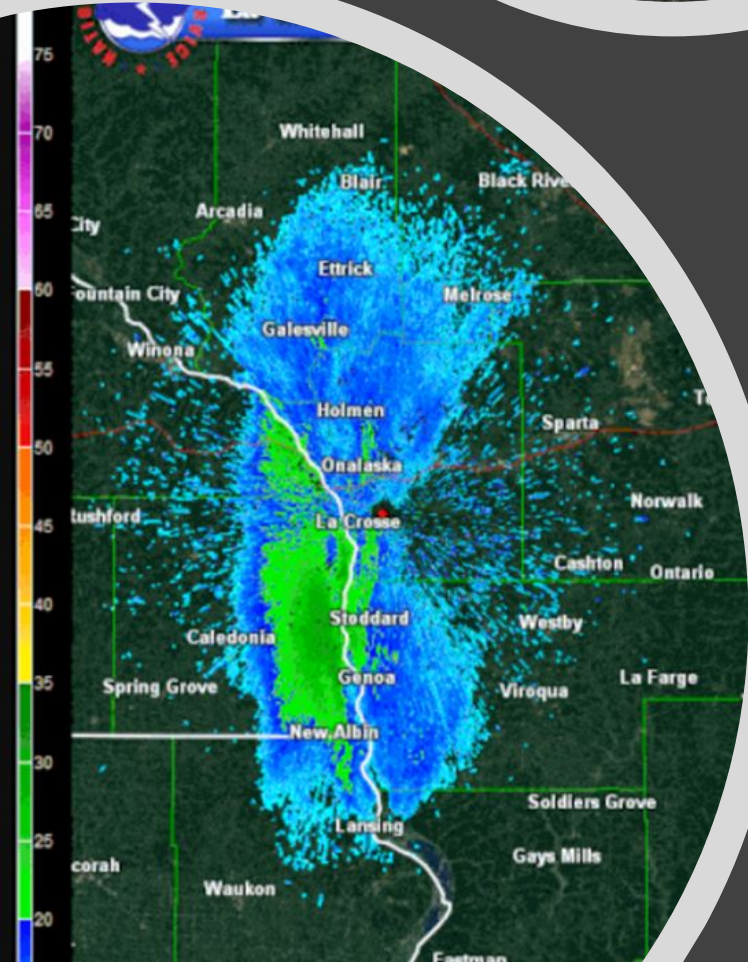




Investigation of Declines in Burrowing Mayfly Populations in the Upper Mississippi River

Shawn Giblin
Mississippi River Water Quality Specialist
Wisconsin Department of Natural Resources



Minneapolis **STAR and Tribune** 1A Metro
 Thursday
 June 25, 1987
 1 Section
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Minneapolis Tribune Co.



Staff Photo by David Brewster

Officer Mike McKenzie examined the slippery mayfly mess Tuesday night on the Interstate Hwy. 494 bridge in South St. Paul.

Some shiver, others rejoice at mayfly blizzard

By Ellen Faley
 Staff Writer

The state dusted off its snowplows and dispatched them to an Interstate Hwy. 494 bridge in South St. Paul Tuesday night, where a slippery mess had piled up on the road, causing two accidents and forcing the State Patrol to close the highway for a short time.

Millions of mayflies attracted by the lights swarmed above the bridge over the Mississippi River about 11:30 p.m., then died after their frantic annual mating ritual.

Their bodies formed up to a foot of slippery goo on the highway, which had to be plowed and then sanded before it was reopened.

Experts forecast that the snowplows will have to stand ready because another enormous batch of adult flies is expected to emerge during the week of July 5 for their day of life.

While the mess might send shivers down some people's spines, biologists and federal officials said people in the Twin Cities should rejoice that the mayflies are back.

Their return in the past two years after a 15-year hiatus signals that the Mississippi River is getting healthier — slowly being transformed from an open sewer back into a natural wonder, experts said.

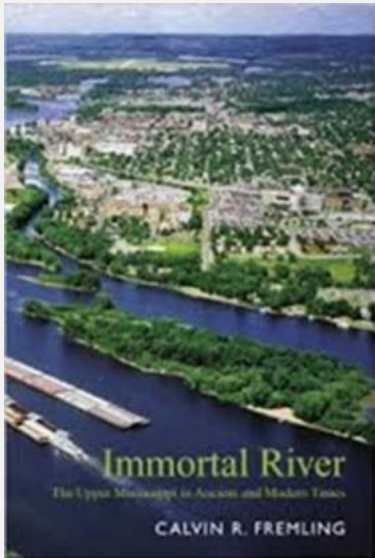
Mayflies continued on page 10A.



Mayflies Return to the Mississippi River: June 1987

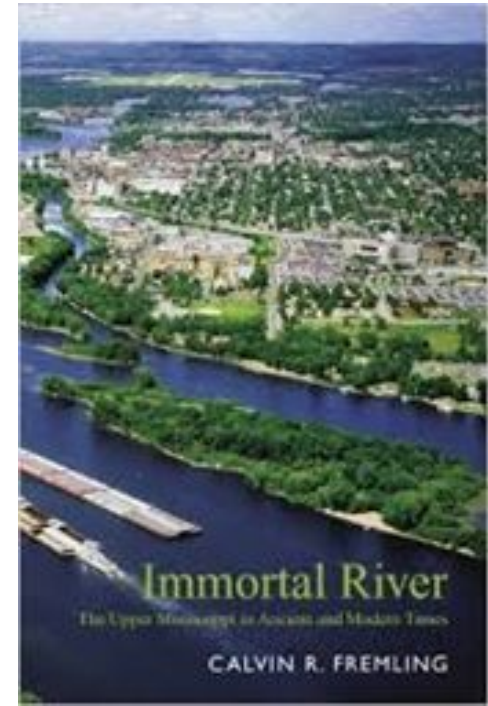
Chapter 19

“Mayflies! What the Hell Good Are They?”



“Burrowing *Hexagenia* nymphs live in sediments where toxins accumulate. They are unable to tolerate anaerobic (no oxygen) conditions, and they can not swim long distances to escape environmental stress. Since they have a relatively long life cycle, the condition of the *Hexagenia* population is a good indicator of water quality over a period of time.”

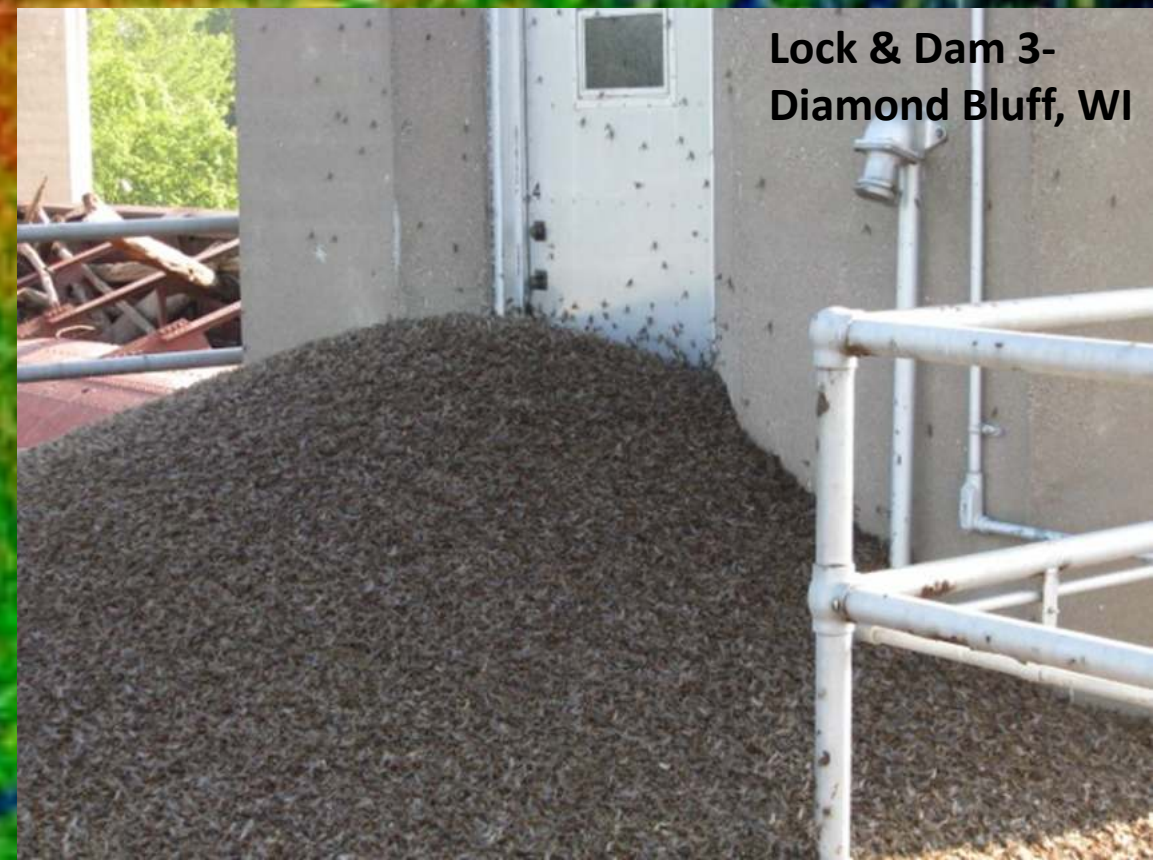
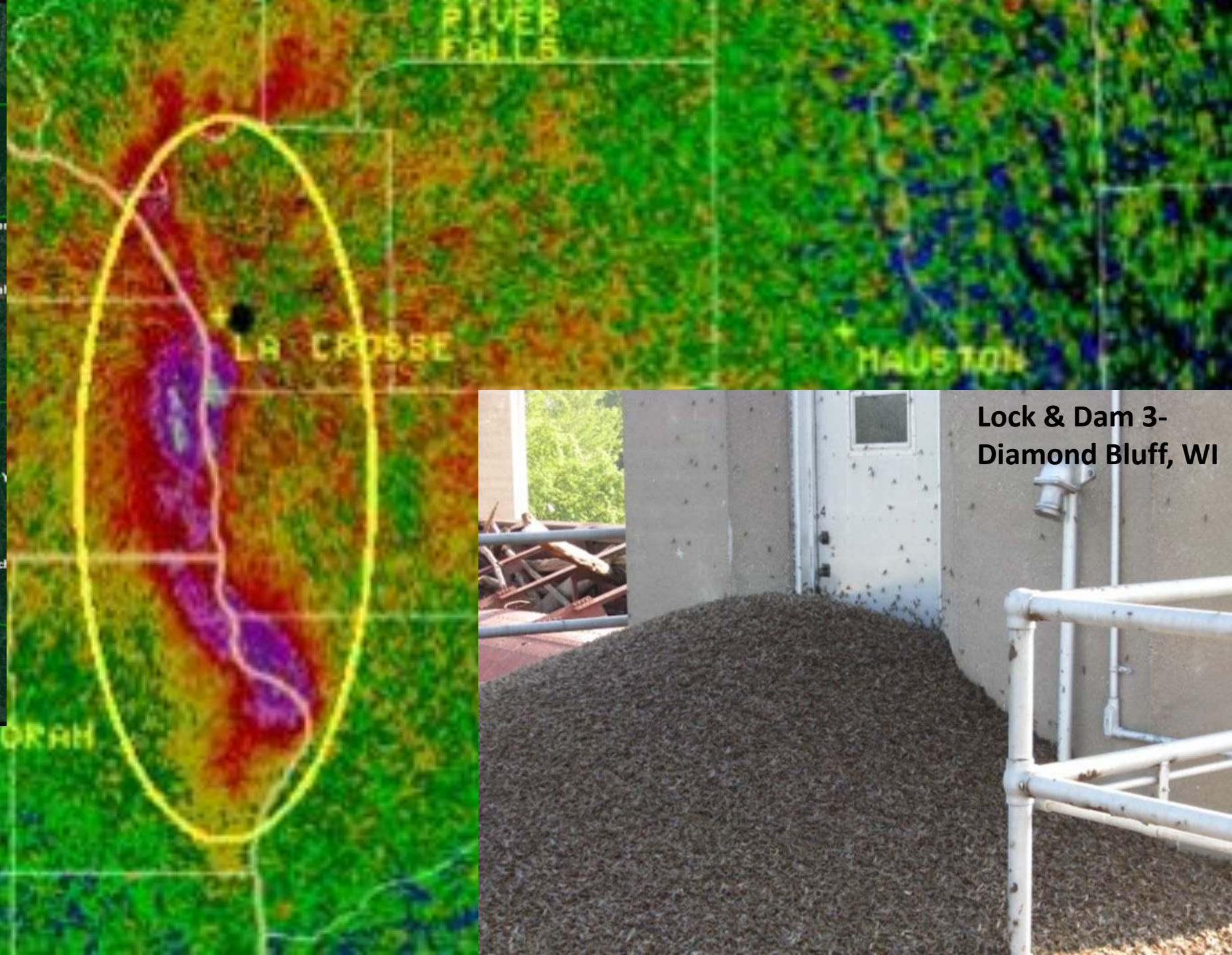
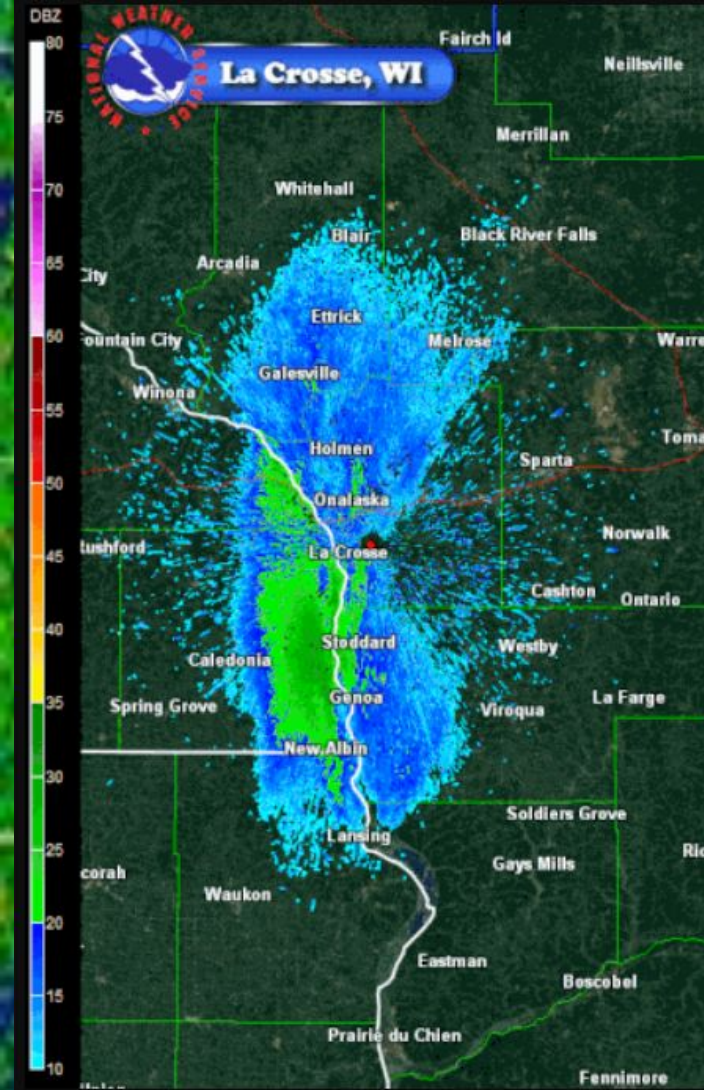
Cal Fremling, *Immortal River*



Burrowing Mayfly Biology

- Two major *Hexagenia* species
 - Largest mayflies in North America
 - Largest hatches in North America occur on Mississippi River and Lake Erie
 - Spend one year as nymphs in burrows on the river bottom
 - Adult life cycle no longer than two days
 - Gravid female lays ~8000 eggs
 - Adults = 104 calories each
 - Annual hatches = trillions of calories
- *Hexagenia limbata*
 - Hatch earlier in season
 - Lighter, yellowish color
 - *Hexagenia bilineata*
 - Hatch later in season
 - Darker color
 - Generally more abundant





Declines in an abundant aquatic insect, the burrowing mayfly, across major North American waterways

Phillip M. Stapanian^{a,b,c,1}, Sally A. Entekhabi^d, Charlotte E. Wainwright^e, Djordje Mirkovic^f, Jennifer L. Tank^g, and Jeffrey F. Kelly^{a,h}

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“History doesn’t repeat itself, but it often rhymes”

— Mark Twain

across many of North America’s largest waterways. The immense scale of mayfly emergences made them a natural spectacle, and reports of the aquatic insects blanketing waterfront cities regularly filled newspaper headlines (12). Deep drifts of mayflies rendered streets impassable until snowplows could clear and grit roadways, and the dense swarms reduced visibility and inhibited water navigation, temporarily halting river transportation (12). These large *Hexagenia* populations were vital for supporting the commercial fishing industry and recreational anglers (13)

D.M. contributed new *Hexagenia* analytical tools; P.M.S. analyzed data; and P.M.S., S.A.E., C.E.W., J.L.T., and J.F.K. wrote the paper.

The authors declare no competing interest.

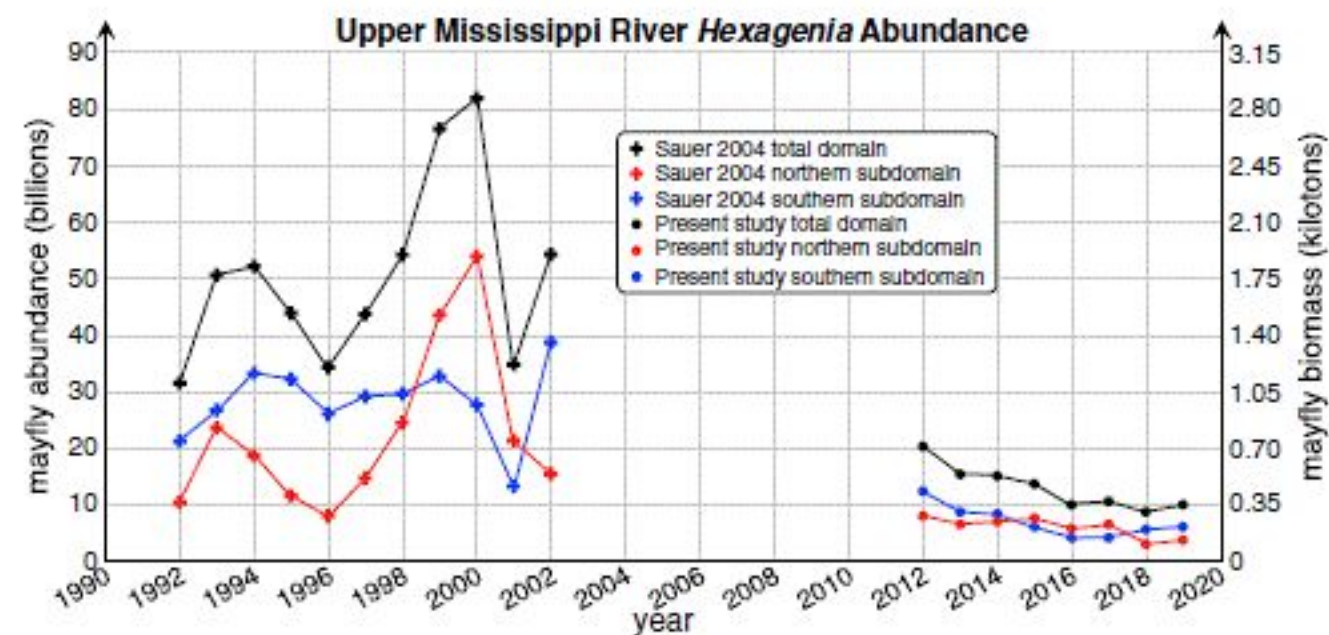
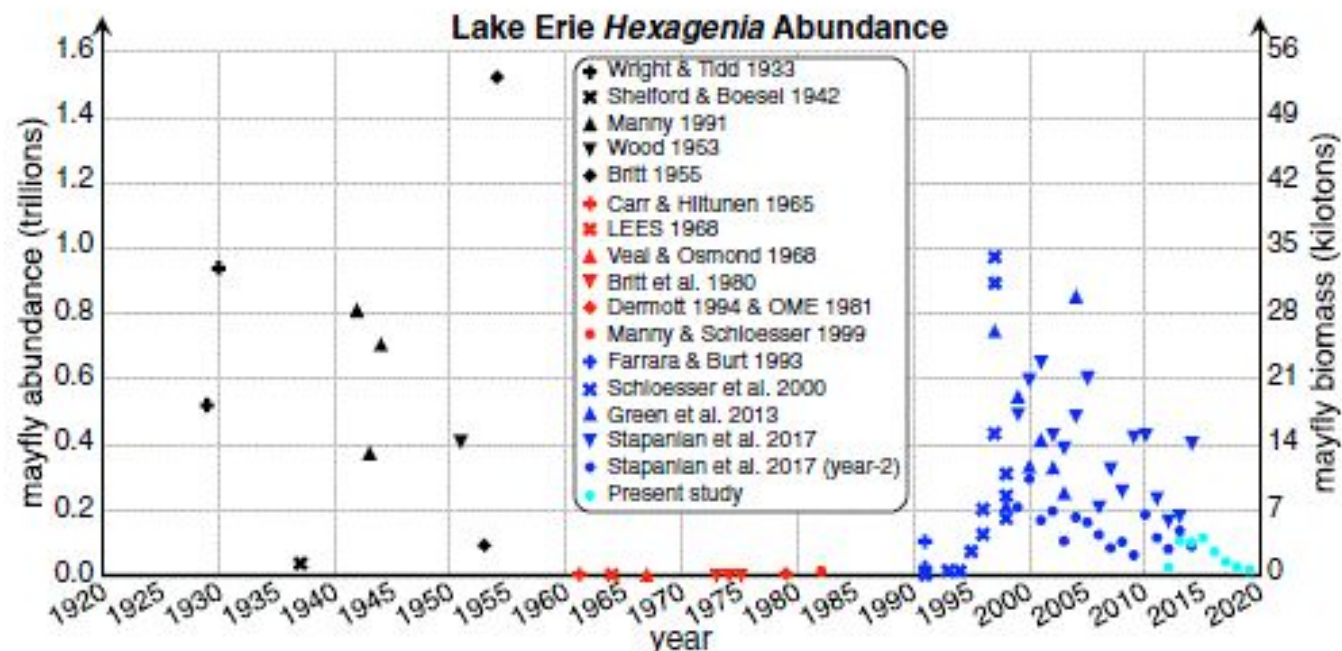
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In recent years, production across both waterways has declined by over 50%

HOME / NEWS / ENVIRONMENTAL NEWS

Mayflies, a keystone species, are on the decline on the Mississippi River

Scientists are interested in predicting the mayflies' timing, but they need the public's help

By Madeline Heim, Milwaukee Journal Sentinel

Jul. 24, 2023 5:00 am, Updated: Jul. 24, 2023 8:37 am



NATIONAL
GEOGRAPHIC



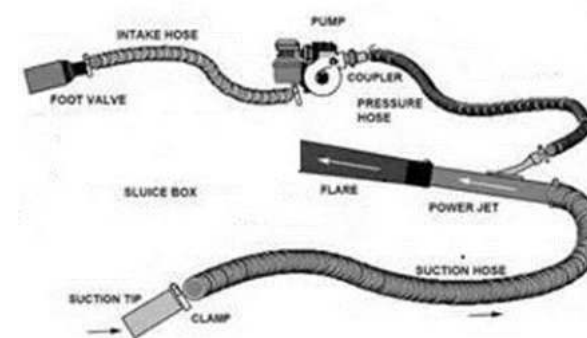
A burrowing mayfly (*Hexagenia limbata*), newly emerged on leaf in Minnesota. Populations in the upper Mississippi Basin dropped 52 percent from 2012 to 2019.

PHOTOGRAPH BY MICHAEL FRANCIS, EARTH SCENES, NAT GEO IMAGE COLLECTION

ANIMALS | NEWS

Mayfly numbers drop by half since 2012, threatening food chain

Mayflies, which form swarms in the billions that are visible on weather radar, are in steep decline, mirroring the plight of insects worldwide.



- Sampling changes occurred and goal of study wasn't to quantify trends.
- Field staff report that *Hexagenia* spp. have become more difficult to collect in recent years.
- Another source of data to consider using “multiple lines of evidence” approach.

Burrowing Mayflies 2023 vs. Historical Abundance

LTRM Field Stations

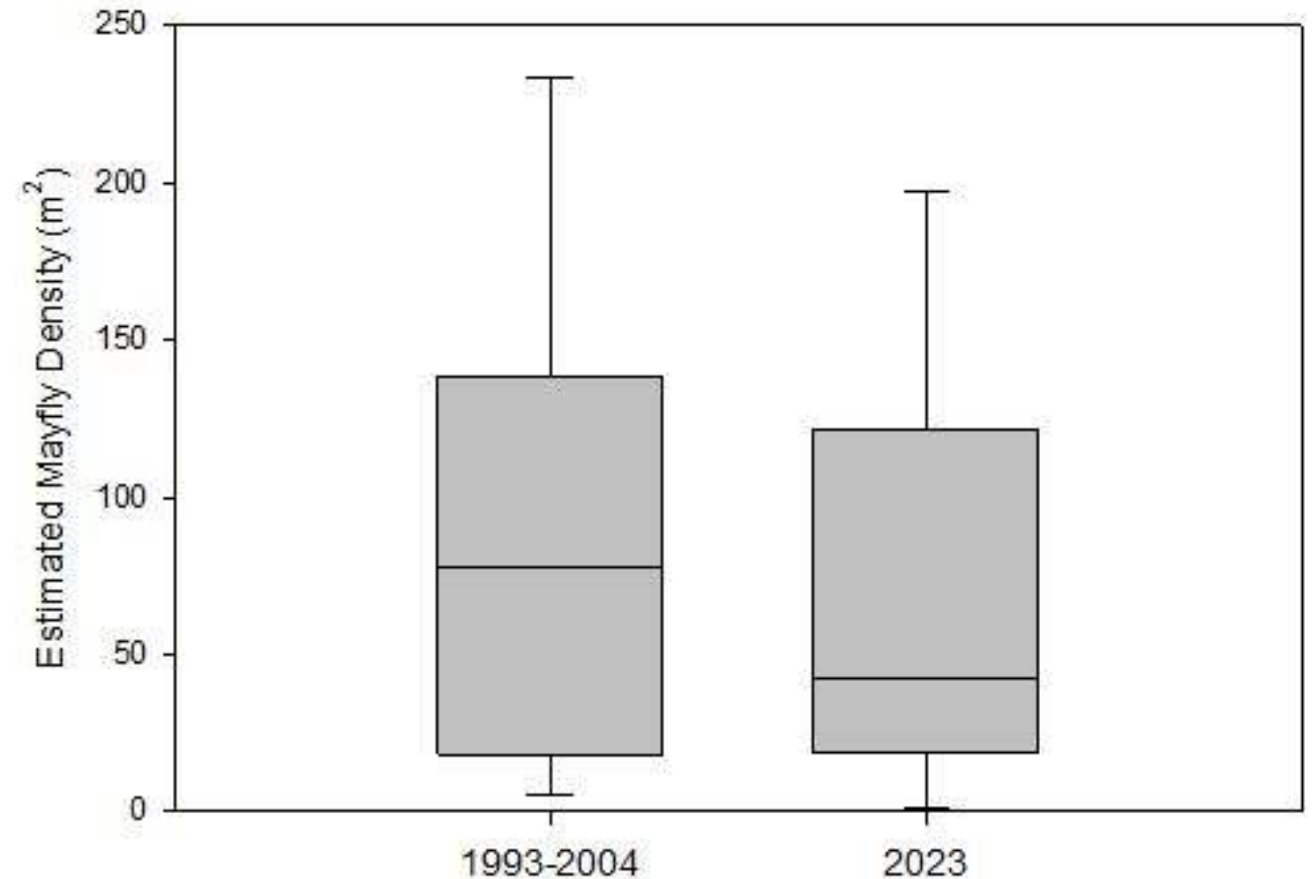


Invertebrate sampling in the LTRM involves lowering a ponar dredge to the bottom of the river and scooping up the soft bottom (usually sand, silt, clay, or a mixture). Sand, silt, and clay are then washed through a screen, leaving the aquatic insects, clams, mussels, and snails behind on the screen. These are identified. Target organisms (mayfly larva, midge larva, fingernail clams, zebra mussels, and Asiatic clams) are counted, and presence/absence is noted on the others.



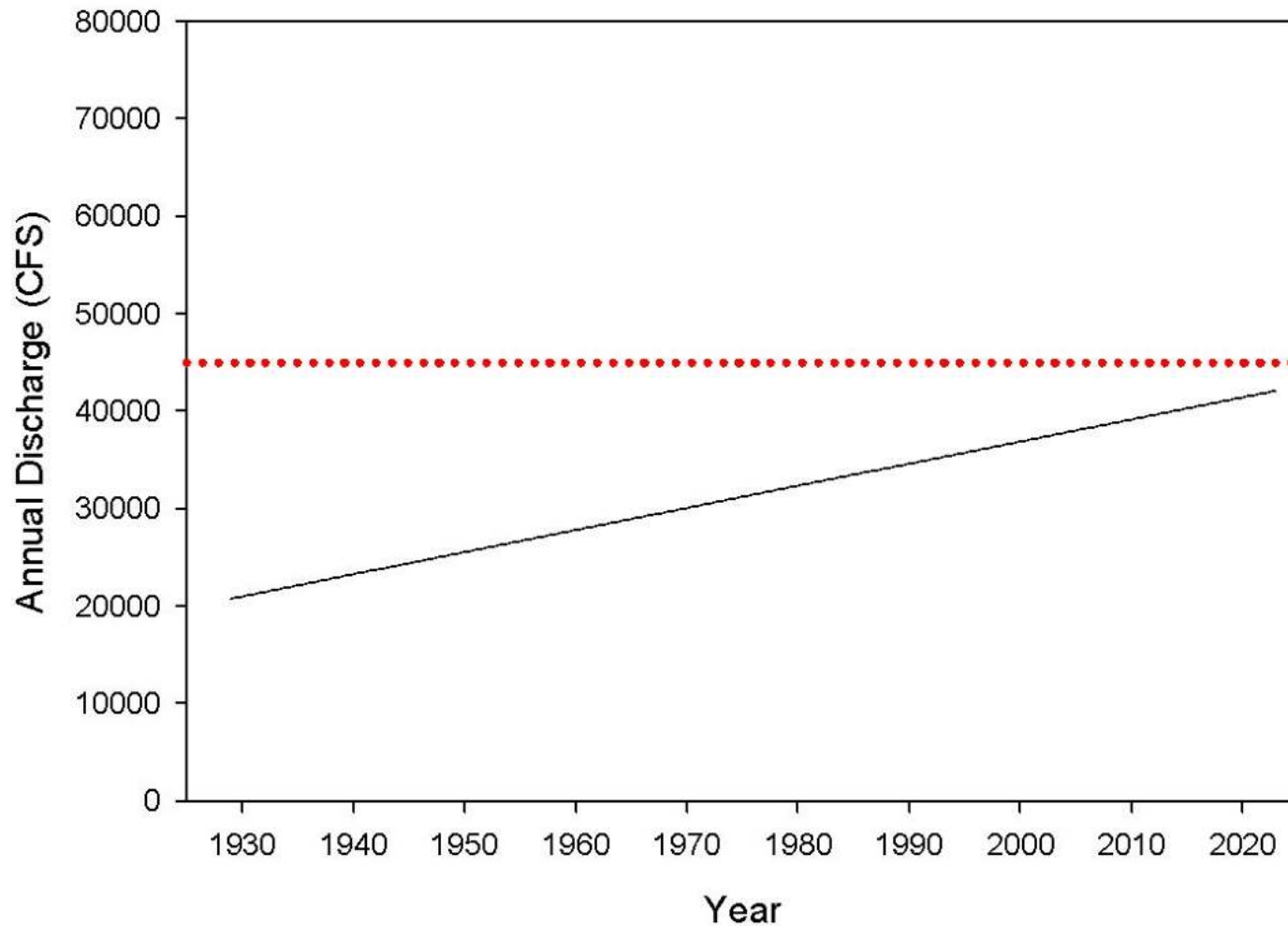
The jaws of the ponar dredge have been opened and mud grabbed from the bottom of the river is being rinsed into a wash screen. The mud will pass through the mesh of the wash screen, leaving the inhabitants of the river bottom behind.

45% Decrease from 1993-2004 to 2023



Title of Project: Assessing long term changes and spatial patterns in macroinvertebrates through standardized long-term monitoring

Mean Annual Discharge Winona 1929-2023



- Substantial increase in mean annual discharge at Winona past 90 years
- Mean annual discharge on the Mississippi River at Winona never exceeded 45,000 CFS during any year from 1929-1980
- The 45,000 CFS threshold has been exceeded 12x since 1980. Seven of these years have been since 2011



Pesticide Prioritization by Potential Biological Effects in Tributaries of the Laurentian Great Lakes

Samantha K. Oliver,^{1,*} Steven R. Corsi,² Austin K. Baldwin,³ Michele A. Nott,⁴ Gerald T. Ankley,⁵ Brett R. Blackwell,⁶ Daniel L. Villeneuve,⁷ Michelle L. Hladik,⁸ Dana W. Kolpin,⁹ Luke Loken,¹⁰ Laura A. DeCicco,¹¹ Michael T. Meyer,¹² and Keith A. Loftis¹

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Abstract: Watersheds of the Great Lakes Basin (USA/Canada) are highly modified and impacted by human activities including pesticide use. Despite labeling restrictions intended to minimize risks to nontarget organisms, concerns remain that environmental exposures to pesticides may be occurring at levels negatively impacting nontarget organisms. We used a combination of organism-level toxicity estimates (in vivo aquatic life benchmarks) and data from high-throughput screening (HTS) assays (in vitro benchmarks) to prioritize pesticides and sites of concern in streams at 16 tributaries to the Great Lakes Basin. In vivo or in vitro benchmark values were exceeded at 15 sites, 10 of which had exceedances throughout the year. Pesticides had the greatest potential biological impact at the site with the greatest proportion of agricultural land use in its basin (the Maumee River, Toledo, OH, USA), with 72 percent compounds or transformation products being detected, 47 of which exceeded at least one benchmark value. Our risk-based screening approach identified multiple pesticide parent compounds in tributaries of the Great Lakes; these compounds included: eight herbicides (metolachlor, acetochlor, 2,4-dichlorophenoxyacetic acid, diuron, atrazine, alachlor, tripyrid, and simazine), three fungicides (chlorothalonil, propiconazole, and carbendazim), and four insecticides (diazinon, fipronil, imidacloprid, and chlorfenvin). We present methods for reducing the volume and complexity of potential biological effects data that result from combining contaminant surveillance with HTS (in vitro) and traditional in vivo toxicity estimates. *Environ Toxicol Chem* 2023;42:367–384. Published 2022. This article is a U.S. Government work and is in the public domain in the USA. *Environmental Toxicology and Chemistry* published by Wiley Periodicals, LLC on behalf of SETAC.

Keywords: Pesticides; neonicotinoids; Laurentian Great Lakes; biological effects; high-throughput screening

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Short Communication

ACUTE AND CHRONIC TOXICITY OF NEONICOTINOIDS TO NYMPHS OF A MAYFLY SPECIES AND SOME NOTES ON SEASONAL DIFFERENCES

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Paul J. Van der Horst,^{1,*} Joanne M. Van Soest,



Lethal and sublethal toxicity of neonicotinoid and butenolide insecticides to the mayfly, *Hexagenia* spp.[☆]

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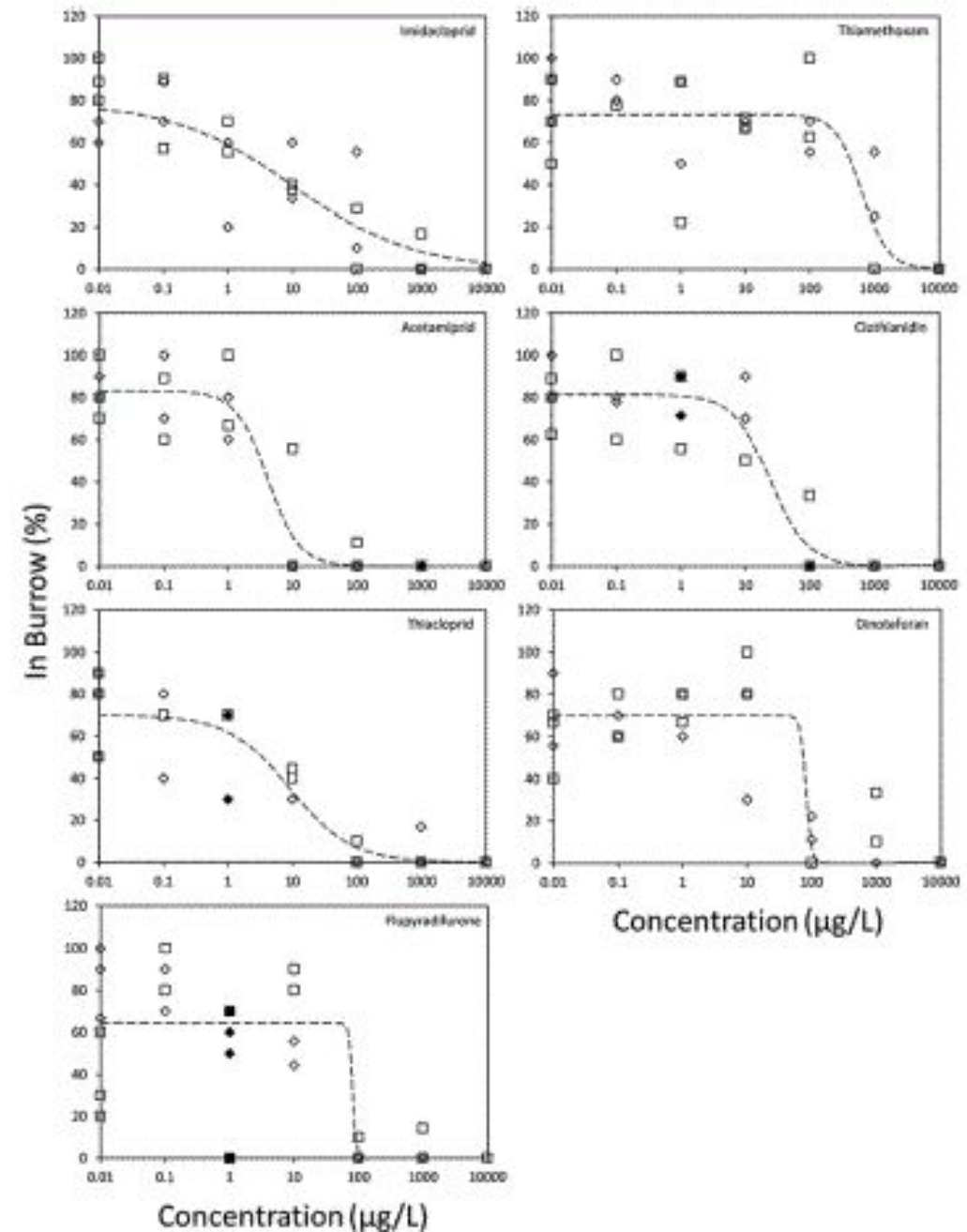
Non-target species

ABSTRACT

Neonicotinoid insecticides are environmentally persistent and highly water-soluble, and thus are prone to leaching into surface waters where they may negatively affect non-target aquatic insects. Most of the research to date has focused on imidacloprid, and few data are available regarding the effects of other neonicotinoids or their proposed replacements (butenolide insecticides). The objective of this study was to assess the toxicity of six neonicotinoids (imidacloprid, thiamethoxam, acetamiprid, clothianidin, thiacloprid, and dinotefuran) and one butenolide (flupyradifurone) to *Hexagenia* spp. (mayfly larvae). Acute (96-h), water-only tests were conducted, and survival and behaviour (number of surviving mayflies inhabiting artificial burrows) were assessed. Acute sublethal tests were also conducted with imidacloprid, acetamiprid, and thiacloprid, and in addition to survival and behaviour, mobility (ability to burrow into sediment) and recovery (survival and growth following 21 d in clean sediment) were measured. Sublethal effects occurred at much lower concentrations than survival: 96-h LC50s ranged from 780 µg/L (acetamiprid) to >10,000 µg/L (dinotefuran), whereas 96-h EC50s ranged from 4.0 µg/L (acetamiprid) to 630 µg/L (thiamethoxam). Flupyradifurone was intermediate in toxicity, with a 96-h LC50 of 2000 µg/L and a 96-h EC50 of 81 µg/L. Behaviour and mobility were impaired significantly and to a similar degree in sublethal exposures to 10 µg/L imidacloprid, acetamiprid, and thiacloprid, and survival and growth following the recovery period were significantly lower in mayflies exposed to 10 µg/L acetamiprid and thiacloprid, respectively. A suite of effects on mayfly swimming behaviour/ability and respiration were also observed, but not quantified, following exposures to imidacloprid, acetamiprid, and thiacloprid at 1 µg/L and higher. Imidacloprid concentrations measured in North American surface waters have been found to meet or exceed those causing toxicity to *Hexagenia*, indicating that environmental concentrations may adversely affect *Hexagenia* and similarly sensitive non-target aquatic species.

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Sublethal effects (behavior and mobility changes well within range of many North American waters)



Aquatic Invertebrate Neonic Concentration Benchmarks Used

Morrissey et al. 2015 (Review Paper):

35 PPT – 199 PPT (Chronic)

≥ 200 PPT (Acute)

EPA Aq. Life Benchmarks (Chronic):

Clothianidin ≥ 50 PPT

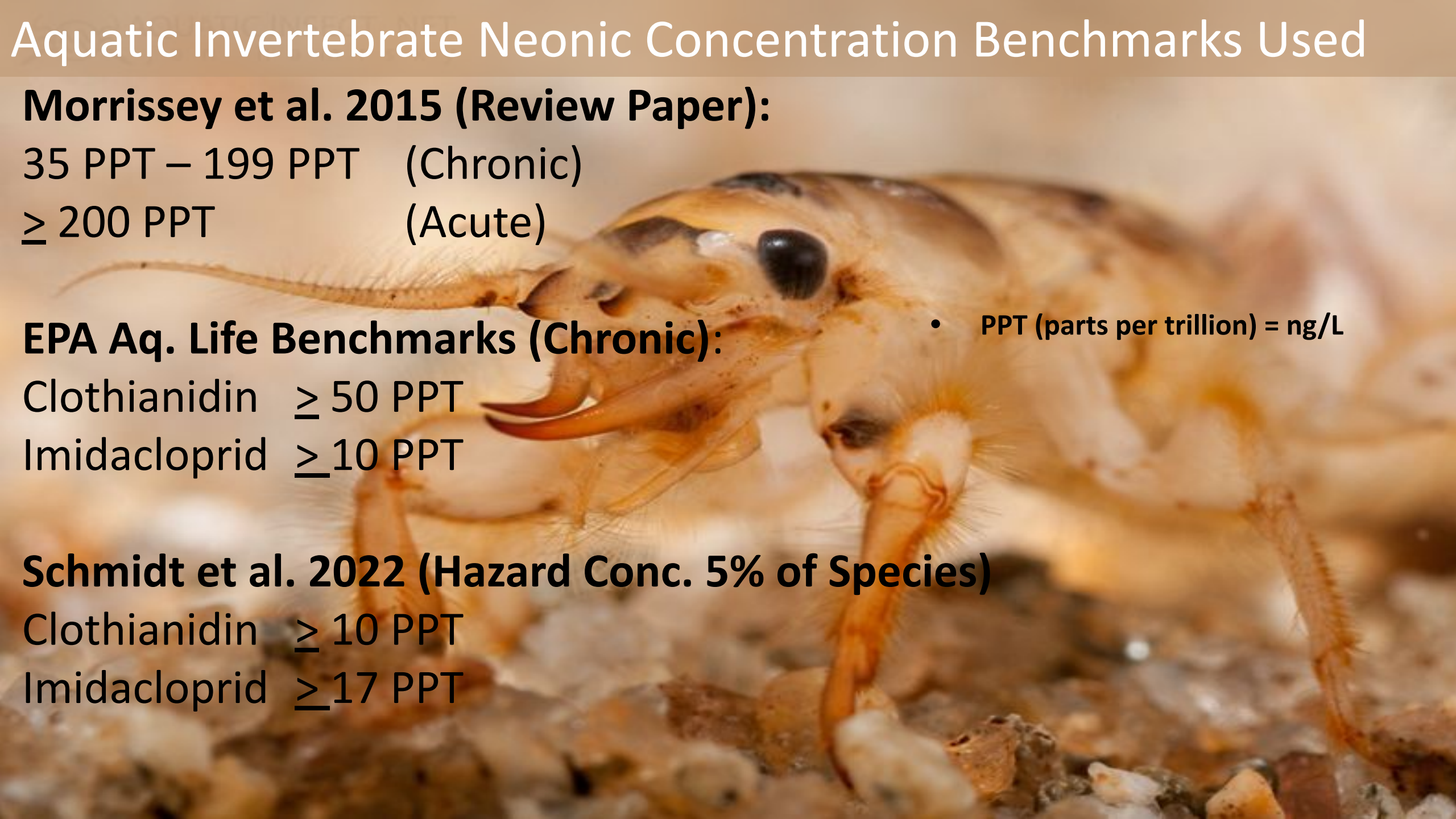
Imidacloprid ≥ 10 PPT

- PPT (parts per trillion) = ng/L

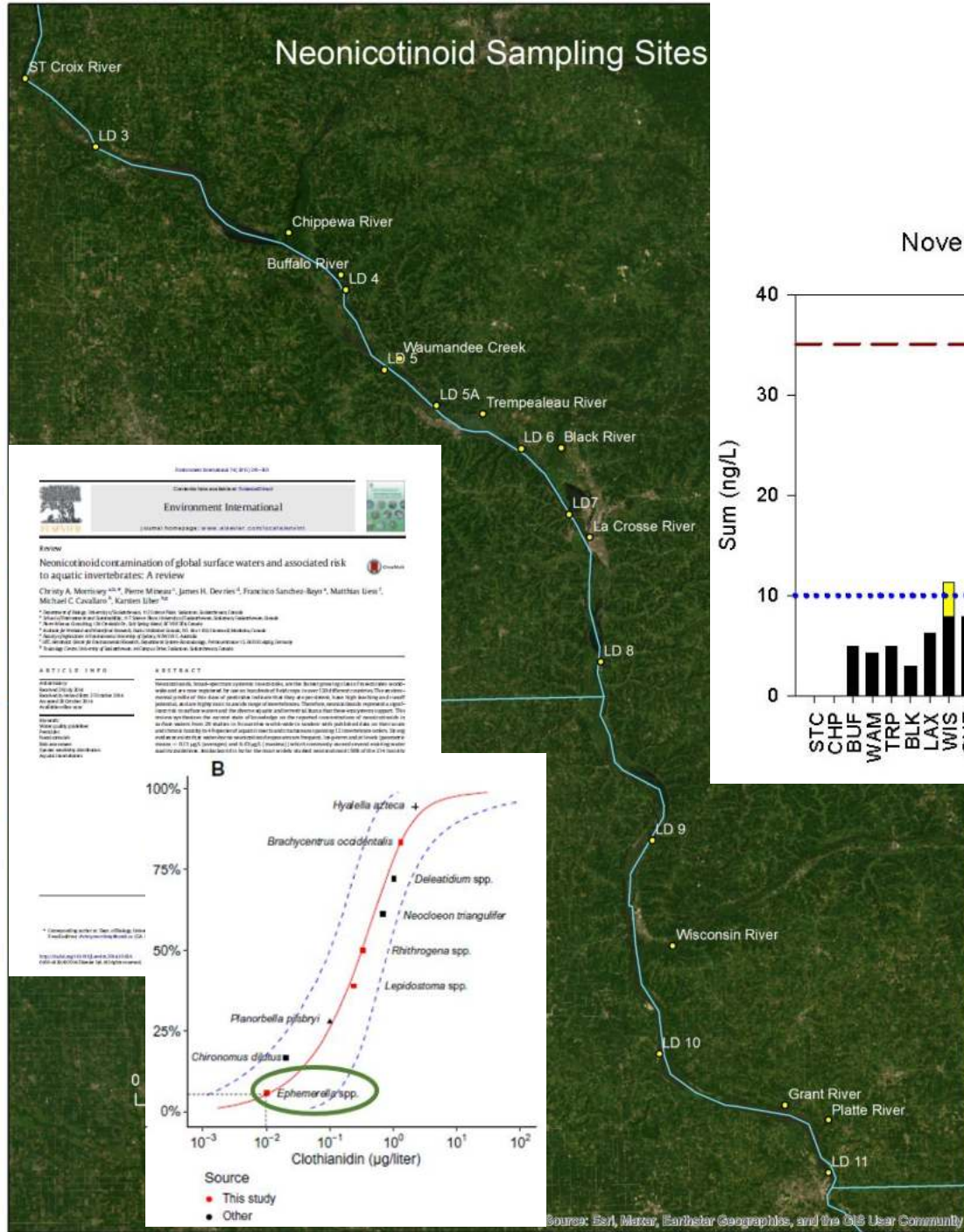
Schmidt et al. 2022 (Hazard Conc. 5% of Species)

Clothianidin ≥ 10 PPT

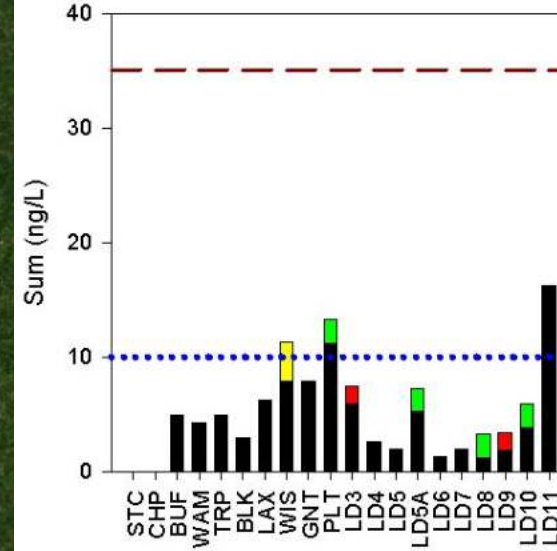
Imidacloprid ≥ 17 PPT



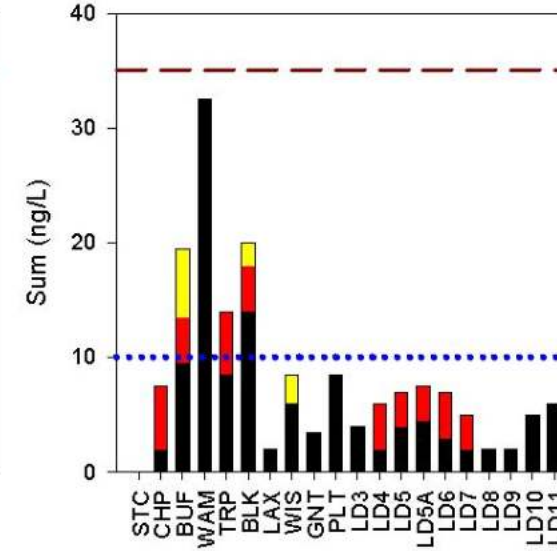
Neonicotinoid Sampling Sites



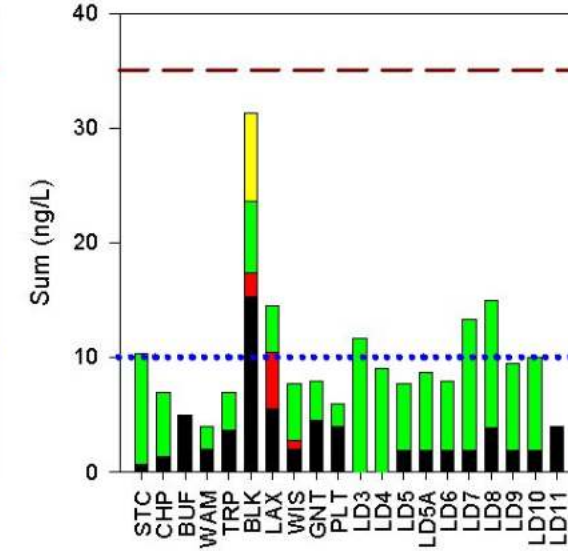
November 2021



May 2022



July 2022



- Neonics detected at 95% of sampling sites
- Non-detects (St. Croix River 2x; Chippewa River 1x)
- 8.3% of samples >10 ng/L clothianidin- hazard concentration 5% of species (Schmidt et. 2022)

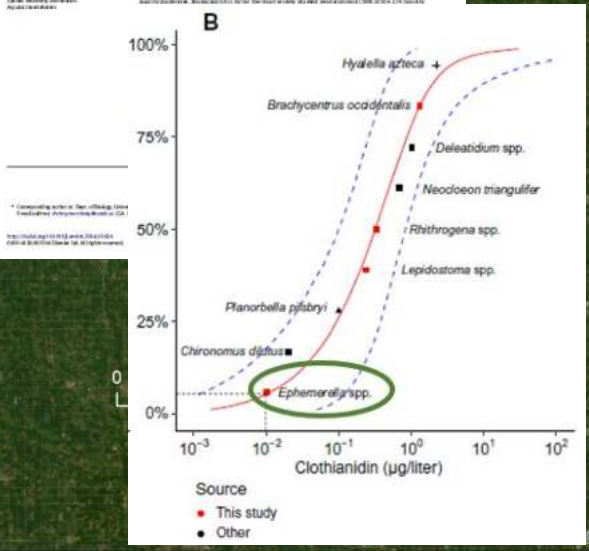


Review
Neonicotinoid contamination of global surface waters and associated risk to aquatic invertebrates: A review
Christy A. Martiny ^{a,*}, Pierre Mineau ^a, James H. Davies ^a, Francisco Sanchez-Bayo ^a, Matthias Lien ^b, Michael C. Crowley ^c, Karen Uller ^d

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ABSTRACT
Neonicotinoid insecticides (neonics) are the most widely used class of insecticides in the world and are a major component of the pest management arsenal in many agricultural and urban settings. The widespread use of neonics has led to their detection in a wide range of aquatic environments. This review synthesizes the current state of knowledge on the reported contamination of neonicotinoids in surface waters from 20 studies in 10 countries, worldwide, in relation to their potential risk to aquatic invertebrates. The review identifies the most commonly reported neonicotinoids in aquatic environments, the most commonly reported species affected, and the most commonly reported adverse effects. The review also identifies the most commonly reported neonicotinoids in aquatic environments, the most commonly reported species affected, and the most commonly reported adverse effects. The review also identifies the most commonly reported neonicotinoids in aquatic environments, the most commonly reported species affected, and the most commonly reported adverse effects.



Source: Bart, Moxer, Earthstar Geographics, and the GIS User Community

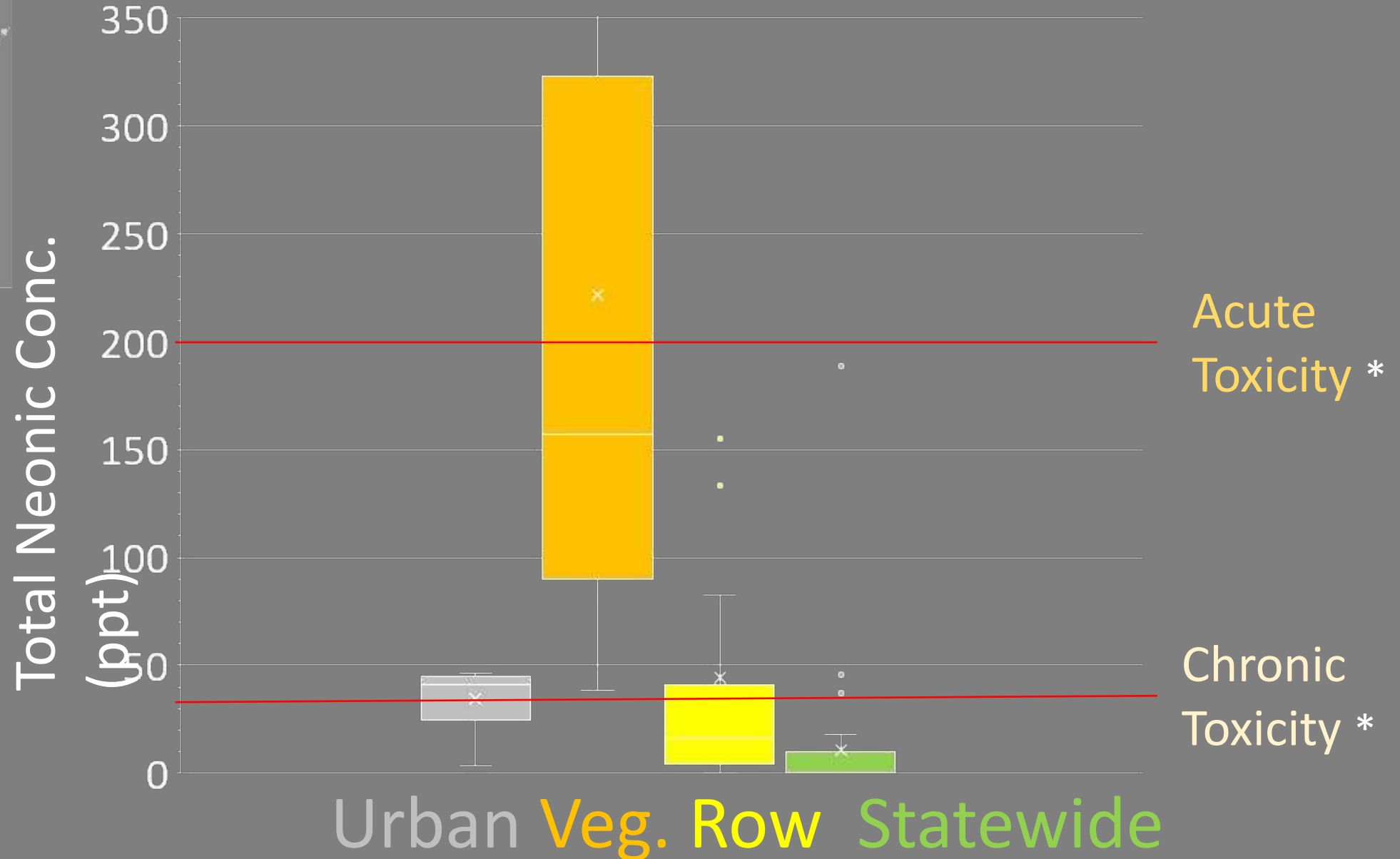
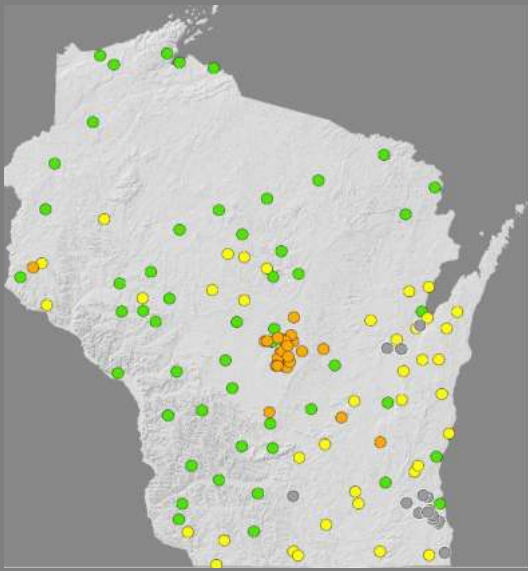
Sampling Design

Land Use Stratification

- Urban n = 11
- Veg. Crops n = 20
- Row Crops n = 39
- Other* n = 48

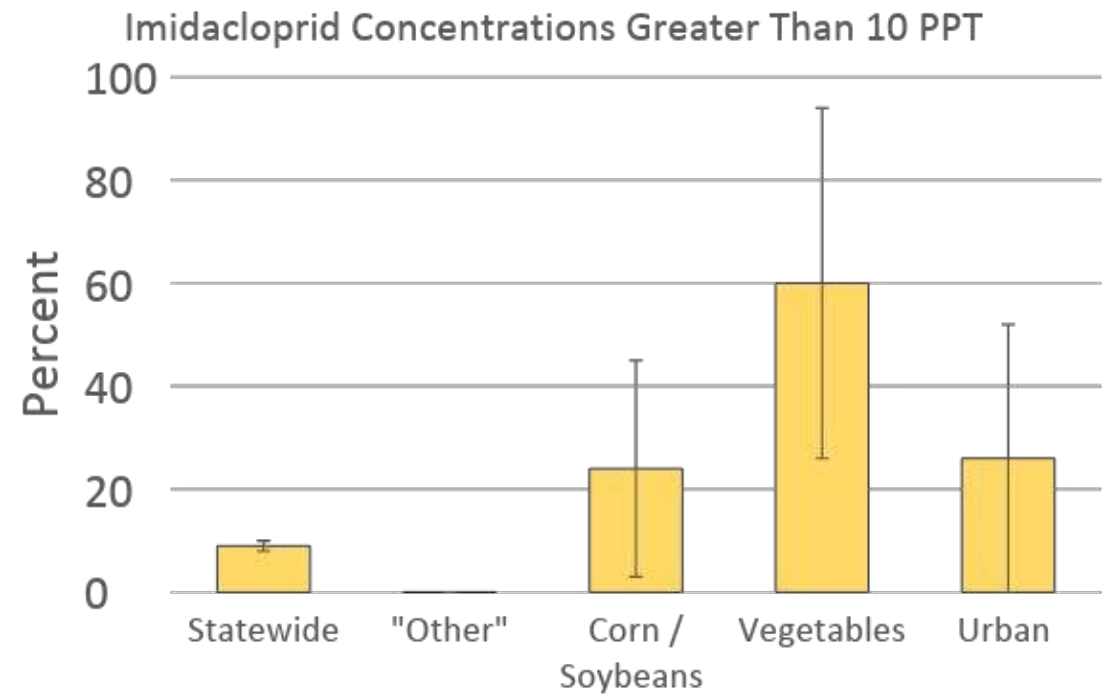
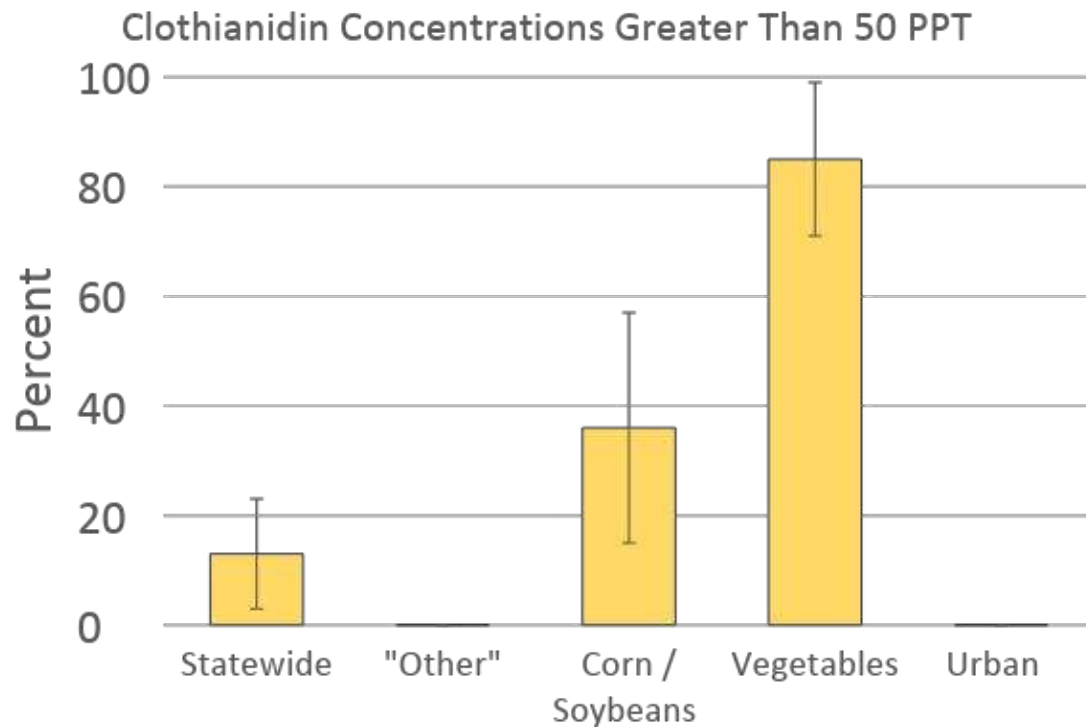
*Land types other than
Urban, Veg. Row Crops

2022 Sampling Results



ppt = ng/L

Wisconsin Streams & Rivers Exceeding EPA Aquatic Life Benchmarks (Freshwater Invert Chronic)

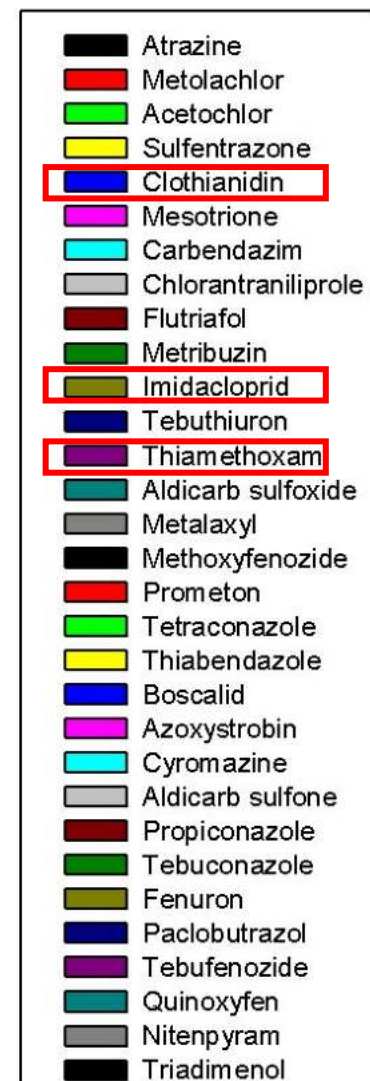
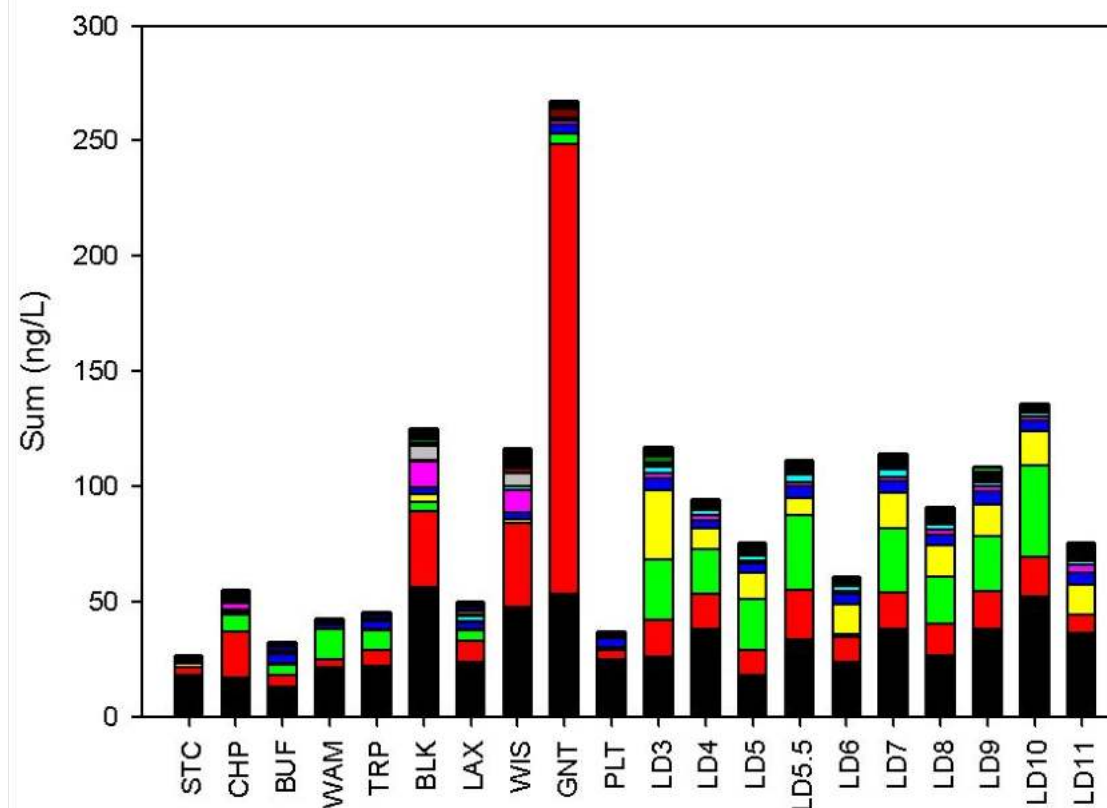
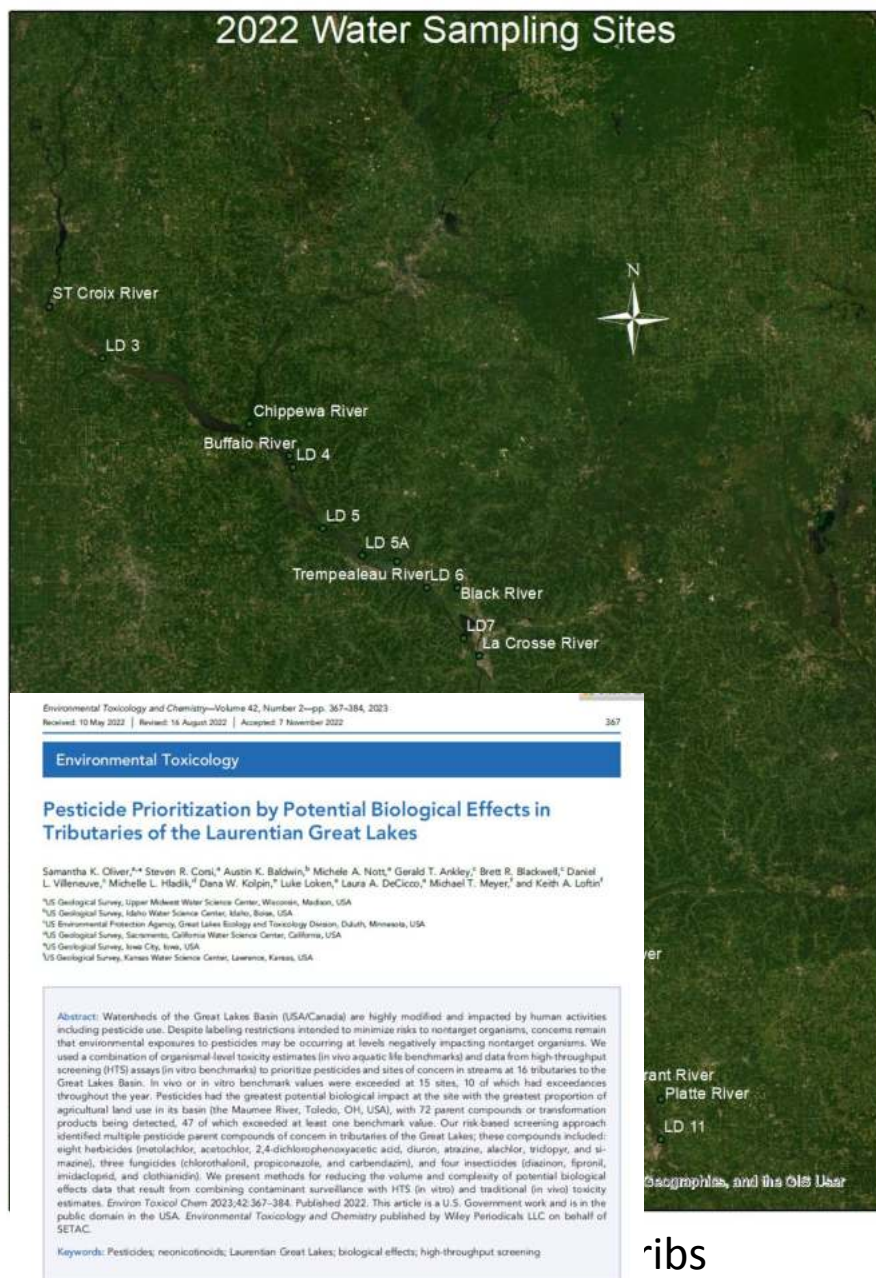


Dominant Watershed Land Use

PPT = ng/L



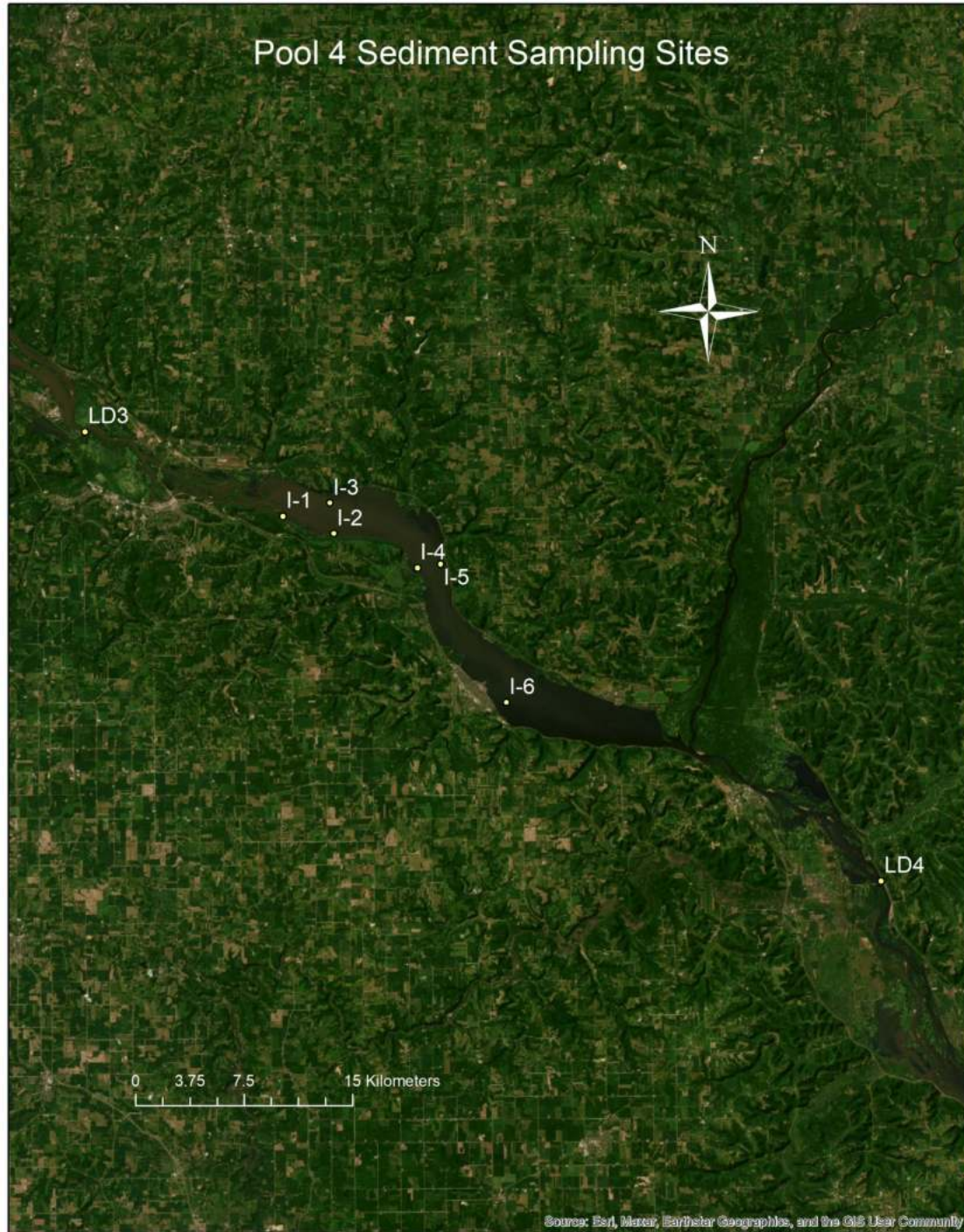
Water Samples Sum Top 31 Compounds



“Our risk-based screening approach identified multiple pesticide parent compounds of concern in tributaries of the Great Lakes; these compounds included: eight herbicides (metolachlor, acetochlor, 2,4-dichlorophenoxyacetic acid, diuron, atrazine, alachlor, triclopyr, and simazine), three fungicides (chlorothalonil, propiconazole, and carbendazim), and four insecticides (diazinon, fipronil, imidacloprid, and clothianidin)”

3 of top 13 detects are neonics

Pool 4 Sediment Sampling Sites



June 2022

Pool 4
Sediment Sites
n=8

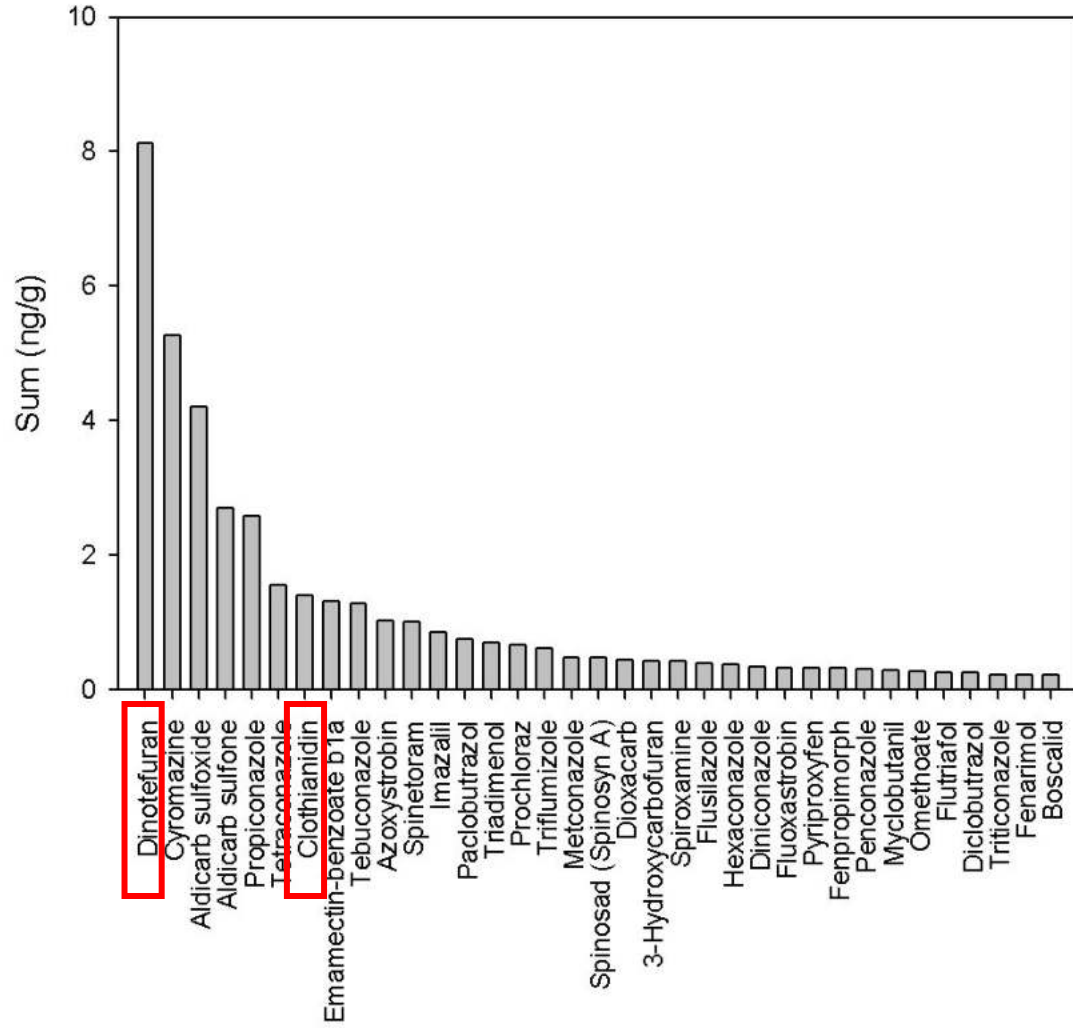
Pool 8
Sediment Sites
n=7

Pool 8 Sediment Sampling Sites

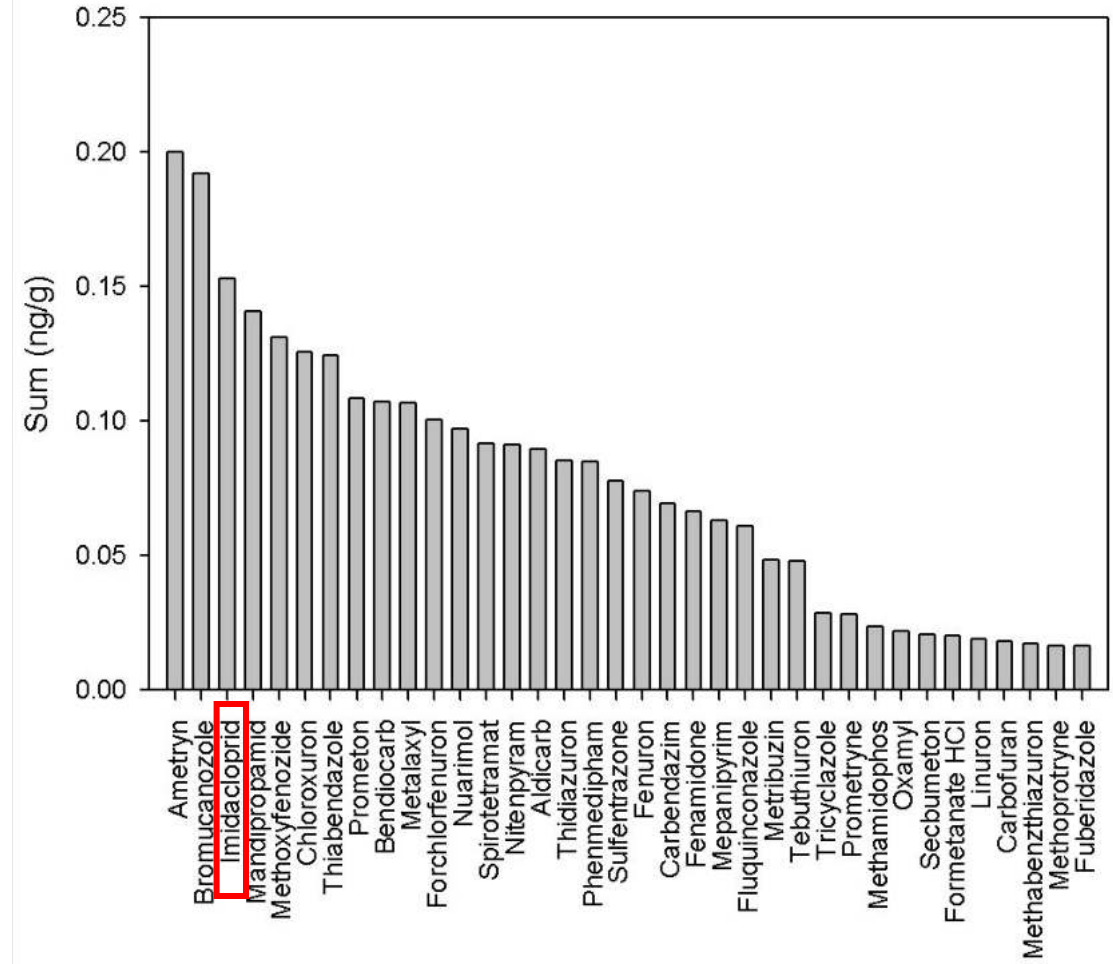


Top 70 Compounds in Sediment

Top 35



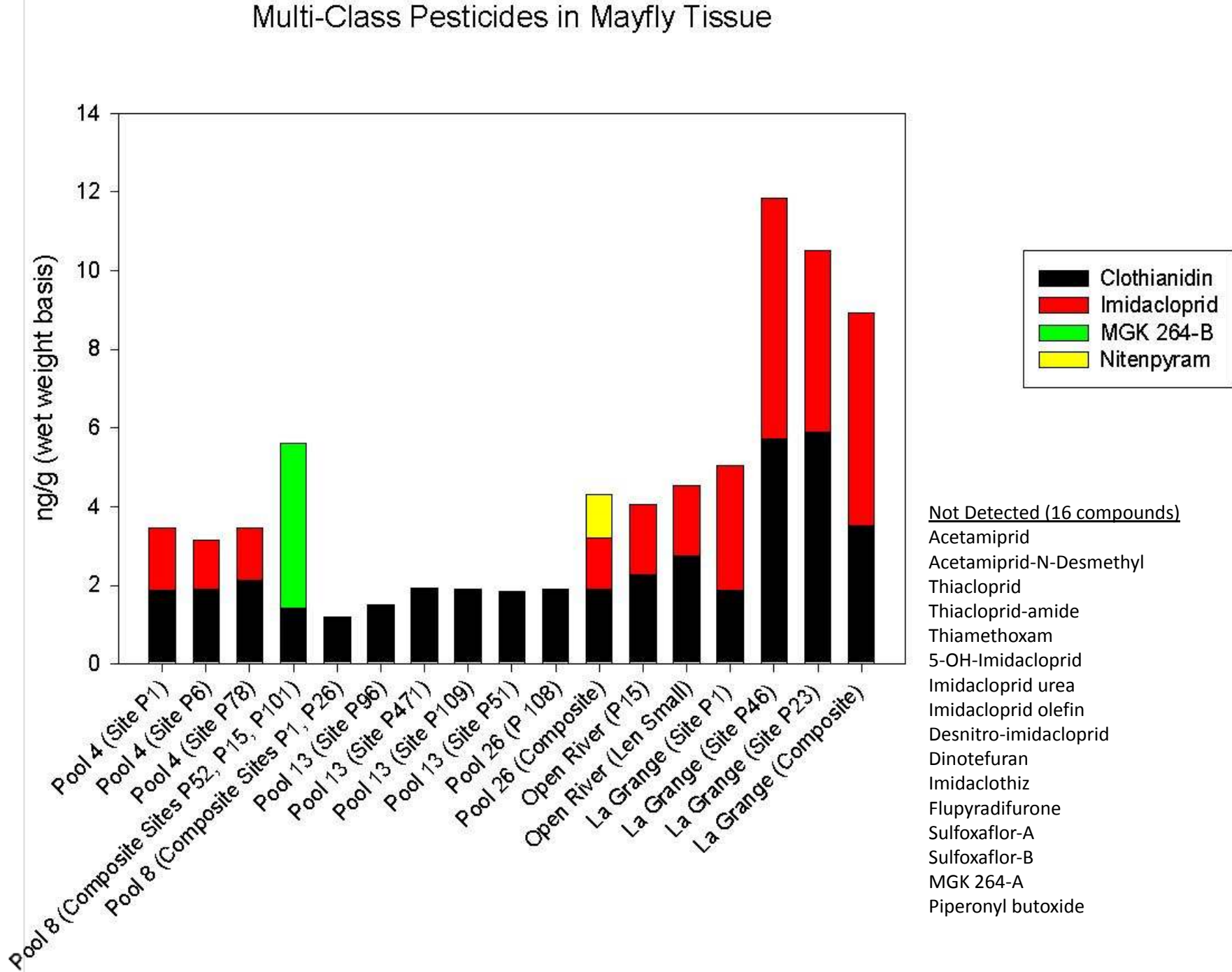
36-70 by Conc.



2023- paired water, sediment, and mayfly tissue samples collected



- Clothianidin detected in mayfly tissue @ 100% of sampling sites
- Imidacloprid detected in mayfly tissue at 59% of sampling sites

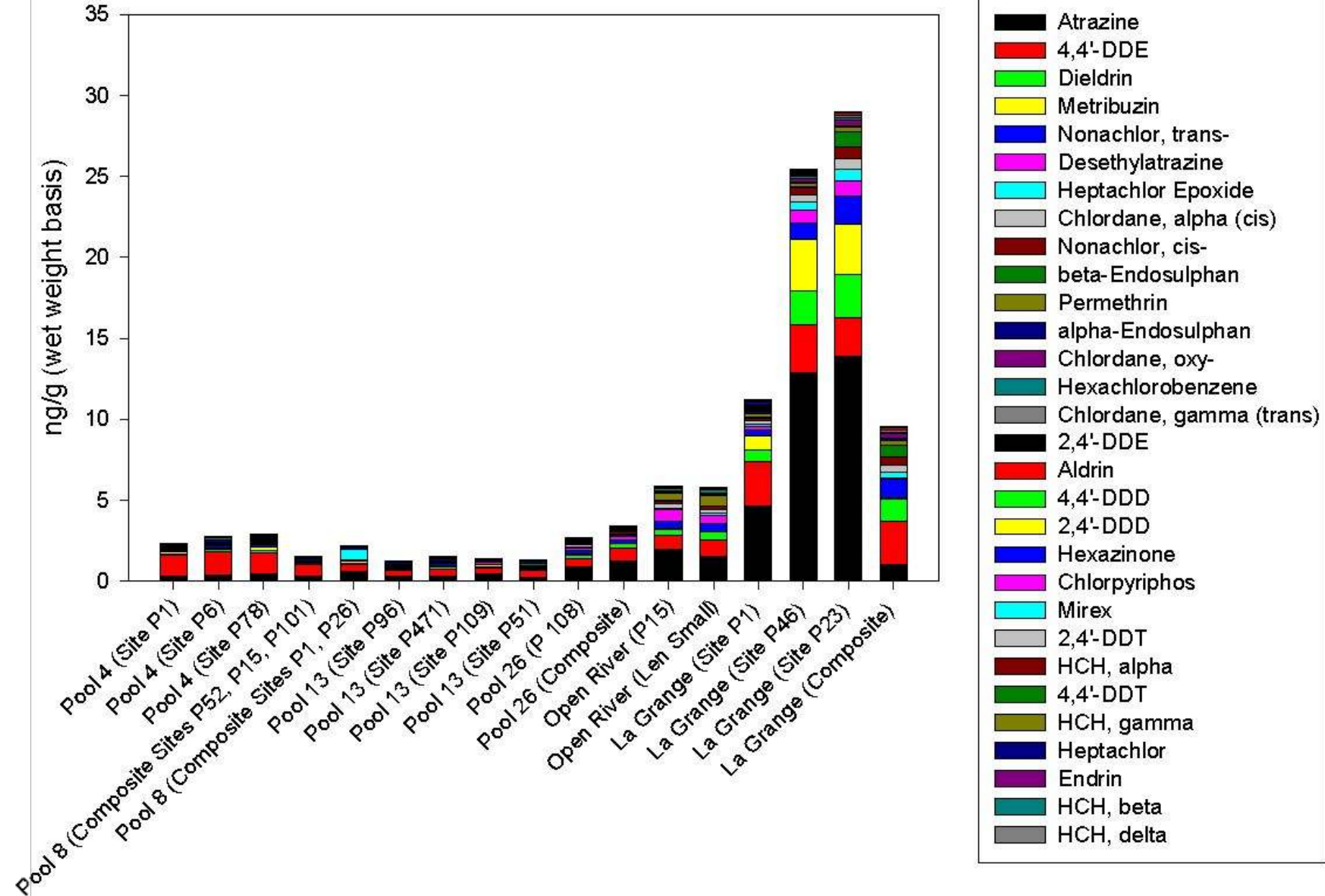


2023- paired water, sediment,
and mayfly tissue samples
collected



- 30 compounds detected in mayfly tissue
- 32 compounds not detected in any sample
- 10 compounds detected in 100% of tissue samples (e.g., atrazine)

Multi-Residue Pesticides in Mayfly Tissue

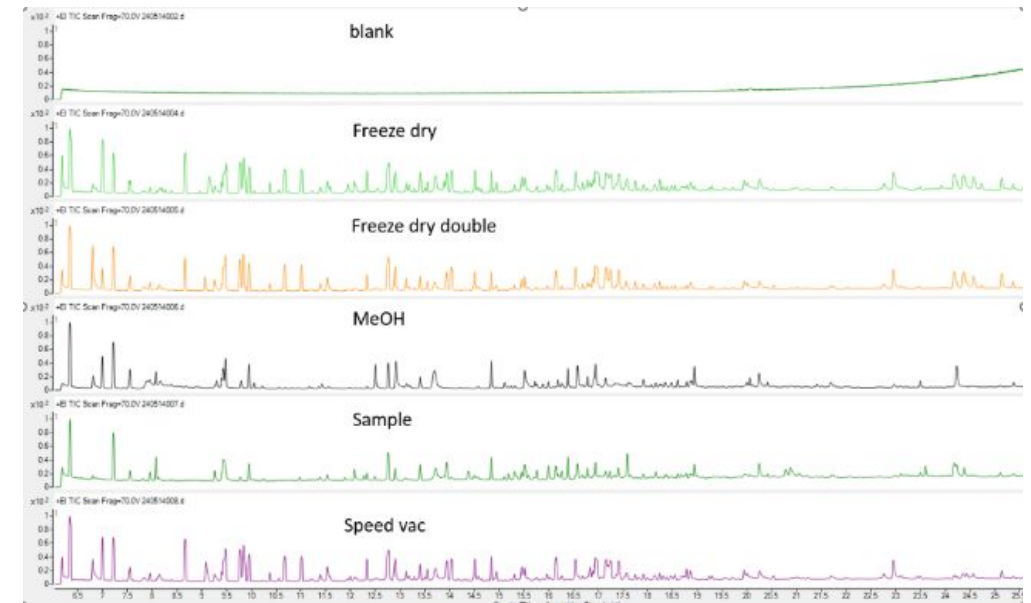
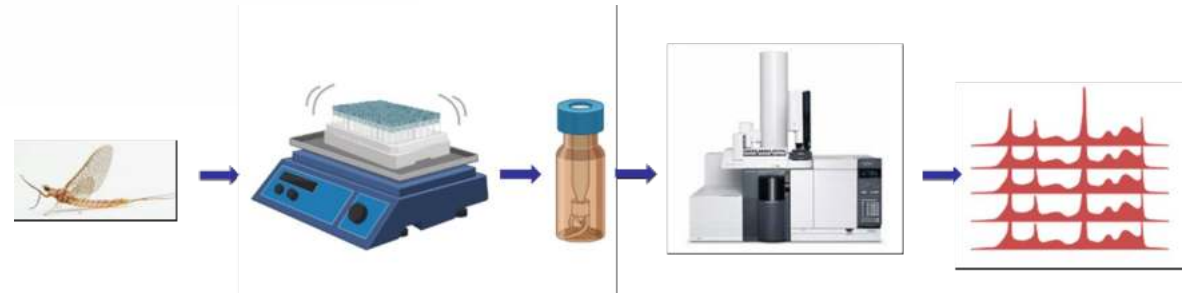


Mayfly Metabolomics



2024- paired water, sediment, mayfly tissue and mayfly metabolomics samples collected

- “Biochemical Snapshot of Stress” occurring at a given time due to a response from a pesticide exposure.
- Paired dataset will allow comparison of stress markers to water and sediment results.



Chou et al., 2017: Thermal stress,
Pomfret et al., 2021: Exposure to naphthenic acid,

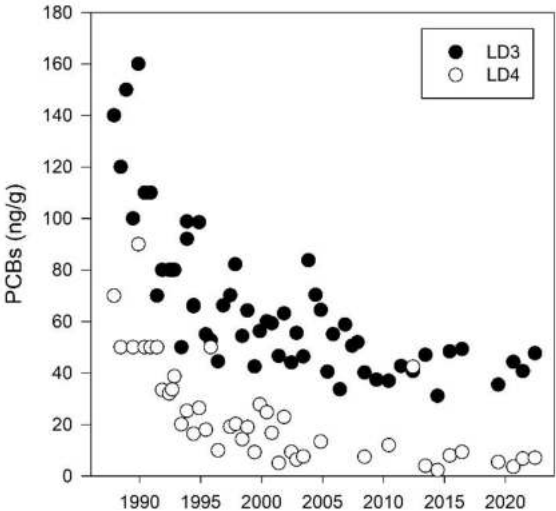
Questions



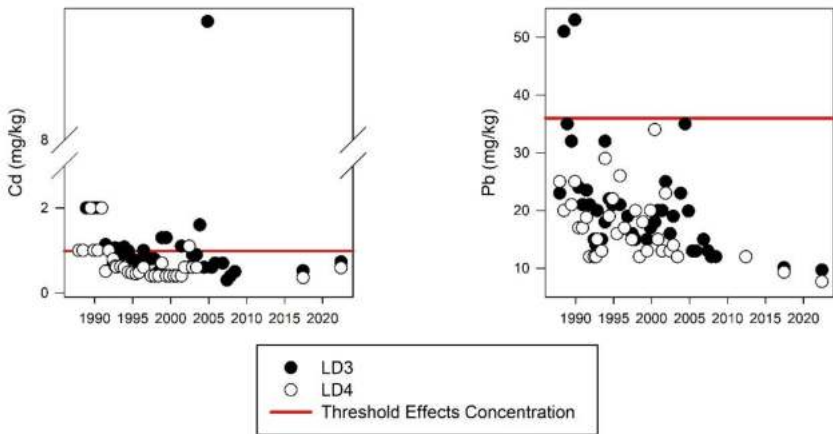
Special Thanks: Jeremy King (WI DNR)
Steve Delain (MN DNR)
Manisha Pant (IL NHS)
Kris Maxson (IL NHS)
All LTRM Staff

Mississippi River Long Term Sediment Trap Contaminant

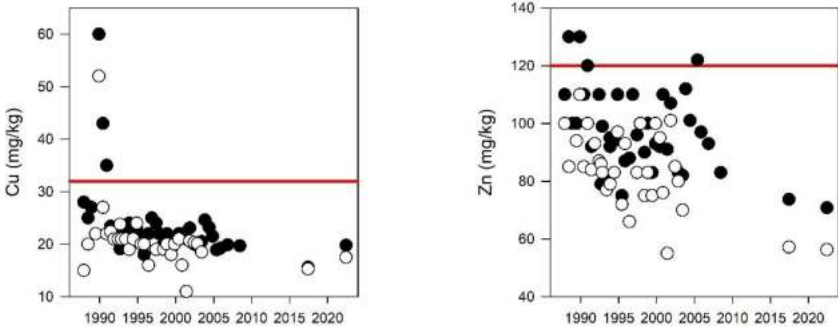
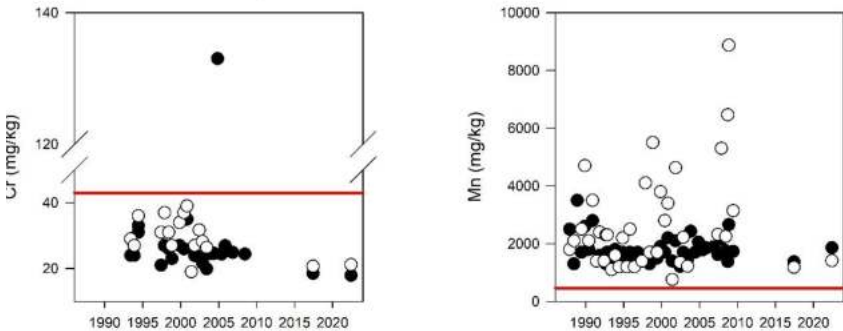
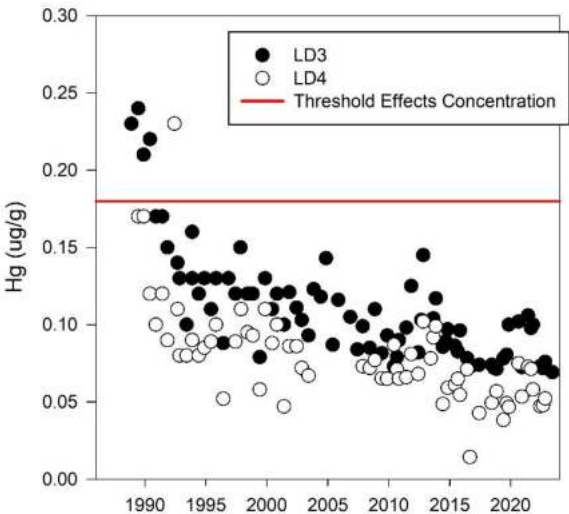
Trends: Lock and Dam 3 and 4 (1987-2023)



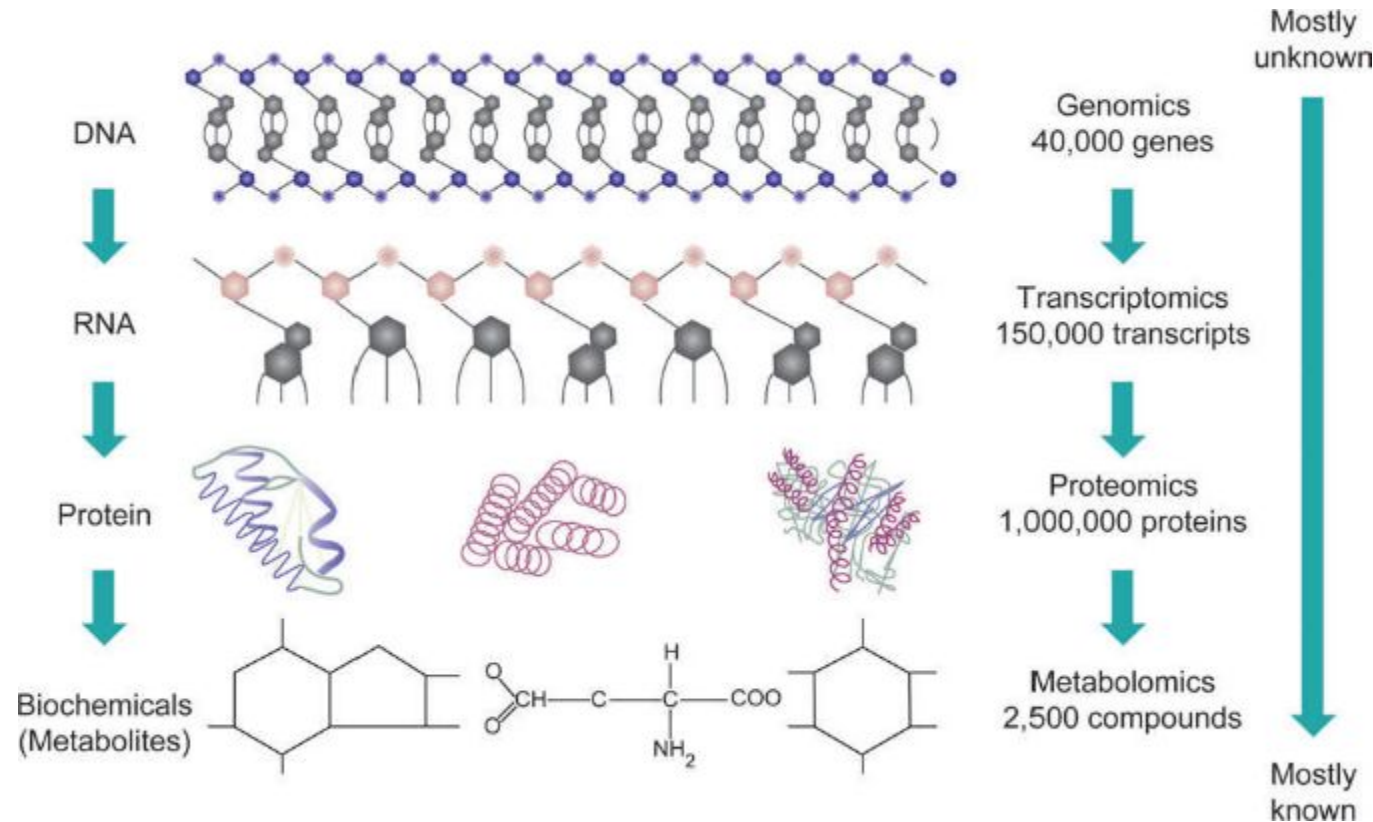
Contaminant Concentrations in Mississippi River Suspended Sediments at LD3 and LD4



PCB ban 1979

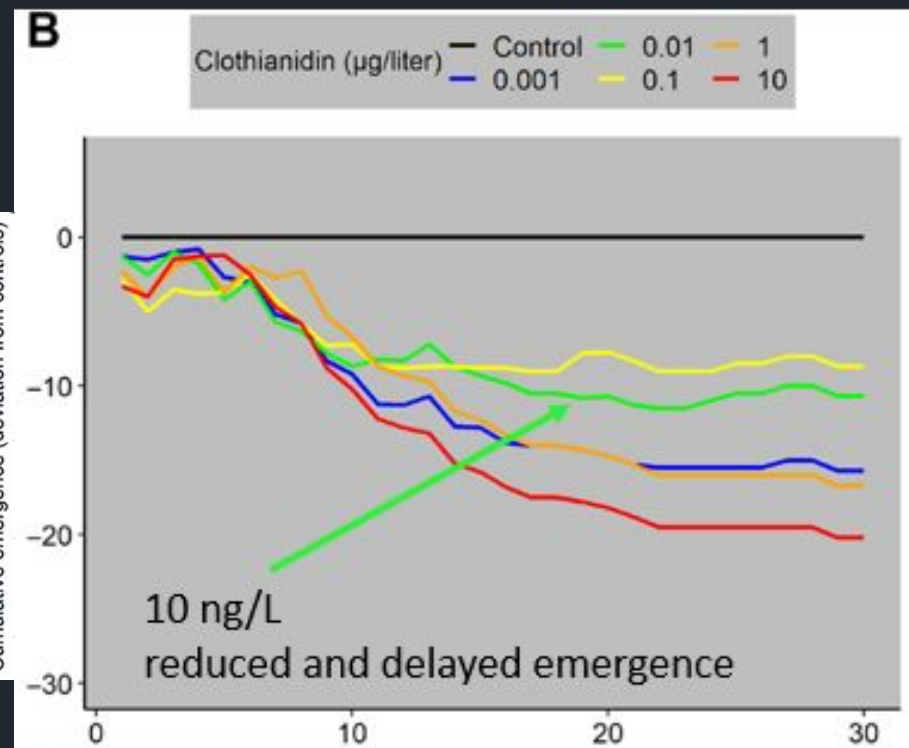
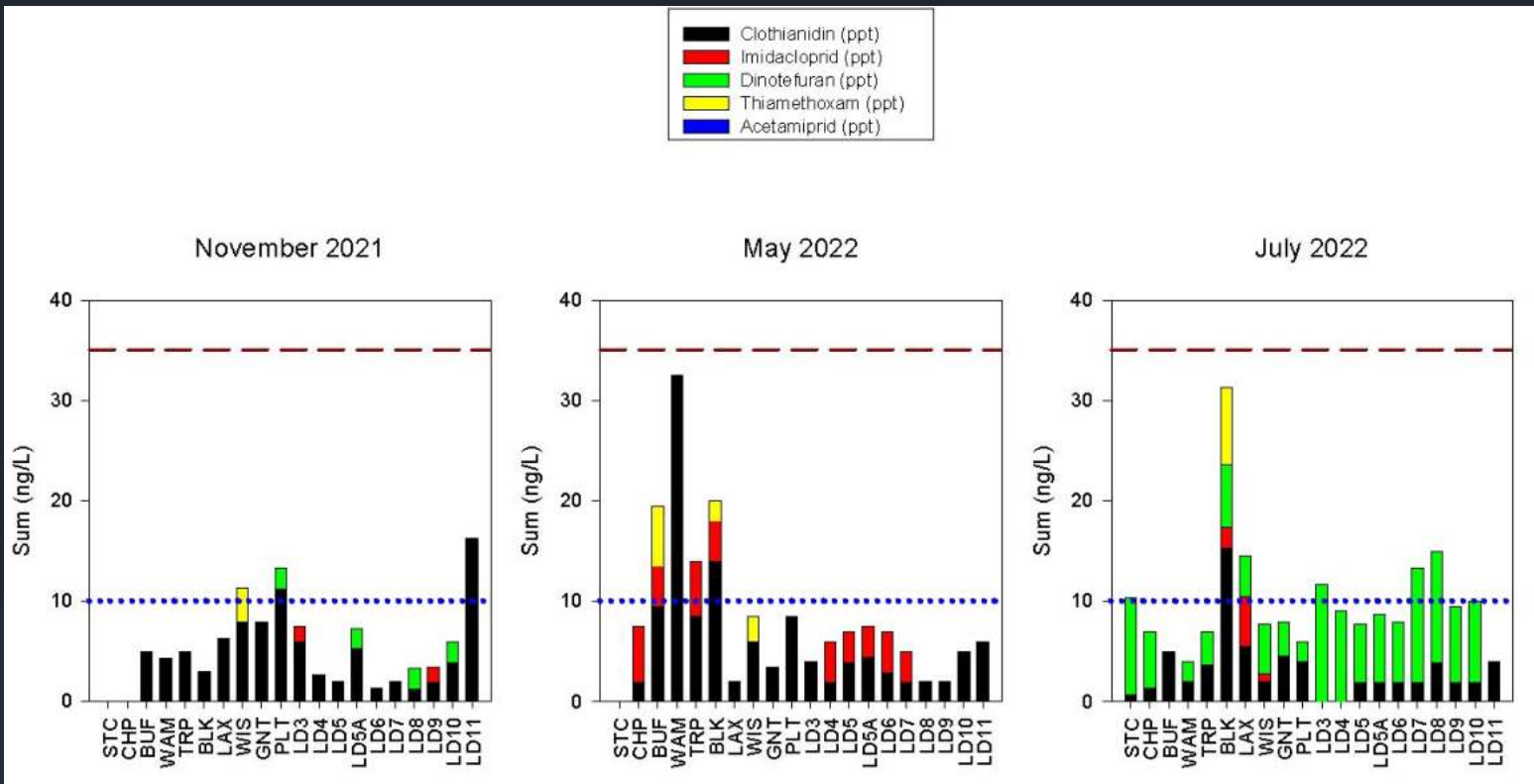


What is Metabolomics?



2024- paired water, sediment, mayfly tissue and mayfly metabolomics samples collected

- “Snapshot” of what is occurring at a given time due to a response from a pesticide exposure, across dose, or any other stressor.
- Mass spectrometry allows numerous metabolites from a single source to be rapidly identified and quantitated.



Schmidt et al., 2022

- We have observed aqueous exposures above this limit (10 ng/L)- 8.3% of samples >10 ng/L clothianidin
- We are observing neonic accumulation in mayfly tissue
- A physiological impact, possibly to growth, fecundity, or emergence
- A feedback loop of decreased emergence that decreases reproduction

Landmark Water Quality Success Stories

- The Clean Water Act passed in response to widespread pollution 1972-

- Objective to make surface waters “fishable and swimmable”

- Required states to establish WQ standards
- Required permits for discharges of pollutants into public waters
- Authorized funding for publicly owned WWTP’s
 - WWTP’s greatly reduced organic pollution from sewage as well as point source loading of trace metals.

- DDT ban 1972

- PCB ban 1979

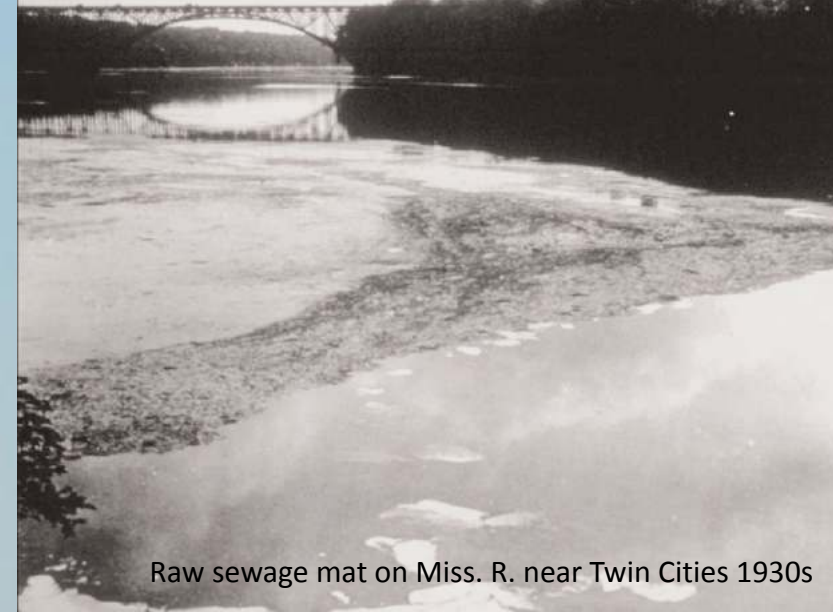
- Phase out of leaded gasoline (1973-1996)

- Lead shot ban (waterfowl hunting) 1991

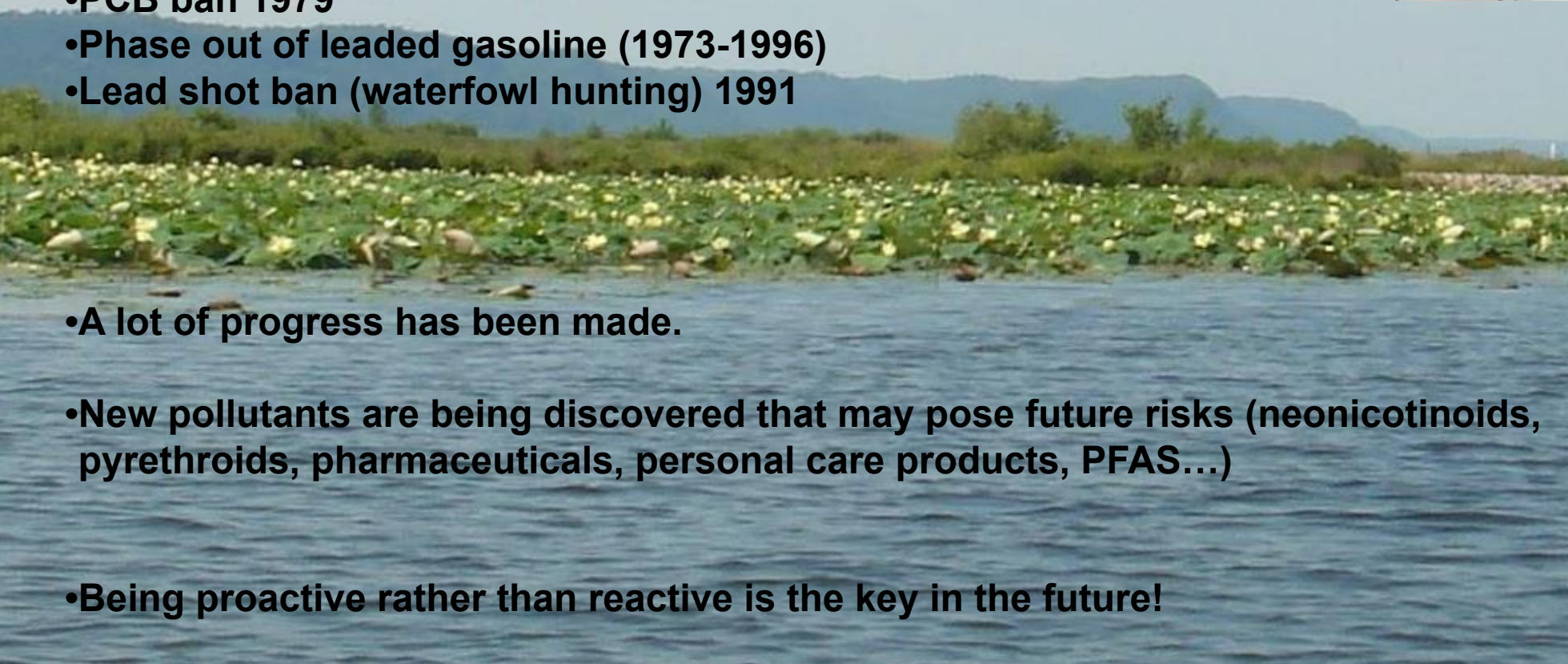
- A lot of progress has been made.

- New pollutants are being discovered that may pose future risks (neonicotinoids, pyrethroids, pharmaceuticals, personal care products, PFAS...)

- Being proactive rather than reactive is the key in the future!



Raw sewage mat on Miss. R. near Twin Cities 1930s



Water Quality Shapes the Health of the River

By Sharon Götts



Above and right: Masses of raw sewage floated in the Mississippi near the Twin Cities in the 1930s.



Our understanding of how rivers work and our interest in healthy rivers have advanced steadily and swiftly over the last century, thanks in part to work done on the Upper Mississippi.

When today's students learn about the Cuyahoga River in the 1950s and 1960s they have difficulty believing that the river was so polluted that it actually started burning. A 1969 article in *Time* described the Cuyahoga as a river that “oozes rather than flows” and in which a person “does not drown but decays.”

When I show students pictures from the 1930s of sewage mats on the Mississippi in the Twin Cities, many think that I am kidding them. I am delighted to show them when I read the passage from Cal Freamling's famous book, *Denuded River*. “The feck, lingering accumulation of raw sewage led the U.S. Bureau of Fisheries to report that during August of 1927 forty-five miles of the river below St. Paul lacked sufficient oxygen to sustain fish life of any kind.” Schoolkids quickly understand that situations like these angered people and motivated them to demand solutions.

Congress passed the Clean Water Act in 1972 with the simple objective of making surface waters “fishable and swimmable” again. The major provisions of the Clean Water Act required states to establish water quality standards, required

permits to discharge pollutants into public waters and authorized funds for publicly owned wastewater treatment plants. America has launched many great ideas. The Clean Water Act and the changes that followed may rank as America's best idea. Other landmark legislation followed, including the DDT ban in 1972, the PCB ban in 1979, the phase-out of leaded gasoline from 1973 to 1996 and the ban on lead shot for waterfowl hunting in 1991.

Fortunately, river scientists had a thorough and well-established monitoring program in place that captured the decline and eventual recovery in unprecedented detail.

Turning the River Around
The science of water quality seems like a collection of meaningless numbers until we show how it affects the people who enjoy the Mississippi River, and the plants, fish and other wildlife that rely on the river for survival.

As we reduced pollutants in the Mississippi River, fish became safer to eat and wildlife recovered. Sediment samples recently collected by the Wisconsin Department of Natural Resources at Lock and Dam 3 (Red Wing, Minn.) and Lock and Dam 4 (Alma, Wis.) found polychlorinated biphenyl (PCB) concentrations about one-quarter to one-fifth of those observed in the late 1960s. The mercury concentration in sediments at Lock and Dam 3 and 4 was roughly half that observed in late 1960s.

In 1972, one active bald eagle nest remained on the Upper Mississippi River Wildlife and Fish Refuge. Current counts

Burrowing Mayflies 2023 vs. Historical Abundance

LTRM Field Stations



Invertebrate sampling in the LTRM involves lowering a ponar dredge to the bottom of the river and scooping up the soft bottom (usually sand, silt, clay, or a mixture). Sand, silt, and clay are then washed through a screen, leaving the aquatic insects, clams, mussels, and snails behind on the screen. These are identified. Target organisms (mayfly larva, midge larva, fingernail clams, zebra mussels, and Asiatic clams) are counted, and presence/absence is noted on the others.

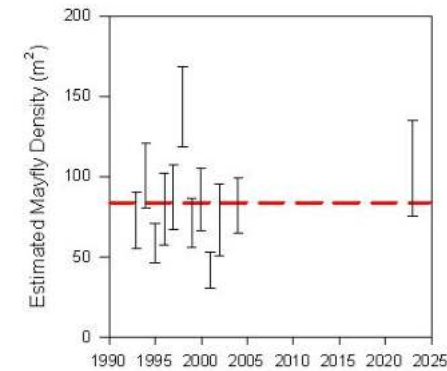


The jaws of the ponar dredge have been opened and mud grabbed from the bottom of the river is being rinsed into a wash screen. The mud will pass through the mesh of the wash screen, leaving the inhabitants of the river bottom behind.

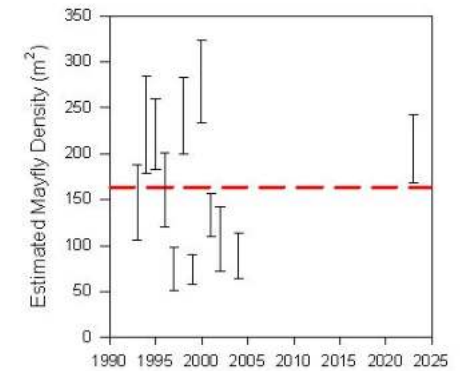
4/6 < long term avg

Title of Project: Assessing long term changes and spatial patterns in macroinvertebrates through standardized long-term monitoring

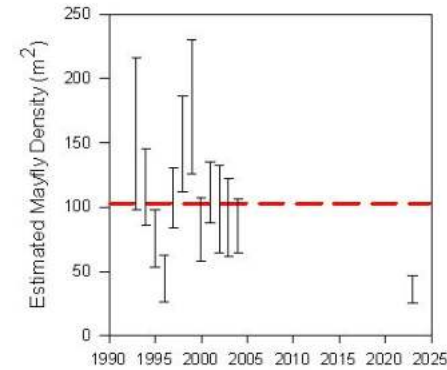
Pool 4 Backwaters



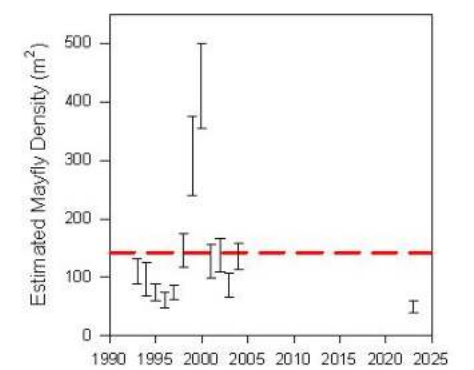
Pool 4 Lake Pepin



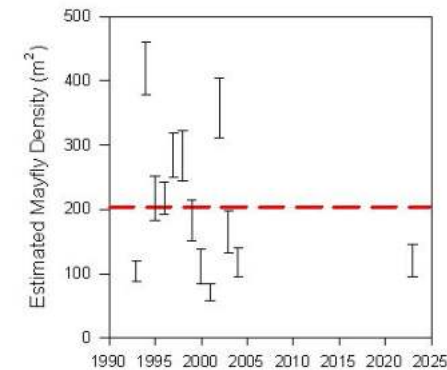
Pool 8 Backwaters



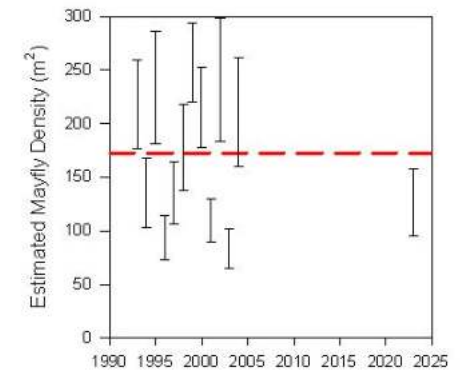
Pool 8 Impounded Area



Pool 13 Backwaters



Pool 13 Impounded Area



Burrowing Mayflies 2023 vs. Historical Abundance

LTRM Field Stations



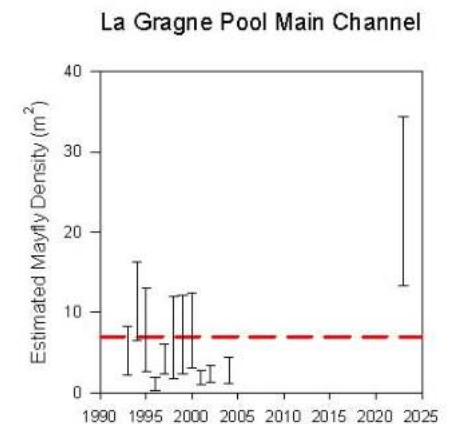
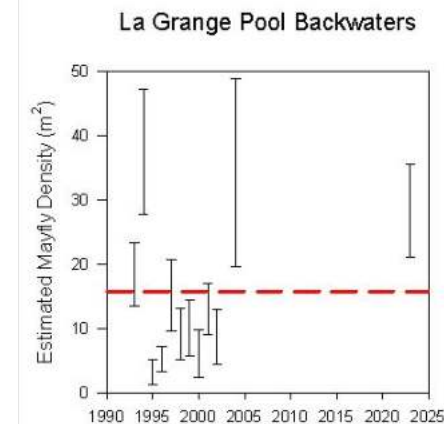
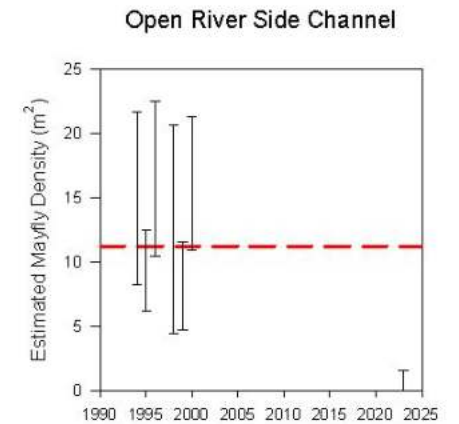
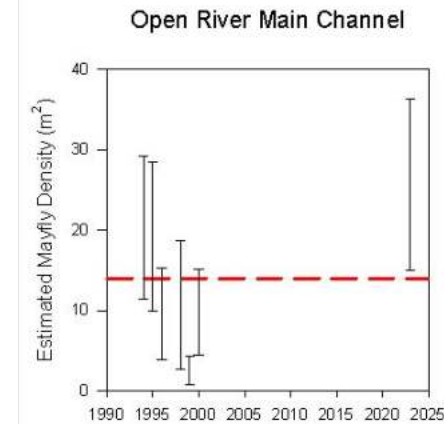
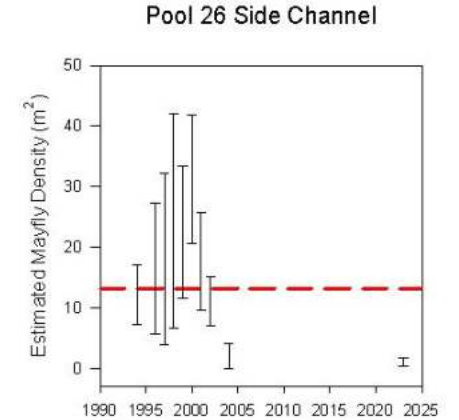
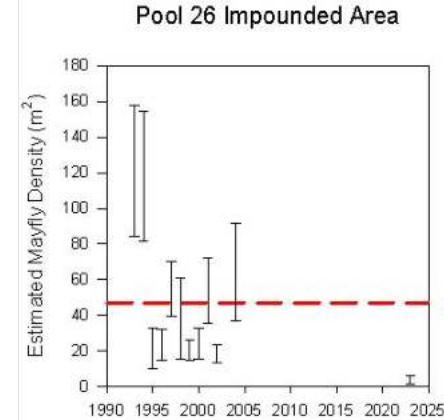
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3/6 < long term avg

Title of Project: Assessing long term changes and spatial patterns in macroinvertebrates through standardized long-term monitoring



Mayfly Extraction Protocol

- Metabolites are extracted using a bi-phasic mixture or aqueous methanol:chloroform. Derivatized and analyzed on a GC-qToF/MS.

