Verification of Ballast Water Treatment Technologies

A research project supported by the USEPA Great Lakes Restoration Initiative

Principal Investigator:

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Associate, Dept. of Immunology and Microbiology

Associate, Center for Molecular Medicine and Genetics

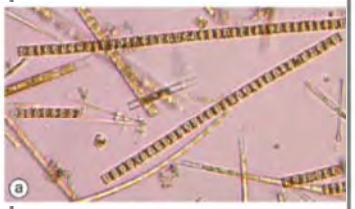
Our Mission:
Science to Serve and Preserve the Value and Beauty of the Great Lakes

Great Lakes are a global resource.
Great Lakes fresh water and its habitats must be protected.
Our goal: Ideas and facts for future action.
Development of new technologies.
Current focus: Invasive species and ballast water technologies

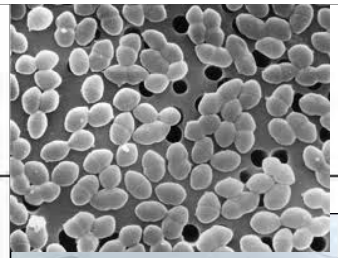
Every year new aquatic invaders threaten Great Lakes' health, recreation, economic activities and ecology

Poster "critters": dreissenid mussels and Asian carp

Many other organisms are of concern

Great Lakes and upper St. Lawrence River 1990-91	Samples from 86 ships ballast tanks 	110 species of zooplankton in 11 phyla. 100 species of bacteria, phytoplankton and protists mainly diatoms and dinoflagellates		Locke *et al.*, 1991, 1993; Subba Rao *et al.*, 1994
Washington state 1991	Samples from ballast water and sediments of 6 Japanese bulk carrier		21 species of phytoplankton and protists (sediment) and at least 8 organisms from ballast water	Kelly 1992, 1993
Gulf of Mexico 1991-1992	Ballast water sampled from 19 ships	5 ships ballast water included *Vibrio cholerae*		McCarthy & Khambaty 1994

From: Pazouki et al., Newcastle University, 2010. Project RP577

Great lakes and St. Lawrence, 1997-98	Ballast water and sediment samples from 28 Transoceanic vessels 	*Fecal Coliforms*, *vibrio cholerae*, *Enterococci* and *Salmonella*		Knight *et al.* 1999
9 Brazilian Ports 2002	99 samples taken from ships calling Brazilian ports	*Vibrio, Fecal Coliforms, E. Coli, Entrocci fecal, V. Cholerae, coliphage, Clostridium*		Anvisa 2003

Many other organisms are of concern

IMO ‘D2’ standards for discharged ballast water

Organism category	Regulation
Plankton, >50 µm in minimum dimension	< 10 cells / m^3
Plankton, 10-50 µm	< 10 cells / ml
Toxicogenic Vibrio cholera (O1 and O139)	< 1 cfu* / 100 ml
Escherichia coli	< 250 cfu* / 100 ml
Intestinal Enterococci	< 100 cfu* / 100 ml

* colony forming unit

IMO timetable: Depending on ship size, BW exchange allowed until 2016; after that only effective BW treatment will be allowed (all ship sizes).

US Environmental Protection Agency

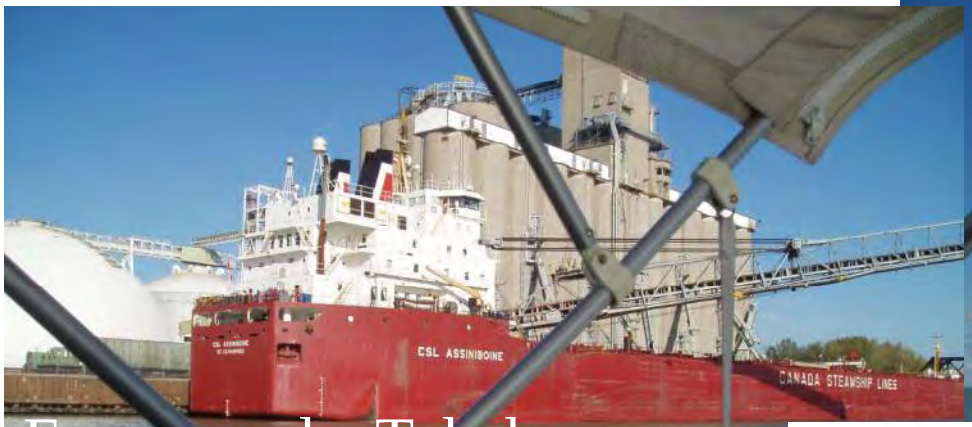
Costs: Invasive species cost the Great Lakes Basin $billions per year, through economic, environmental, and control costs impacts. Pathogens threaten human health and fish.

2010 Aquatic Invasive Species goals of the USEPA **Great Lakes Restoration Initiative**:

1. **Predict** them: forecast high-risk sites, species, effects, and restoration or prevention opportunities
2. **Stop** them: Develop and verify ballast water treatment systems
3. **Detect** them: Systematic early detection and threat assessment
4. **Control** them: Prevention, eradication, and control

Nearby ports threaten our waters

Great Lakes shipping



For example, Toledo, a major port of the Great Lakes, and
Near the "fishiest waters of the Great Lakes," western Lake Erie

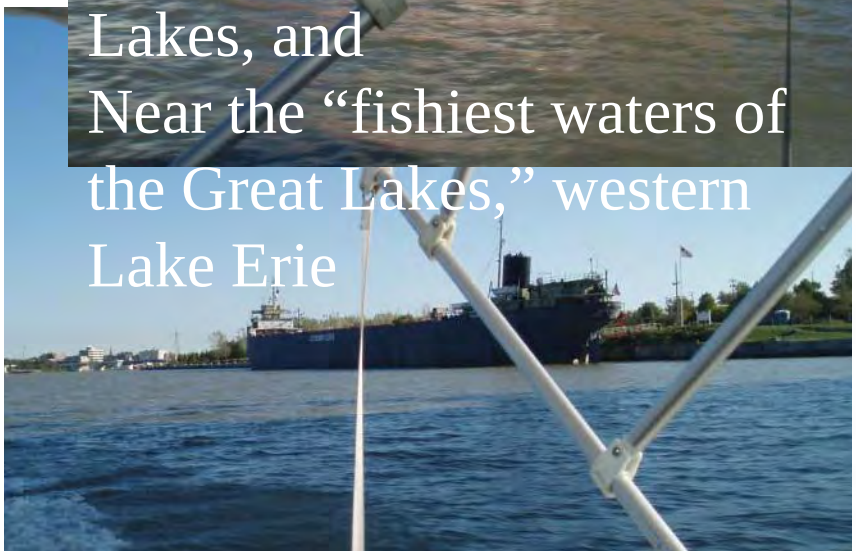


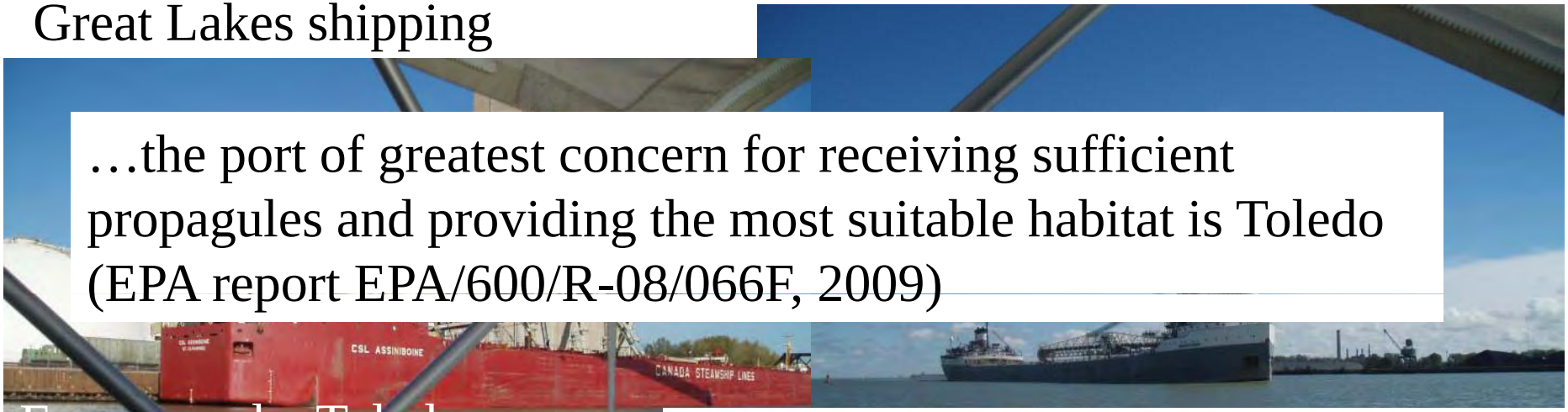
Table E-2. Ballast water discharges at U. S. Great Lakes during 2006-2007. In

Original source of ballast water from outside the Great Lakes

US Great Lake Port	Tanks Discharged	Volume Discharged (metric tons)	Vessels Discharging
Duluth	407	184,844	58
Toledo	85	65,335	13
Superior	50	78,085	10
Green Bay	18	5,984	4
Gary	17	11,154	4
Milwaukee	11	10,768	2
Oswego	8	1,239	5
Chicago	7	17,916	3

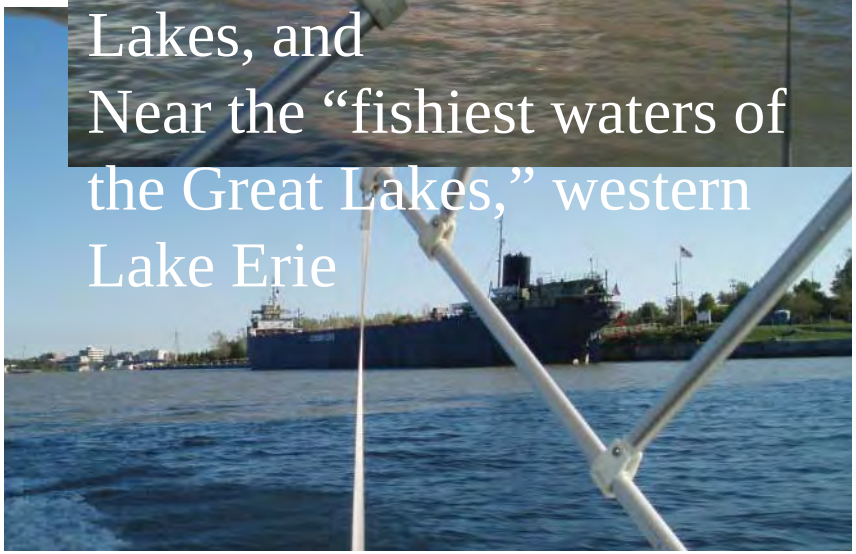
Nearby ports threaten our waters

Great Lakes shipping



…the port of greatest concern for receiving sufficient propagules and providing the most suitable habitat is Toledo (EPA report EPA/600/R-08/066F, 2009)

For example, Toledo, a major port of the Great Lakes, and
Near the "fishiest waters of the Great Lakes," western Lake Erie



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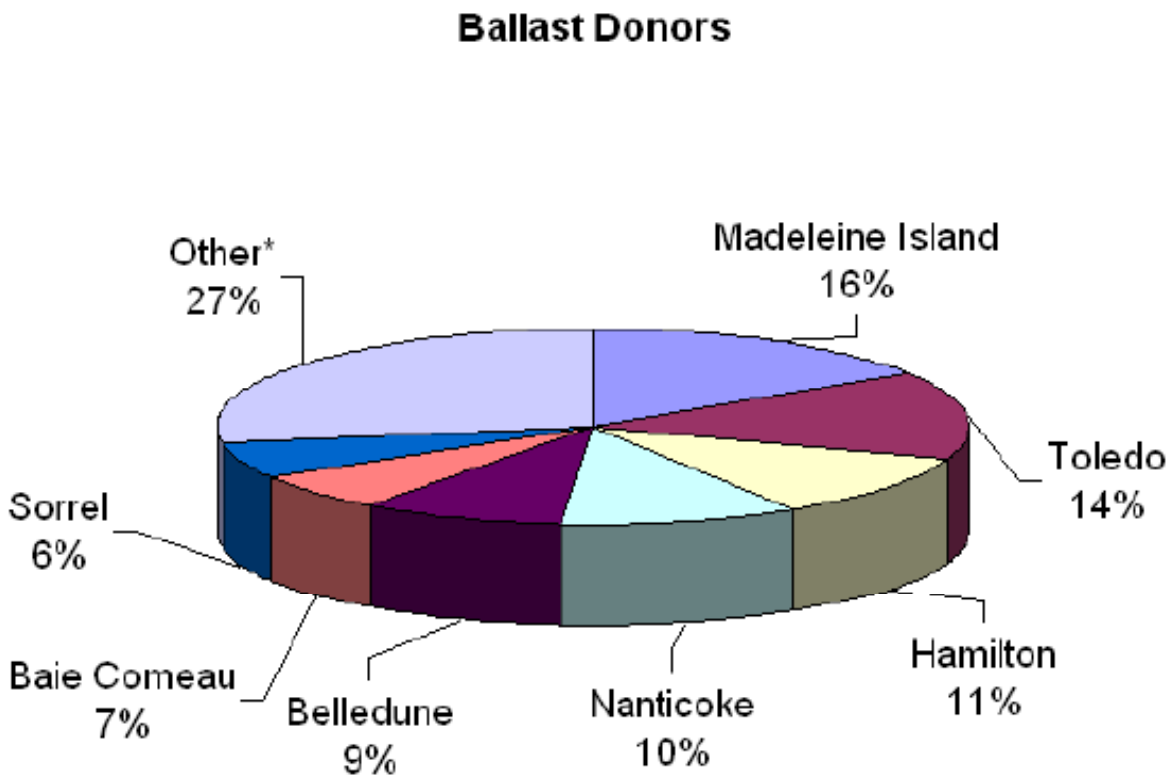
Toledo is also a ballast water donor



(MT- Metric Tons of Ballast Water)

*Other includes 20 ports with ballast water volumes less than 274,356 MT

(Data for Canada Shipping Lines, only)

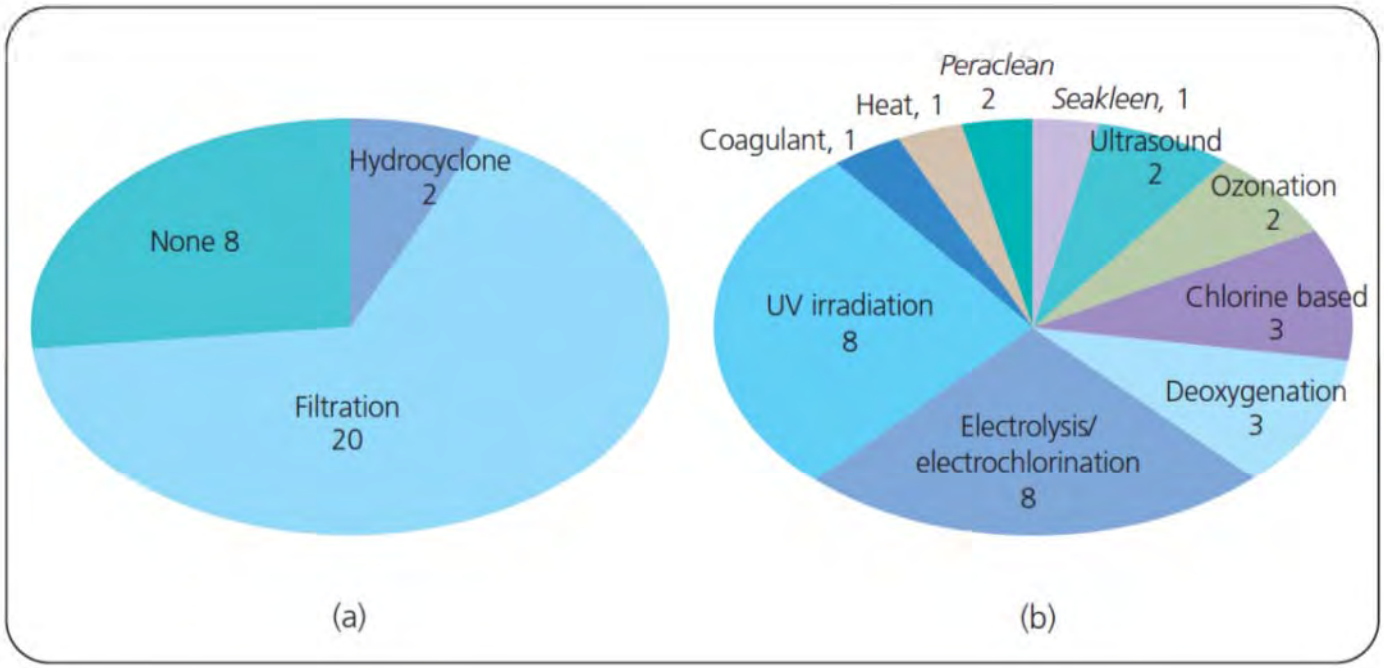
EPA GLRI's goals for Ballast water treatments

Many treatment systems in development.

Can they meet EPA's GLRI goals?

- "virtual elimination of living organisms from discharges" Includes not only the "poster critters" but also microscopic algae and bacteria, and
- "development of verification…of shipboard treatment systems."

Treatment systems include:



From Lloyd's Register, 2008 *Ballast Water Treatment Technology Current Status*

Summary of treatment technology options for (a) physical pre-treatment, and (b) disinfection

RamLab GLRI ballast water project

Project Title: Verification of Ballast Water Treatment Technologies

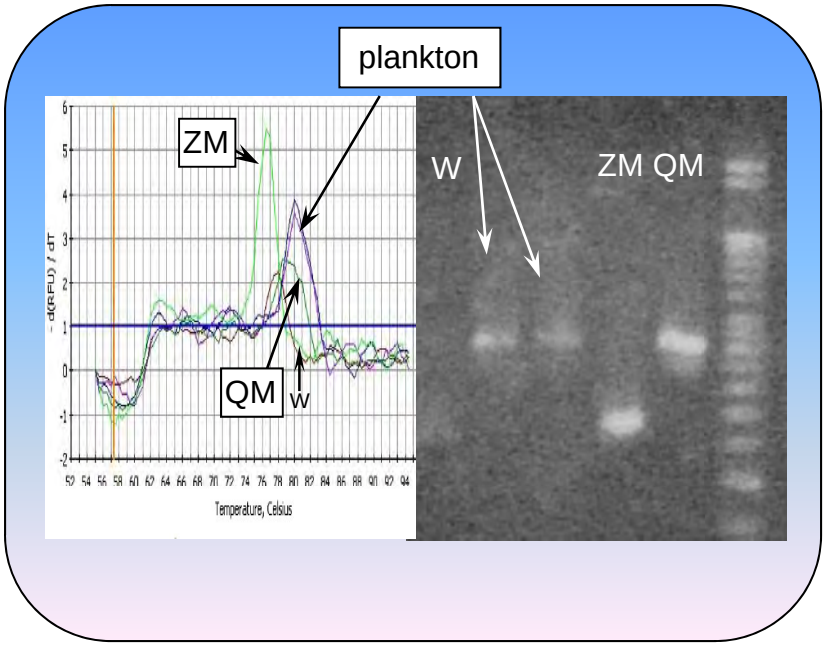
Principal Investigaor: Jeffrey Ram, WSU

Collaborating Investigator: Sarah Bailey, DFO, Canada

Overall goals: Develop MOLECULAR and MICROSCOPIC methods to differentiate LIVE-FROM-DEAD over a broad range of potential INVASIVE and PATHOGENIC species, applicable to a range of treatment systems.

Expected milestones:

- Test live/dead molecular methods. DNA sequence data for risk analysis
- Test and compare FlowCAM and other fluorescence microscopic methods
- Design Standard Operating Protocols for shipboard sample collection and preservation for land-based analysis
- Conduct shipboard verification tests



RamLab GLRI Milestone One

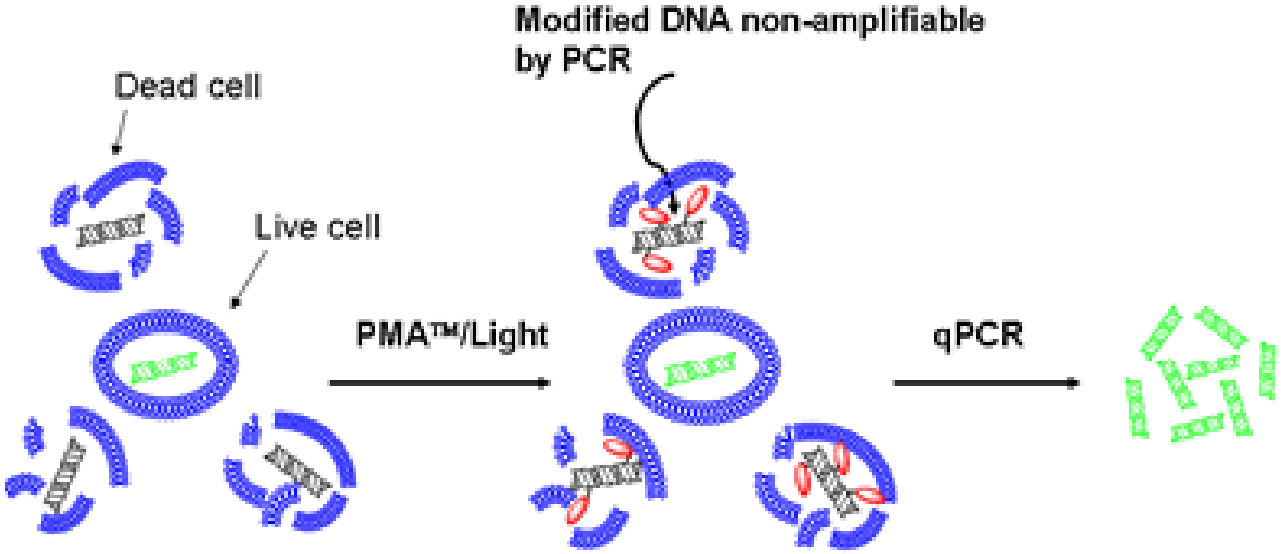
WAYNE STATE UNIVERSITY

- **Test live/dead molecular methods. DNA sequence data for risk analysis**

PMA-PCR

PMA™ for Selective Detection of Live Pathogens by qPCR

PMA = propidium monoazide



Ref.: Nocker, A., Cheung, C.Y., and Kamper, A.K. (2006). Comparison of propidium monoazide with ethidium monoazide for differentiation of live vs. dead bacteria by selective removal of DNA from dead cells. J. Microbio Meth. *67(2), 310-320.*

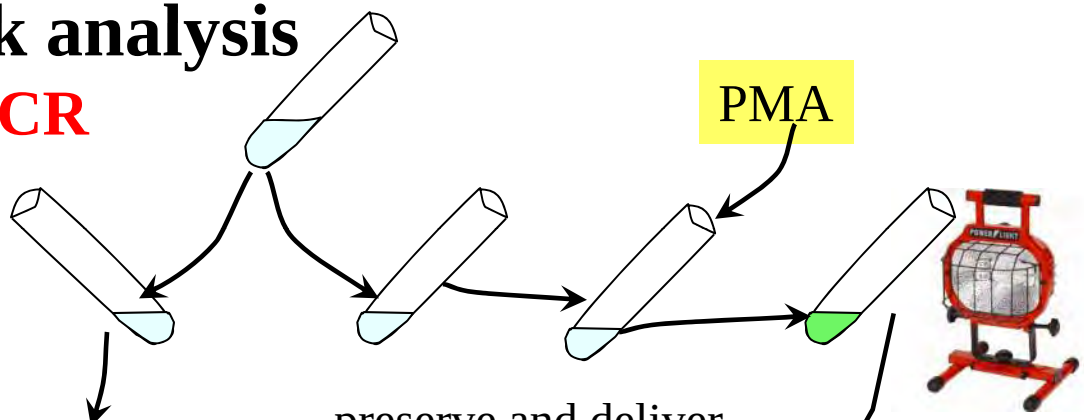
RamLab GLRI Milestone One

WAYNE STATE UNIVERSITY

PHYSIOLOGY Established 1913

•Test live/dead molecular methods. DNA sequence data for risk analysis

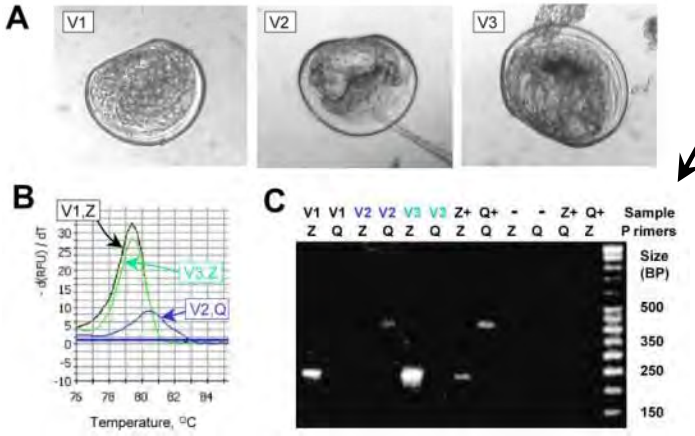
PMA-PCR



PCR will use prokaryotic universal primers (for bacteria) and eukaryotic universal or subgroup (algae, protozoans, etc.) specific primers.

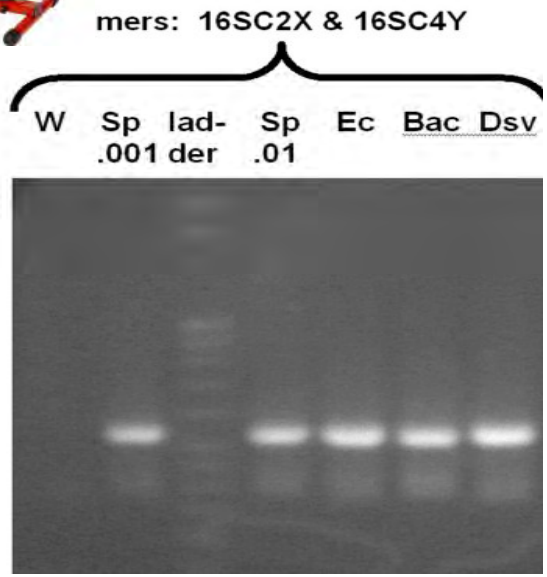
Test with a variety of specific organisms varying treatment techniques as needed.

PCR,
With universal or species specific primers



…..AGGCCTAG…

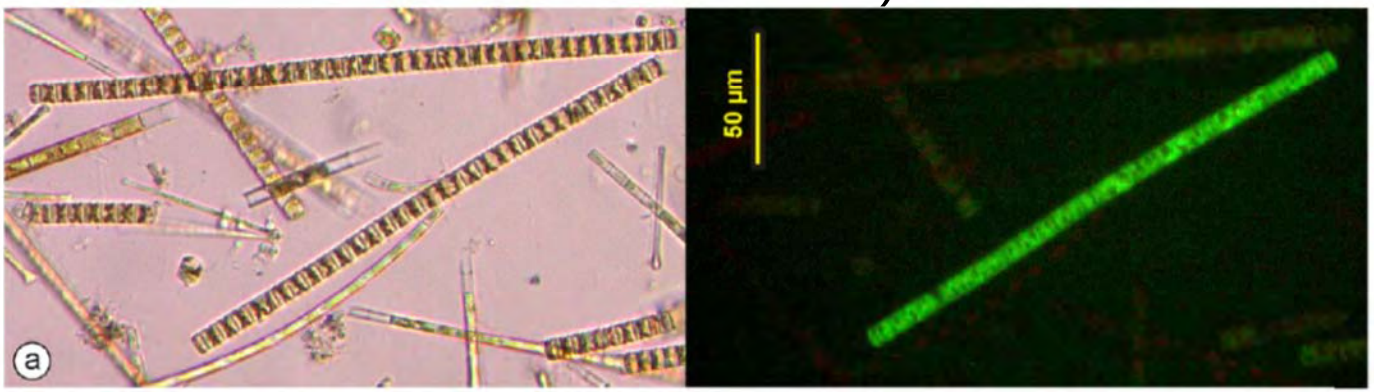
determine sequence



RamLab GLRI Milestone Two

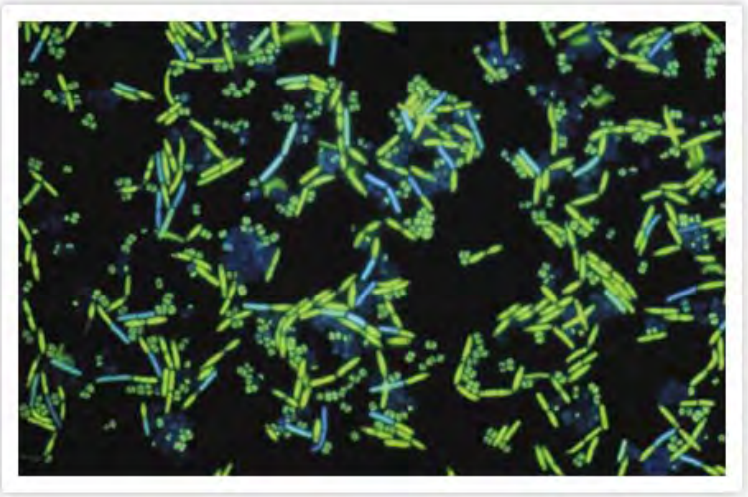
•Test and compare FlowCAM and other fluorescence microscopic methods (Sarah Bailey, DFO Canada)

•Stain only live cells (e.g., fluorescein diacetate (live esterase releases fluorescent fluorescein)



FDA stained mixture of live and dead phytoplankton. Left,: bright field; right, fluorescence (from Reavie et al., J Gr. Lakes Res. 36:540-547, 2010).

•Stains that stain only dead cells (e.g. SYTOX Green—penetrates permeable membranes and fluoresces with DNA). Illustrated with *Micrococcus/Bacillus* mix



RamLab GLRI Milestone Three

•Design Standard Operating Protocols for shipboard sample collection and preservation for land-based analysis

Adapt laboratory methods to shipboard sample collection and treatment, based on results of Milestones One and Two

Methods will take into account shipboard conditions:

- Stability
- Lack of sophisticated equipment and personnel
- Limited time
- Large sample volumes compared to lab experiments

Prepare QAPP based on above criteria,

To achieve desired sample collection, processing, COC documentation, analytical procedures upon receipt, and data analysis, to assure precision, accuracy, representativeness, completeness, and comparability.

RamLab GLRI Milestone Four

- Conduct shipboard verification tests
  - Will work with ECOLOGIQ (Delivering eco-friendly solutions...for life.)
    A multinational biotechnology company, with headquarters in Michigan and partners in Canada
  - Will test our verification systems with Ballaclean™, Ballaclean Plus™, or other BW treatment technologies in shipboard tests with shipowners in Canada.

    Ballaclean™ is a deoxygenation based method.

    Ballaclean Plus™ is a multi-component approach, combining filtration, chlorination, and neutralization prior to discharge
  - Alternative or supplementary shipboard tests in collaboration with Sarah Bailey and others.

Pilot Study on Early Detection in Toledo Harbor

25 lb bottom dredge

Jeffrey Ram, Wayne State prof in physiology, microbiology, & molecular genetics)

0.5 mm mesh sieve

Thanks to:

Funding agencies: EPA, Sea Grant, HOW, NSF

Excellent collaborators and students: Donna Kashian, Sarah Bailey, Carl Guething, Dick Gala, Pedro Tan and JJ Antakli (EcologiQ), Aos Karim, Payel Acharya, Sonal Purohit