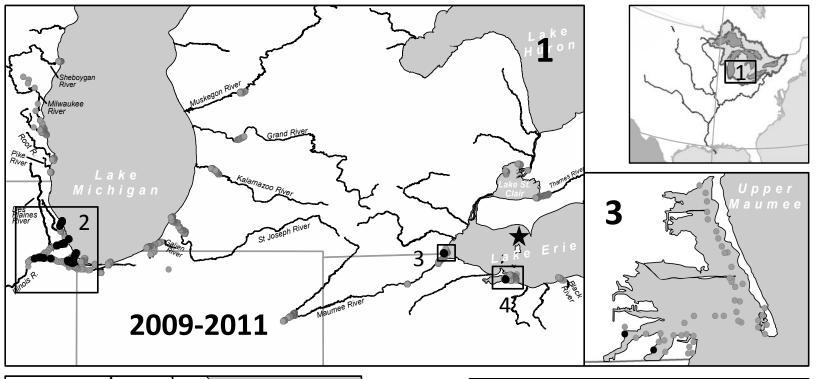
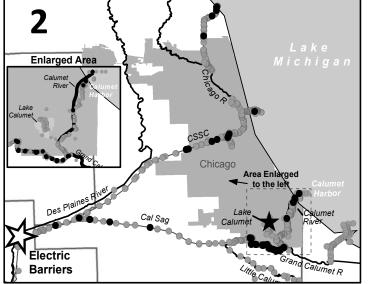
### **Environmental DNA surveillance**in the Great Lakes

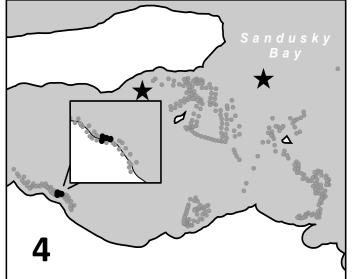


- GLRI on Great Lakes surveillance
- GLFI on Great Lakes bait trade
- GLFT on methodology and calibration
- Recommendations & comments

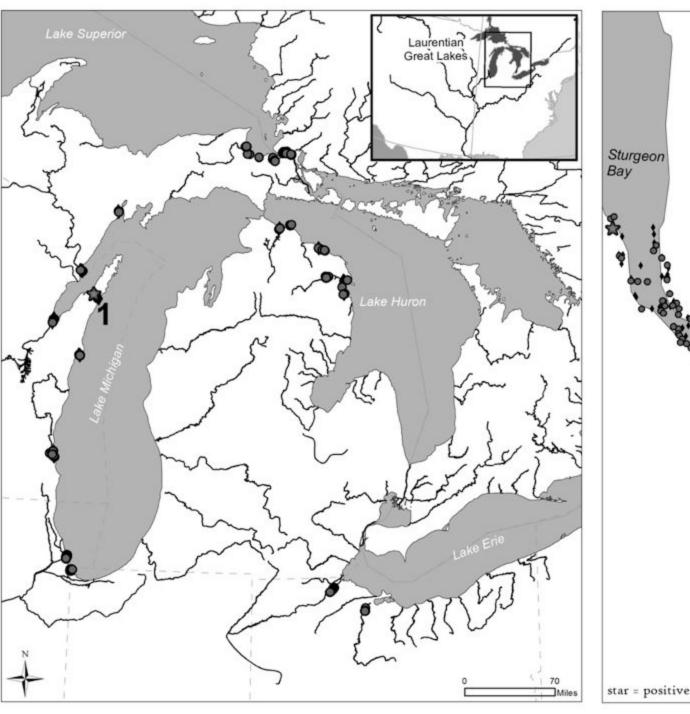


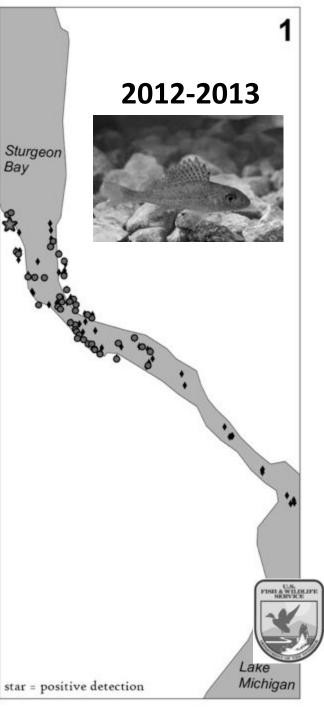












### Evaluation of the Great Lakes Bait Trade as a Pathway for Invasive Species Introductions







Lucas Nathan at Central Michigan University (Soon to be at University of Connecticut)

# **Bait Shop Locations Year Sampled** 2012 2013 2012 and 2013

### **Positive Detections Species Detected** Silver Carp Round Goby Tubenose Goby Rudd Goldfish

#### **Bait trade conclusion's and comments**

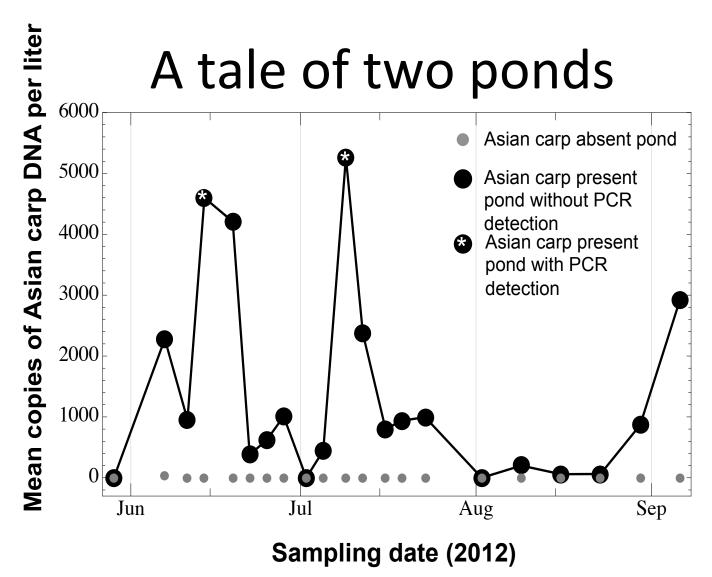
- Total # detections:
  27/576 (≈4.7%)
- Regional patterns
- Total bait trade contamination?
- Final report to EPA submitted



## GLFT methodology & calibration

**Cameron Turner University of Notre Dame** 

Great Lakes Fishery Trust



**Figure 1:** Time series of Asian carp qPCR estimated DNA concentrations for a pond with Asian carp (black dots) and our control pond (gray circles). qPCR always detected Asian carp DNA in the Asian carp present pond and on all but one occasion, the control pond was absent Asian carp DNA. The PCR assay only detected Asian carp DNA twice throughout the study (Black dots with white asterisk).

#### A tale of many ponds with variable fish density

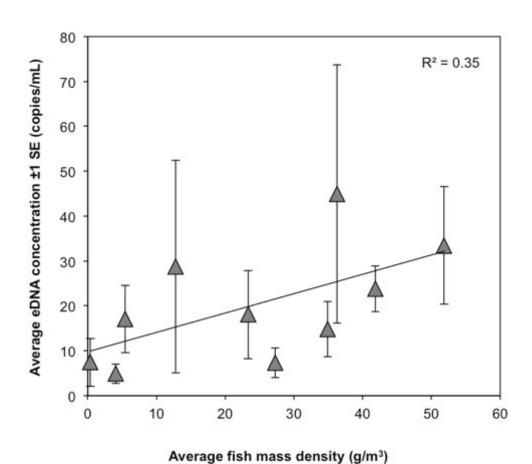


Figure 2: Average Asian carp DNA concentration (copies/mL) as a function of Average fish mass density across 10 experimental ponds for the study period (5/30/2012 to 10/16/2012) using precipitation collection method. There is a positive correlation between fish mass density and Asian carp DNA concentration (P<0.05), but much of the variability in the relationship is unexplained and attributed to differences in environmental conditions. The range of fish numbers per pond was 1to 27.

Conclusions – there is a positive correlation between fish mass density and Asian carp DNA concentration...and the PCR assay is less sensitive to detection of Asian carp DNA than the qPCR assay



#### **Recommendation 1**

Laboratories conducting eDNA research should have separation between eDNA extraction and PCR amplification

Mifflin TE (2007) Setting up a PCR laboratory. Cold Spring Harbor protocols. Vol. 2007. p. pdb.top14.

#### **Recommendation 2**

 Increase the sensitivity of Asian carp detection using the qPCR assay

Turner, Cameron R. et. al. Submitted. Improved methods for capture, extraction and assay of Asian carp eDNA.

Wilcox, Taylor M., Kevin S. McKelvey, Michael K. Young, Stephen F. Jane, Winsor H. Lowe, Andrew R. Whiteley, and Michael K. Schwartz. "Robust detection of rare species using environmental DNA: the importance of primer specificity." *PloS one* 8, no. 3 (2013): e59520.

#### **Recommendation 3:**

- Change to using polycarbonate track etched filter (PCTE) filters with CTAB extractions
  - Cheaper
  - More DNA recovered
  - Reduced contamination risk (less handling)
- Notre Dame's eDNA group will be moving to this method but will adjust sample volume

Turner, Cameron R. et. al. Nearing submission. Improved methods for capture, extraction and assay of Asian carp eDNA.

#### **Comment 1: Volume matters**

- Single Large or Several Small (SLOSS)
- Unclear results from CAWS using precipitate or centrifuge methods
- 2-L sample clearly performed better than surface collected 15ml and 50 ml samples
- Need to resolve "how many 15 or 50 ml samples" to take to equal one 2-L sample

### Comment 2: "When" matters

- Water volume
- Water quality
- Life history of target organism
- Degradation rates
- Behavior of target organism
- Inhibitors
- Repeated and seasonal sampling is needed for inferences in some locations and for some species

## Comment 3: Increased sensitivity requires increase vigilance to reduce chance of contamination

Assay sensitivity may result in dramatic changes in collection procedures

A dialog is needed about what contamination means and does not mean

Best practices are being developed for the different assays, collections, extractions, and communication of results

#### **People**

Jerde, Chadderton, Mahon, Lodge, Tucker, Turner, Barnes, Nathan, Renshaw, Budny, Uy, Deines, Galaska, Corush, Gantz, Feeks, Xu, Miller, Mysorekar







Protecting nature. Preserving life."

