

# Department of the Interior

## Alternative Approaches to Ecotoxicological Testing and Assessment



# Mission

Protect and manage the Nation's natural resources and cultural heritage

Provide scientific and other information about those resources

Honor trust responsibilities & commitments to American Indians, Alaska Natives and affiliated island communities



# Some Applied Ecotoxicological Research Limited regulatory authority on “chemicals”

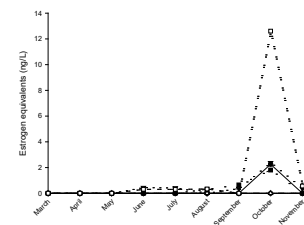
1. Research with direct application to natural resource management
2. Environmental contaminant biomonitoring
3. Natural Resource Damage Assessment
4. Chemicals for invasive species control
5. Alternatives to “lead shot” used in hunting

Embrace 3R's



# Testing Environmental Samples for Endocrine Activity *In Vitro*

- Substrate-free bioluminescent yeast bioassays
  - Commercially available yeast strains
    - Estrogenicity
    - Androgenicity
    - Cytotoxicity
- Cost-effective screening of environmental water sample extracts
- 96-well plate format



# Effects-directed Analysis of Endocrine-active Chemicals



1) Sediment and water collected from locations with immunocompromised and/or intersex fish

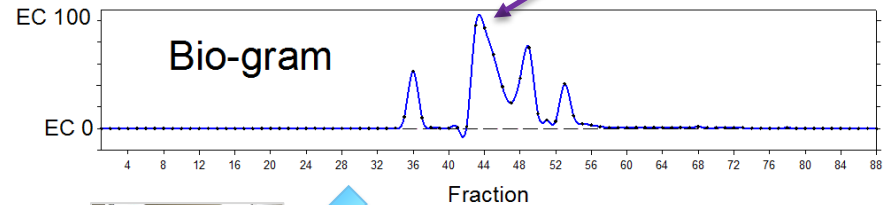


2) Extracts are screened with reporter cell bioassays



3) Bioactive extracts fractionated and tested with reporter cell bioassays

Bioactivity

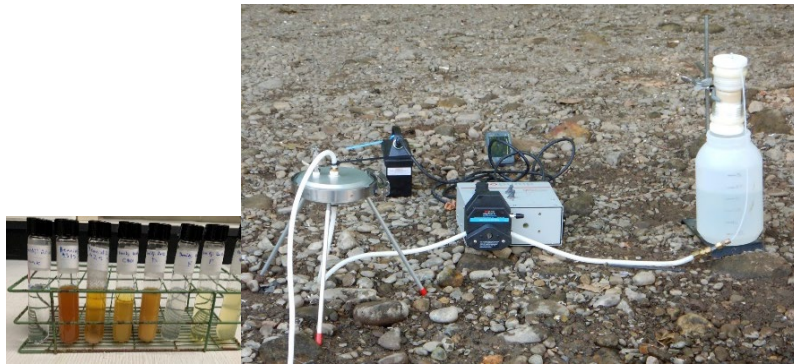


5) Further testing of the chemical suspects in higher tier assays



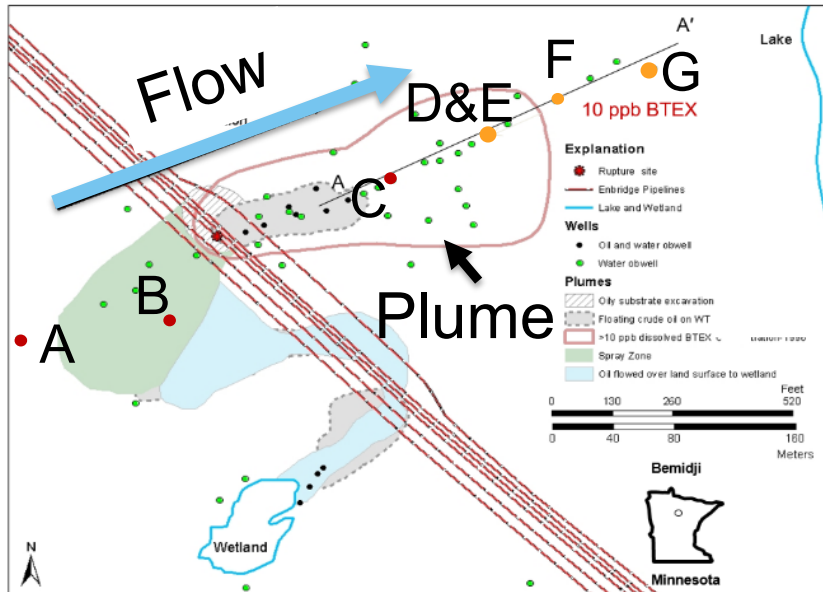
4) Bioactive fractions analyzed by UPLC-QTOF-MS for chemical suspects

# Testing for PAH Metabolites in Oil-contaminated Groundwater



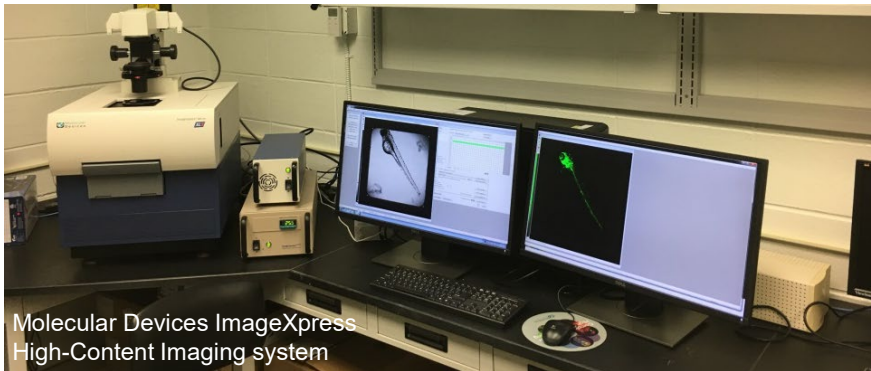
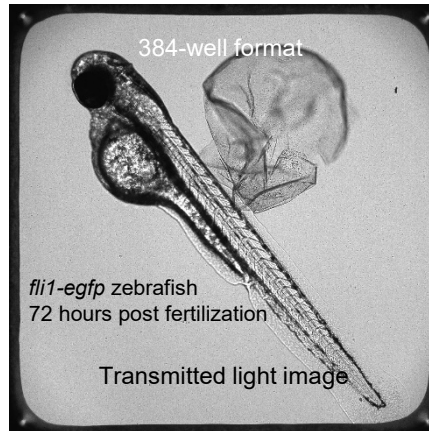
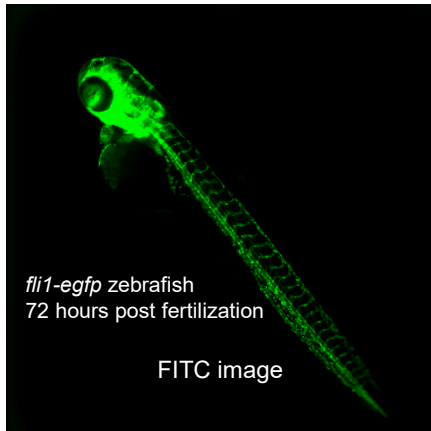
Large-volume water sampler provides enough sample for in vitro and in vivo testing

In vitro reporter assays track movement of PAH-type activity in groundwater plume



Sample Location	PAH-type activity, Human cells	PAH-type activity, Rat cells
A	/	/
B	/	/
C	+++	+
D	++	/
E	/	/
F	++	+
G	++	/

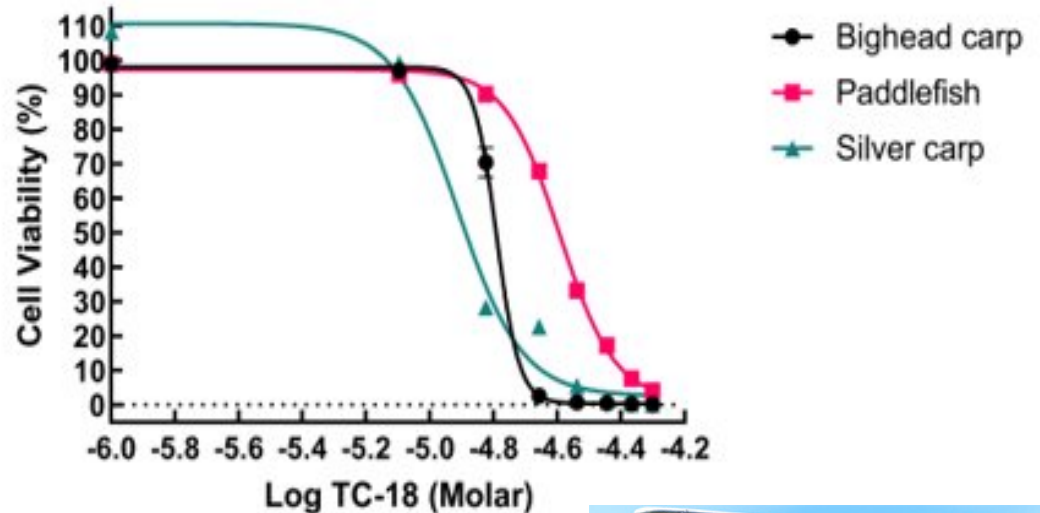
# High Content Screening



- Developmental cardiovascular toxicity assay at 72 hpf
  - Pericardial area
  - Intersegmental vessel area
  - Heart rate
- Targeted assessment of toxicity
  - LC50 and mode of action
- Rapid image acquisition, data extraction and analysis
- Utilizes pre-feeding fish embryos in microtiter plate format to **reduce** animal use, test compound & labor

# Toxicant Prioritization and *In Vitro* Toxicity Screening

- *In vitro* fish cell lines
  - Target (invasive BHC) and endangered non-target (Paddlefish)
  - Gill cell cytotoxicity screening Example: Paddlefish sensitivity without using live animals





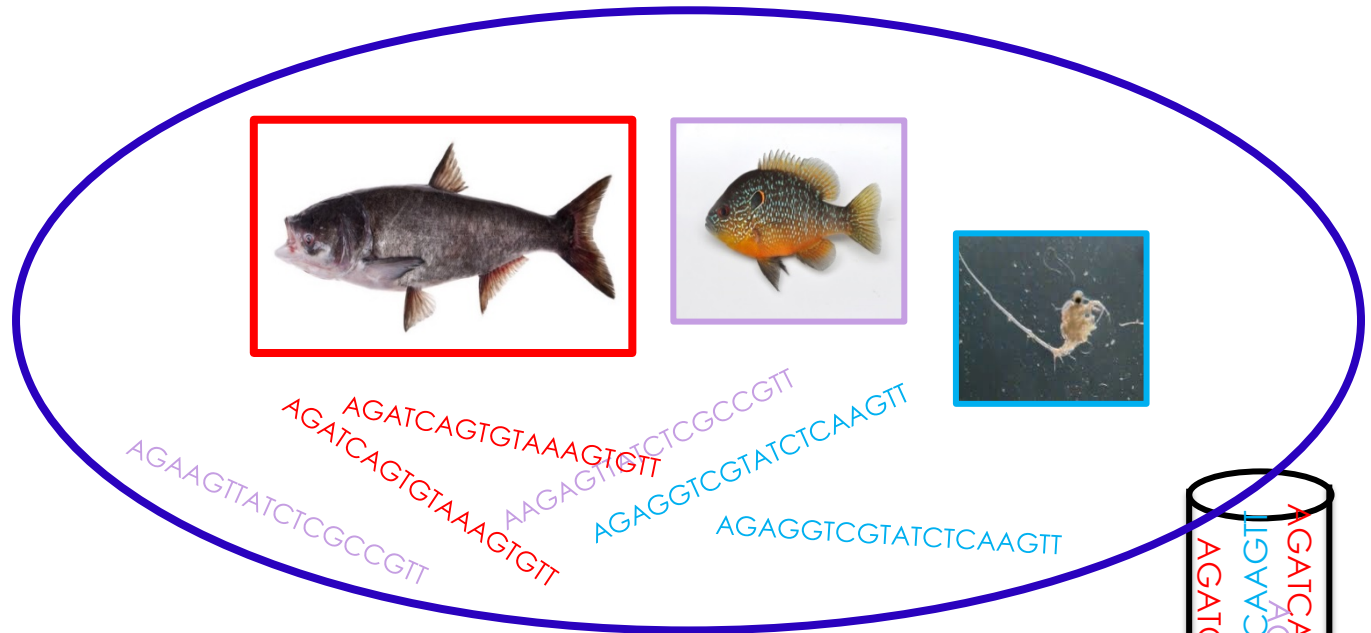




## What is eDNA ?

Pawlowski et al. (2020)–  
 “The total pool of DNA  
 isolated from  
 environmental samples.”

A non-invasive genetic  
 method for surveying  
 biotic diversity



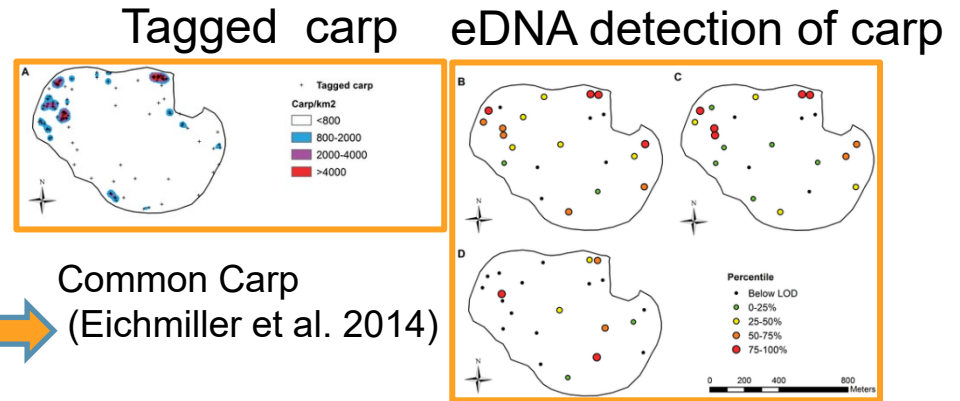
- Sloughing of epithelial cells
- Released gametes





# How is it being used?

1. Species Monitoring and Surveying
2. Ecological Questions
3. Estimate Population Location and Size



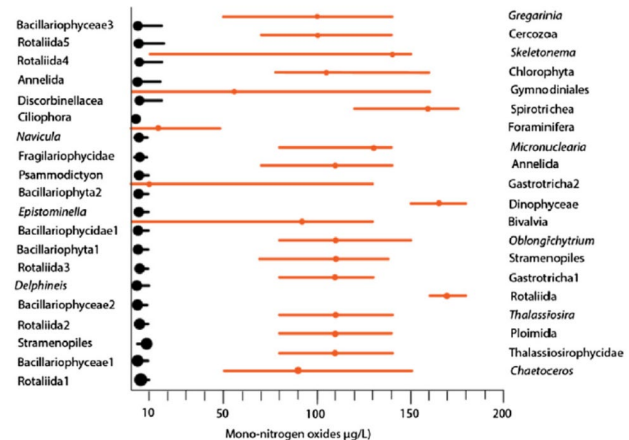
4. Contaminants



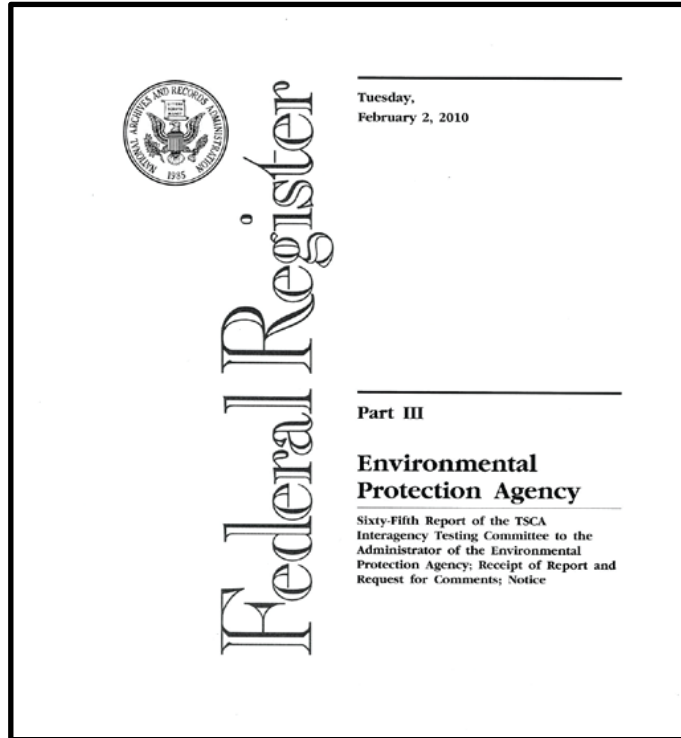
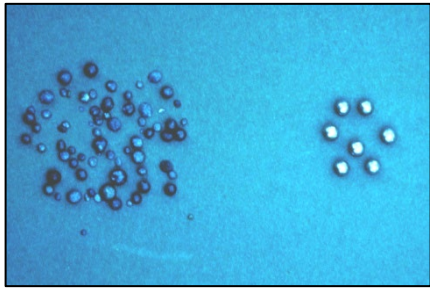
## Threshold Indicator Taxa Analysis (TITAN): Mono-Nitrogen Oxides

5. "Ecology of eDNA" – what affects the physical state and detection of eDNA

Benthic Metabarcoding  
 (Chariton et al. 2015)



# Registration of Non-toxic Shot



- Lead shot replacements:
- iron (steel)
  - iron-tungsten
  - bismuth-tin
  - copper-clad iron
  - corrosion-inhibited copper
  - tungsten-bronze
  - tungsten-iron
  - tungsten-matrix
  - tungsten-nickel-iron
  - tungsten-polymer
  - tungsten-tin-bismuth
  - tungsten-tin-iron
  - tungsten-tin-iron-nickel

- Bottom Line – many shot types registered using existing information, risk assessment and no toxicity test (harmonized with Canada)
- interest expressed by European Chemicals Agency

# Challenge...

extrapolating toxic/therapeutic effects  
among diverse groups of species

- Diverse exposure pathways
- Differences in life stage, life history and behavior
- Differences in ADME among species that can affect toxicity

