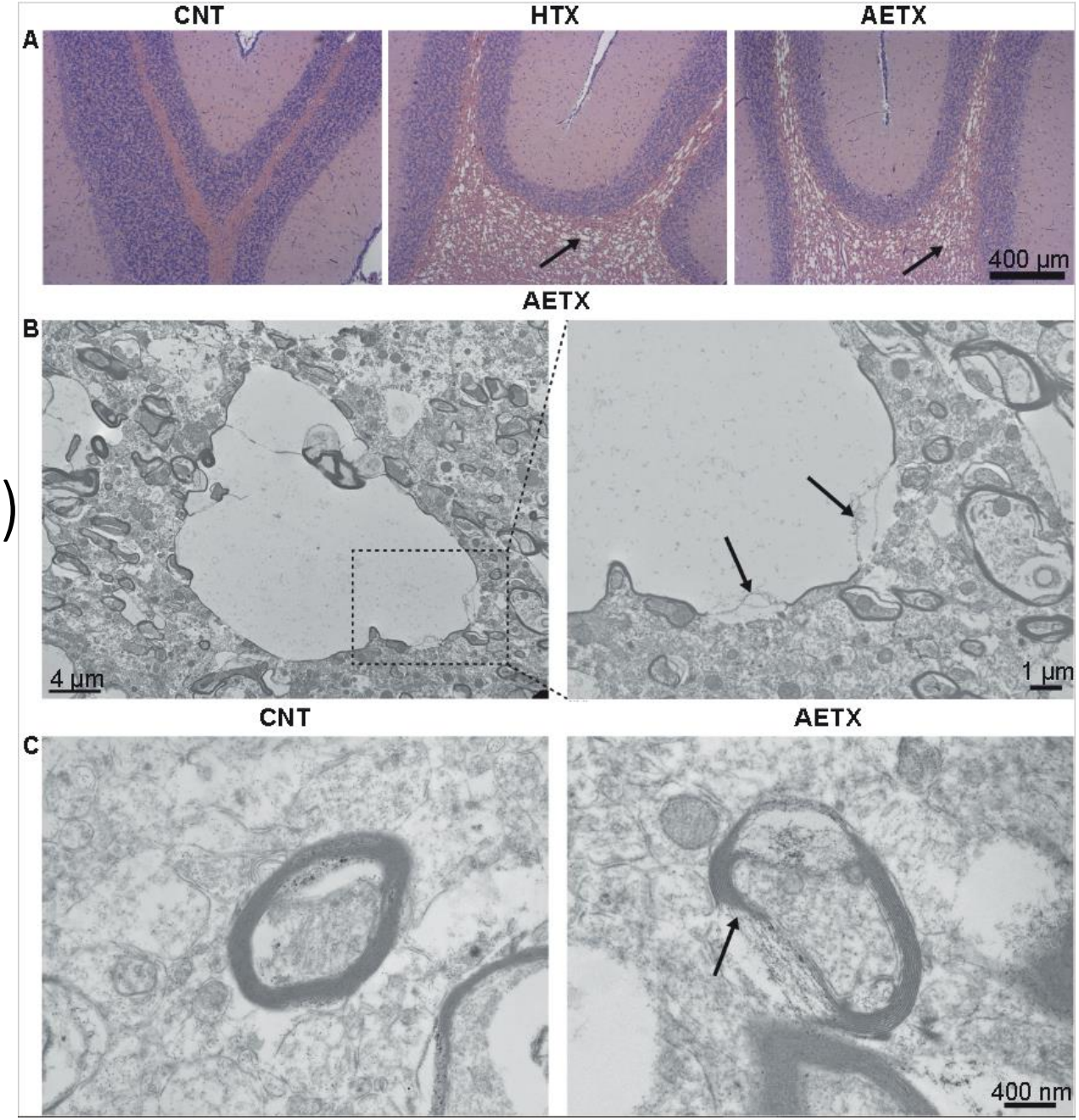




Chickens exposed to Aetokthonotoxin develop VM



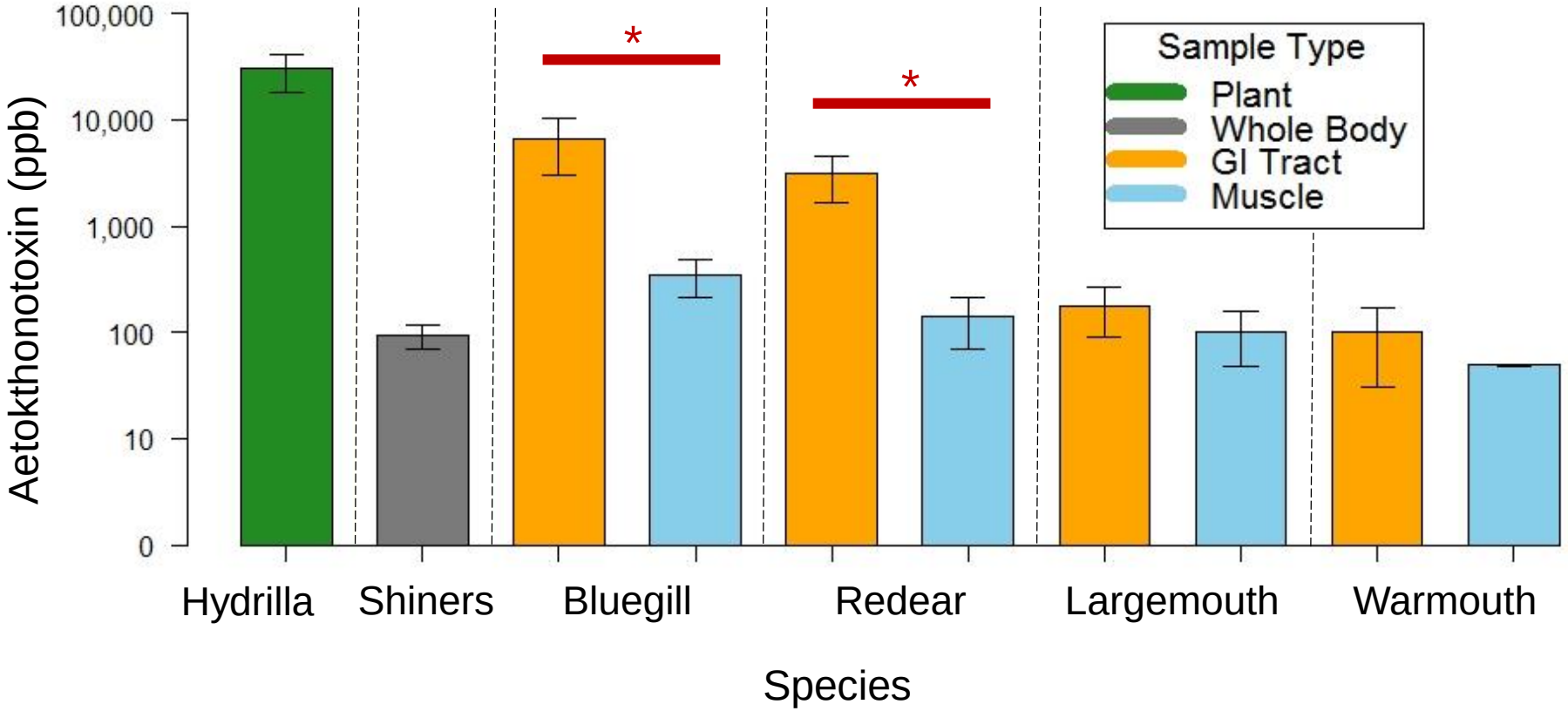
Transmission Electron Microscopy(TEM) confirmed intramyelenic edema from AETX exposure



Aetokthonotoxin in wild fish tissue

The University of Georgia
WARNELL
SCHOOL OF FORESTRY & NATURAL RESOURCES

MS Thesis
Alex Pelletier



Aetokthonotoxin in wild coot tissue

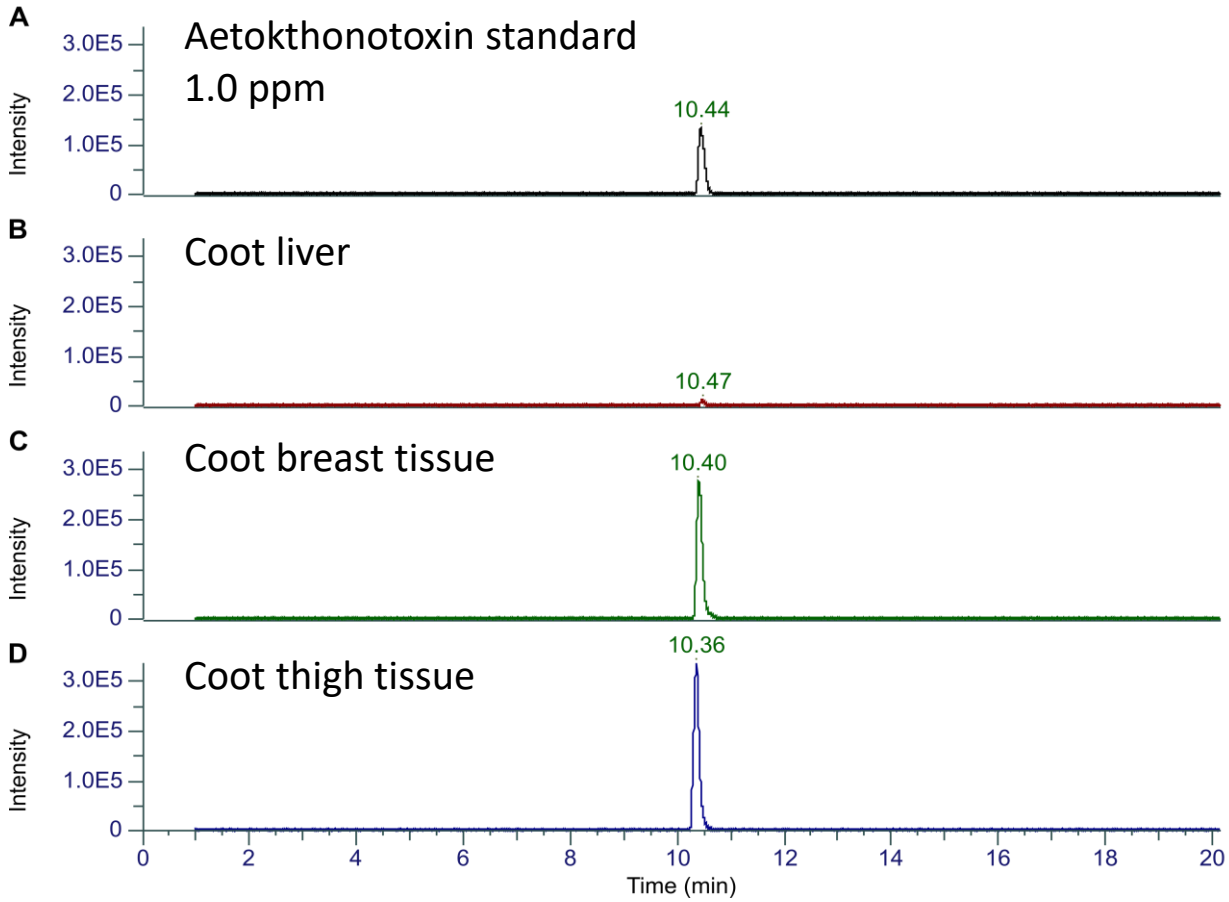
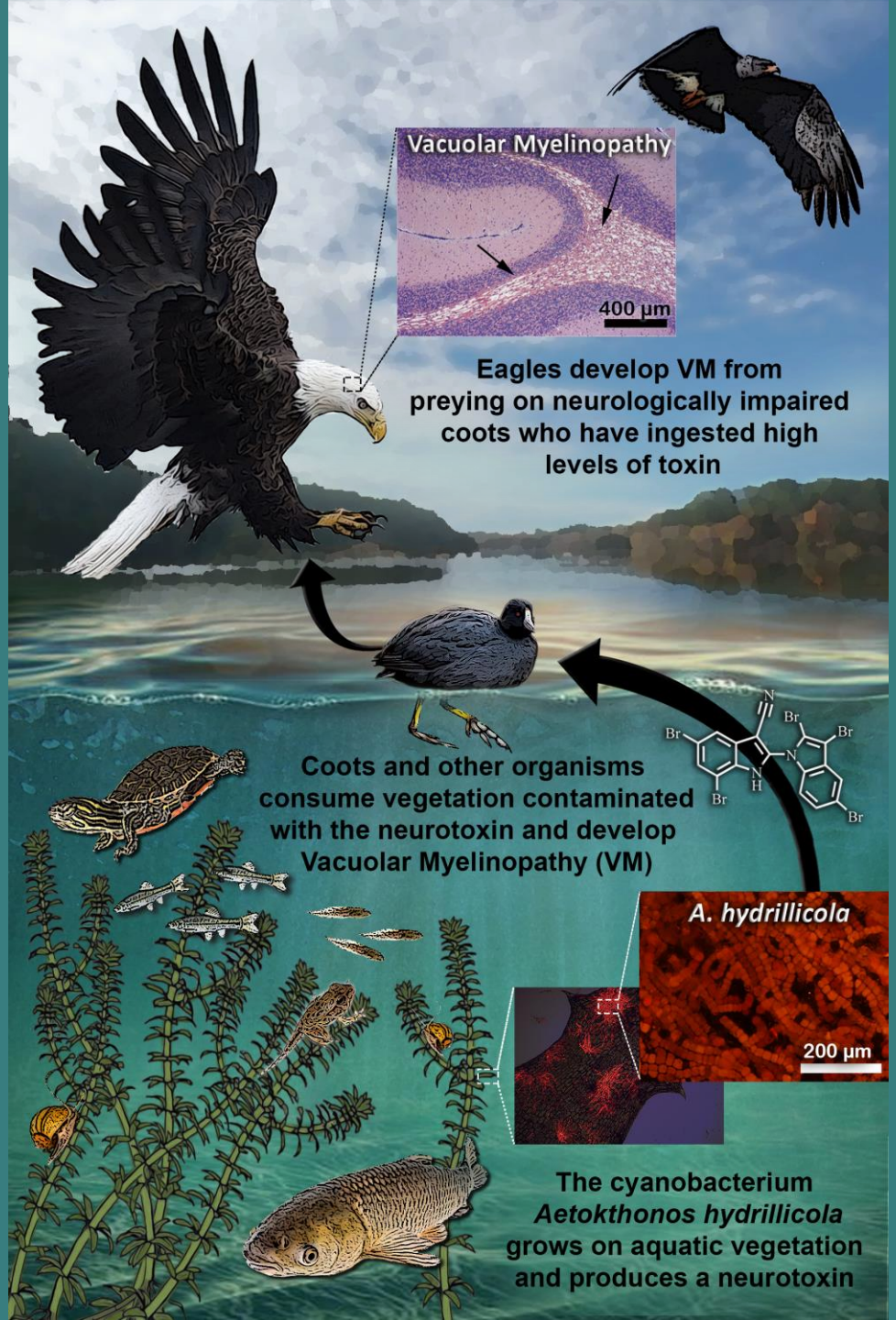


Fig. S5. Tissue screening for aetokthonotoxin (AETX) of wild American Coots (*Fulica americana)* succumbed to Vacuolar Myelinopathy (VM). A targeted mass spectrometry analysis (HPLC-SRM-MS) was carried out to selectively screen the tissues for AETX presence. Chromatograms show the SRM traces of most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

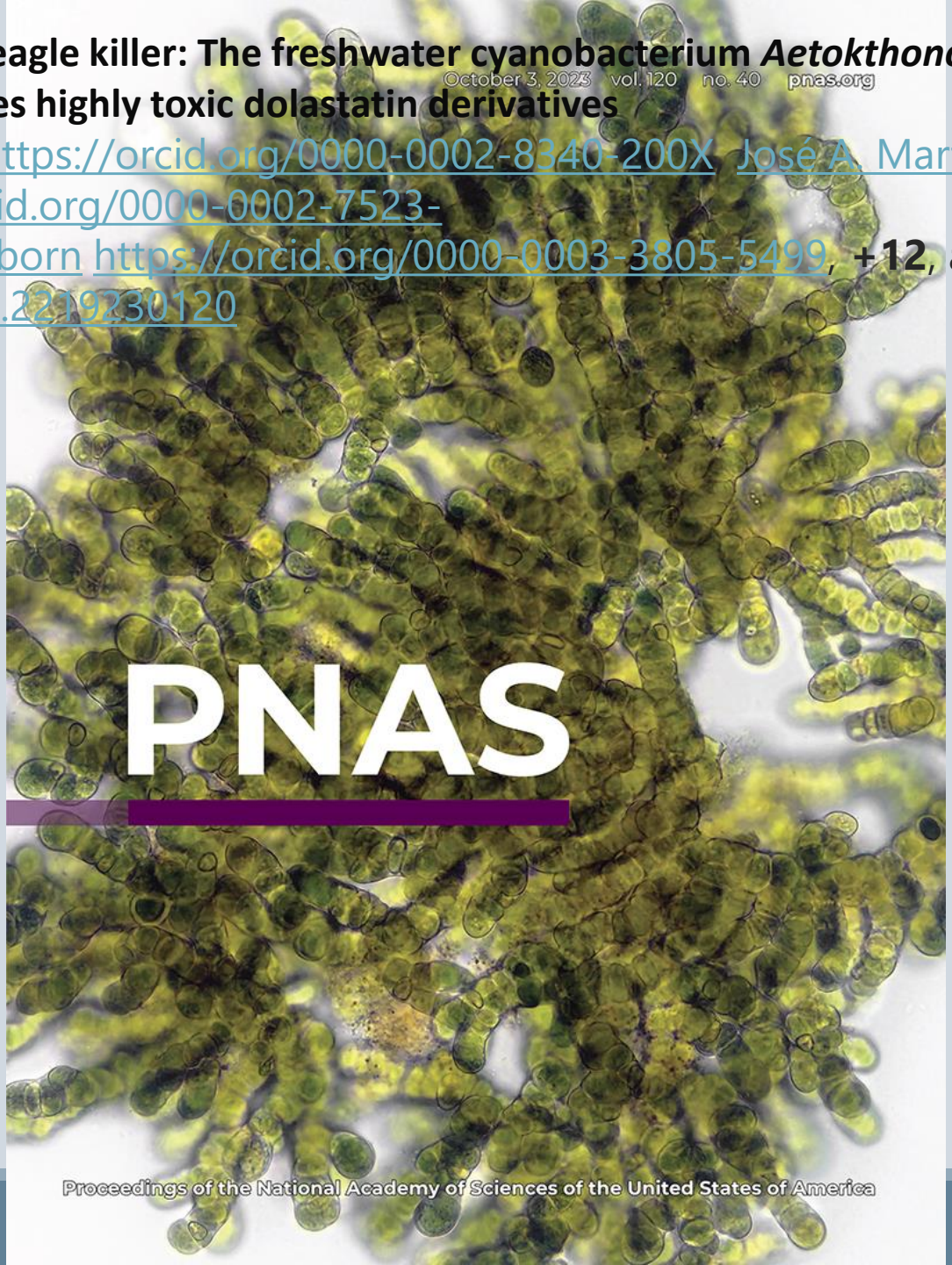
Breinlinger S, T Phillips, B Haram, J Mareš, JA Martínez Yerena, P Hrouzek, R Sobotka, WM Henderson, P Schmieder, SM Williams, JD Lauderdale, HD Wilde, W Gerrin, A Kust, C Wagner, B Geier, M Liebeke, H Enke, THJ Niedermeyer, SB Wilde. (submitted 10/29/20). Hunting down the eagle killer: A novel cyanobacterial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



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

More than just an eagle killer: The freshwater cyanobacterium *Aetokthonos hydrillicola* produces highly toxic dolastatin derivatives

Markus Schwark https://orcid.org/0000-0002-8340-200X, José A. Martínez Yerena https://orcid.org/0000-0002-7523-822X, Kristin Röhrborn https://orcid.org/0000-0003-3805-5499, **+12**, and Timo H. J. Niedermeyer .2219230120



PNAS RESEARCH ARTICLE | BIOCHEMISTRY

More than just an eagle killer: The freshwater cyanobacterium *Aetokthonos hydrillicola* produces highly toxic dolastatin derivatives

Markus Schwark[a,1], José A. Martínez Yerena[b,c,d,1], Kristin Röhrborn[a,2], Pavel Hrouzek[d], Petra Divoká[d], Lenka Štenclová[b], Kateřina Delawská[c,d], Heike Enke[e], Christopher Vorreiter[f], Faith Wiley[g,3], Wolfgang Sippl[f], Roman Sobotka[c,d], Subhasish Saha[d,4], Susan B. Wilde[h], Jan Mareš[b,c,d,5], and Timo H. J. Niedermeyer[a,i,5]

Edited by Wilfred van der Donk, University of Illinois at Urbana-Champaign, Urbana, IL; received April 13, 2023; accepted August 14, 2023

Cyanobacteria are infamous producers of toxins. While the toxic potential of planktonic cyanobacterial blooms is well documented, the ecosystem level effects of toxigenic benthic and epiphytic cyanobacteria are an understudied threat. The freshwater epiphytic cyanobacterium *Aetokthonos hydrillicola* has recently been shown to produce the "eagle killer" neurotoxin aetokthonotoxin (AETX) causing the fatal neurological disease vacuolar myelinopathy. The disease affects a wide array of wildlife in the southeastern United States, most notably waterfowl and birds of prey, including the bald eagle. In an assay for cytotoxicity, we found the crude extract of the cyanobacterium to be much more potent than pure AETX, prompting further investigation. Here, we describe the isolation and structure elucidation of the aetokthonostatins (AESTs), linear peptides belonging to the dolastatin compound family, featuring a unique modification of the C-terminal phenylalanine-derived moiety. Using immunofluorescence microscopy and molecular modeling, we confirmed that AEST potently impacts microtubule dynamics and can bind to tubulin in a similar matter as dolastatin 10. We also show that AEST inhibits reproduction of the nematode *Caenorhabditis elegans*. Bioinformatic analysis revealed the AEST biosynthetic gene cluster encoding a nonribosomal peptide synthetase/polyketide synthase accompanied by a unique tailoring machinery. The biosynthetic activity of a specific N-terminal methyltransferase was confirmed by in vitro biochemical studies, establishing a mechanistic link between the gene cluster and its product.

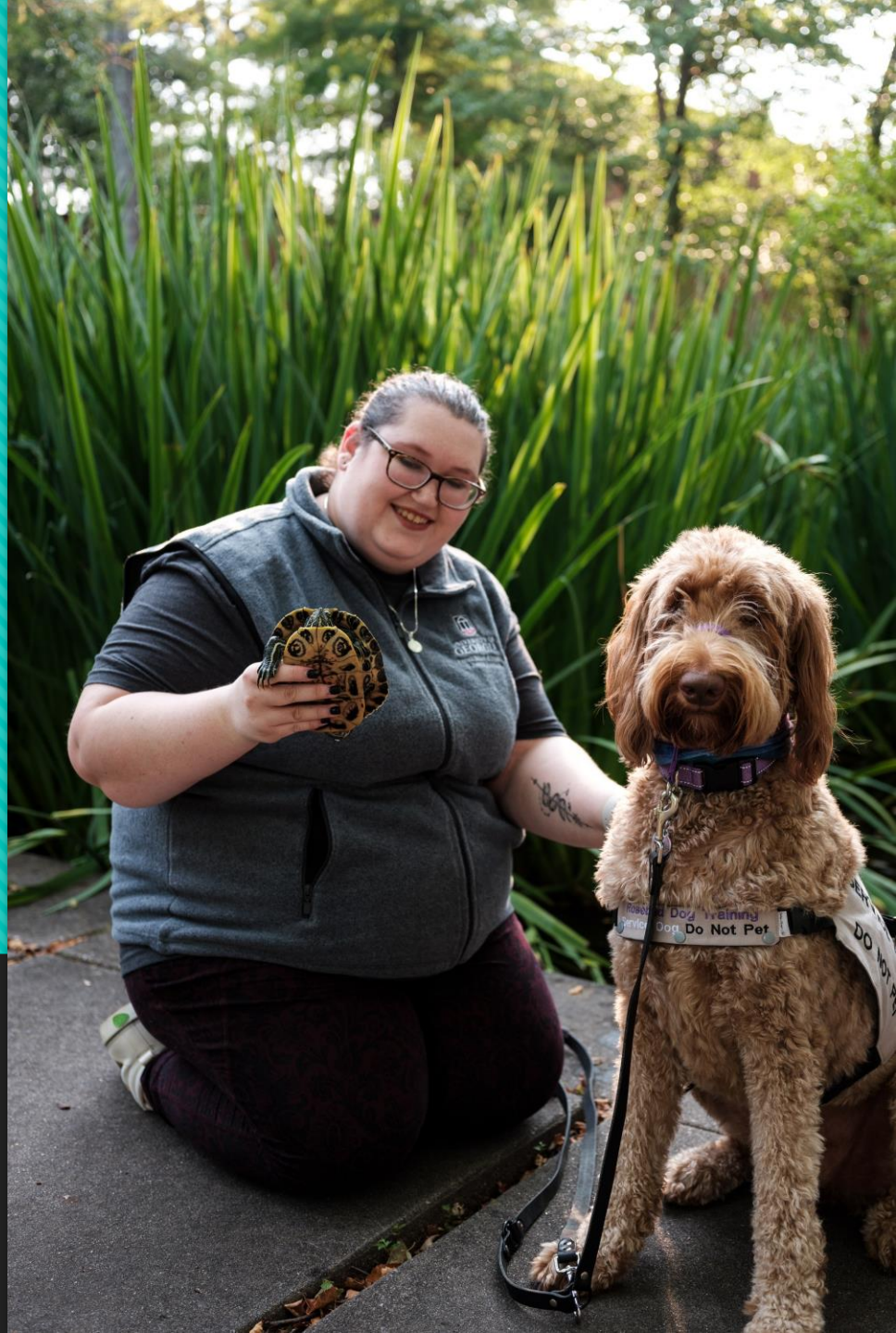
cyanotoxin | cytotoxicity | aetokthonostatin | dolastatin | biosynthesis

Significance

Cyanotoxins have adverse effects on ecosystems. Our understanding of their potential risk has recently been expanded by the discovery of aetokthonotoxin, produced by the cyanobacterium *Aetokthonos hydrillicola* growing on invasive plants. Via trophic transfer, it acts as a neurotoxin causing mortality in animals including top predators like bald eagles. Closer examination of *A. hydrillicola* revealed that it also produces highly toxic dolastatin derivatives. *A. hydrillicola* is the only known cultured cyanobacterium producing dolastatin derivatives, which

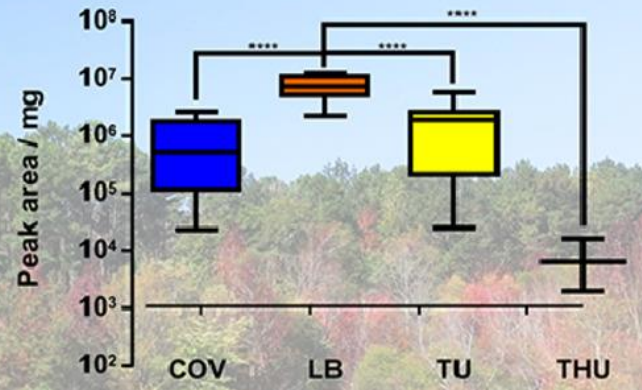
Outreach Education Teaching about VM with animals

Ryann Heninger, MS
August 2024

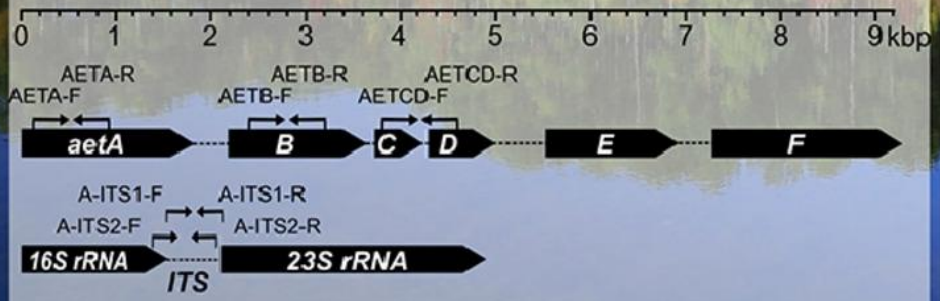


Occurrence of aetokthonotoxin producer in natural samples - a PCR protocol for easy detection

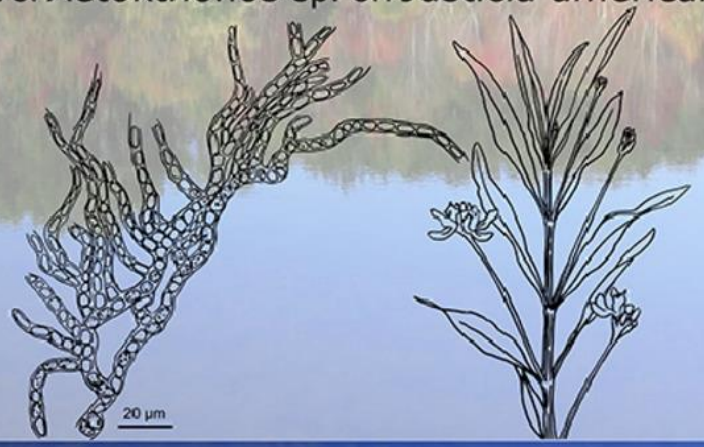
Distribution of AETX at different localities



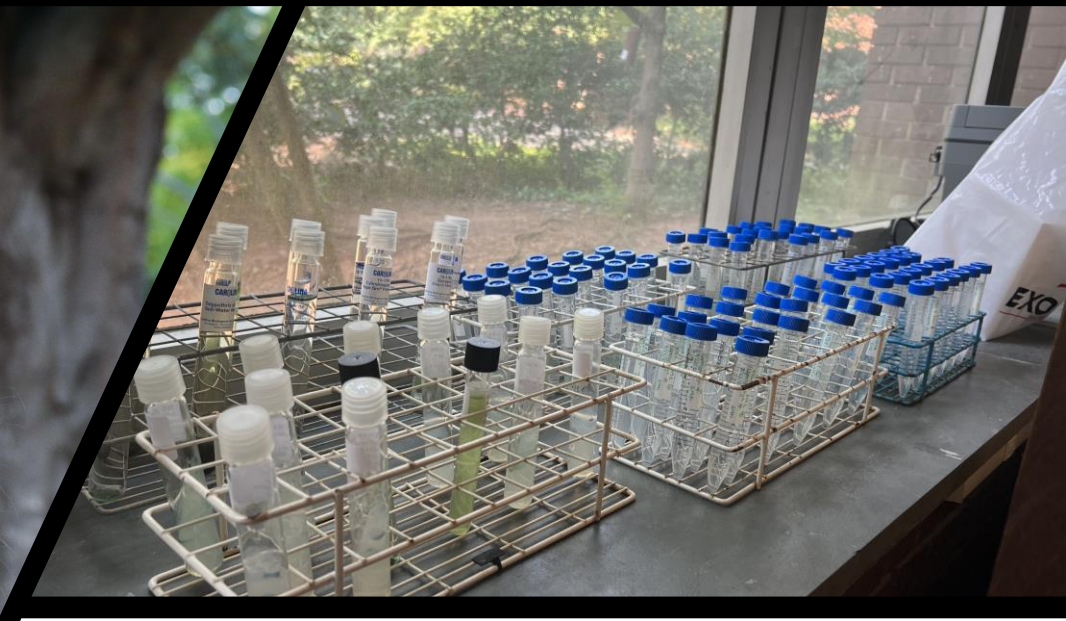
PCR primers designed in the present study

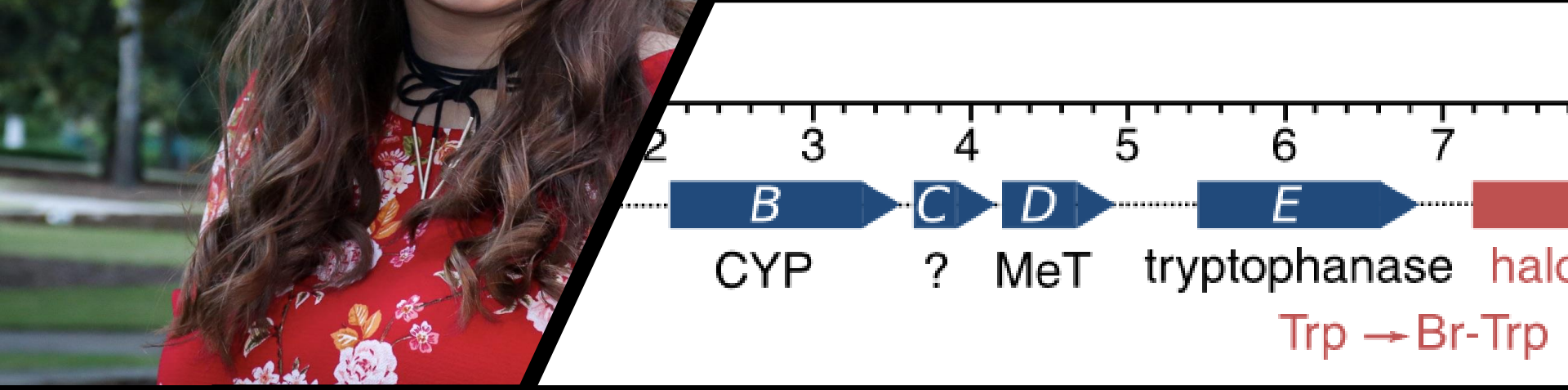


Novel *Aetokthonos* sp. on *Justicia americana*



Erika Klar. LAMP development
M.S. December 2024
emk70547@uga.edu

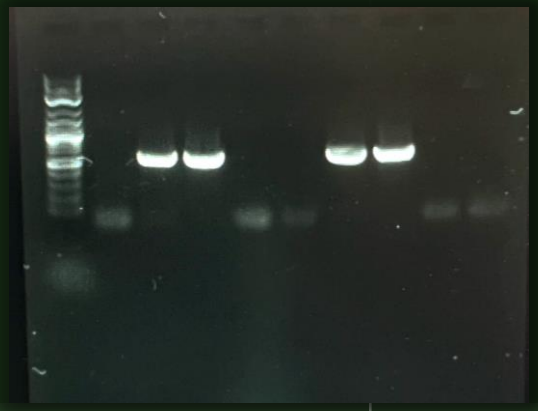




Molecular Assay

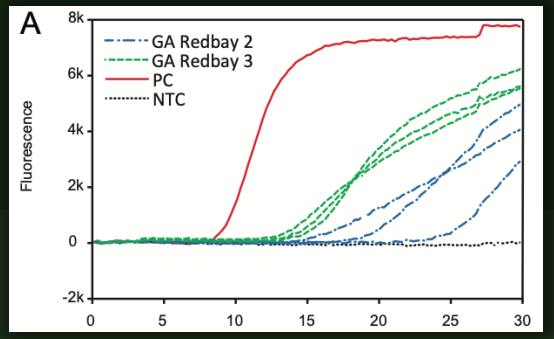
PCR (Polymerase Chain Reaction)

- Not Portable
- Specific, 1 target region
- Time Consuming



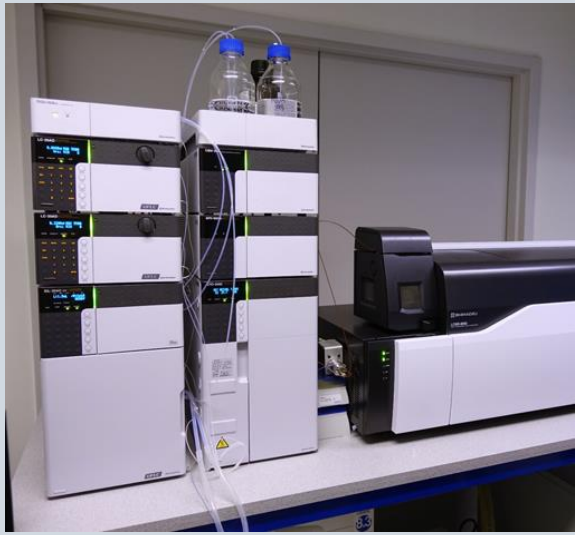
LAMP (Loop Mediated Isothermal Amplification)

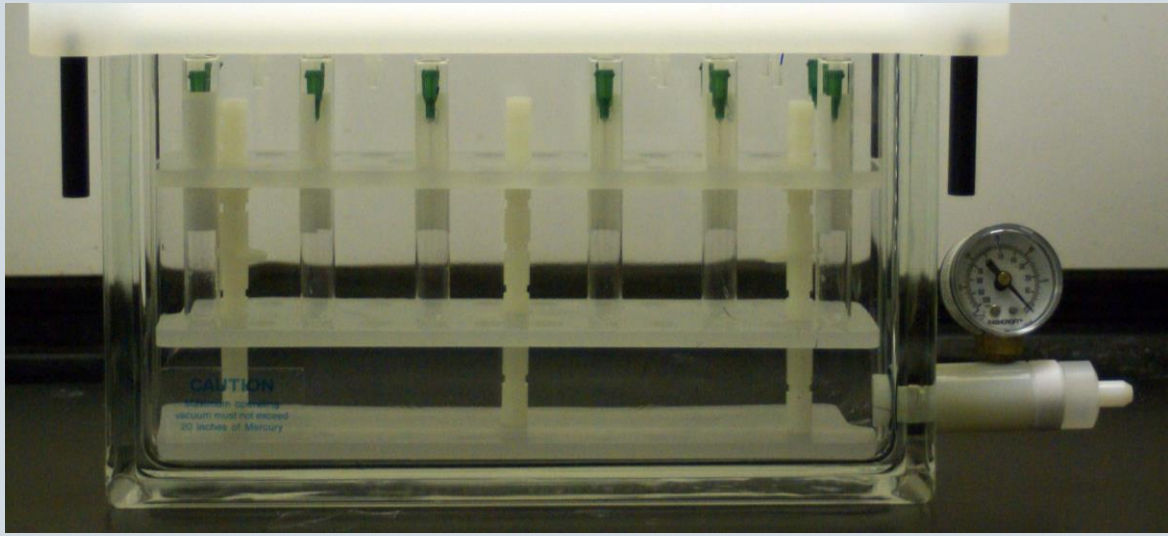
- Portable
- More specific, useful for field extractions, 6-8 target regions
- Rapid



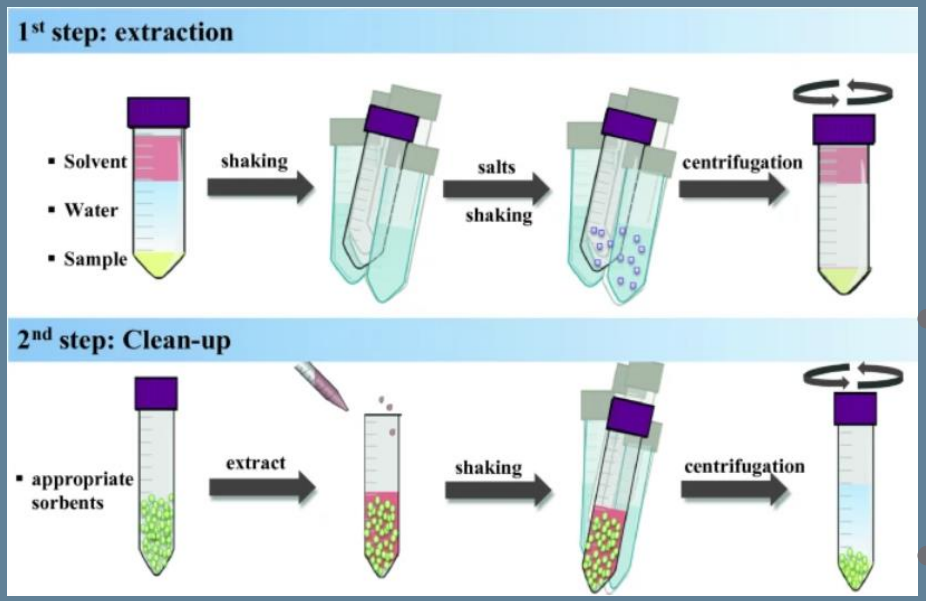
AETX Extraction

- 1.0 g skeletal muscle or liver – homogenize
- Organic solvent extraction
- Solid Phase Extraction (SPE)
- Liquid chromatography with tandem mass spectrometry (LC – MS/MS)



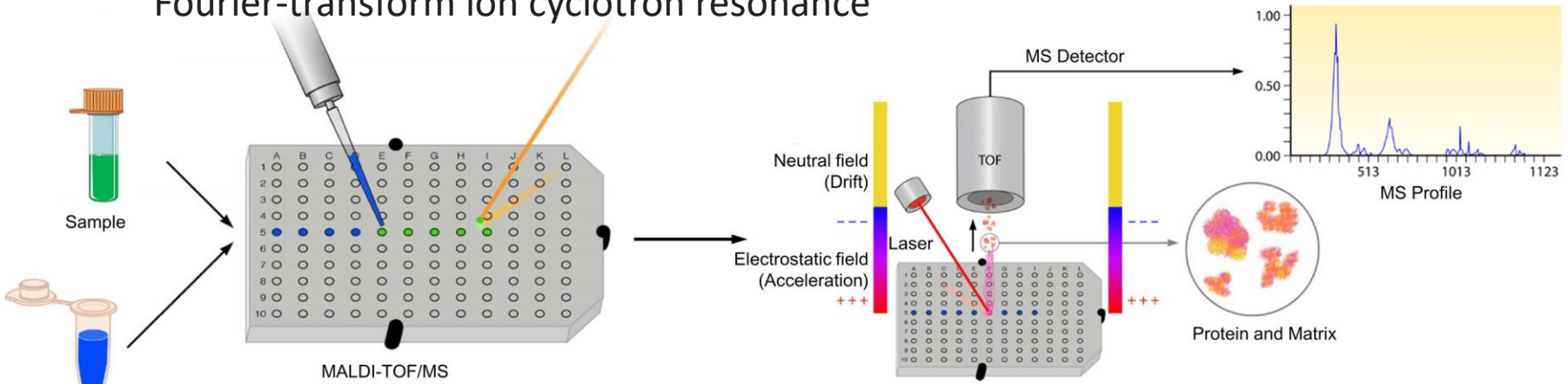


Seth McWhorter, PhD 2025
Moving Forward Faster…



- QuEChERS Sample Preparation (Quick, Easy, Cheap, Effective, Rugged, Safe)
- MALDI (Matrix Assisted Laser Desorption / Ionization)

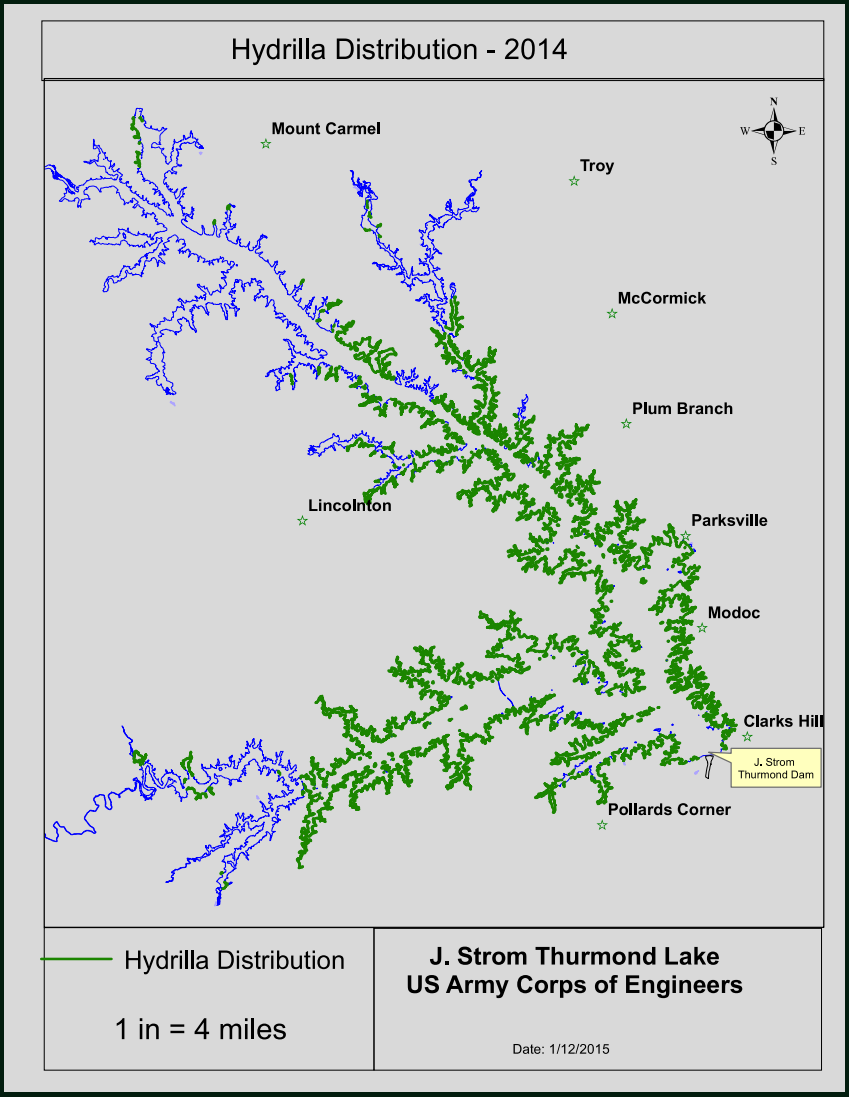
Fourier-transform ion cyclotron resonance



Grant NIH S10 OD025118
Bruker Solarix XR 12 T FTICR MS

Expanding Food Chain

Lethargic beaver recovered from AVM positive site during late fall



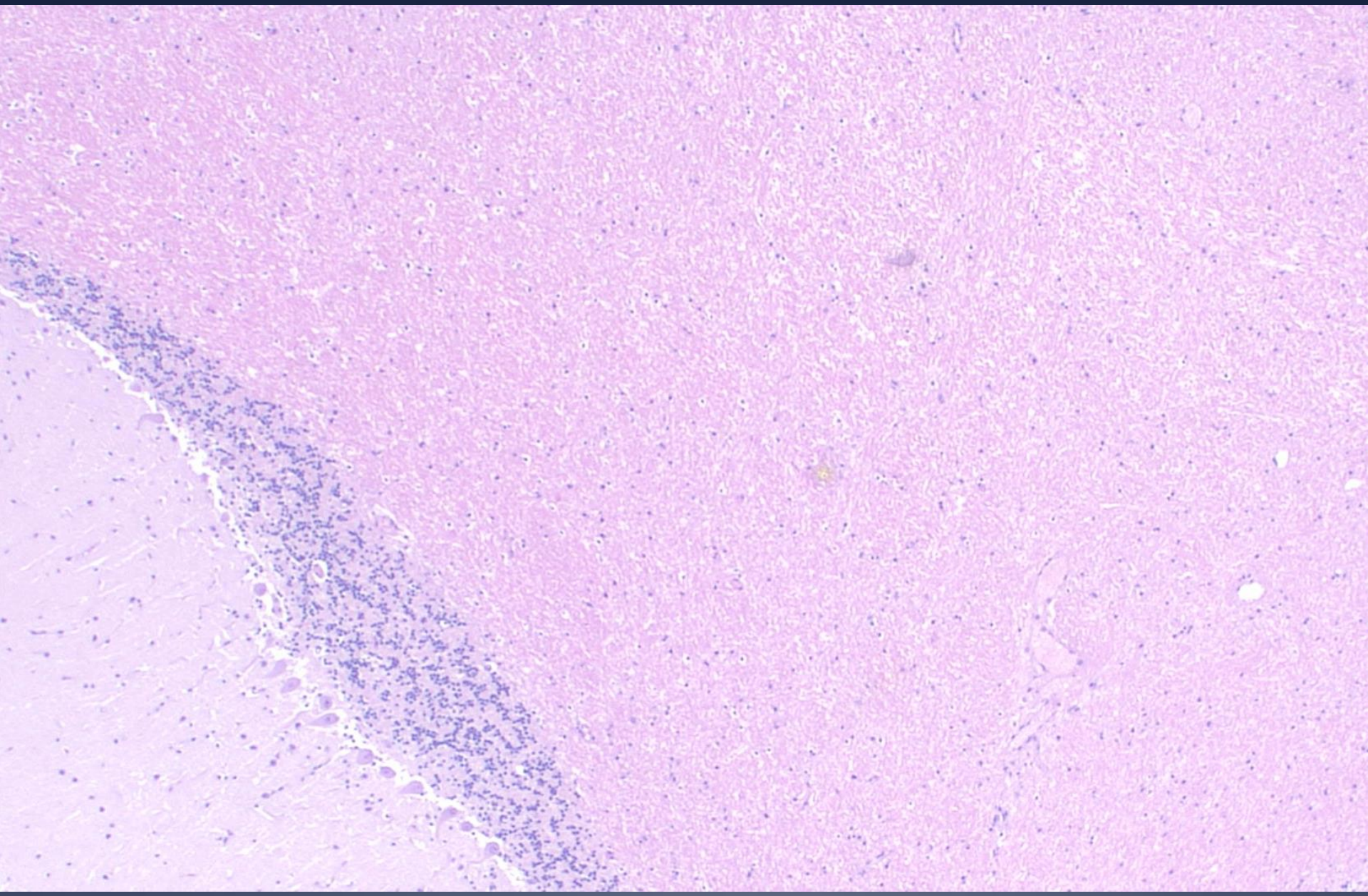
J. Strom Thurmond Reservoir

Gut contents of neurologically impaired beaver

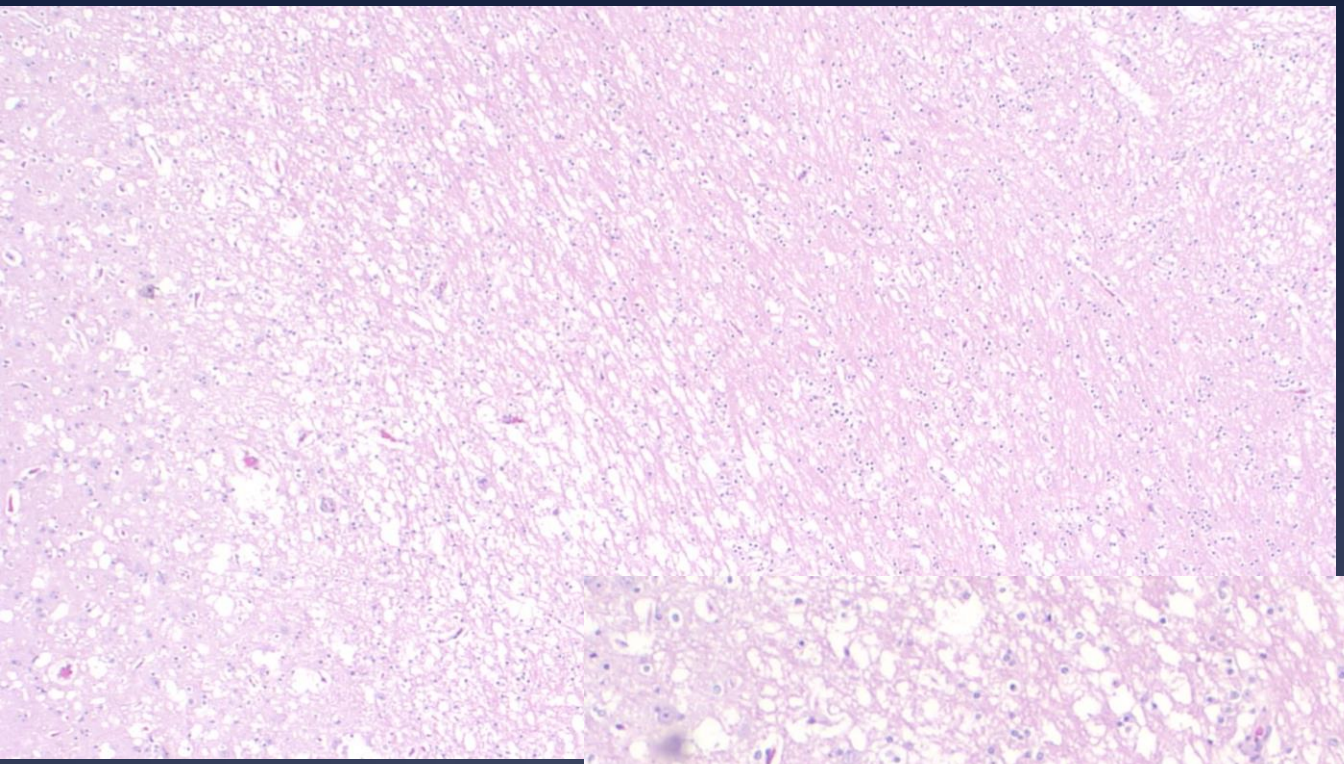
Hydrilla spine

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

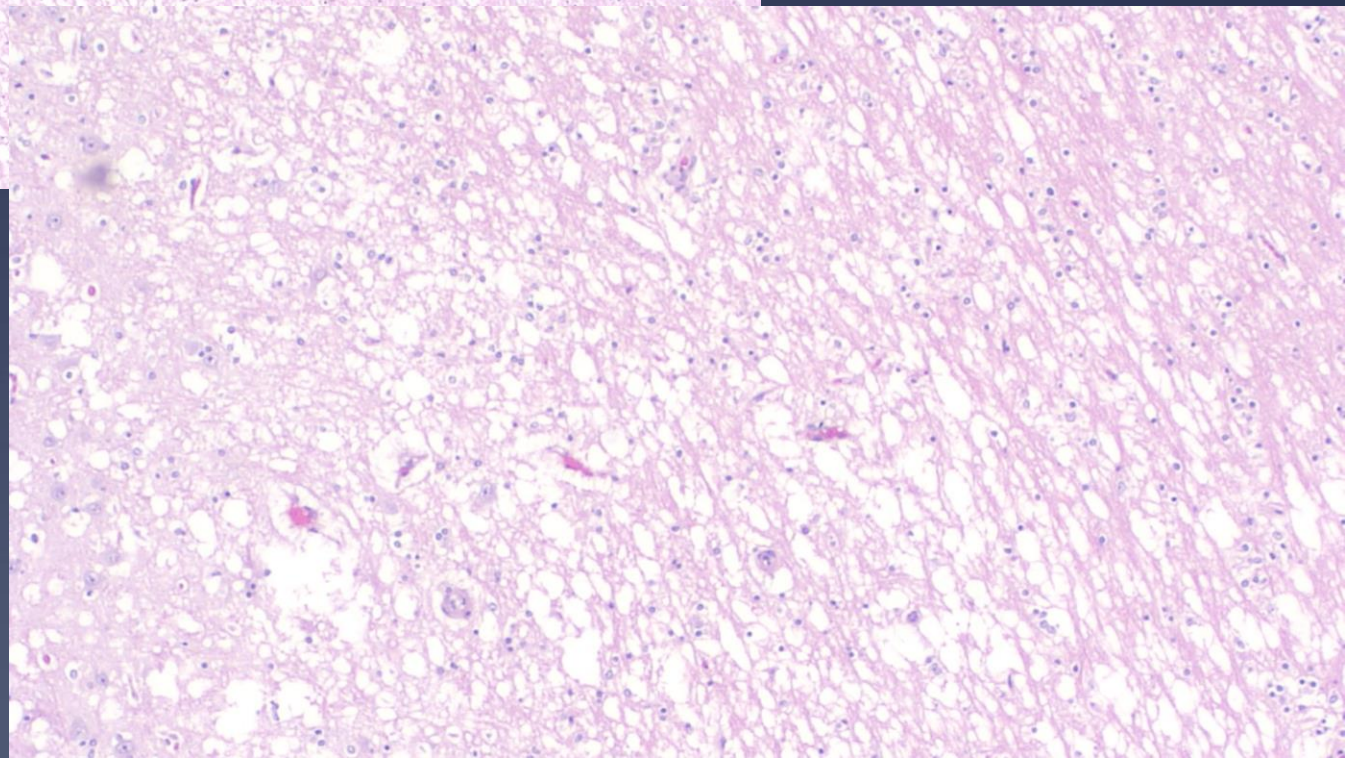
Normal beaver histology cerebrum



White matter vacuolation



cerebrum



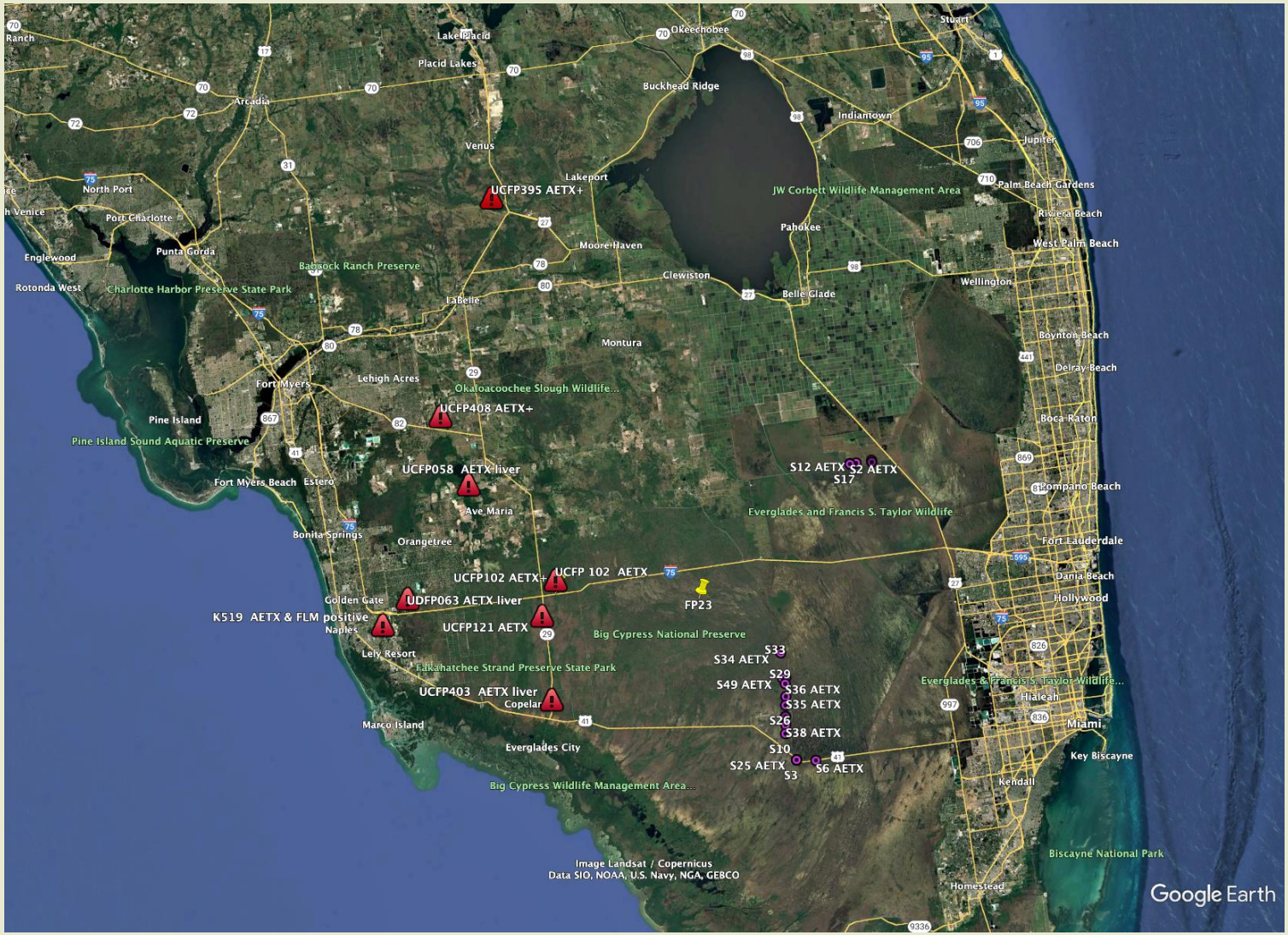
cerebellum

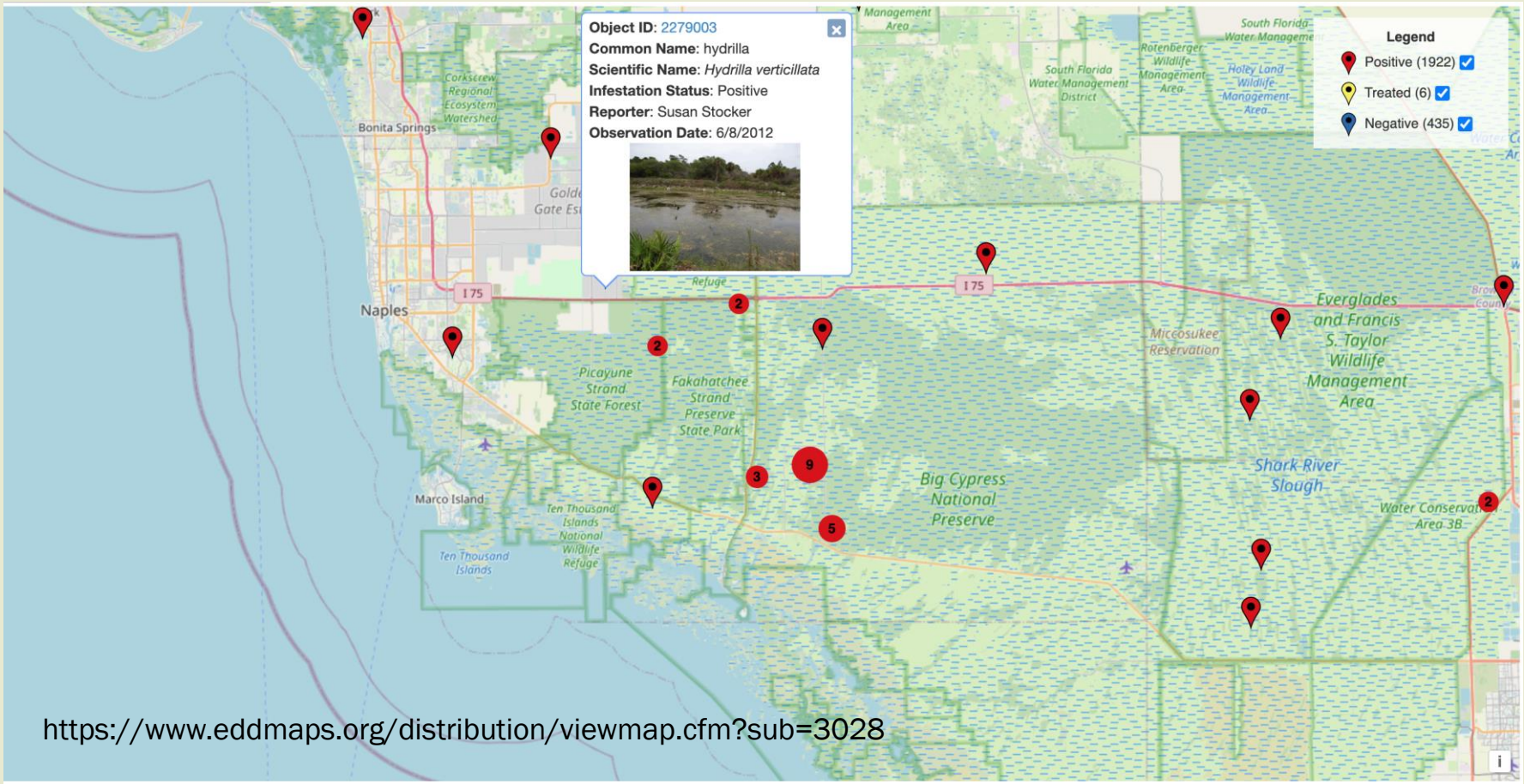
A mysterious neurological disease is afflicting endangered Florida panthers

A disease known as feline leukomyelopathy has likely stricken 48 panthers and bobcats, leading to concerns about the impact on Florida's state animal.

Florida Panther (*Puma concolor coryi*) and Bobcat (*Lynx rufus*)

- Feline Leukomyelopathy (FLM) **unknown cause** since 2017
- Symmetric degradation of axons and myelin in CNS
- 75 cases: 31 panthers, 44 bobcats
- Tissues sent to Wilde Lab for AETX analysis


UCFP395 AETX+
UCFP408 AETX+
UCFP058 AETX liver
UCFP102 AETX+
UCFP 102 AETX
UDFP063 AETX liver
K519 AETX & FLM positive
UCFP121 AETX
UCFP403 AETX liver
FP23
S12 AETX
S2 AETX
S17
S33
S34 AETX
S29
S49 AETX
S36 AETX
S35 AETX
S26
S38 AETX
S10
S25 AETX
S3
S6 AETX
Lakeport
Venus
Placid Lakes
Lake Placid
Okeechobee
Buckhead Ridge
Indiantown
Stuart
Jupiter
Palm Beach Gardens
Riviera Beach
West Palm Beach
Wellington
Boynton Beach
Delray Beach
Boca Raton
Pompano Beach
Fort Lauderdale
Dania Beach
Hollywood
Hialeah
Miami
Key Biscayne
Kendall
Homestead
Biscayne National Park
Everglades & Francis S. Taylor Wildlife...
Everglades and Francis S. Taylor Wildlife...
JW Corbett Wildlife Management Area
Pahokee
Belle Glade
Clewiston
Moore Haven
Babcock Ranch Preserve
Charlotte Harbor Preserve State Park
Punta Gorda
Port Charlotte
North Port
Englewood
Rotonda West
Arcadia
LaBelle
Montura
Lehigh Acres
Fort Myers
Okaloacoochee Slough Wildlife...
Pine Island
Pine Island Sound Aquatic Preserve
Fort Myers Beach
Estero
Ave Maria
Bonita Springs
Orangetree
Golden Gate
Naples
Lely Resort
Big Cypress National Preserve
Fakahatchee Strand Preserve State Park
Copelar
Marco Island
Everglades City
Big Cypress Wildlife Management Area...
Image Landsat / Copernicus
Data SIO, NOAA, U.S. Navy, NGA, GEBCO
Google Earth



https://www.eddmaps.org/distribution/viewmap.cfm?sub=3028

Please cite the EDDMapS as:

EDDMapS. 2024. Early Detection & Distribution Mapping System. The University of Georgia - Center for Invasive Species and Ecosystem Health. Available online at http://www.eddmaps.org/; last accessed October 23, 2024.


Plants of Florida


0 1 2 3 4 5 6 7 8 9 10
cm
copyright reserved
ILLINOIS
Plants of Florida
Hydrocharitaceae
Hydrilla verticillata (L.f.) Caspary
Florida. Monroe County
25° 45.566' North Latitude, 80° 59.805' West Longitude
Loop Road west of Pinecrest, Big Cypress Nature Preserve, UTM Zone 17, 500644mE, 2849030mN.
J.L. Crane & Payson M. Pallah 98-491
19 May 1998
J.L. Crane (ILLS)
137914
S00068086
Illinois Natural History Survey (ILLS)

Cassidy Brown, MS
AETX and Wood ducks

Tobias Haymes, MS
Mammals and VM

WHO, WHAT, WHERE, WHEN, HOW?

Graduate Students Vacuolar Myelinopathy Research

Wednesday, April 2

Tobias Elliott Haymes

Master of Science Thesis Defense

"Aetokthonotoxin in Mammals: An Experimental and Exploratory Analysis"

Thursday, April 10

Jeffrey Cullen

Doctoral Dissertation Defense

"Exposure Assessment and Trophic Distribution of Aetokthonotoxin in a Freshwater System"

Friday, April 4

Cassidy Gabrielle Brown

Master Thesis Defense

"Wood Duck (AIX SPONSA) Artificial Nest Box Success and Productivity Assessment Across the Piedmont of Georgia"


Coot 526 ppb


40 ppb


Bald eagle 13 ppb


Bobcat 100 ppb


Florida Panther 1023 ppb

Preliminary maximum AETX concentration in fish and wildlife tissues


Wood duck eggs 40 ppb


14 ppb

Python tail 14 ppb


Beaver 198 ppb


Coyote 8 ppb


Warmouth sunfish 120 ppb


bluegill sunfish 80 ppb


Mouse trial 1000 ppb

Hydrilla AETX concentration >5000 ppb


Sediment AETX concentration 18 ppm

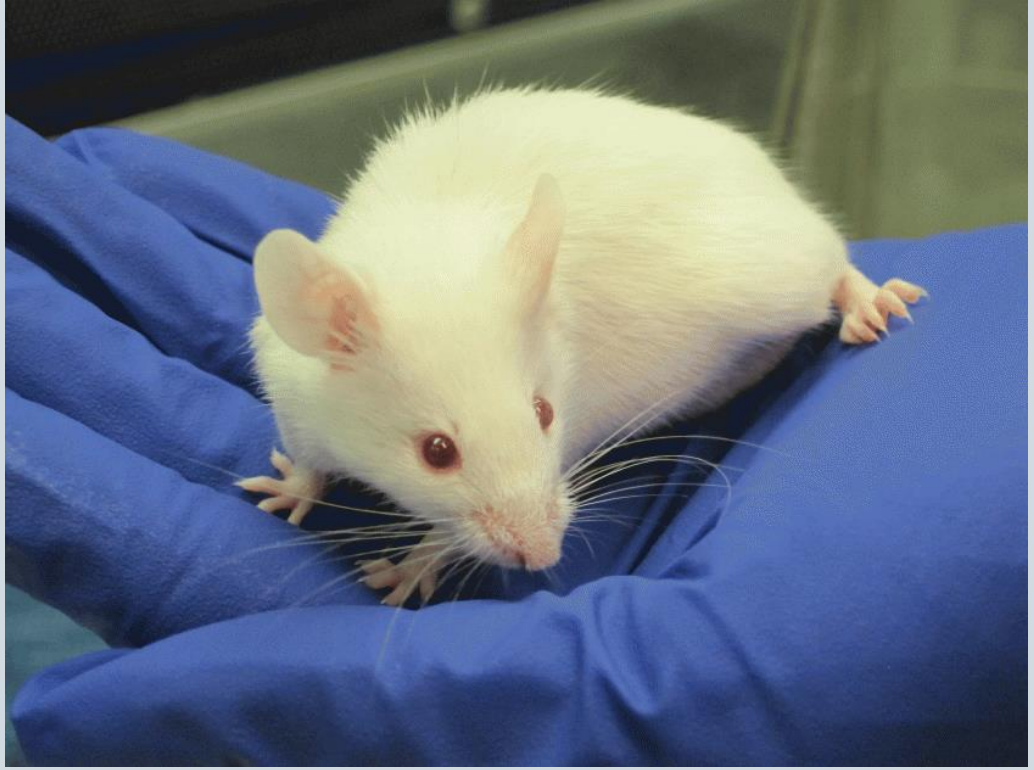
Laboratory Mouse Trials

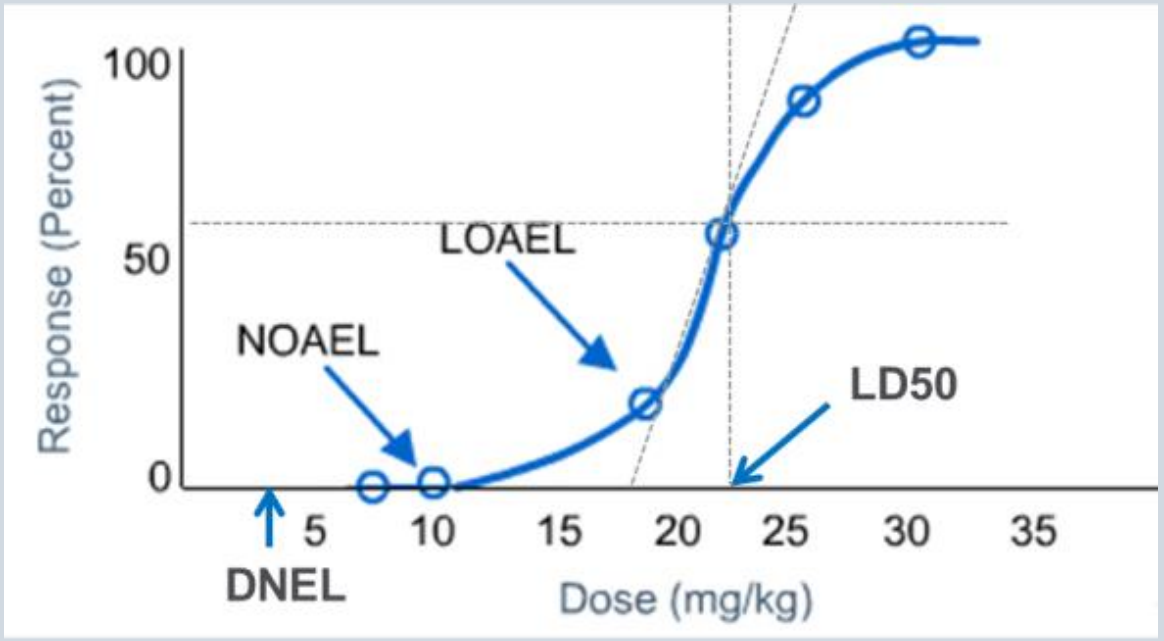
- 14 d gavage trial

Crude extract (AETX +AEST+…)

Control

5, 10, 20 ppm AETX





Initial Limit Test AETX

- 3 dose levels + control – 0, 5, 10, 20 mg/kg by weight AETX
- Include crude extract
- Oral gavage
- 2 mice per group
- Monitored 2x daily for 14 days
- Necropsy, fix brain tissue for EM
- Histological examination - SCWDS

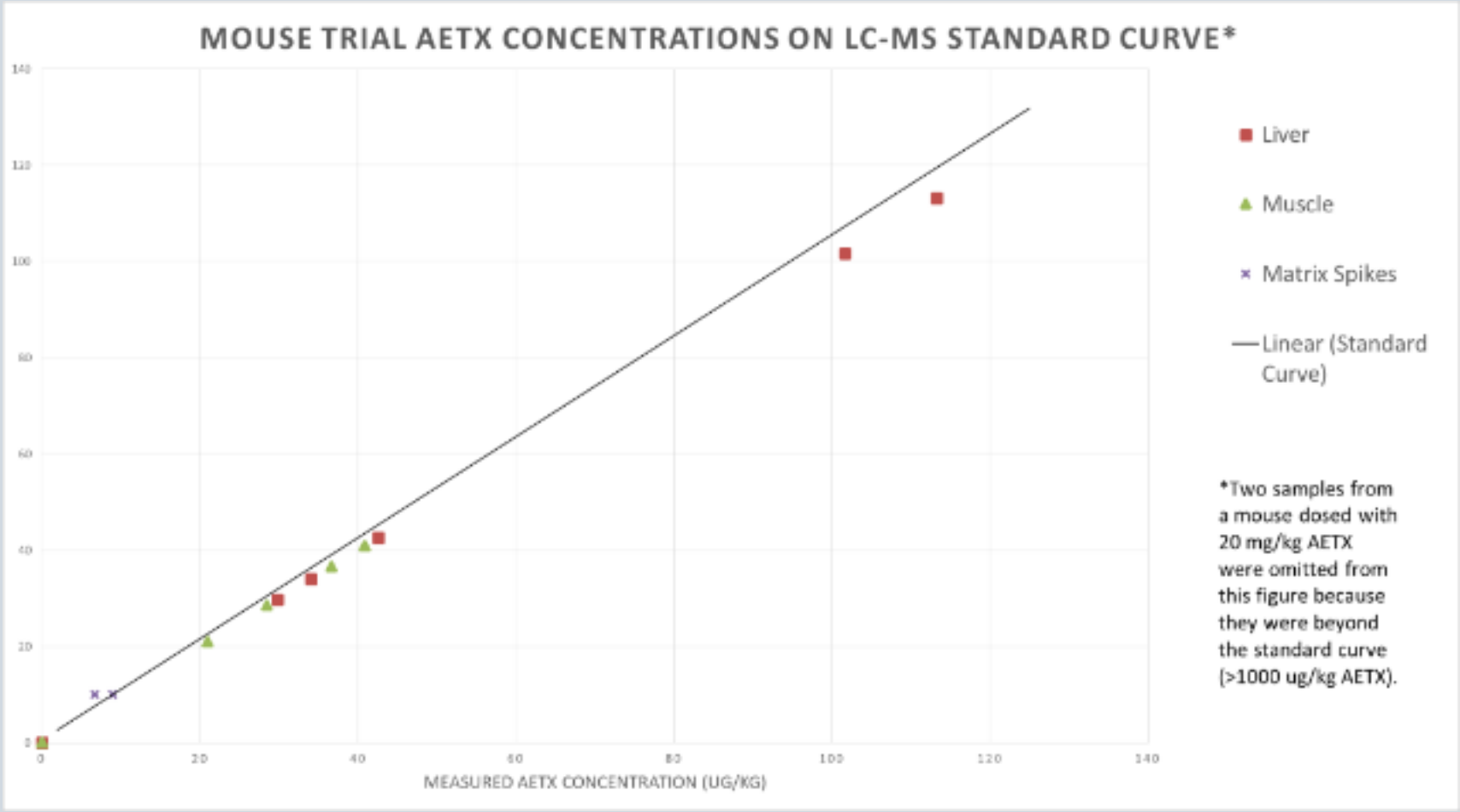
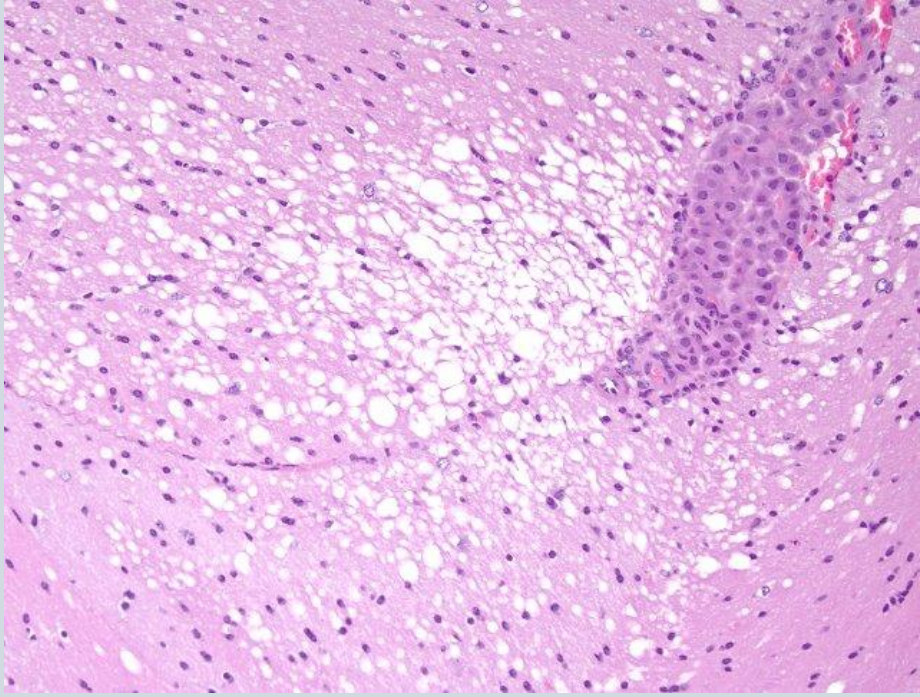


Figure 2. Plot of aetokthonotoxin (AETX) concentrations found in mice tissues (muscle and liver) after a 2-week acute oral toxicity trial. Values obtained using a modified QuEChERS extraction protocol followed by high-performance liquid chromatography with tandem mass spectrometry (Agilent Triple-Quadropole LC-MS). Mice were dosed with 5, 10, and 20 mg/kg AETX. The concentrations seen from a 20 mg/kg-dosed mouse were far above the standard curve (>1000 ug/kg), and were omitted from the figure for visual purposes.

Control

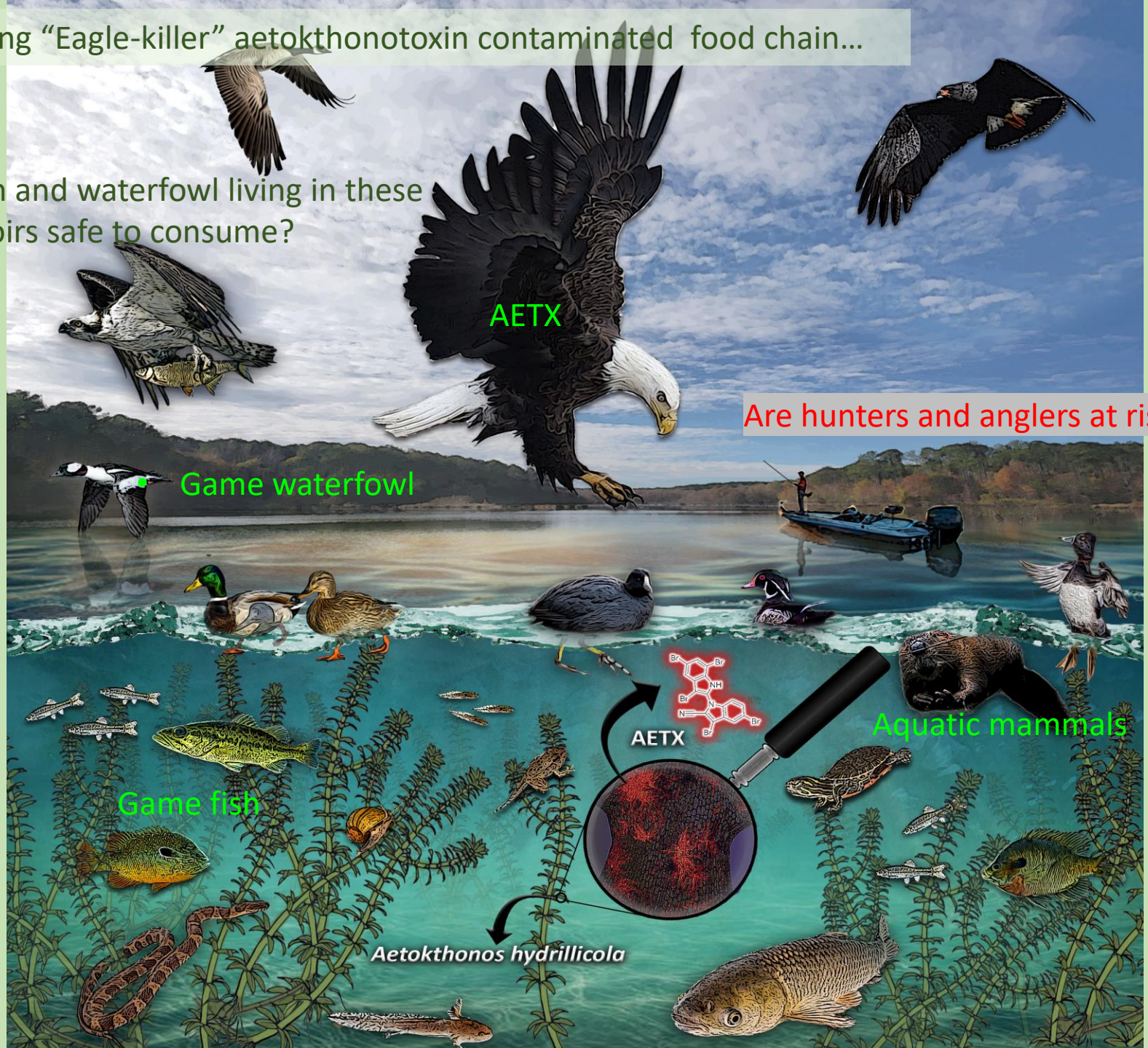


10 mg/km AETX

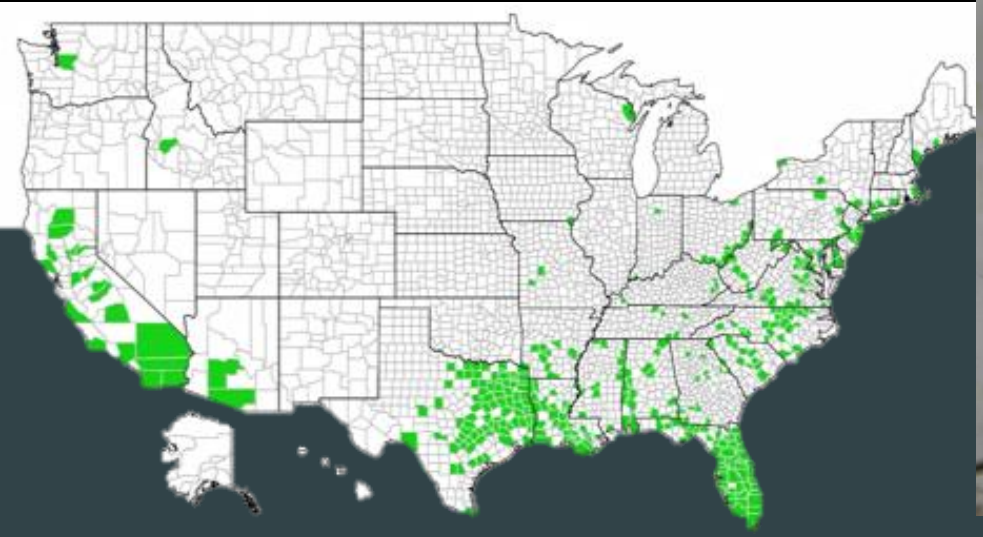
Figure 1. Histologic sections of mouse brains, at similar levels, stained with hematoxylin and eosin. Figure A shows a mouse dosed with a solvent control which has no vacuolation of white matter. In contrast, Figure B shows a mouse dosed with 10 mg/kg aetokthonotoxin (AETX) that has severe vacuolation throughout the white matter of the hippocampus and optic tract.

Expanding “Eagle-killer” aetokthonotoxin contaminated food chain…

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



Contact Information
swilde@uga.edu



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

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University of Georgia
Athens, GA 30602-2152

Cell: 706-247-5912
swilde@uga.edu

Acknowledgements

The University of Georgia WARNELL SCHOOL OF FORESTRY & NATURAL RESOURCES

Wilde Lab UGA

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United States Environmental Protection Agency

Department of Cellular Biology, Franklin College of Arts and Sciences, University of Georgia

Martin-Luther-Universität Halle-Wittenberg

U.S. Fish & Wildlife Service, Department of the Interior

John Carroll University

UF IFAS University of Florida

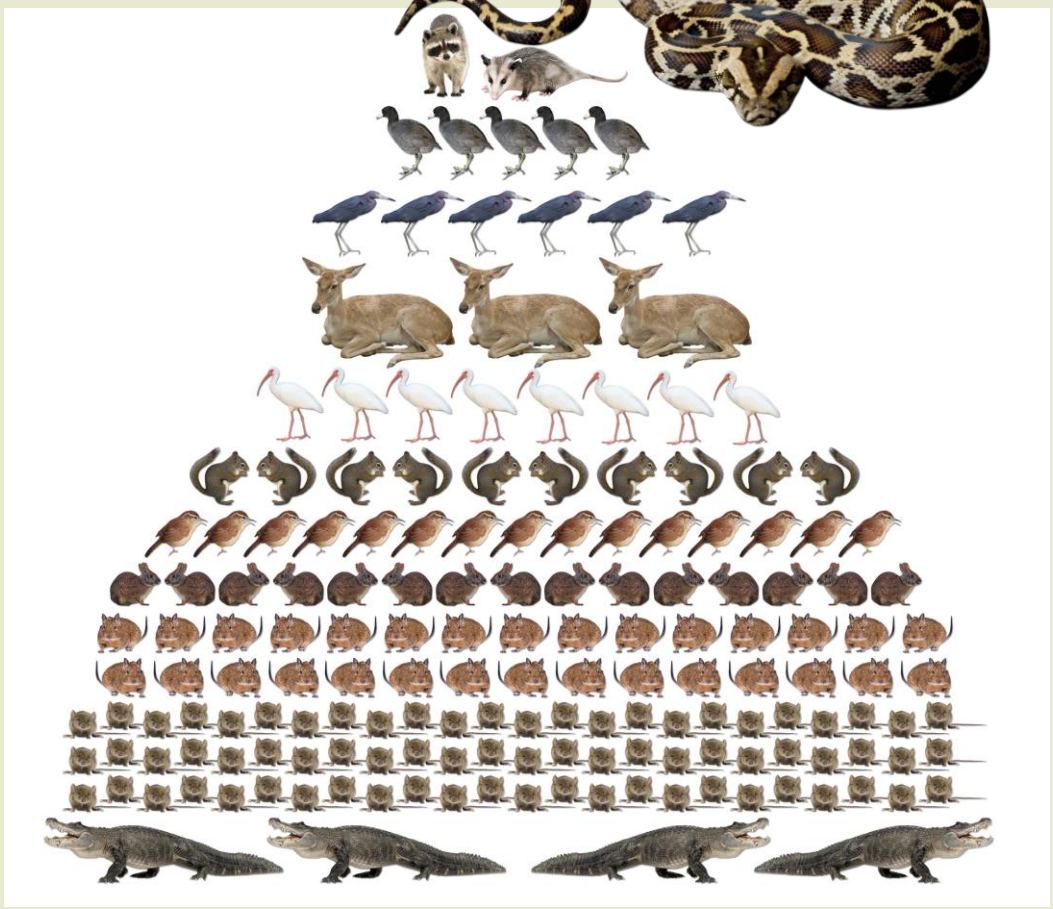
Florida Fish and Wildlife Conservation Commission

South Carolina Department of Natural Resources DNR

Georgia Department of Natural Resources, Wildlife Resources Division

Gulf & South Atlantic Regional Panel on Aquatic Invasive Species

Gulf States Marine Fisheries Commission



Source: Skip Snow, Everglades National Park and Dr. Stephan Secor, University of Alabama.