BALLAST WATER MONITORING PROJECTS: eDNA OF TARGET ORGANISMS, AND THE ROLE OF INDICATIVE MONITORING

Allegra Cangelosi, Penn State University

and Jeffrey Ram, Wayne State University

WHEN CAN eDNA DELIVER CLUES ON VITAL STATUS?

Experiments to determine drivers for persistence/extinction of the eDNA signal of a target invader in natural Great Lakes water.

PROJECT TEAM

- Allegra Cangelosi, PSU Behrend
- Ivor Knight, PSU Behrend
- Matt Gruwell, PSU Behrend
- Mary Balcer, Independent Consultant
- Multi-Disciplinary Project Advisors

ARE THERE INSTANCES IN WHICH eDNA DETECTION SIGNALS RECENTLY ALIVE ORGANISMS?

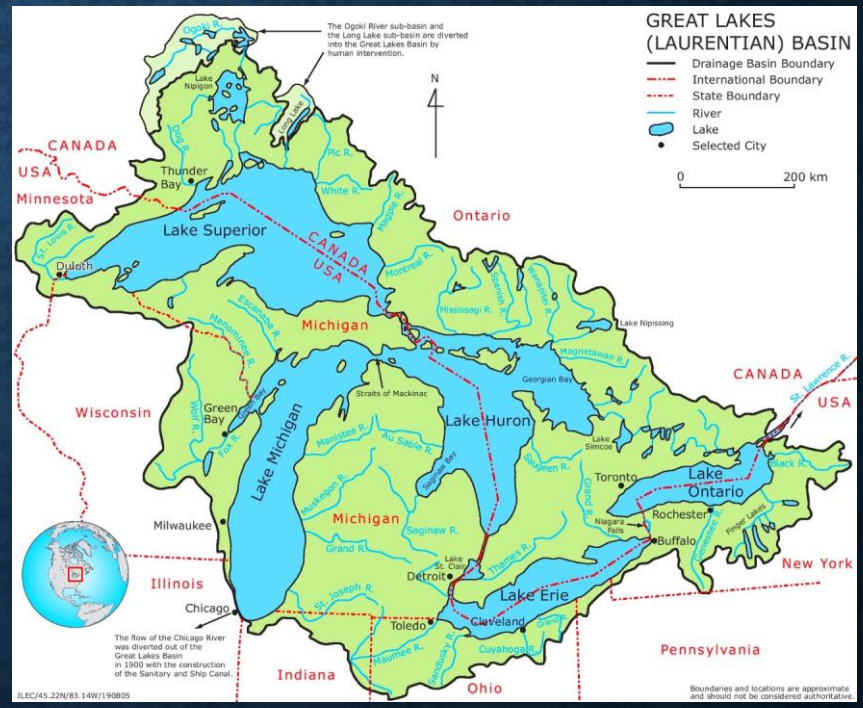
Persistence of eDNA Signal as Influenced by Characteristics of Species and Matrix

Port of Erie, PA in the North American Great Lakes

Photo Credit: CuriseMapper.com

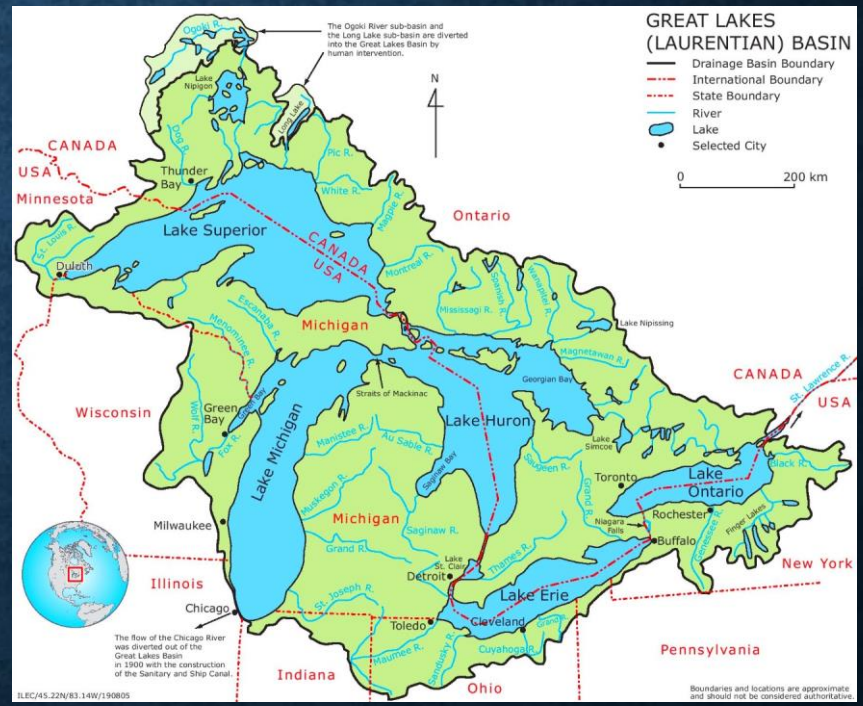
NIS DISTRIBUTION TARGETS FOR EARLY DETECTION BW/HARBOR MONITORING

- Not yet detected or established in the GL but on a Watch List.
- Distribution localized to a single Great Lakes harbor.
- Distributed in lower lakes but not yet Lake Superior.



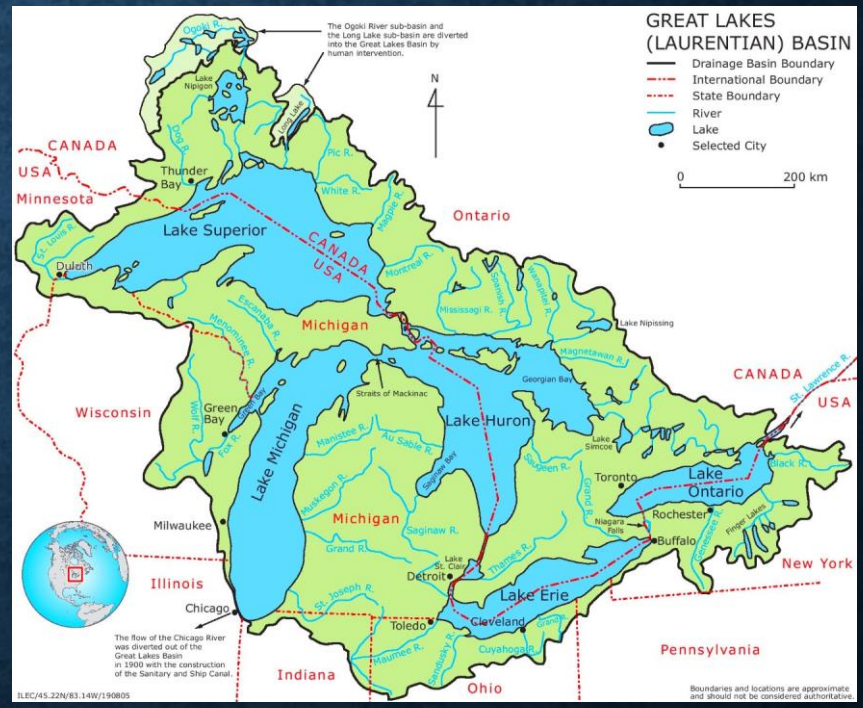
OPPORTUNITIES TO PROTECT GL FROM TARGET NIS THROUGH EARLY DETECTION AND MONITORING OF BW AND HARBORS

- Not yet detected or established in the GL but on a Watch List.
- Distribution localized to a single Great Lakes harbor.
- Distributed in lower lakes but not yet Lake Superior.



OPPORTUNITIES TO PROTECT GL FROM TARGET NIS THROUGH EARLY DETECTION AND MONITORING OF BW AND HARBORS

- Not yet detected or established in the GL but on a Watch List.
- Distribution localized to a single Great Lakes harbor.
- Distributed in lower lakes but not yet Lake Superior.



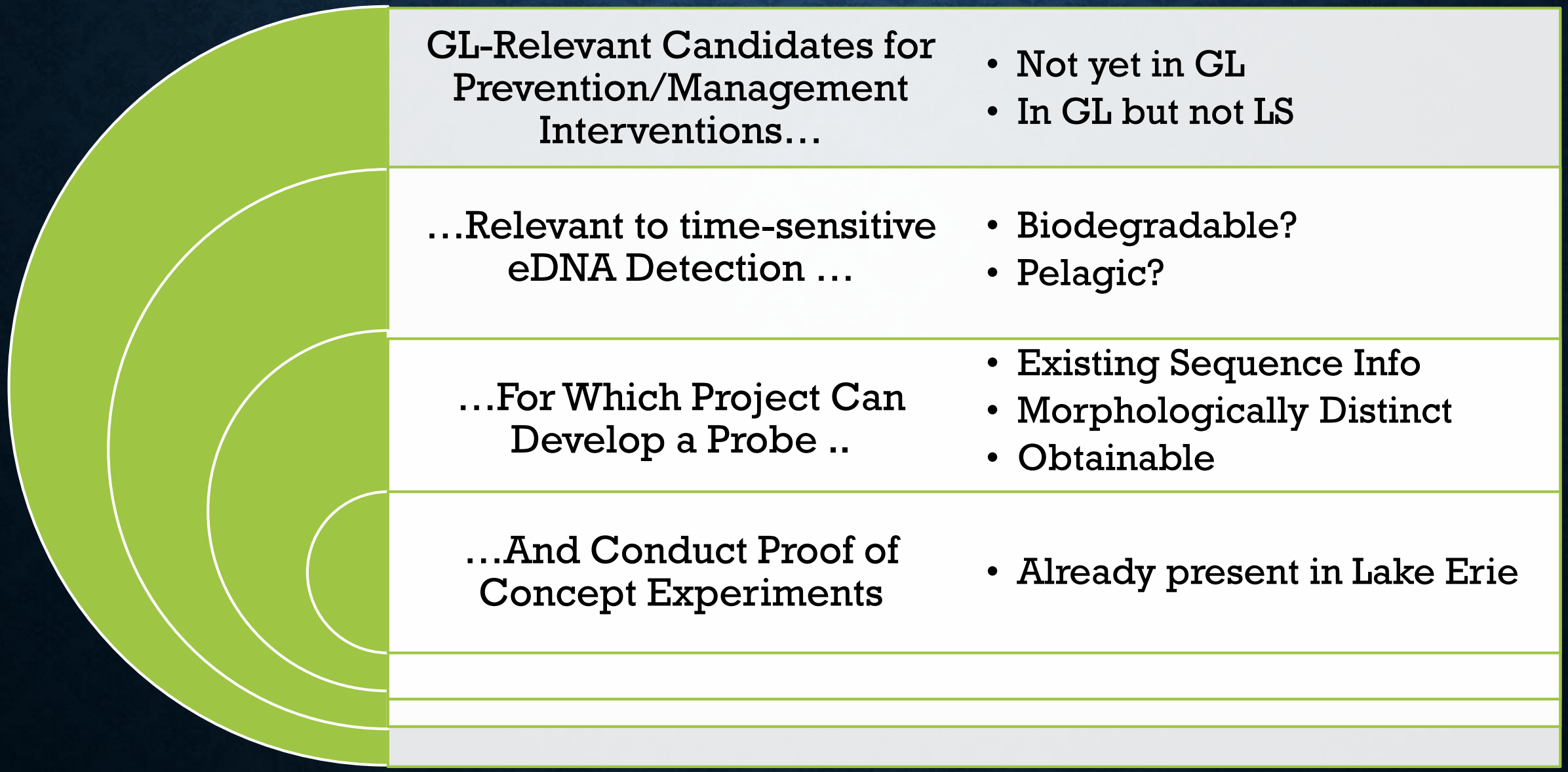
PROJECT DELIVERABLES

- Inventory Known eDNA Sequences for Target NIS
- Develop Probe for a Short List of Organisms
- Conduct Exploratory Experiments at Mesocosm Scale
- Design Larger More Definitive Assays – More TOs/Collaboration
- Conduct Outreach to Great Lakes Regional, National and International Stakeholders and Scientific Community

PROJECT PLAN: TIME FRAME AND DELIVERABLES

- **Inventory Known eDNA Sequences for Target NIS**
- Develop Probe for a Short List of Organisms
- Conduct Exploratory Experiments at Mesocosm Scale
- Design for Larger More Definitive Assays – More TOs/Collaboration
- Conduct Outreach to Great Lakes Regional, National and International Stakeholders and Scientific Community

TIERS OF TARGET ORGANISMS



INVENTORY OF KNOWN EDNA FOR TARGET GL PLANKTON - RESOURCES

- Great Lakes Aquatic Nonindigenous Species Information System (GLANSIS)
- Barcode of Live (Life) Database (BOLD)
- Genomic Databases

PRELIMINARY OUTPUT - WATCHLIST

Group	Family	Scientific Name	Common Name	Continent of Origin
Crustaceans-Mysids	Mysidae	Limnomysis benedeni	Caspian slender shrimp	Europe
Crustaceans-Mysids	Mysidae	Paramysis (Mesomysis) intermedia	opossum shrimp	Europe
Crustaceans-Mysids	Mysidae	Paramysis (Metamysis) ullskyi	opossum shrimp	Europe
Crustaceans-Mysids	Mysidae	Paramysis (Serrapalpisis) lacustris	opossum shrimp	Europe
Crustaceans-Cladocerans	Podonidae	Cornigerius maeoticus maeoticus	water flea	Europe
Crustaceans-Cladocerans	Podonidae	Podonevadne trigona ovum	water flea	Europe
Crustaceans-Cladocerans	Daphniidae	Daphnia cristata	water flea	Europe
Crustaceans-Cladocerans	Sididae	Diaphanosoma fluviatile*	water flea	South America
Crustaceans-Copepods	Pseudodiaptomidae	Calanipeda aquaedulcis	calanoid copepod	Europe
Crustaceans-Copepods	Temoridae	Heterocope appendiculata	calanoid copepod	Europe
Crustaceans-Copepods	Temoridae	Heterocope caspia	calanoid copepod	Europe
Crustaceans-Copepods	Cyclopidae	Cyclops kolensis	cyclopoid copepod	Europe
Crustaceans-Copepods	Ectinosomatidae	Ectinosoma abrau	harpacticoid copepod	Europe
Crustaceans-Copepods	Leptastacidae	Paraleptastacus spinicaudus triseta	harpacticoid copepod	Europe
Crustaceans-Copepod	Leptastacidae	Paraleptastacus Wilsoni	Harpacticoid Copepod	North America
Rotifers	Brachionidae	Brachionus leydigii	wheel animal	Unknown
Rotifers	Trochosphaeridae	Filinia cornuta	wheel animal	Unknown
Rotifers	Filiniidae	Filinia passa	wheel animal	Unknown

NIS NOT CURRENTLY ESTABLISHED IN ALL LAKES

Group	Family	Scientific Name	Common Name	Continent of Origin	Superior	Huron	Michigan	Erie	Ontario
Crustaceans-Mysids	Mysidae	Hemimysis anomala	bloody red shrimp	Eurasia	2018 u	2008 c e u	2007 e	2006 c e	2006 e u
Crustaceans-Cladocerans	Bosminidae	Eubosmina maritima	a cladoceran	Eurasia		1992 e	1988 e	1991 e	
Crustaceans-Cladocerans	Cercopagidae	Cercopagis pengoi	fishhook waterflea	Asia	2003 c	2002 e	1999 e	2001 e	1998 c e
Crustaceans-Cladocerans	Daphniidae	Daphnia galeata galeata	waterflea	Eurasia				1980 e	
Crustaceans-Cladocerans	Daphnidae	Daphnia lumholtzi	a waterflea	AustralAsia				1999 c e	
Crustaceans-Cladocerans	Sididae	Diaphanosoma fluviatile	a cladoceran	South/Central America			2018 u	2015 u	
Crustaceans-Copepods	Cyclopidae	Cyclops strenuus	Cyclopoid copepod	North America	1972 e				
Crustaceans-Copepods	Cyclopidae	Megacyclops viridis	Cyclopoid copepod	Europe					
Crustaceans-Copepods	Cyclopidae	Mesocyclops pehpeiensis	Cyclopoid copepod	Asia				2016 e	
Crustaceans-Copepods	Cyclopidae	Thermocyclops crassus	Cyclopoid copepod	Europe, Africa	2018 u		2017 e	2014 e	
Crustaceans-Copepods	Diaptomidae	Skistodiaptomus pallidus	calanoid copepod	North America		1974 e		1973 e	1967 e
Crustaceans-Copepods	Canthocamptidae	Heteropsyllus nr nunni	a harpacticoid copepod	Unknown		2007 e	1996 e		
Crustaceans-Copepods	Ameiridae	Nitokra hibernica	a harpacticoid copepod	Eurasia	2018 u	1998 e	2001 e	2001 e	
Crustaceans-Copepods	Diosaccidae	Schizopera borutzkyi	a harpacticoid copepod	Europe	2018 u		1988 e	2003 e	

PROJECT PLAN: TIME FRAME AND DELIVERABLES

- Inventory Known eDNA Sequences for Target NIS
- **Develop Probe for a Short List of Organisms**
- Conduct Exploratory Experiments at Mesocosm Scale
- Design for Larger More Definitive Assays – More TOs/Collaboration
- Conduct Outreach to Great Lakes Regional, National and International Stakeholders and Scientific Community

PROBE DEVELOPMENT TARGET ORGANISMS

- Watch List/Limited-GL Distribution NIS …
- …That have unique shapes *and* are obtainable, *for example:*
  - *Cercopagis*
  - *Daphnia lumholtzi*
  - *Cornigerius maeoticus?*
  - *Podonevadne trigona?*
  - *Paraleptastacus?*
- Others?

PROBE DEVELOPMENT TARGET ORGANISMS

- Watch List/Limited-GL Distribution NIS …
- …That have unique shapes *and* are obtainable, *for example:*
  - *Cercopagis*
  - ***Daphnia lumholtzi***
  - *Cornigerius maeoticus?*
  - *Podonevadne trigona?*
  - *Paraleptastacus?*
- Others?

PROJECT PLAN: TIME FRAME AND DELIVERABLES

- Inventory Known eDNA Sequences for Target NIS
- Develop Probe for a Short List of Organisms
- **Conduct Exploratory Experiments at Mesocosm Scale**
- Design for Larger More Definitive Assays – More TOs/Collaboration
- Conduct Outreach to Great Lakes Regional, National and International Stakeholders and Scientific Community

HEMIMYSIS SELECTED FOR INITIAL ERIE EXPERIMENTS

- *Hemimysis anomola* is an introduced AIS not yet established in all of the Great Lakes
- eDNA for this species has been identified and genetic probes are available
- Locally abundant, relatively easy to collect
- Readily IDed and sorted in the field
- Project Bench Experiments showed likely eDNA signal degradation in lab conditions

H. anomala : **6 – 13 mm** FW shrimp. Native to FW margins of Black, Azov, and eastern Ponto-Caspian Seas. First GL record 2006 (SE Lake Ontario & channel from Muskegon Lake to Lake Michigan). Lake Erie 2007. Lake Huron 2008

MESOCOSM EXPERIMENTS – AQUARIUM BASED

Round 1

- Twenty-four aerated exposure chambers with 20L of Lake Erie water
- Four treatments: DNA-Exposed Water
  - With and Without Organisms;
  - Low and High BOD.
- Outcome: Noisy but encouraging. eDNA signal largely disappeared in 1-3 days, both BOD levels.

Round 2

- Just lake water (one BOD condition) + one eDNA inoculum approach/amount
- 10 tanks + multiple samples per tank/time point
- Double sample volume (20 mL)
- Added a qPCR analytical technique
- Results not yet available

PROJECT PLAN: TIME FRAME AND DELIVERABLES

- Inventory Known eDNA Sequences for Target NIS
- Develop Probe for a Short List of Organisms
- Conduct Exploratory Experiments at Mesocosm Scale
- **Design for Larger More Definitive Assays – More TOs/Collaboration**
- **Conduct Outreach to Great Lakes Regional, National and International Stakeholders and Scientific Community**

BALLAST WATER MONITORING PROJECTS:

THE ROLE OF INDICATIVE MONITORING

Allegra cangelosi, Penn state University

And Jeffrey Ram, Wayne State University

PRESENTATION OUTLINE

- Project Background
- Purposes for Ballast Water Monitoring
- Practicum Output
- For Additional Details

PROJECT BACKGROUND

- Purpose: Provide a Resource for Regulators, Resource Managers, Ship Owners, Monitoring Tool Developers and Other Stakeholders to Support Protection of the Great Lakes through Effective and Informed Indicative Monitoring of Ballast Water;
- Output: Two Hands-On Interactive Practicums (Erie, PA and Superior, WI) to Demonstrate (and Troubleshoot) Indicative Monitoring Devices in Great Lakes water, and a Guidebook.
- Support: This project was made possible by a grant to WSU from the Great Lakes Protection Fund.

PURPOSES FOR INDICATIVE MONITORING OF BW AND HARBORS

- Compliance Monitoring of BW Discharge Against the USCG/IMO Standard
- Informal (Pre-Compliance) Monitoring Against the USCG/IMO Standard
- Assessments of Programmatic Participation and Effectiveness
- Risk Assessments for Target Organisms of Concern

VESSEL INCIDENTAL DISCHARGE ACT 2018

REGULATORY-RELATED MONITORING

USCG

STATES

- New US BWDS, **Implementing Regulations (**2022)
  - States can (consistent with rule):
    - **Conduct compliance monitoring,**
    - Propose, **with justification,** and **enforce** with EPA approval:
      - No Discharge Zones;
      - Additional Ship Equipment Requirements (8-state consensus);
      - Additional BMPs in GL (5-state consensus).

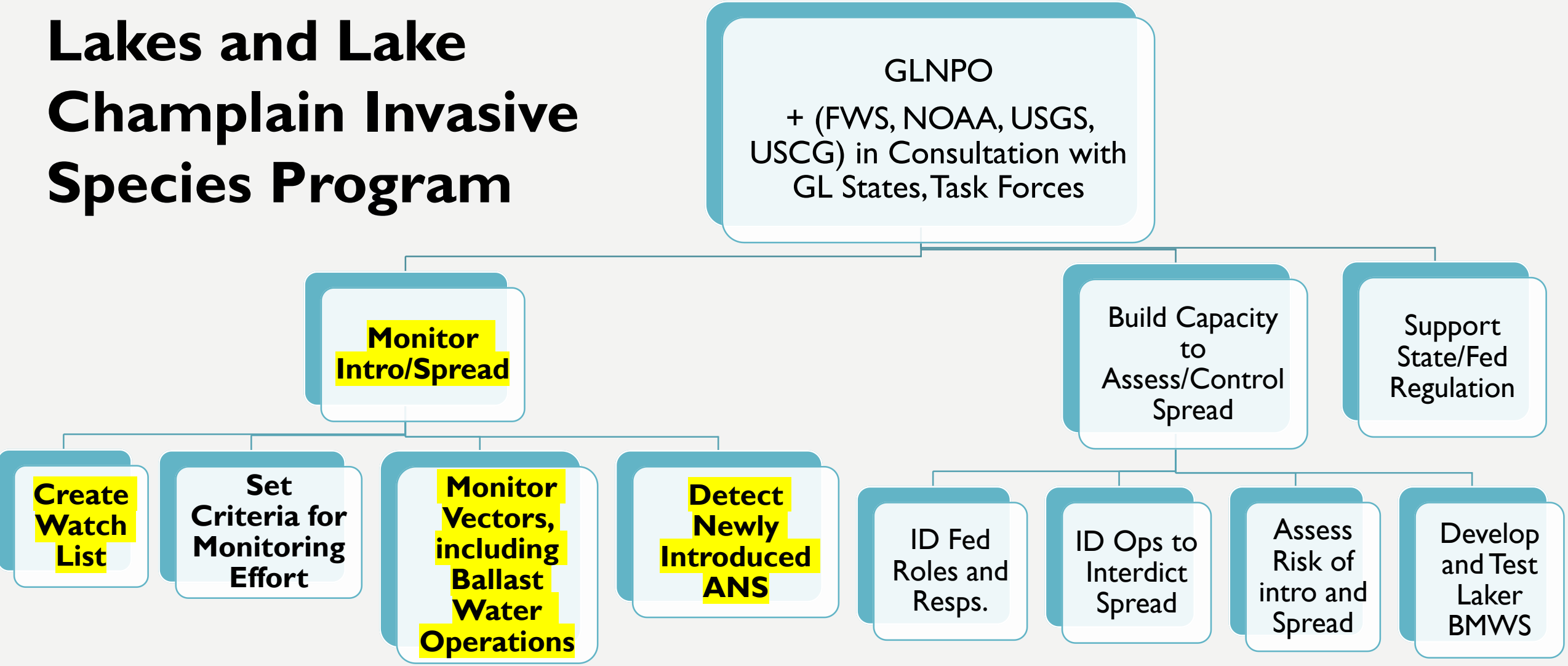
VIDA: INTERGOVERNMENTAL RESPONSE FRAMEWORK

"IN GENERAL.—The [USCG], in consultation with the [EPA] .. shall establish a **framework for Federal and intergovernmental response** to ...the **introduction, spread, and establishment** of [ANS]... '

"BALLAST DISCHARGE RISK RESPONSE.—The [EPA] in coordination with the [USCG] …shall establish a **risk assessment** and response framework using **ballast water discharge data** and [ANS] **monitoring data** for...:

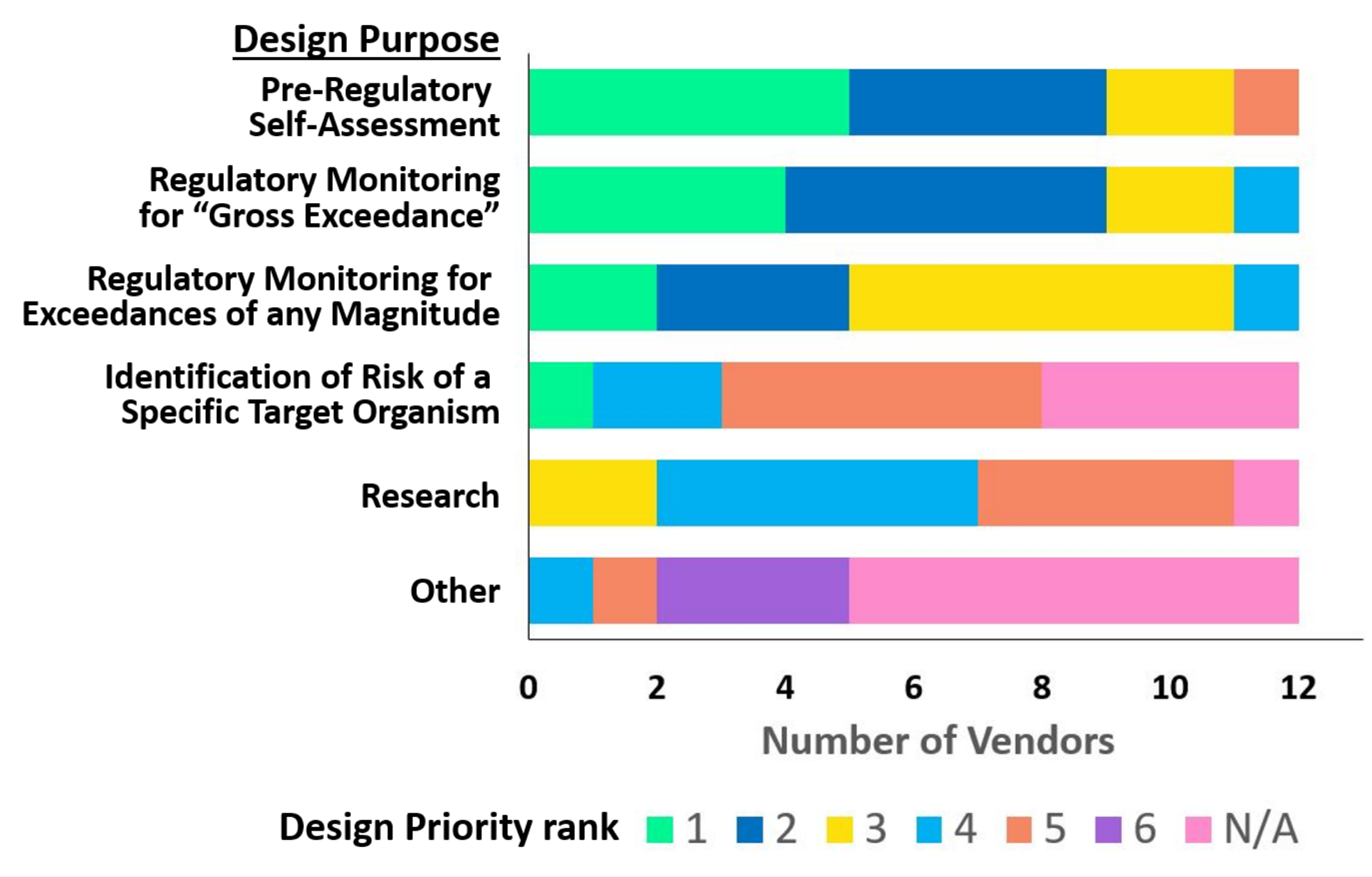
- **identifying and tracking populations** of [ANS];
- **evaluating the risk** of any [ANS] population …establishing and spreading in waters of the United States or waters of the contiguous zone; and '
- establishing emergency [BMPs] that may be deployed rapidly, in a local or regional manner, to respond to **emerging [ANS] threats**"

VIDA 2018: Great Lakes and Lake Champlain Invasive Species Program

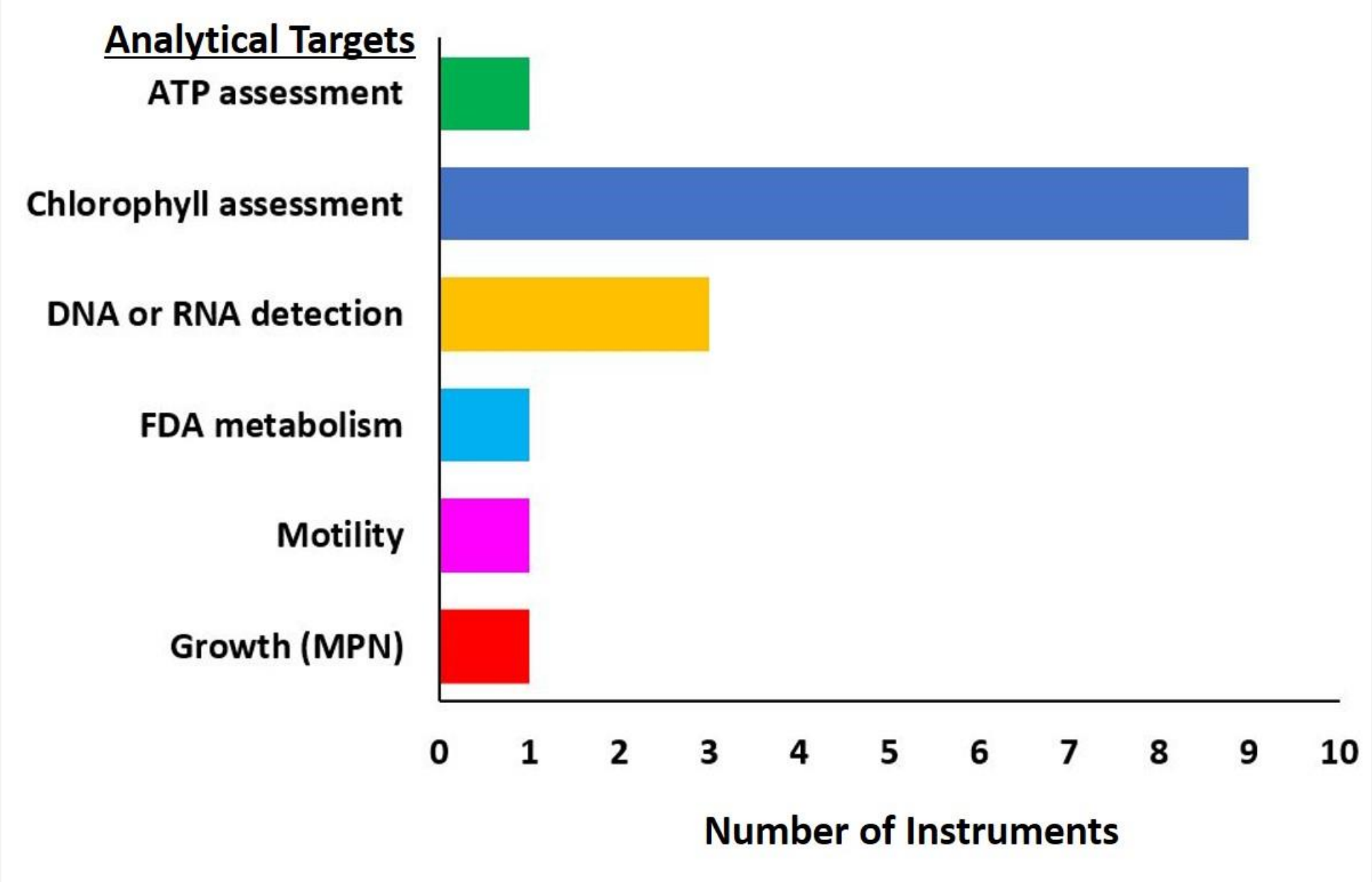


PRACTICUM OUTPUT

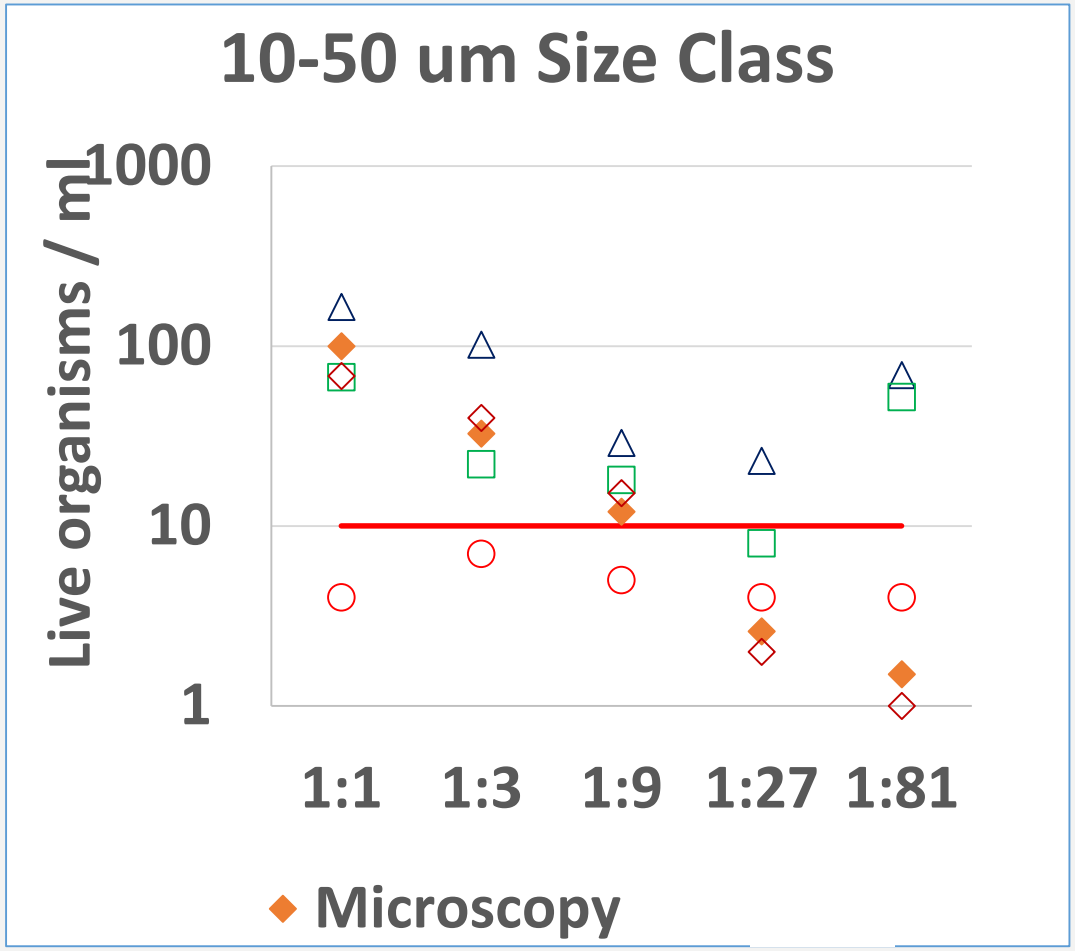
Vendor priorities in designing indicative monitoring tools for ballast

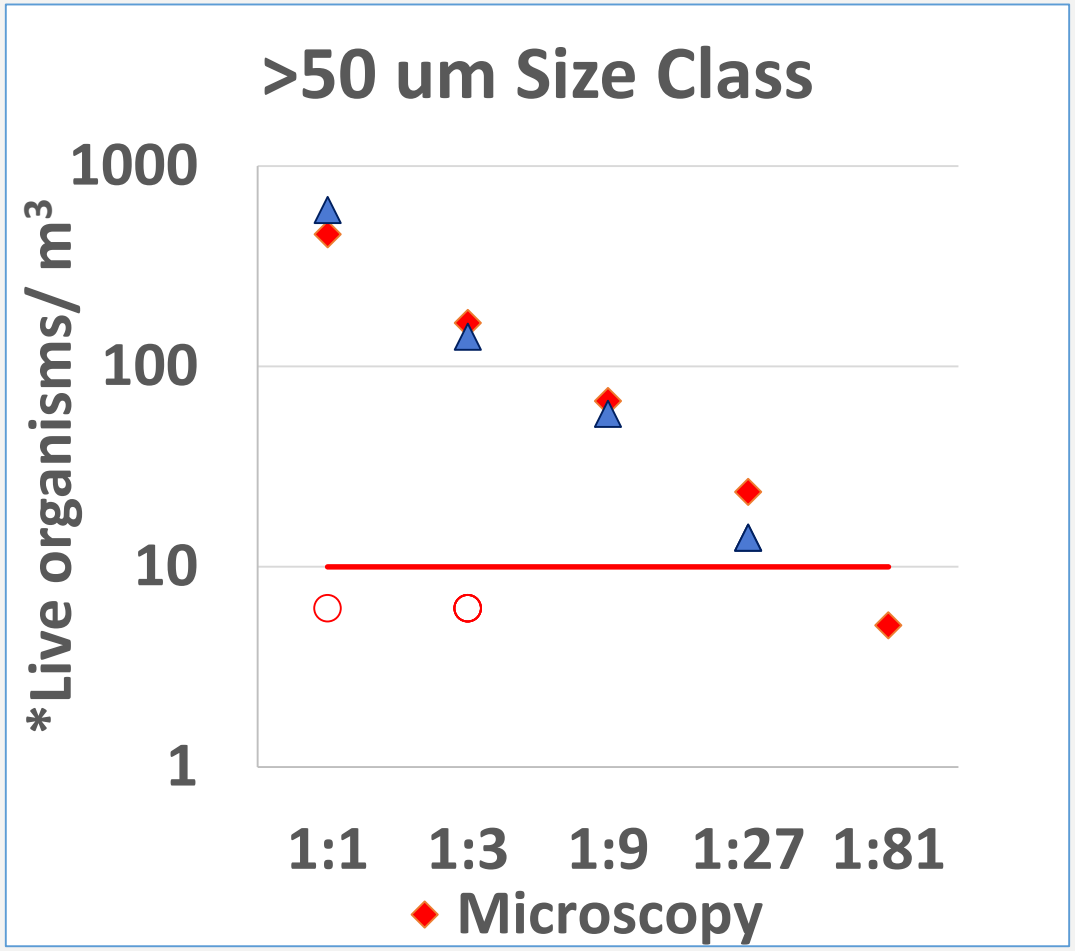


Technology approaches used by various vendors



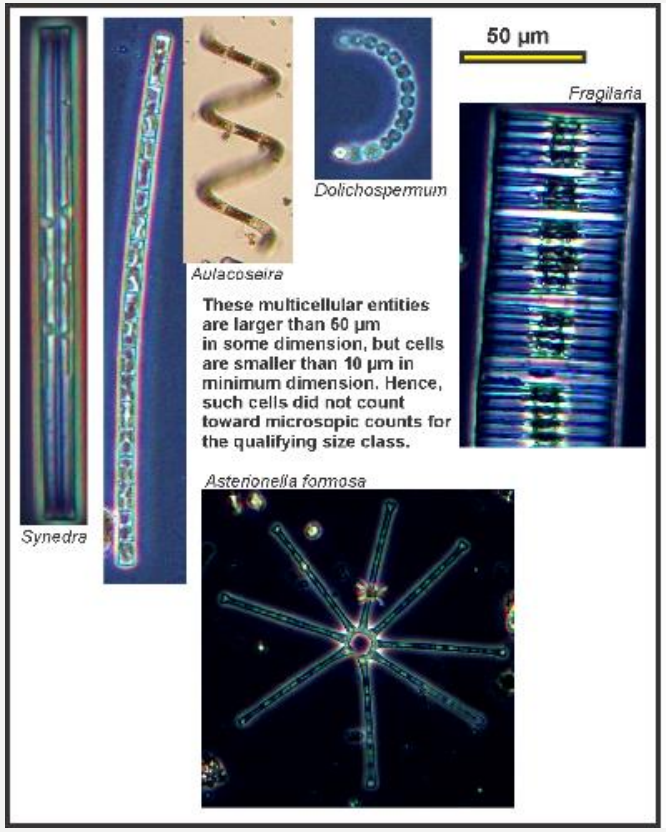
ERIE: MICROSCOPY AND INDICATIVE TOOLS,

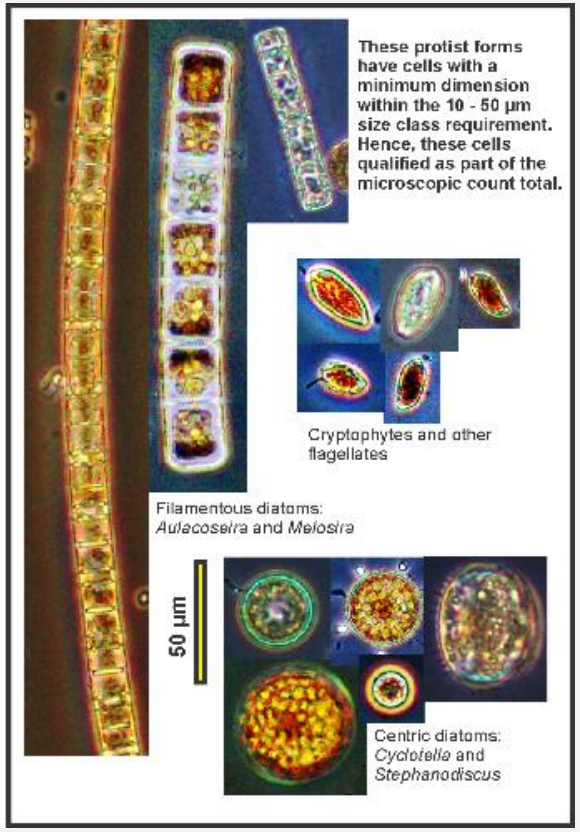




*>50 um size organisms were provided "as if" the sample had already been concentrated 1,000 times down from 1 m3 (i.e. 1000 L) to 1 L.

REASONS FOR DISCREPANCIES





In General:

- ❑ Not a Precision Process
- ❑ Not all Vendors Fully Equipped
- ❑ Time-Limitations

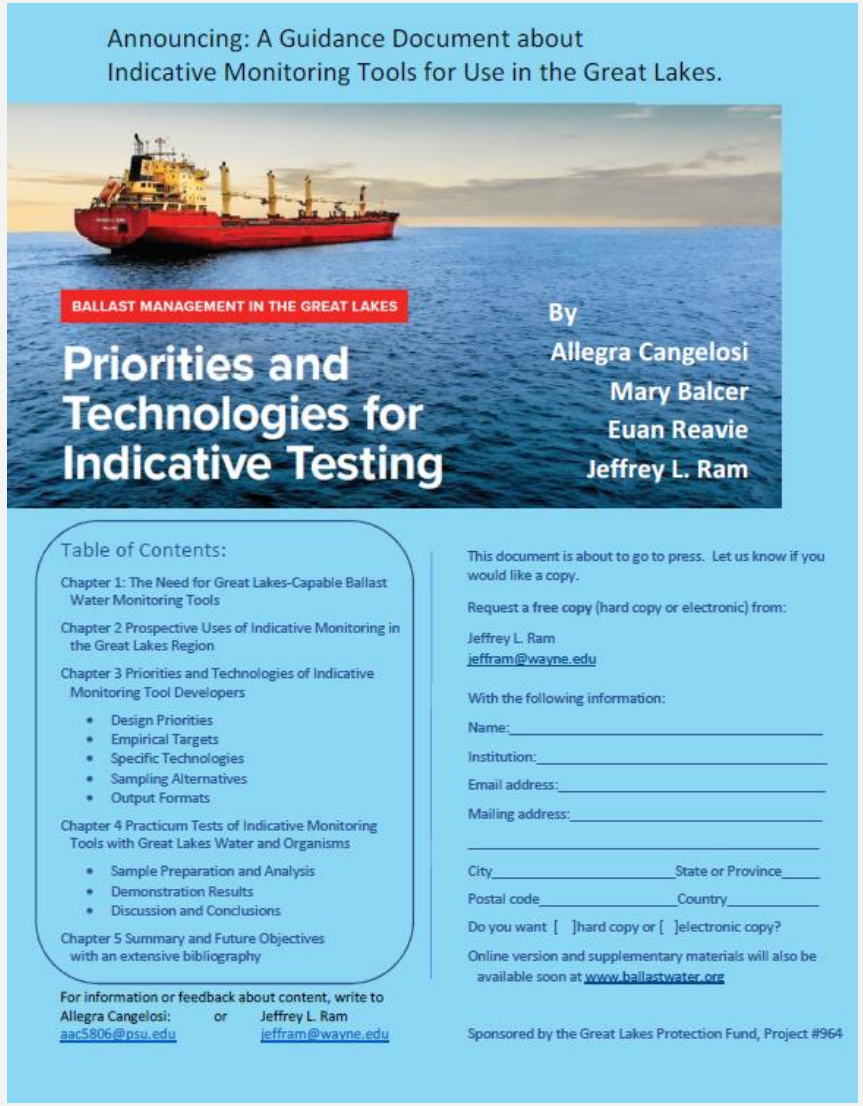
Great Lakes-Relevant:

- ❑ Matrix Characteristics (High DOCs, Coloration)
- ❑ Biota Characteristics: colonial algae & soft-bodied zooplankton near the size class boundary

FOR ADDITIONAL DETAILS

This document is about to go to press. Let us know if you would like a copy.
Request a **free copy** (hard copy or electronic) from:
Jeffrey L. Ram
jeffram@wayne.edu
With the following information:
Name:_____
Institution:_____
Email address:_____
Mailing address:_____

_____
City_____State or Province_____
Postal code_____Country_____
Do you want [ ]hard copy or [ ]electronic copy?
Online version and supplementary materials will also be available soon at www.ballastwater.org
Sponsored by the Great Lakes Protection Fund, Project #964



For information or feedback about content, write to Allegra Cangelosi: or Jeffrey L. Ram
aac5806@psu.edu jeffram@wayne.edu

Thank you!

Questions?