# Fish Flesh and Fresh Produce as Sources of Microcystin Exposure to Humans

**Stuart A. Ludsin (**ludsin.1@osu.edu**),**
**Jiyoung Lee, Seungjun Lee, Manju Manubolu**
**Jay F. Martin, Lindsay Collart, & Ken M. Riedl**

# Acknowledgments


- Monetary Support

OhioHigherEd
Department of Higher Education

OHIO DEPARTMENT OF NATURAL RESOURCES
DIVISION OF WILDLIFE

SPORT FISH RESTORATION

- In-kind support

Ohio Environmental Protection Agency

# Background & Objectives

- Lake Erie is becoming more eutrophic (Scavia et al. 2014)
  - Cyanobacteria blooms are larger & more frequent




# Background & Objectives

- Cyanobacteria blooms threaten ecosystem services
  - Safe beaches for swimming
  - Tourism & property values
  - Safe drinking water





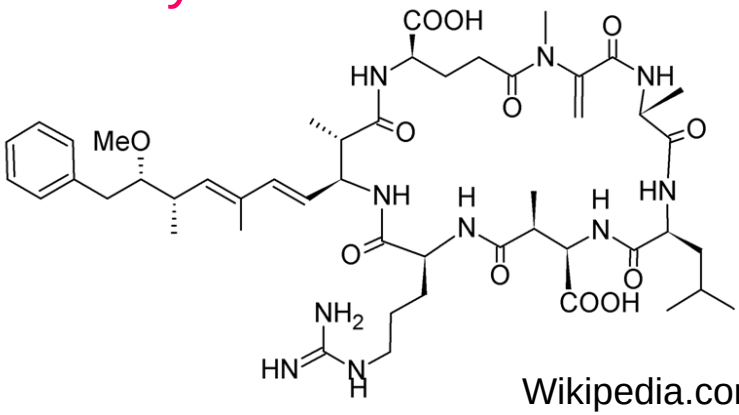
**Driven by Climate Change, Algae Blooms Behind Ohio Water Scare Are New Normal**

*Climate change and increased runoff are triggering more potentially toxic blooms.*

# Background & Objectives

- Cyanobacteria may threaten food safety through algal toxin accumulation in edible tissues


Microcystin L-R




Wikipedia.com

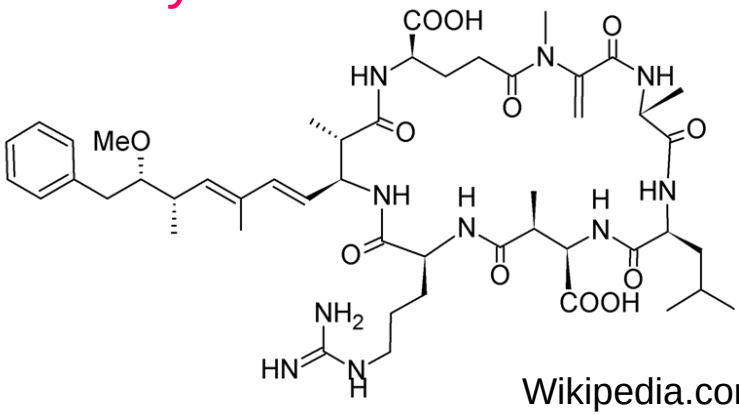
## Key Questions

- Are fish caught during the cyanobacteria season safe to consume?
- Are vegetables irrigated with cyanobacteria-contaminated water safe to consume?

# Background & Objectives

- Cyanobacteria may threaten food safety through algal toxin accumulation in edible tissues


Microcystin L-R




Wikipedia.com

## Key Questions

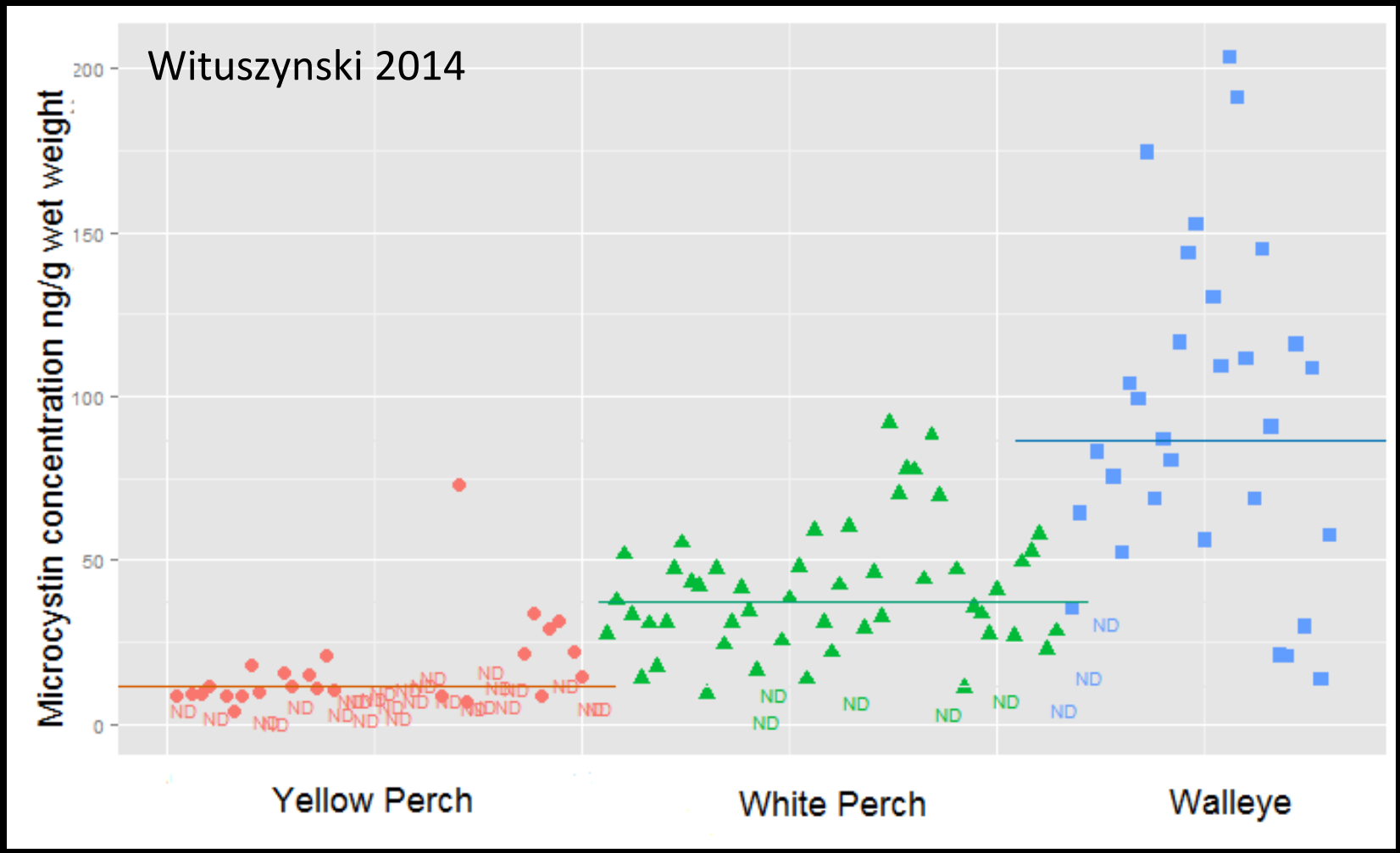
- Are fish caught during the cyanobacteria season safe to consume?
- Are vegetables irrigated with cyanobacteria-contaminated water safe to consume?

# Are fish caught during the cyanobacteria season safe to consume?



# Are fish caught during the cyanobacteria season safe to consume?

- Microcystin can accumulate in edible tissues of Lake Erie fish (Poste et al. 2011; Wituszynski 2014)



# Are fish caught during the cyanobacteria season safe to consume?

- Microcystin concentrations in Lake Erie fish quantified with Enzyme-Linked Immunosorbent Assay (ELISA)

- ELISA results are problematic
  - Quantitatively unreliable because their cross-reactivity with the fish tissue matrix can cause false positive results (Carmichael & An 1999)
  - Viewed more as a screening tool (i.e., presence-absence) (Geis-Asteggiante et al. 2011)

- Need better methods to quantify microcystins in fish

# Are fish caught during the cyanobacteria season safe to consume?

- OSU has been developing more quantitative methods
  - Account for cross-reactivity with fish tissues
  - Unbind microcystins from fish tissues so can be quantified
- Includes development of more reliable digestion, extraction & analytical techniques
  - LC-MS/MS: Liquid chromatography-tandem mass spectrometry
  - MMPB: 3-methoxy-2-methyl-4-phenylbutyric acid
- Offer a better means to measure microcystins in fish
  - Individual congeners (e.g., MC-LR) & total microcystins

OhioHigherEd
Department of Higher Education

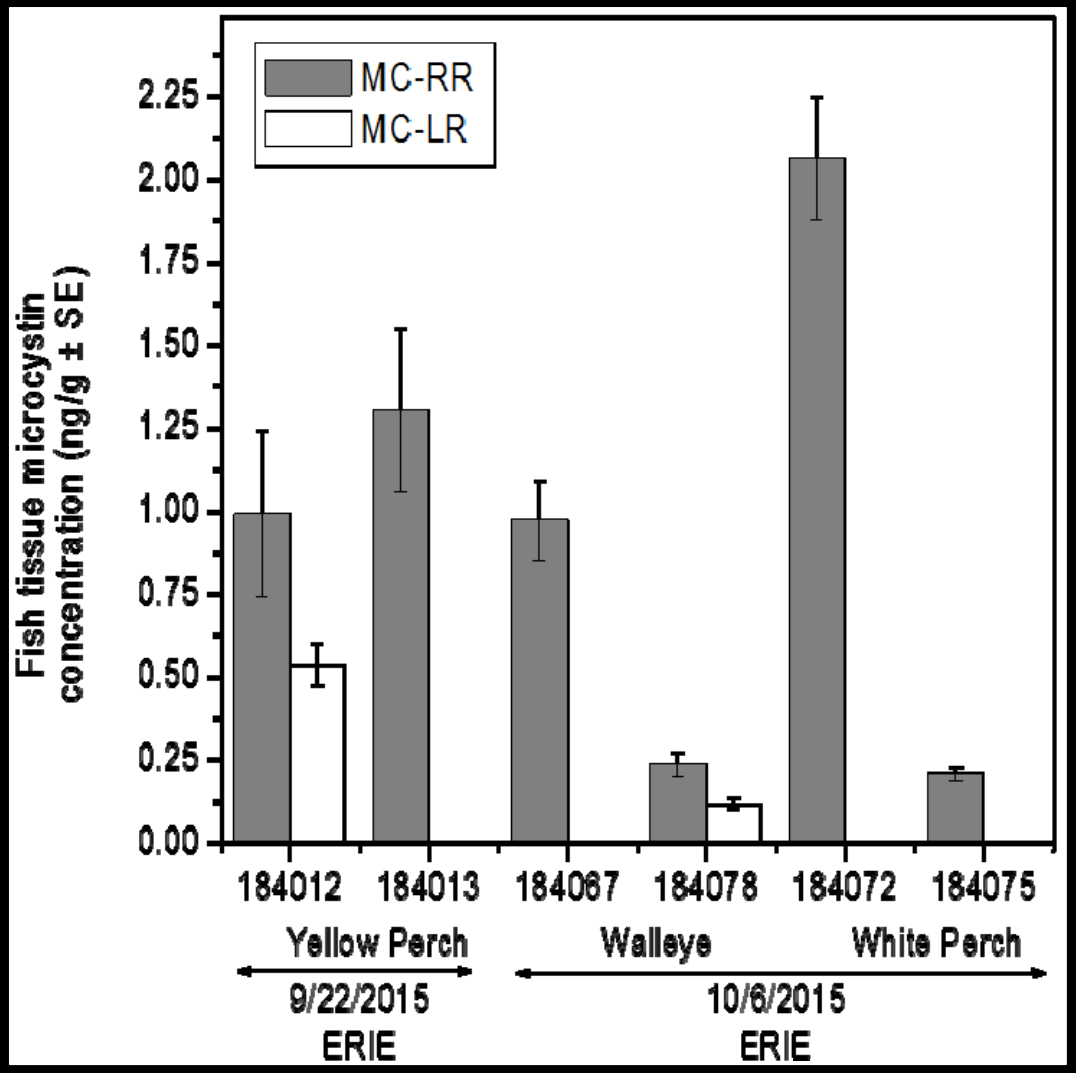
# Are fish caught during the cyanobacteria season safe to consume?

- To date, we have successfully developed & used LC-MS/MS to quantify MC-LR and MC-RR in fish
- We are currently working on developing MMPB capabilities (expect to be complete in 2017)
- An inter-laboratory comparison is being planned to verify the accuracy of our methods
- Our methods will be used to quantify microcystins in Lake Erie fish before, during & after the bloom season

OhioHigherEd
Department of Higher Education

# Are fish caught during the cyanobacteria season safe to consume?

- Preliminary LC-MS/MS results from 2015 found no microcystins in most Lake Erie fish
- MC-LR or MC-RR > 0
  - 0 of 12 white bass
  - 2 of 13 white perch
  - 2 of 12 yellow perch
  - 2 of 12 walleye
- Fish levels are low relative to WHO consumption guidelines (10x-100x less)
- But, fish collected during peak bloom period not yet analyzed



# Are vegetables irrigated with cyanobacteria-contaminated water safe to consume?

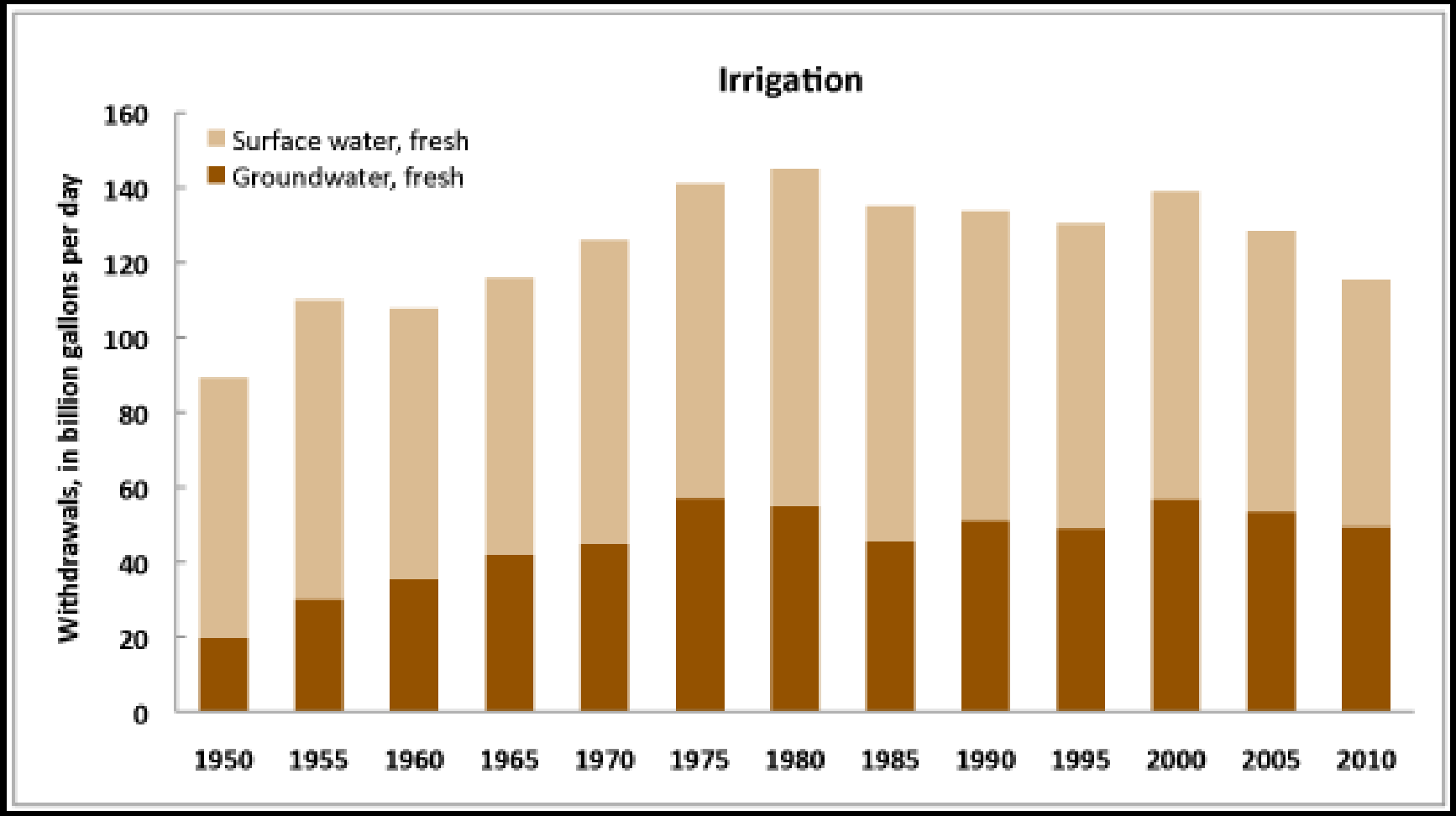
www.civileblog.com/

www.nespal.org

http://impeller.net/

# Are vegetables irrigated with cyanobacteria-contaminated water safe to consume?

- Surface water irrigation is a common practice




http://water.usgs.gov/watuse/wuir.html

# Are vegetables irrigated with cyanobacteria-contaminated water safe to consume?

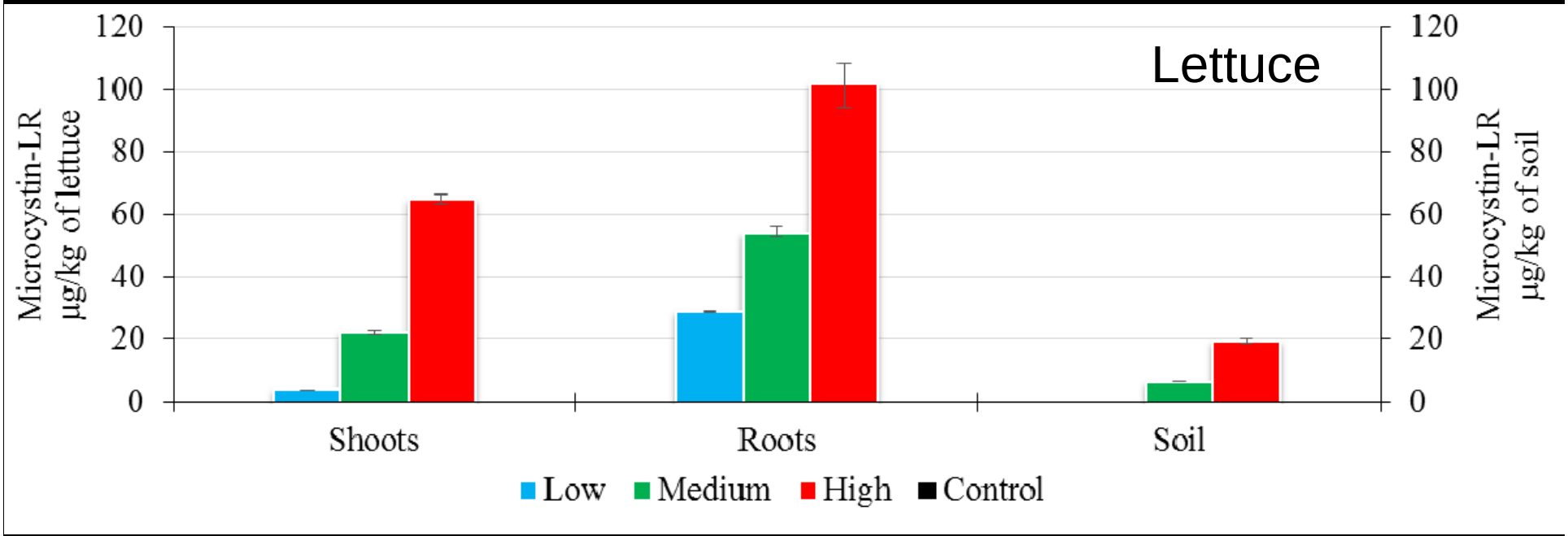
- Surface water irrigation is a common practice
- Microcystins can accumulate in edible tissues of plants (Mohamed & Shehri 2009, Hereman & Bittencourt-Oliveira 2012)
- Microcystins can impair crop growth (Bittencourt-Oliveira 2014)
- Key Questions remain:
  - How does microcystin accumulation vary with crop type?
  - How do the effects of microcystin vary among crop types?
  - How stable are microcystins in soils?
  - Can microcystins accumulate to levels that may threaten human health?

# Are vegetables irrigated with cyanobacteria-contaminated water safe to consume?

- Controlled laboratory experiment
  - 4 plant types (lettuce, green beans carrots, tomatoes)
  - 4 microcystin treatments with environmentally relevant levels (MC-LR: 0, 1, 5, 10 ug/L)
  - Inoculations occurred 3 times per week
  - 3 replicate sets of 10 plants per set
  - Harvest at 11 weeks of age
- Response variables
  - MC levels measured with ELISA: roots, shoots/leaves, soil
  - Crop quality (Length, mass, leaf color, number & diameter of leaves or beans)

# Are vegetables irrigated with cyanobacteria-contaminated water safe to consume?

- Microcystin accumulated in edible portions of all plants (e.g., the shoots/leaves of lettuce), as well as their associated soils.
- Microcystin accumulated in a dose-dependent pattern.

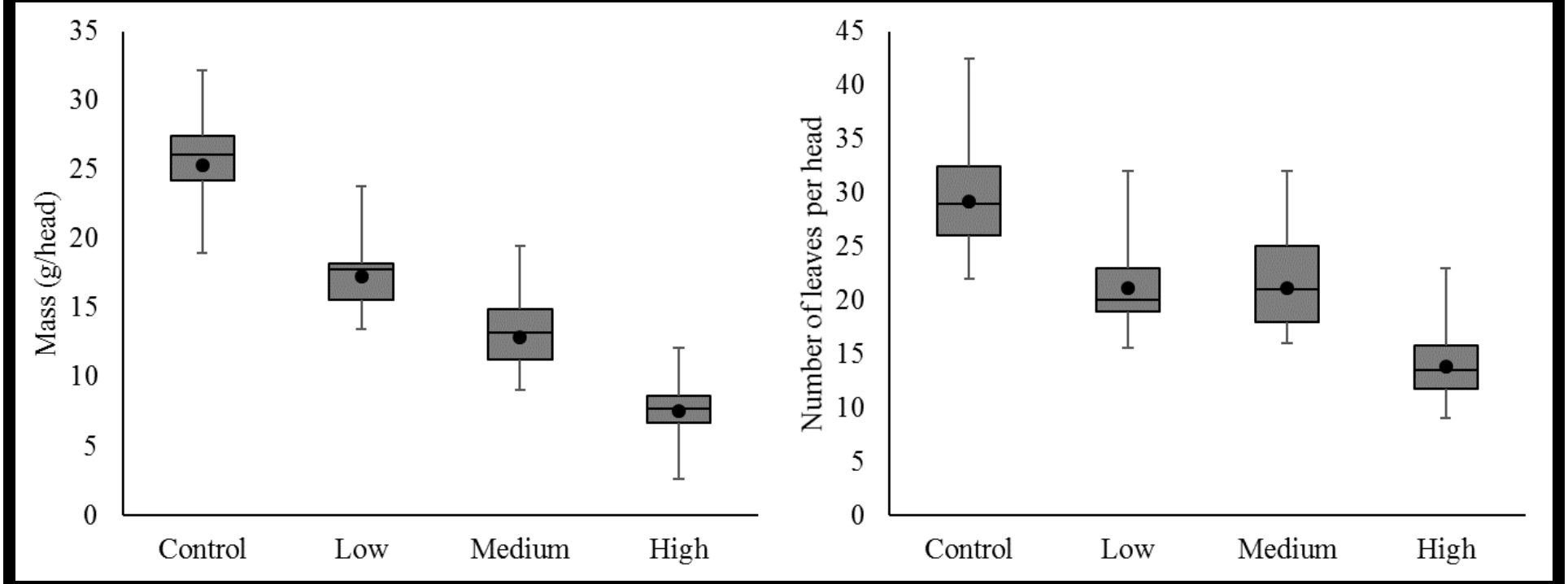



Seungjun Lee & Jiyoung Lee, unpublished data

# Are vegetables irrigated with cyanobacteria-contaminated water safe to consume?

- Microcystin negatively affected the quality and production of all vegetables

Lettuce




Seungjun Lee & Jiyoung Lee, unpublished data

# Are vegetables irrigated with cyanobacteria-contaminated water safe to consume?

- Risk is high relative to USEPA (chronic reference dose) & WHO (total daily intake) consumption guideline
- Single meal in single day
  - 25 kg child eats 40 g lettuce & 7.5 g carrots
  - 60 kg adult eats 80 g lettuce & 15 g carrots
- Risk in Lake Erie's watershed remains unknown

Seungjun Lee & Jiyoung Lee, unpublished data

# Take-Home Messages

- **Are fish caught during the cyanobacteria season safe to consume?**
  - Too early to tell for certain
    - Fish collected post-bloom did not have MC-LR/MC-RR, or had levels that were non-threatening
    - Processing of fish across the 2015 & 2016 bloom seasons is needed (results will emerge this fall)
  - Recommend not eating more than one fish meal per week from Lake Erie (per current consumption guidelines for other contaminants)
    - Safe even when using over-inflated ELISA MC results that were ~100-fold higher than our LC-MS/MS results

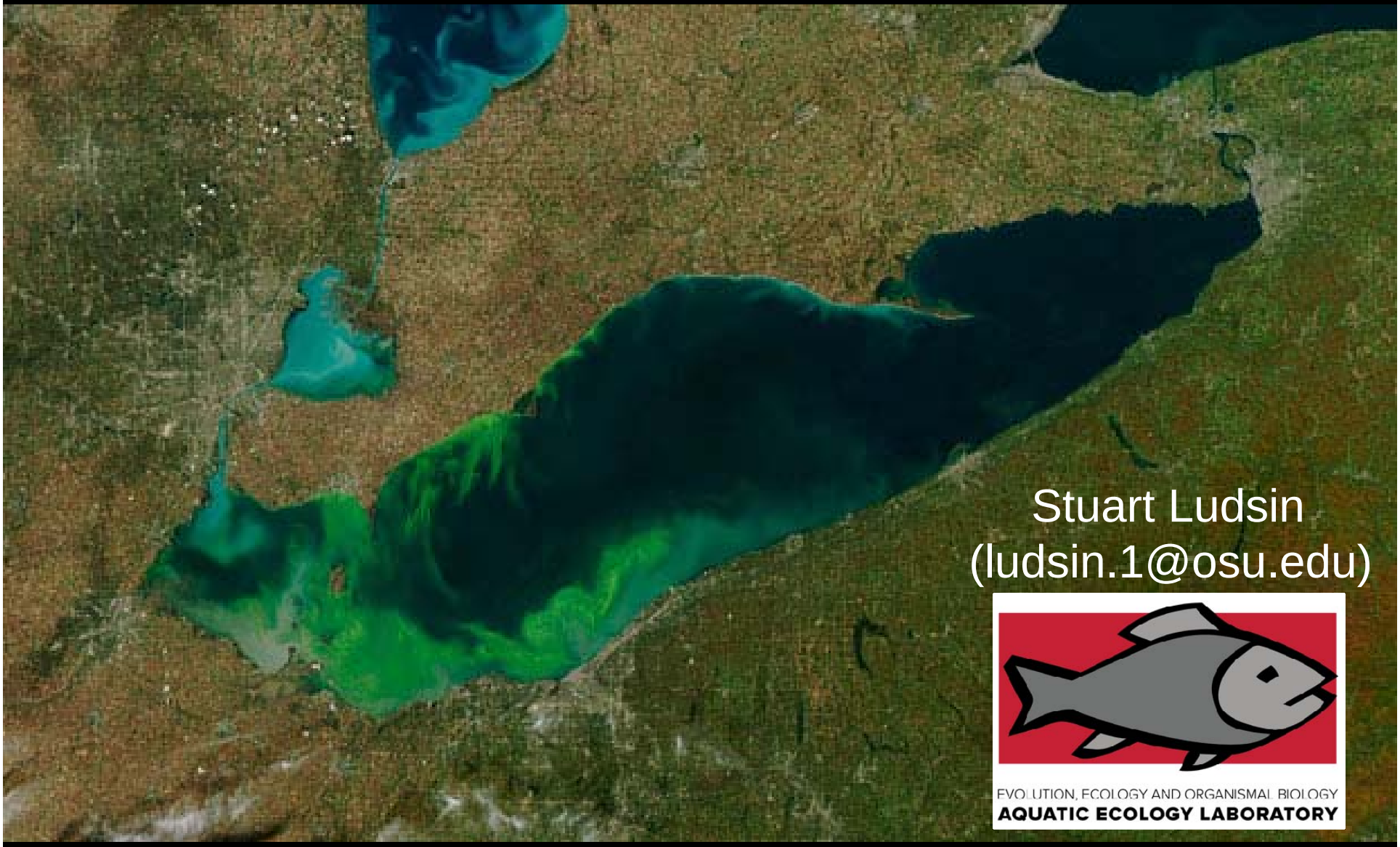
# Take-Home Messages

- **Are vegetables irrigated with cyanobacteria-contaminated water safe to consume?**
  - If microcystins are in irrigation water, it will accumulate in both edible plant tissues and the soil
    - This relationship was dose-dependent
  - Microcystin-LR had negative effects on crop productivity
    - The causal mechanism of this relationship remains uncertain
  - More information is needed to understand the risk to posed to humans
    - Uncertain where and when surface waters are used for irrigation
    - Uncertain if microcystins are in groundwater sources
    - Uncertain how reliable ELISA is in quantifying microcystins in plant tissues (i.e., same issues with fish?)

# Take-Home Messages

- Given the potential for algal toxins to accumulate in edible tissues of plants and animals:
  - We recommend continued monitoring of microcystins in water and fish and plant tissues across the cyanobacteria bloom season
  - We support efforts to minimize factors that promote cyanobacteria blooms
- Such efforts will not only help reduce risk to human health, but also benefit the many other ecosystem services that Lake Erie and its watershed provides

# Thanks for your Attention



Stuart Ludsin
(ludsin.1@osu.edu)

EVOLUTION, ECOLOGY AND ORGANISMAL BIOLOGY
AQUATIC ECOLOGY LABORATORY

# Are fish caught during the cyanobacteria season safe to consume?

- Preliminary results from 2015 found no microcystin in most Lake Erie fish

Tolerable doses to microcystin-LR in relation to frequency and duration of exposure

| Temporal pattern of exposure and ensuing Tolerable Intake (TI) | Assumptions | Tolerable Intake per kg | Tolerable Intake for a 10 kg child | Tolerable Intake for a 75 kg adult | Guideline value for food (μg $kg^{-1}$) | |
|---|---|---|---|---|---|---|
| | | | | | AF = 1 | AF = 0.2 |
| Acute TI | NOAEL of 250 μg/kg and day, extrapolation factors of 100 (Fromme et al., 2000) | 2.5 μg per kg and single exposure | 25 μg per single exposure | 190 μg per single exposure | Adult: 1900, Child: 250 | Adult: 380, Child: 50 |
| Seasonal TDI | NOAEL of 0.4 μg/kg and day, extrapolation factors of 100 (Chorus and Bartram, 1999, adapted) | 0.4 μg per kg and day | 4 μg per day | 30 μg per day | Adult: 300, Child: 40 | Adult: 60, Child: 8 |
| Lifetime TDI | NOAEL of 0.4 μg/kg and day, extrapolation factors of 100 and uncertainty factor of 10 (Chorus and Bartram, 1999) | 0.04 μg per kg and day | 0.4 μg per day | 3 μg per day | Adult: 30, Child: 0.4 | Adult: 6, Child: 0.08 |