Northwest **Power** and **Conservation** Council

August 4, 2015

MEMORANDUM

TO: **Fish and Wildlife Committee**

FROM: **Kerry Berg**

SUBJECT: **Update on Threat of Invasive Mussels and Other Species of Concern and Briefing on New Genetic Tools for Early Detection.**

BACKGROUND:

Presenters: Gordon Luikart, Flathead Lake Biological Station.

Summary: Gordon Luikart will provide an update on his research related to invasive species including information on early detection of invasive mussels. Below is some information from the Flathead Lake Biological Station website. For more information go to http://flbs.umt.edu/ .

Gordon Luikart

Education:

- Ph.D., 1997, University of Montana, Organismal Biology and Ecology
- M.S., 1992, University of Montana, Zoology
- B.S., 1988, Iowa State University, General Biology with minor in Animal Ecology

851 S.W. Sixth Avenue, Suite 1100
Portland, Oregon 97204-1348
www.nwcouncil.org

Steve Crow
Executive Director

503-222-5161
800-452-5161
Fax: 503-820-2370

Research Interests:

My general research interests are in ecology, population genetics, and conservation biology. (view my C.V.). The primary focus of my research is the application of genetics to the conservation of natural and domesticated populations (video). I work at FLBS and in the Montana Conservation Genomics Laboratory (MGCL) at the University of Montana (UM) with my colleagues, Fred Allendorf, Robb Leary, and Steve Amish. My research applies the principles and tools of population genetics to fish, wildlife, and a variety of other species, including nasty invasive species. (AIS video). Our lab has established exchange programs and collaborations between the University of Montana and the University of Porto in Portugal (CIBIO-UP) with Portuguese colleagues (e.g., Albano Beja-Pereira) to promote international education, research, and conservation. We have exciting collaborations with Clint Muhlfeld at the US Geological Survey, and Mike Schwartz at the Rocky Mountain Research Station. Many of our projects combine field sampling with the use of molecular genetic markers and novel data analysis approaches to understand and monitor landscape connectivity, adaptation to climate change, population viability, and biodiversity conservation. For more information, visit my Research pages.

Flathead Lake Biological Station

The Flathead Lake Biological Station (FLBS) is a center and academic department within the University of Montana system. We are an ecological research and education center located on Flathead Lake in the Rocky Mountains near Glacier National Park. For over 100 years, we have conducted college courses, graduate programs, and research focused on the Crown of the Continent ecosystem.

Students and researchers from around the world continue to come to FLBS to learn about ecology and limnology from experts in the field. We conduct year-round novel research to help people live in a healthy, sustainable environment and help policy-makers make informed decisions.

Relevance: The 2014 Fish and Wildlife Program supports preventing the introduction of non-native and invasive species in the Columbia River Basin, and the suppression or eradication of non-native and invasive species. Aggressively addressing non-native and invasive species to preserve program effectiveness is listed as one of the emerging priorities in the program.

Dreissenid mussel threats and eDNA monitoring to help prevent invasion of Flathead Lake & the CRB

Flathead Lake Biological Station
The University of Montana

Gordon Luikart; Professor, Conservation genetics

Dreissenid Mussels are among the most harmful of all aquatic invasive species

Zebra mussel

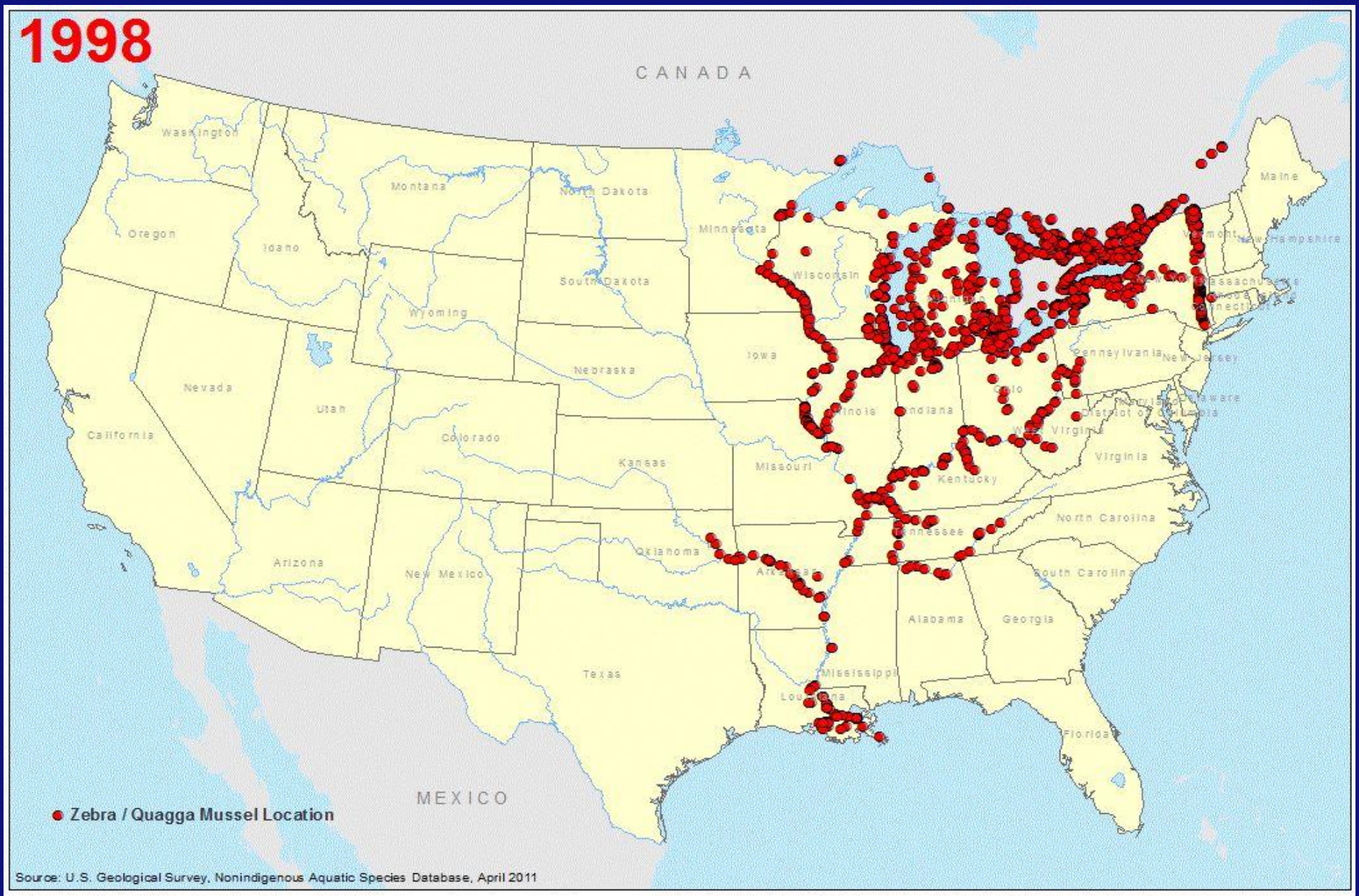
(*Dreissena polymorpha*)

Quagga mussel

(*Dreissena bugensis*)

Slides from Tom Bansak

http://search.tb.ask.com/search/video.jhtml?searchfor=zebrea+mussel+video+flathead+lake+biological+station&cb=Y6&pg=GGmain&p2=%5EY6%5Exdm003%5ES13124%5Eus&n=781B64D9&qid=960b0bfddc3b48e88d1b0aa2cac0a998&ss=sub&pn=1&st=hp&ptb=07BA9734-4417-4098-ABFD-1E8442C1201D&tpr=hpsb&si=CKWfwN_CisYCFROTfgodmgsAlg&vidOrd=1&vidId=MMT9qgAkAVk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Arizona
Colorado
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North Dakota
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Oklahoma
Texas
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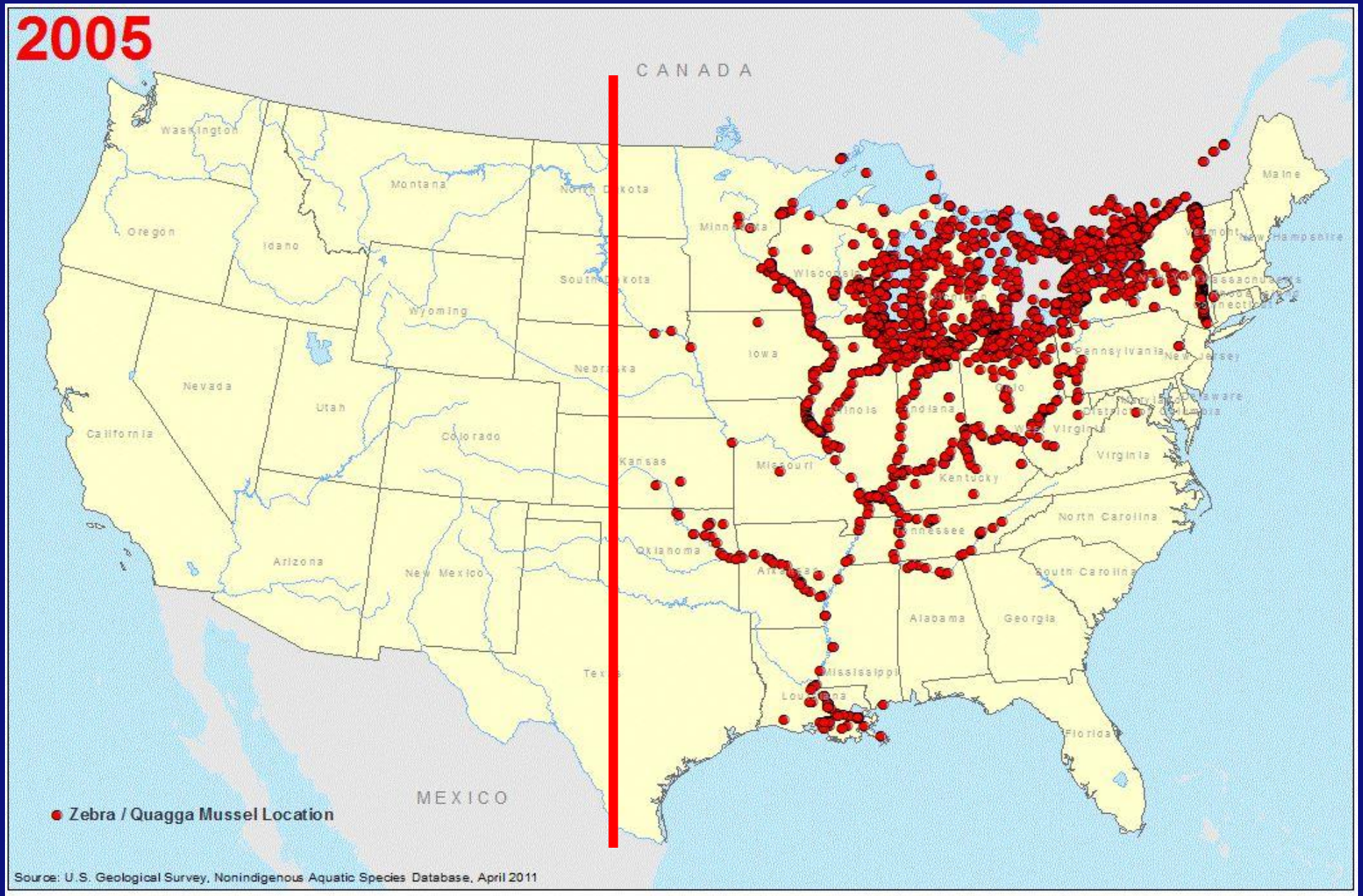
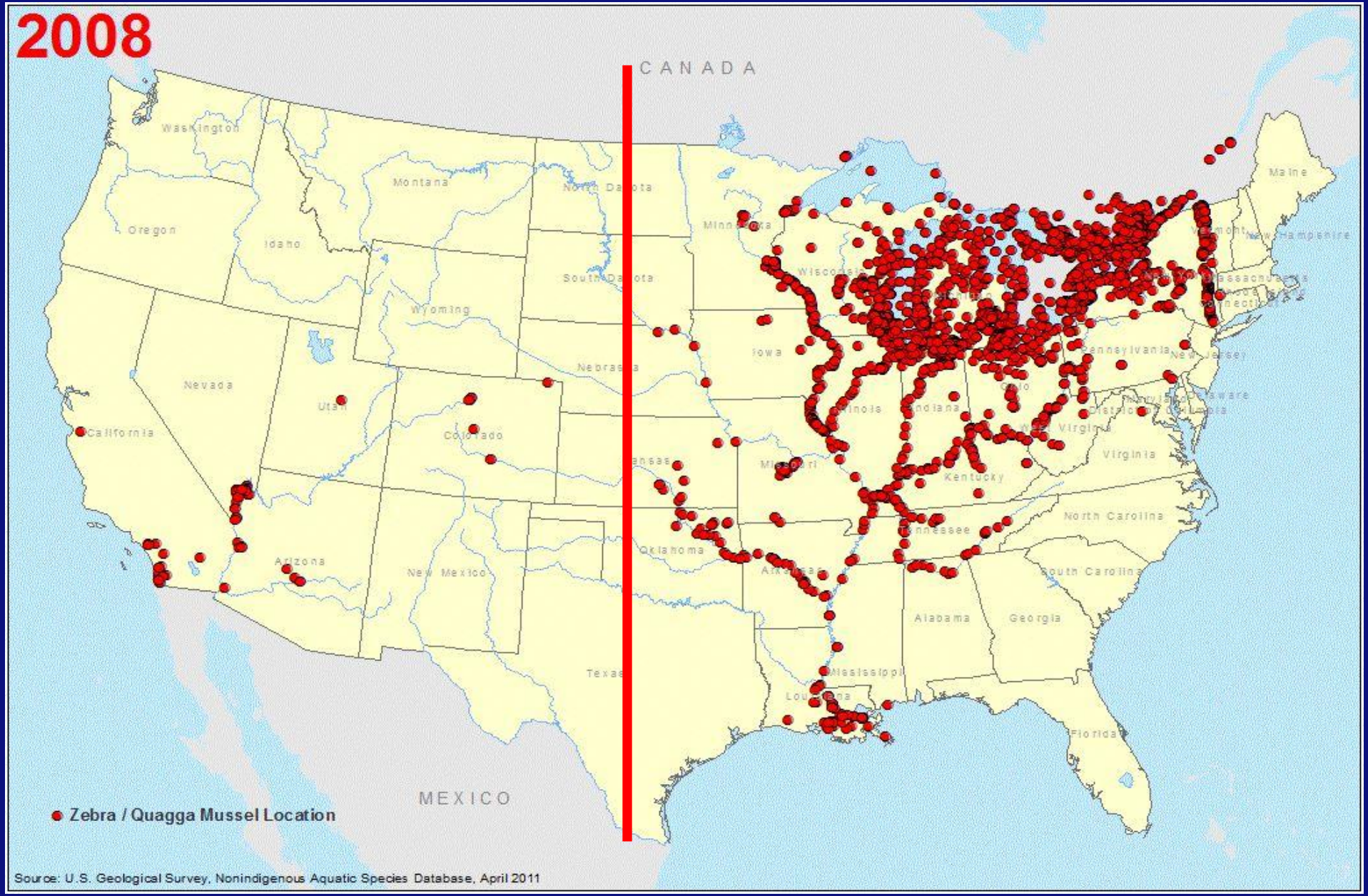
Louisiana
Wisconsin
Illinois
Michigan
Indiana
Ohio
Kentucky
Tennessee
Mississippi
Alabama
Georgia
Florida
South Carolina
North Carolina
Virginia
West Virginia
Pennsylvania
New York
Maine
Vermont
New Hampshire
Massachusetts
Connecticut
Rhode Island
New Jersey
Delaware
Maryland
MEXICO
Zebra / Quagga Mussel Location
Source: U.S. Geological Survey, Nonindigenous Aquatic Species Database, April 2011


2005
CANADA
MEXICO
Zebra / Quagga Mussel Location
Source: U.S. Geological Survey, Nonindigenous Aquatic Species Database, April 2011


2008
CANADA
MEXICO
Zebra / Quagga Mussel Location
Source: U.S. Geological Survey, Nonindigenous Aquatic Species Database, April 2011

2012

Zebra and Quagga Mussel Sightings Distribution

Dreissena polymorpha and D. rostriformis bugensis

- Zebra mussel occurrences
- Quagga mussel occurrences
- Both species occurrences
- Zebra mussels eradicated

Map produced by the U.S. Geological Survey, Nonindigenous Aquatic Species Database, December 10, 2012.

Dreissenid Mussel Biology

Wide temperature tolerance of 1-30 degrees Celsius. Quagga mussels are more cold-tolerant than zebra mussels and can live on soft substrates, allowing them to inhabit deeper water and more habitat (Rosenberg et a1. 1994).

Eggs are fertilized externally in the water column. Females can produce 30,000-**1 ,000,000 eggs annually**.

Fertilized eggs become free-floating (pelagic) veligers that use available calcium in the water to form minute bivalve shells.

The veligers then attach to available substrates using byssal threads.

Ecological Effects of Zebra/Quagga Mussels

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  - Cause crash in fish populations

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- Cover all available substrate

Impacts of Zebra/Quaggas on People

- Clog intake pipes and distribution networks for municipal, agricultural and power plant water supplies

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  - > $3 billion spent on control by power producers in Great Lakes between 1993-1999
    - Manual removal, chemicals, electrical current, screens, drawdowns

Impacts of Zebra/Quaggas on People

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  - > **$3 billion** spent on control by power producers in Great Lakes between 1993-1999
    - Manual removal, chemicals, electrical current, screens, drawdowns
    - Costs passed on to rate payers for electricity and water
  - **> $89 million** annual mitigation costs estimated for Columbia River Hydropower infrastructure (Independent Economic Analysis Board, 2010)
  - > **$95 million** estimated costs including annual maintenance costs for power, irrigation, water supply and recreation in Idaho alone! (Idaho Aquatic Nuisance Task Force, 2009)

Impacts of Zebra/Quaggas on People (cont)

- Decreased recreation and angling opportunities (and associated economic revenue)
  - Fish population crashes
  - Water bodies closed to recreation
  - Beaches unusable due to mussel shells

Outline

1. The problem: AIS & Zebra mussels threaten the economy and ecosystems

2. What is environmental (e) DNA? How can eDNA help?

3. Future technologies to facilitate early detection
   - Sampling (targeting convergence zones, autonomous)
   - DNA PCR testing (digital PCR, sensitivity, specificity)

Environmental DNA (eDNA): DNA present in the environment

Facilitates early detection of invasives allowing managers to quarantine and treat initial infestations before they spread.

ZEQUANOX®
Eradication, Christmas Lake, Minnesota

http://www.startribune.com/minnesota-lake-first-to-use-new-product-to-kill-zebra-mussels/274420131/

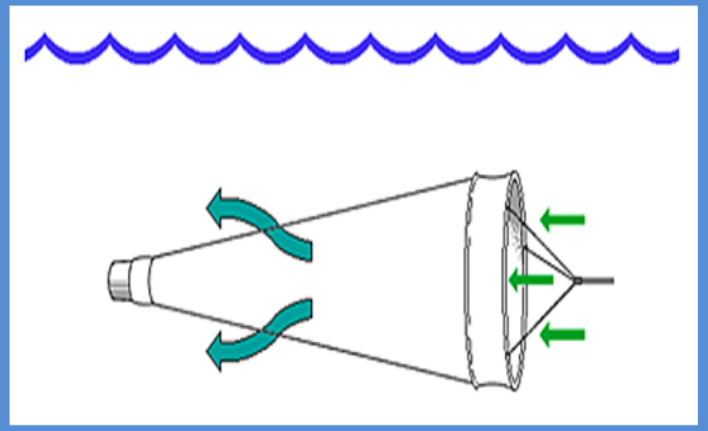
Even if eradication fails, the financial benefits of early detection are substantial. For example, hydroelectric facilities would likely have 1–3 years to prepare for costs associated with shutting down turbines for cleaning structures, screens, etc. (IEAB 2010).

Aquatic organisms release DNA into the water

Feces
Urine
Tissue/cells
Mucous
Gametes
Larvae
Seeds
Plant material

Sampling methods collect the free DNA and sloughed cells from water

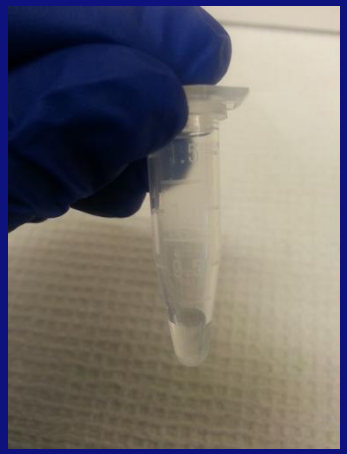
In the field:



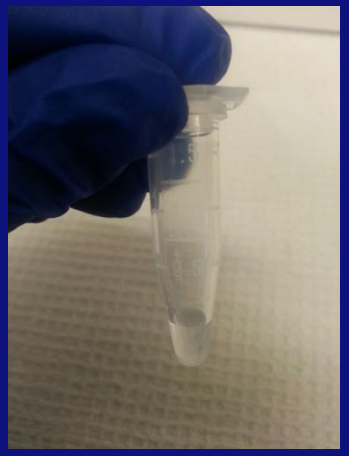
Tow net sampling

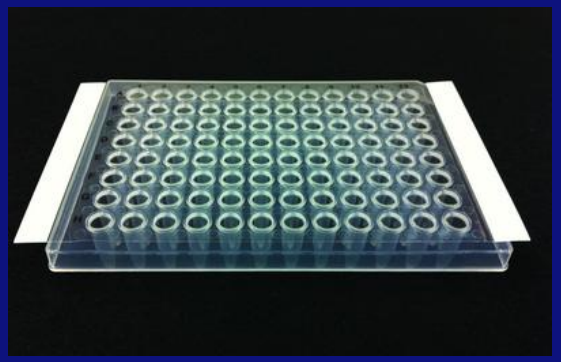
filters **2,840 liters** of lake water over a 40 meter tow to generate one eDNA sample

At the lab:



At the lab:





Real-time PCR Detection Assay

Real time (rt) PCR Detection Assays

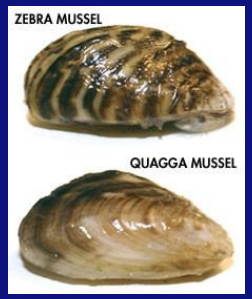
Allow us to detect eDNA from one or more target organisms

Note: Polymerase Chain Reaction (PCR) amplifies a species-specific DNA sequence

We designed & tested 2 new PCR assays:

1. Native vs invasive mollusk assay (genus identification)

VS



General 'Native' mollusk DNA sequence

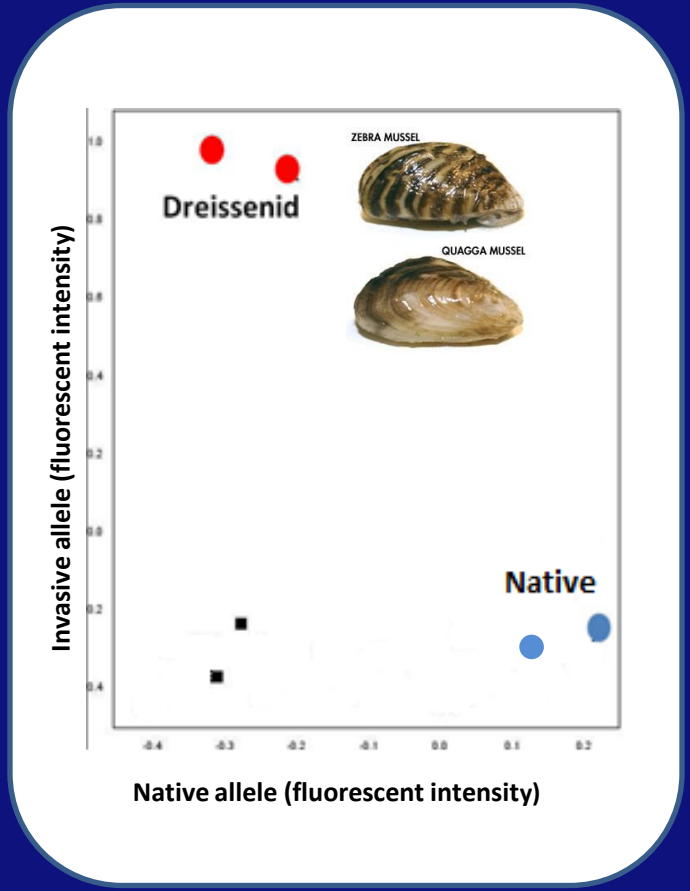
Dreissenid DNA sequence

2. Zebra vs Quagga species identification assay

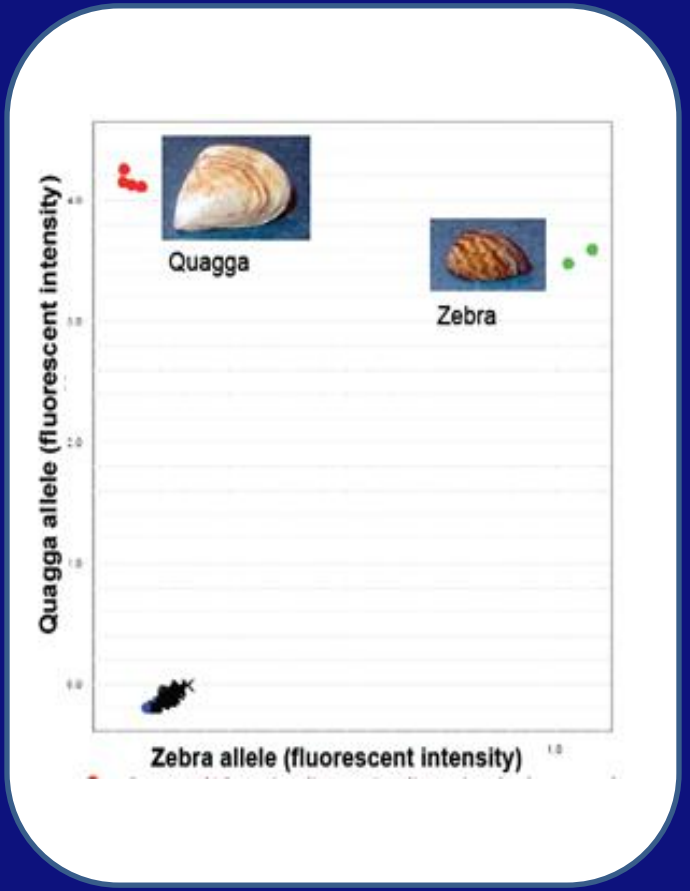
VS

Assay Testing

Native/Invasive mollusk assay



Zebra/Quagga assay



These PCR assays are taxon specific

Assay Testing

Sensitivity – the DNA concentration at which the target organism is reliably detected

In 100s of independent tests per assay:

All assays reliably detected DNA quantities down to
1 picogram DNA per PCR reaction

Equivalent to:
<1 Zebra or Quagga mussel larva
(or a few cells)

Field Sampling 32 sites with collaborators

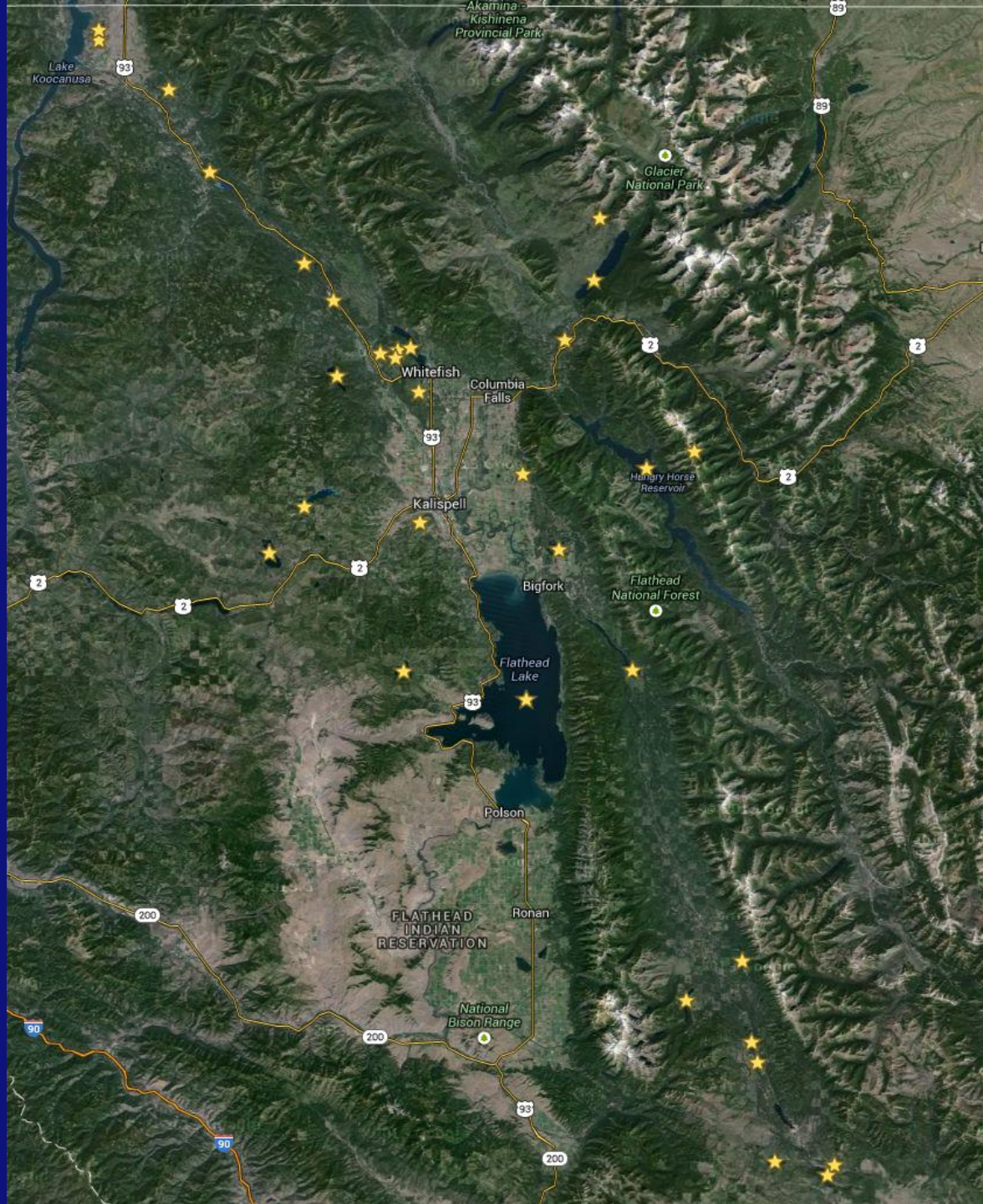
USGS science for a changing world

FOREST SERVICE U S DEPARTMENT OF AGRICULTURE

Clearwater Resource Council

WEED DISTRICT MISSOULA COUNTY & EXTENSION

Whitefish Lake INSTITUTE



Results

No Dreissenids detected in any Montana lakes

Detected *Native* mollusk sequence in 28/32 lakes

Outline

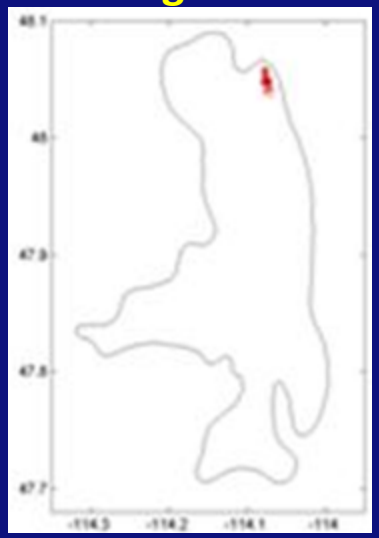
1. The problem: AIS & Zebra mussel threaten the economy and ecosystems

2. What is environmental (e) DNA? How can eDNA help?

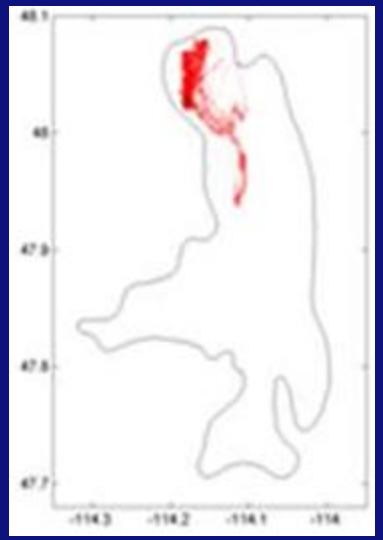
3. Future research & technologies improving early detection
   - Sampling (targeting convergence zones or eddys, autonomous remote sampler)
   - DNA PCR testing (digital PCR, sensitivity, specificity)

Water flow measurement allows targeting of hot spots for AIS and eDNA sampling

Bigfork



Sommers



Simulated drift patterns of veligers released from boat launch locations around Flathead Lake, Montana (upper Columbia River Basin).

Swan River

Map of measured horizontal flow vectors for the Swan River, Montana (in the upper Columbia River Drainage). Yellow arrows represent current speed and direction. Red oval is an eddy and sampling hotspot.

Figures from Mark Lorang, FLBS

Autonomous qPCR field instruments built for **Continuous Early-Warning**

Size of sampler:
11” Diameter, 11” Long

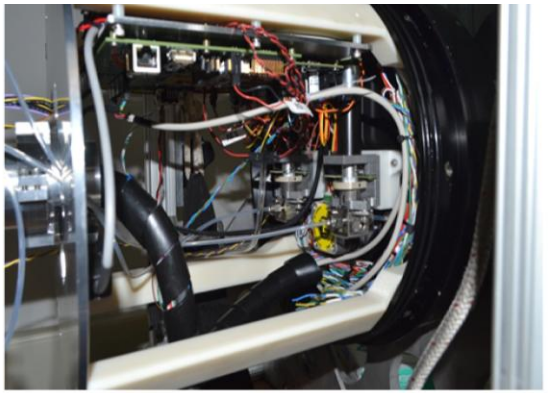
Temperature:
Function: 2 to 37 °C
Shipping: 0-50 °C

Energy:
nominal 2-20W.

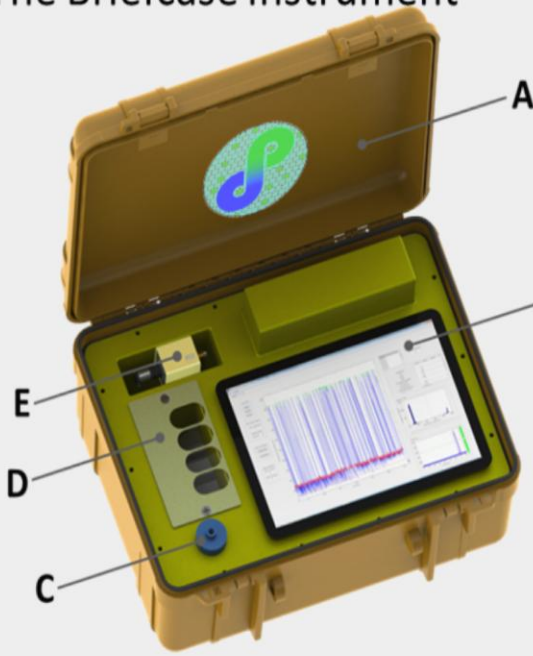
Field Testing:
At FLBS in 2017.

Field Deployment on AUV

The Embedded Instrument



The Briefcase Instrument



A) The portable brief-case format with external power & recharge outlet, B) The tablet PC with control and data analysis GUI, C) The sample injection port, D) The rapid-replace consumable reagent bay, and E) The target primer library.

Slides from Cody Youngbull

Conclusions/Recommendations

1. Identify hotspots for eDNA sampling
2. Expand eDNA monitoring using available real time (rt) qPCR technology
3. Test and deploy different autonomous sampling instruments, and digital qPCR
4. Finalize rapid response plans to include eDNA (from experienced labs)

Divergent mtDNA lineages of goats in an Early Neolithic site, far from the initial domestication areas

PNAS | October 17, 2006

Fernandez et al.

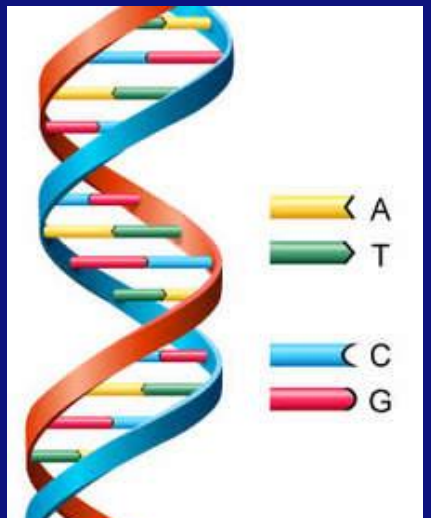
Noninvasive genetic sampling: look before you leap

TREE vol. 14, no. 8 August 1999

Taberlet et al.

Salmonid connectivity, genomics, and climate-change adaptation research

Thank you!



eDNA PCR in Idaho's rapid response plan

IDAHO RAPID RESPONSE PLAN FOR EARLY DETECTION OF DREISSENID MUSSELS

(A supplement to the Columbia River Basin Rapid Response Plan)

Initially drafted 11/06/09; Draft edits 5/26/2015

Objective 1: Verify

Purpose: Confirm suspected identification of the Dreissenid species.

Lead entity: ISDA.

A waterbody will be identified as "Suspect" for Dreissenid mussels if:

1) Settled adult Dreissenid mussels are found and verified by two qualified experts OR

2) Dreissenid mussel veligers are found and confirmed utilizing **BOTH** of the following methods:

- Microscopy identification of a sample from a qualified expert and concurrence from a second qualified expert: (EcoAnalysts, Bureau of Reclamation ("BOR"), Portland State University ("PSU") **AND**
- PCR (genetic) identification of a sample by a qualified expert and concurrence from a second qualified expert: (Pieces Labs, BOR)

A waterbody will be considered "Positive" for Dreissenid mussels if specimens are verified through the above protocol during two separate sampling events.

Assay Testing

Specificity- ability to detect the target organism

- Positive Controls: Tow net samples spiked with mussel veligers, milfoil tissue, or DNA from target species

- Negative controls: Included known-negative eDNA samples and H_2O

For all assays,
positive controls amplified and negative controls did not

Preventing Contamination

- Frequent field blanks, negative controls for each extraction and PCR
- eDNA extraction and PCR conducted in separate labs

Verifying Results

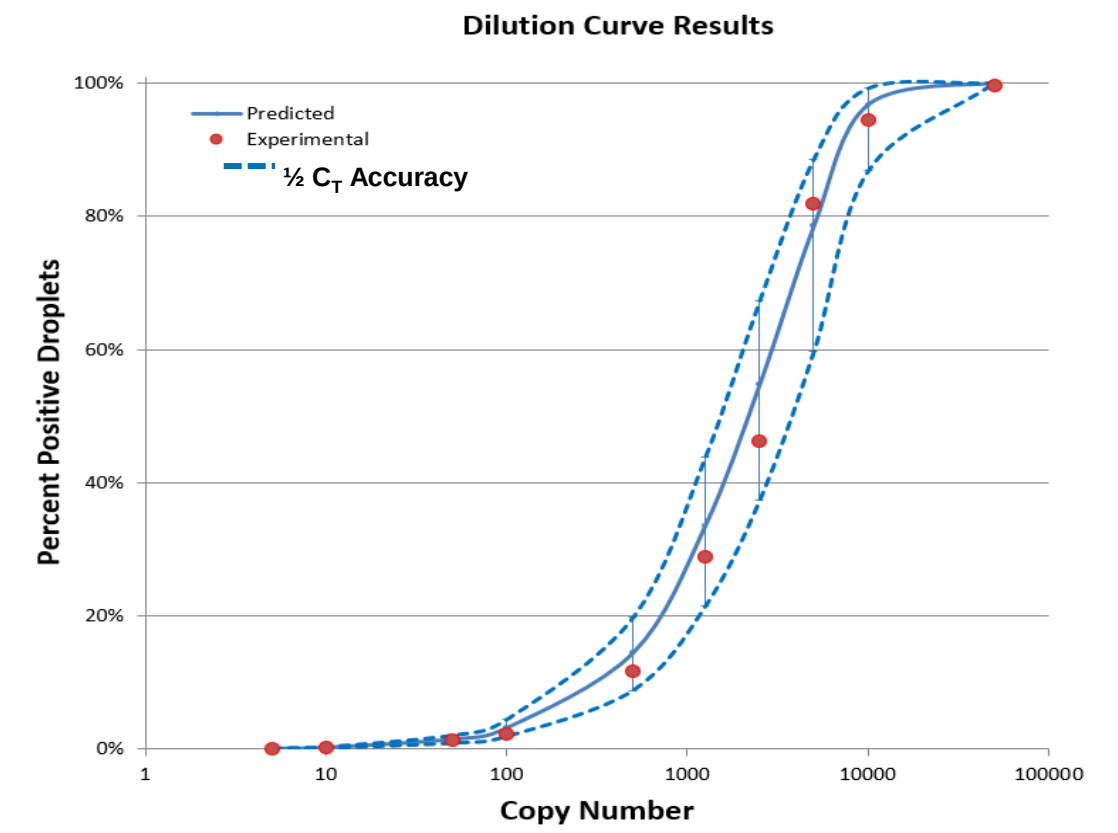
- 3 independent PCR tests run per sample
- Native species acted as internal positive controls
- Positive controls on each plate
- 5% of samples were re-extracted and re-analyzed

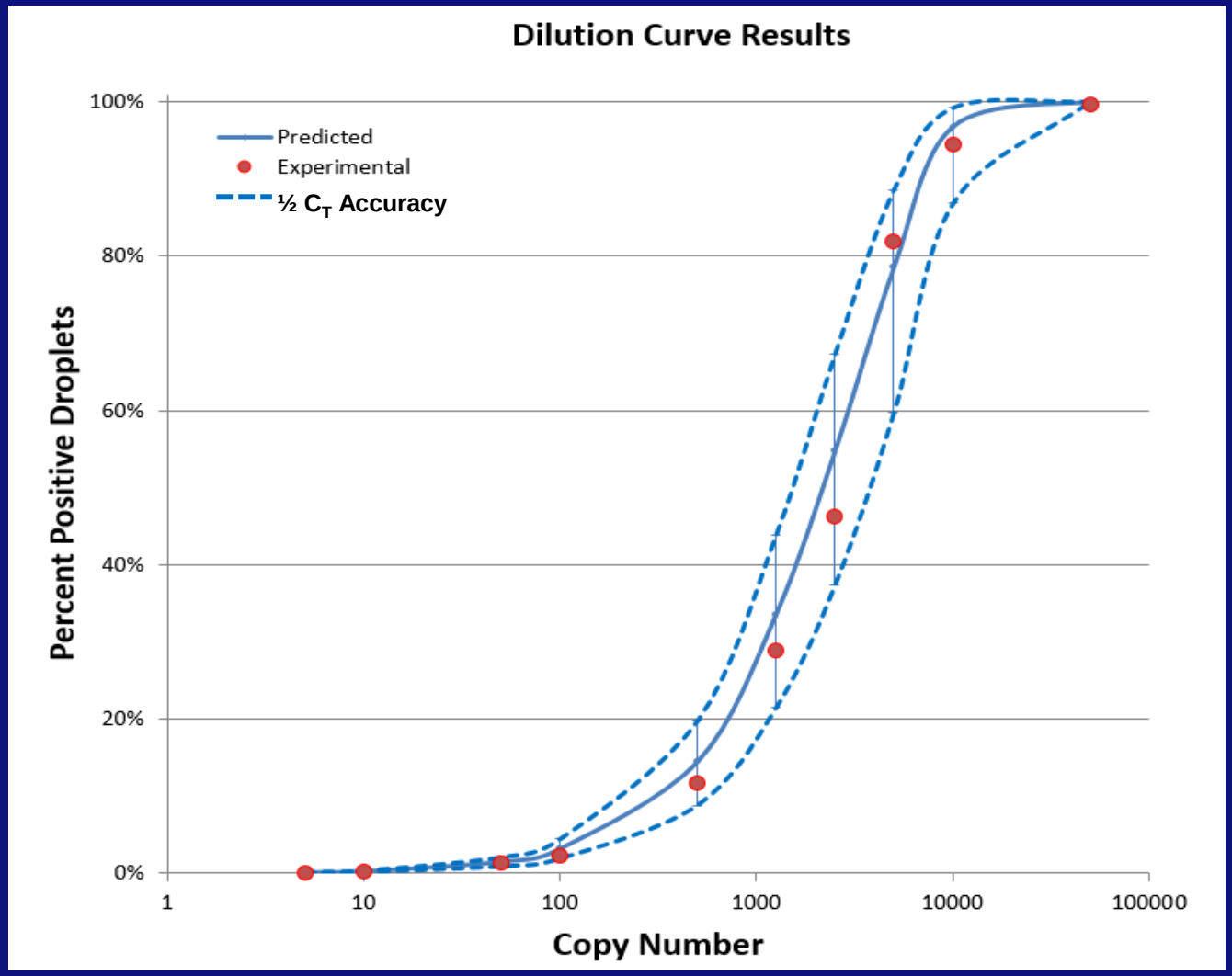
Next-Generation, Autonomous Bioanalytical Field Instrument

Dr. Cody Youngbull

Arizona State University and Flathead Lake Biological Station

Funded by the Gordon and Betty Moore Foundation, the National Science Foundation, and the State of California




Dilution Curve Results
Predicted
Experimental
½ C_T Accuracy
Percent Positive Droplets
100%
80%
60%
40%
20%
0%
1
10
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1000
10000
100000
Copy Number

Ecological impact (fishery impacts):

Management of Biological Invasions (2014) Volume 5, Issue 3: 287–302
doi: http://dx.doi.org/10.3391/mbi.2014.5.3.12

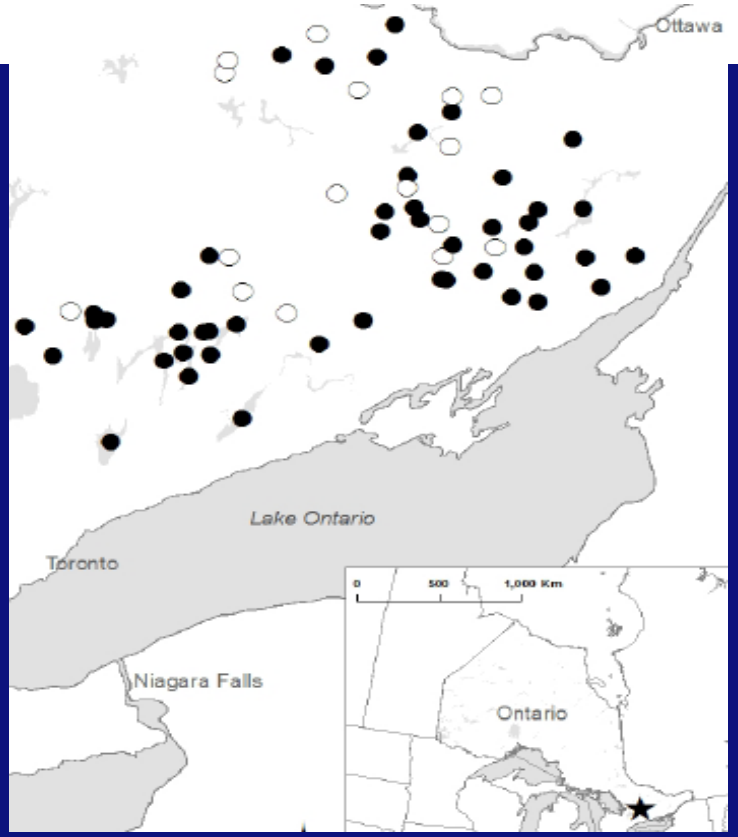
Open Access

Proceedings of the 18th International Conference on Aquatic Invasive Species (April 21–25, 2013, Niagara Falls, Canada)

Research Article

An empirical analysis of the consequences of zebra mussel invasions on fisheries in inland, freshwater lakes in Southern Ontario

Condition, growth, and relative abundance of game fish significantly varied among lakes with and without zebra mussel.



“Devastating changes are documented in ecosystems invaded by zebra mussels (Strayer, 2009).”

Zebra mussels that appeared in the Hudson River in 1991 constitute >50% of all biomass in the river (Strayer et al., 1999).

Phytoplankton biomass in the river fell by 80%, the pelagic part of the food web withered.

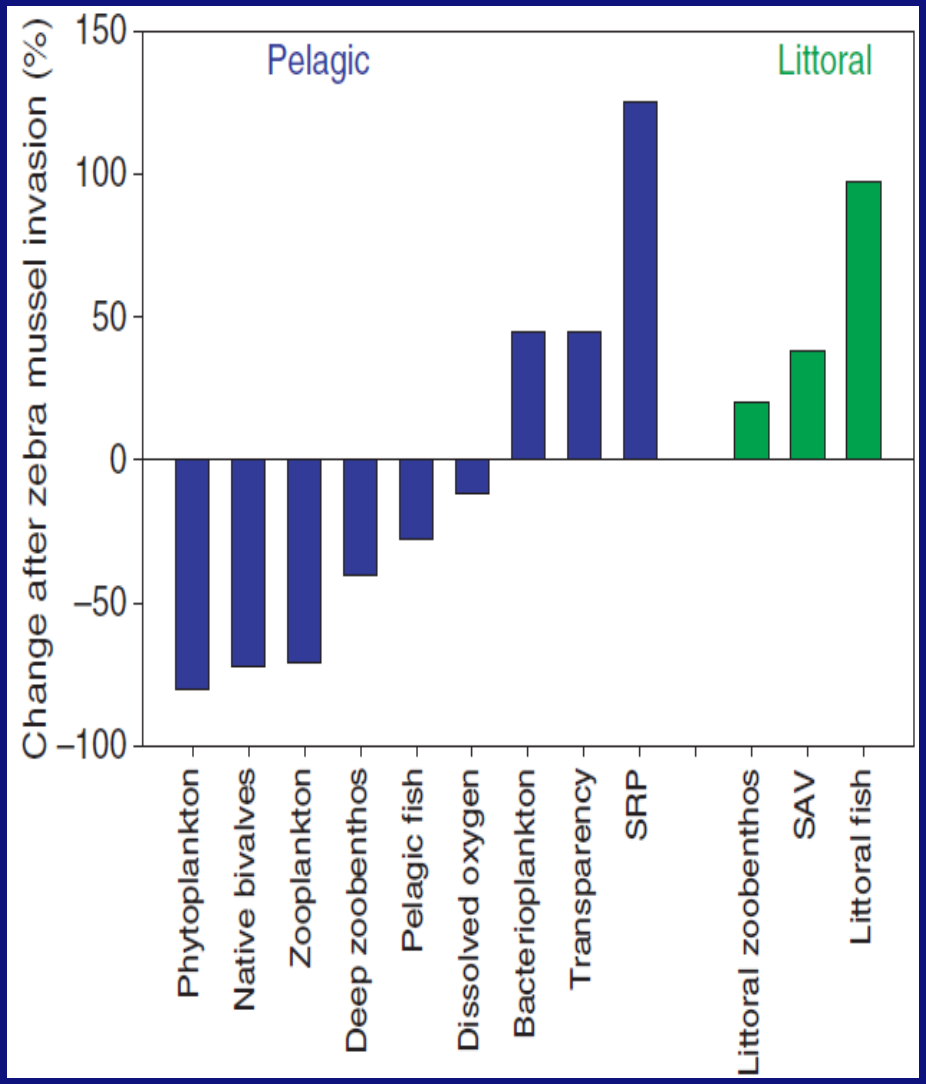
Freshwater Biology (2010), **55** (Suppl. 1), 152–174 doi:10.1111/j.1365-2427.2009.02380.x

Alien species in fresh waters: ecological effects, interactions with other stressors, and prospects for the future

DAVID L. STRAYER
Cary Institute of Ecosystem Studies, Millbrook, NY, U.S.A.

SUMMARY

1. Biological invasions are numerous in fresh waters around the world. At least hundreds of freshwater species have been moved outside of their native ranges by vectors such as ballast water, canals, deliberate introductions, and releases from aquaria, gardens, and bait buckets. As a result, many bodies of fresh water now contain dozens of alien species.



Summary of the effects of the zebra mussel on the Hudson River ecosystem

Early detection of invasive species can allow managers to quarantine and treat initial infestations before they spread.

"Early detection is a critical step: The earlier cancer is identified, the greater the patient's chance of survival."

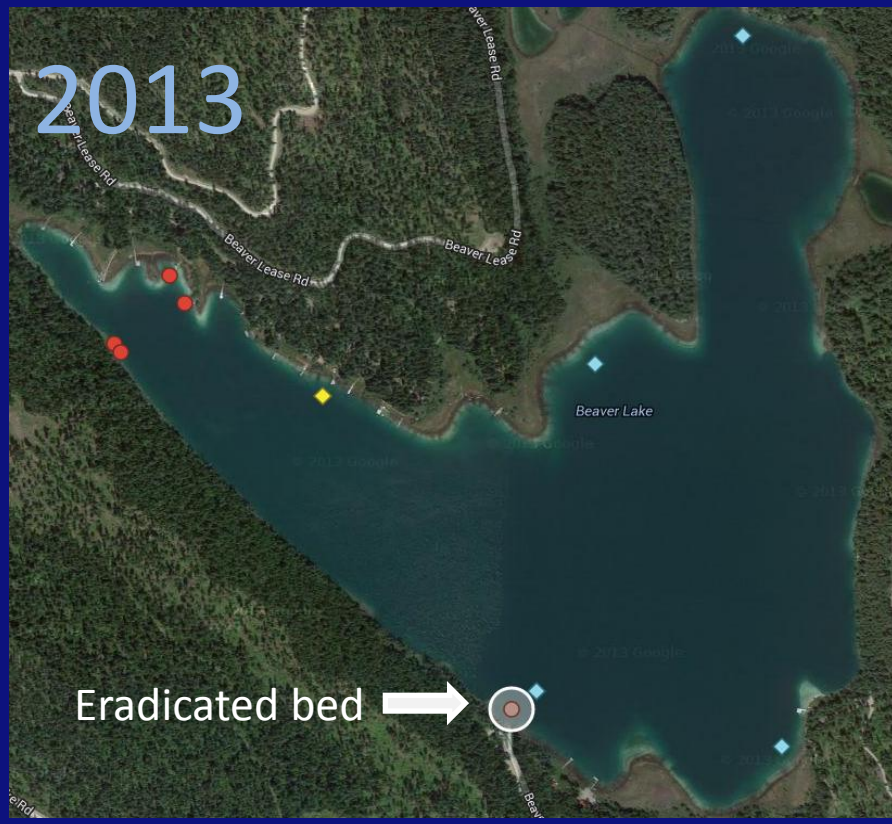
Aquatic Invasive Species: Lessons from Cancer Research

The medical community's successes in fighting cancer offer a model for preventing the spread of harmful invasive species

Adam Sepulveda, Andrew Ray, Robert Al-Chokhachy, Clint Muhlfeld, Robert Gresswell, Jackson Gross and Jeff Kershner

American Scientist, Volume 100

Detected Eurasian milfoil in Beaver Lake.



- Eurasian milfoil bed
- Positive eDNA sample
- Negative eDNA sample

Recent new invasion

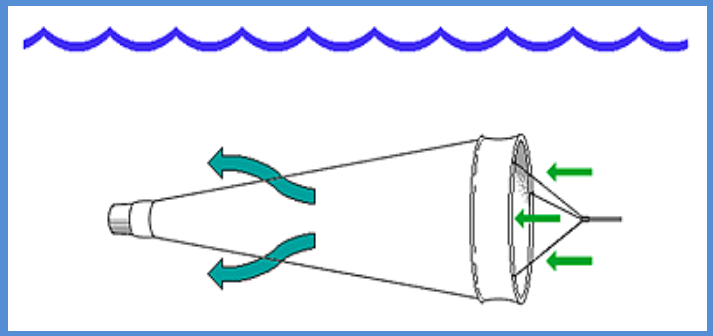
One Milfoil detection assay

Northern/Eurasian milfoil assay

Northern (native) milfoil

VS

Eurasian milfoil



Tow net sampling
filters **2,840 liters** of lake water over a 40 meter tow to generate one eDNA sample

Field methods for sampling eDNA

Sampled during peak biomass production of target

Sampled near likely introduction sites

Collected samples using a plankton tow net

Lab methods

Tested using the Northern/Eurasian milfoil assay and the Native/Invasive mollusk assay

Saved half of sample for potential microscopy