Environmental DNA Projects: Aquatic Invasive Species

Zebra/Quagga Mussels

The Problem:

Zebra and quagga mussels (*Dreissena polymorpha* and *D. rostriformis*) are two of the world's most problematic biological invaders (Figure. 1a). They are colonizing and devastating lakes and rivers across North America and might soon invade Montana and the Columbia River drainage. These mussels are prolific spreaders and cost the U.S. billions of dollars each year. They can clog water intakes and damage equipment by attaching to boat motors and hard surfaces (Figure.1b). They can collapse fisheries, smother native mussels and crayfish (Figure.1c), and litter beaches with their sharp shells that cut the feet of children and pets. Adult zebra mussels can survive out of water for days, attached to boat hulls or fishing equipment, and thus can be spread easily and widely among lakes and regions.

Figure.1(a). Zebra & Quagga Mussels

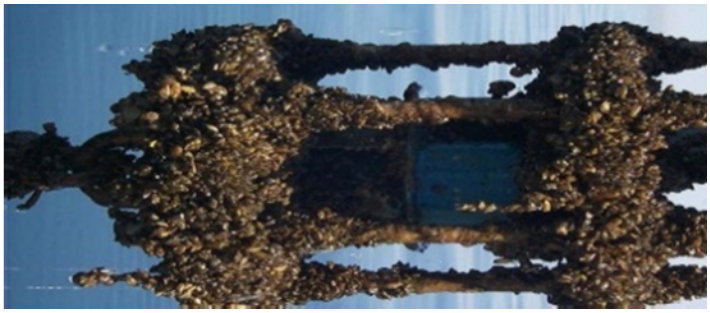

Figure.1(b) Encrusted Research Equipment

Figure.1 (c) A crayfish encrusted with zebra mussels leading to a slow death

Zebra/Quagga Mussels

Solutions:

DNA Tests to Prevent Zebra Mussel Invasion

Our PCR-based tests provide sensitive and rapid identification of zebra/quagga mussels from water samples, which is crucial for the early detection of the species' presence to prevent their spread. Success of control strategies (e.g., quarantine or removal of problem boats from exposed/infected docking areas, chemical treatment of waters) on Dreissenid populations increases with the speed of detection and management actions to minimize spread and population growth or establishment (Hosler, 2011). Eradication is possible (Figure. 2) if detection is early enough.

Early detection is increasingly feasible thanks to recent advances in genetic technologies for environmental DNA (water) sampling and detection of tiny organisms (larvae) or cells sloughed from a target species using DNA extracted from environmental samples (Beja-Pereira et al. 2009; Blanchet 2012). Surprisingly, relatively little research has been published on the sensitivity and reliability of genetic methods for detection of Dreissenids. We have developed and are refining field sampling protocols and DNA tests for the early-

detection of *Dreissena* taxa from plankton tow samples from multiple lakes and streams in Montana and the Pacific North West.

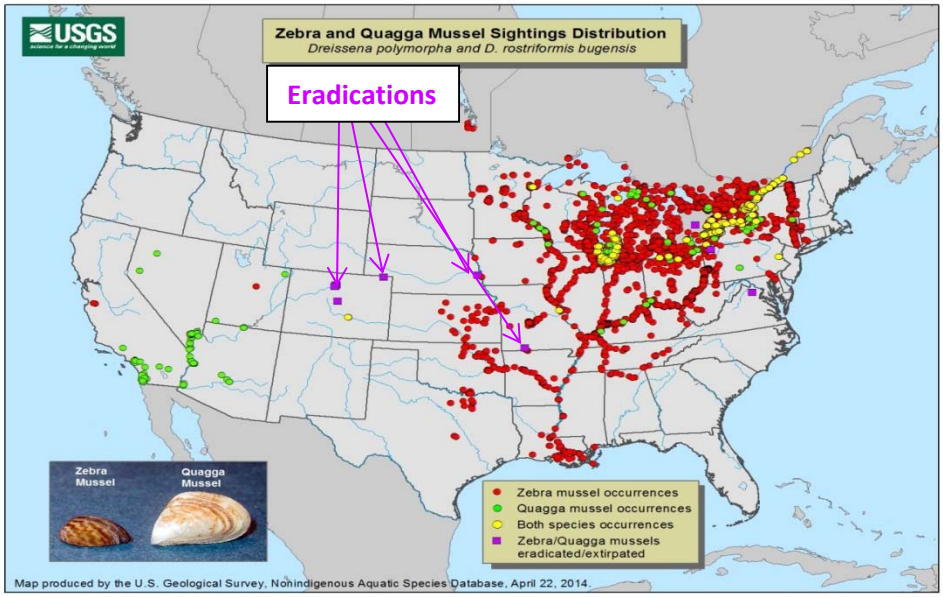

Figure 2. Locations of zebra and quagga mussel occurrences (red and green, respectively), and occurrence of both species (yellow). The arrows and nine purple squares are locations where eradication was successful. From USGS zebra/quagga web page (April, 2014).

Eurasian watermilfoil

The Problem:

Eurasian watermilfoil (EWM) is an "exotic" aquatic plant (Figure.1) and is native to Europe, Asia and Northern Africa. Eurasian watermilfoil was first discovered in North America in the 1940s and has spread throughout the country (https://www.pca.state.mn.us). Figure 2 shows the geographic distribution of Eurasian watermilfoil in the US.

Eurasian watermilfoil grows and spreads really fast which reduces the amount of light that reaches into the lake and kills off other native aquatic plants. Eurasian watermilfoil's dense growth makes it difficult for invertebrates and other organisms that eat fish to survive (Figure.3). Eurasian watermilfoil pushes out many native plants and animal species while provides habitat for non-native fish (e.g. Bass, Pike) that pray on native fish (e.g. salmonids**). It has cost millions in some lakes alone to maintain access to boat launches and docs.** It has dropped property value around 8% to 16% (Horsch and Lewis 2008; Cornell University Report, 2011). Private and Government sources in Washington spend about $1,000,000 per year on Eurasian watermilfoil control. Minnesota, Wisconsin, Vermont, New York, and British Columbia also spend similar amounts per year to control Eurasian watermilfoil infestations (Washington State Department of Ecology).

Figure.1. Eurasian watermilfoil

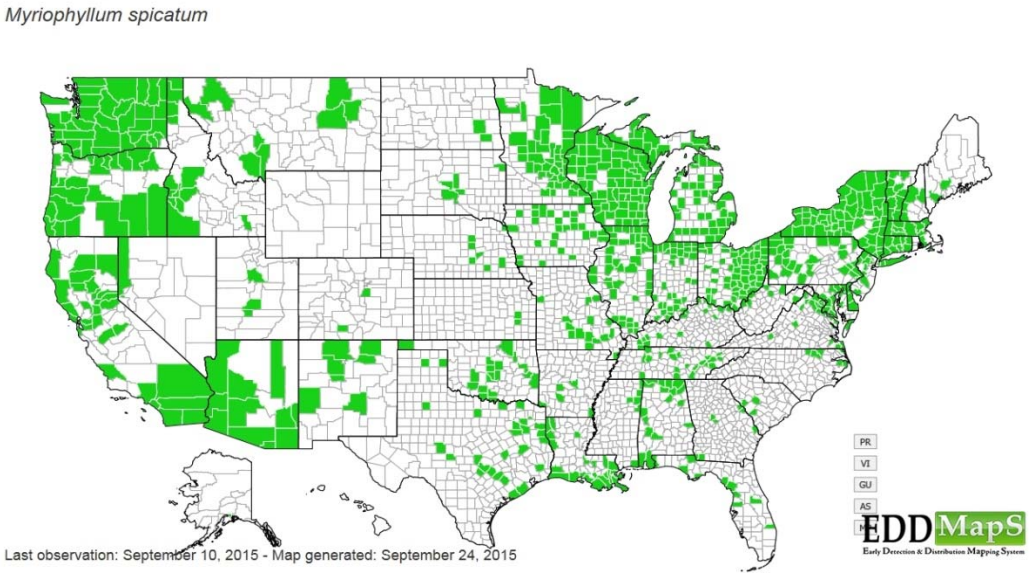

Figure.2. Geographic Distribution of Eurasian watermilfoil in the United States of America

Figure.3. Eurasian watermilfoil Lake Infestation (Michigan Sea Grant: www.miseagrant.umich.edu)

The Solution

eDNA test to prevent Eurasian watermilfoil (*Myriophyllum spicatum)* Invasion

Environmental DNA (eDNA) detection is a promising new technology for early detection to locate small founder populations of invasive species, making control and eradication more feasible without the high physical and monetary costs of late-detection invasions (e.g. Bohmann et al. 2014; Goldberg et al. 2015).

Every year, thousands of recreational boats enter Flathead Lake. Some boats have been exposed to and may carry dangerous aquatic invasive species (AIS) from other lakes, including the most damaging species such as Eurasian watermilfoil (EMF). Control and eradication of EMF has been successful in lakes such as Beaver Lake in Montana. Our Lab has been testing eDNA samples for early detection of EWM since 2014. Below is the map showing sampling locations for EWM from Flathead Lake and surrounding lakes in Montana (Figure 4). At some locations many samples are taken twice per season (e.g. 10 locations around Flathead Lake) (when enough funding exists for the testing). Figure 5 shows an example of real time PCR Assay for early detection of Eurasian watermilfoil.

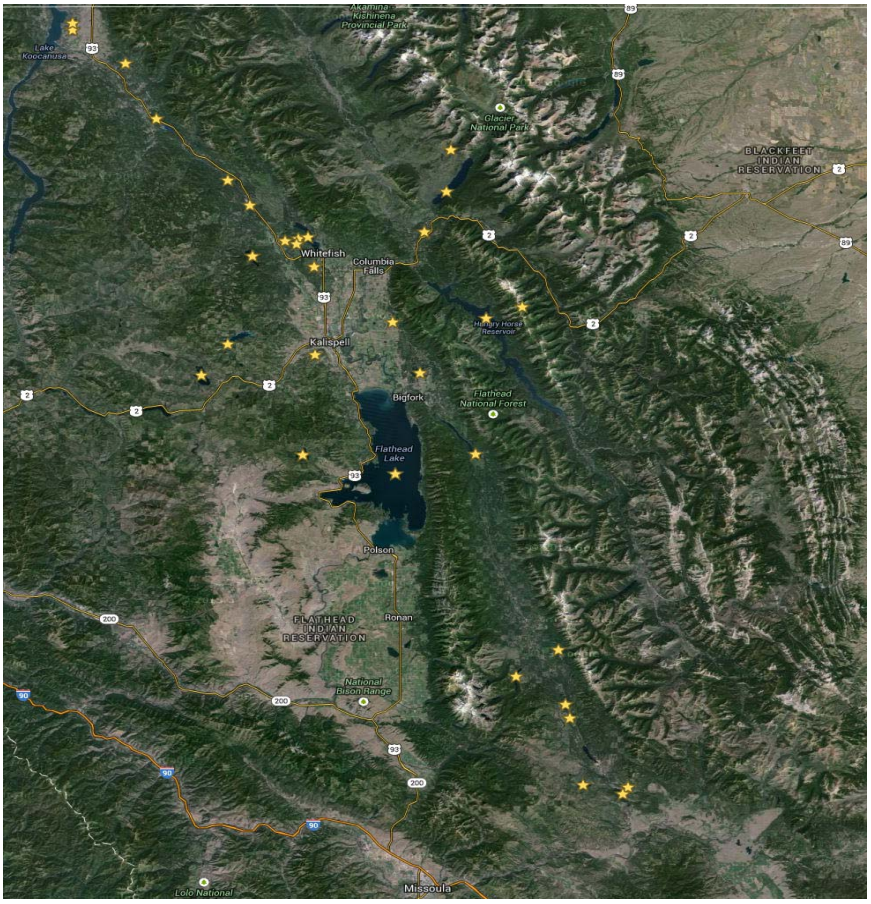

Fig.4. Sampling locations for Eurasian watermilfoil from Flathead Lake and surrounding lakes in Montana. At some locations many samples are taken twice per season (e.g. 10 locations around Flathead Lake) (when enough funding exists for the testing)

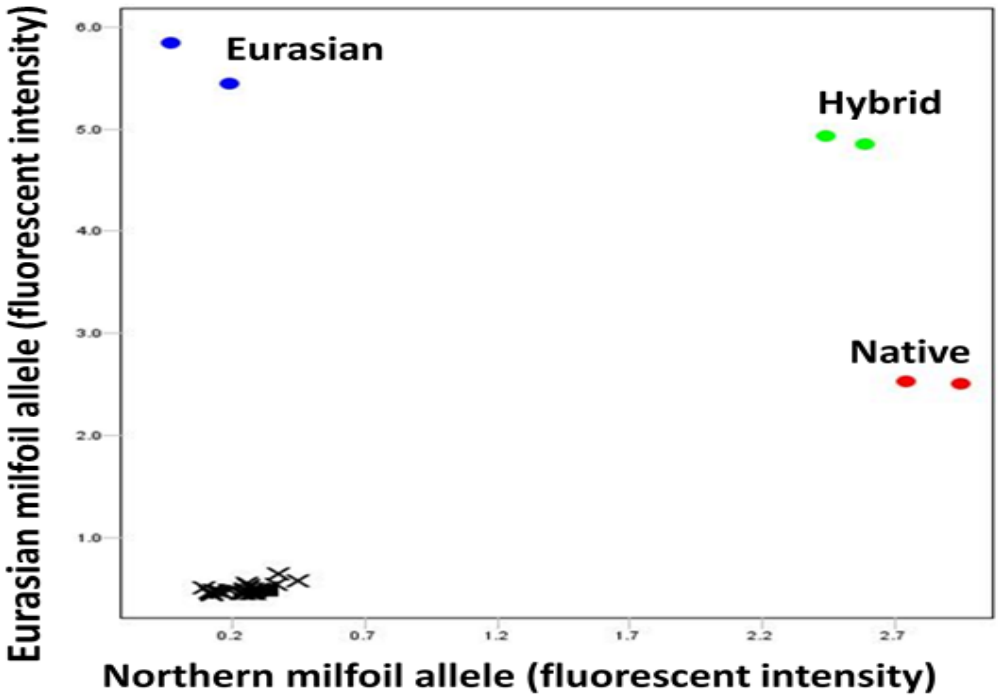

Fig.5. Real-time PCR amplification plot showing fluorescent signal from Eurasian (blue), native (red), and hybrid (green) milfoil DNA. Negative controls, which contain no milfoil DNA, cluster near the graph's origin.

Development of field eDNA sampling protocol

Our Lab has designed high volume eDNA sampling protocol for Lakes, Rivers and Streams which includes designing new equipment for collecting eDNA samples from large volume of water.

Plankton tow: Plankton tow net is a 1.52 meter-long net with 64 micron mesh with opening that is 30 centimeters wide attached to a Dolphin™ bucket (Figures 1 and 2). It has the capacity to filter approximately 7,000 liters (L) per sample by towing the net a 100 meter distance at each site. The net is bleached between each sampling site to ensure no transmission of aquatic invasive species between sites and no DNA cross-contamination between samples from different sites.

Figure.1. Environmental DNA sample collection by plankton tow net (left) and by filter (right)

Figure.2. Environmental DNA sample collection by plankton tow net

Current Projects:

Monitoring for aquatic invasive species: Working with numerous collaborators (WLI, USFS, USGS, CRC, & concerned citizens), we began monitoring for AIS using eDNA 3 years ago in waterbodies around western Montana. Using plankton tow nets and filters…

Development of lake sampling protocols: comparison of filter versus plankton tow nets

Development of a high volume stream sampler:

Biomass and stream sampling technique comparison:

Bull trout density field tests:

Development of autonomous eDNA sampler:

Available and in progress Environmental DNA

Aquatic Invasive Species

1) Milfoil
2) Pondweed: Curly Leaf Pondweed (*Potamogeton crispus*) (in Progress)
3) Zebra mussels
4) Quagga mussels

Fish:

1) Rainbow trout
2) Westslope cutthroat trout
3) bull trout
4) brook trout
5) small mouth bass (in progress)
6) Walleye (in progress)
7) Northern pike (in progress)

eDNA Laboratory Services

- Testing water samples for eDNA from invasive, non-native, and rare or cryptic native species