Molecular Genetic Tools for Assessing the Status and Vulnerability of Aquatic Resources

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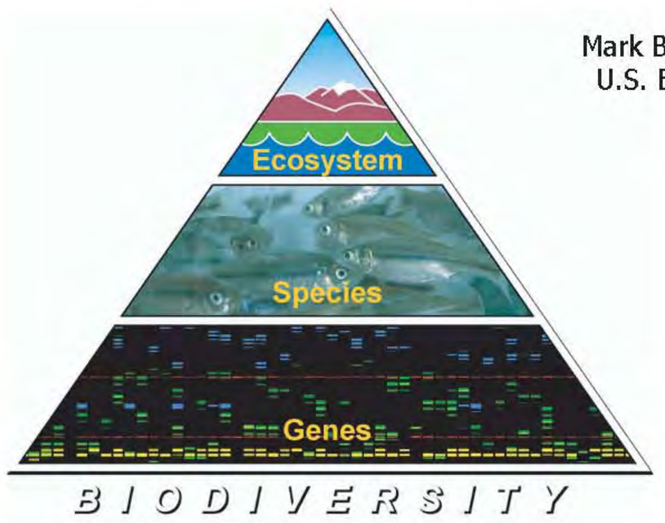


ORD's Ecological Research Program is investing in development of genetic methodologies to supplement and improve existing ecological indicators for aquatic ecosystems.

The EPA is currently exploring six research areas that can improve the science of ecological assessment through incorporation of genetic methods are under active investigation:

(1) development of accurate and precise methods for biological identification of aquatic species and subspecies.

(2) delineation of ecological assessment units through analysis of genetic structure across multiple species.

(3) assessment of changes in genetic diversity as an indicator of present and historical environmental condition.

(4) assessment of genetic diversity at diagnostic loci and across the genome as an indicator of vulnerability to environmental perturbations.

(5) integrated assessments to link landscape-level stressors to population-level outcomes.

(6) Development of methods for assessing baseline ecological conditions via genetic analysis of archival samples.

1. IMPROVED METHODS FOR SPECIES IDENTIFICATION AND ENUMERATION

RELEVANCE:

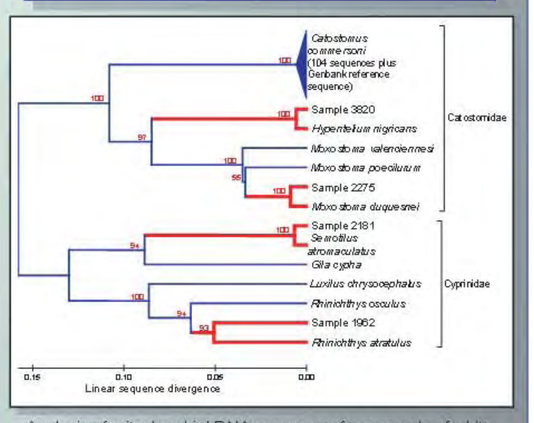
- Understanding of ecological condition depends on accurate description of species assemblages
- DNA provides the most accurate and precise information on species identity
- EPA needs efficient and transferable "DNA ID", "DNA barcoding" or " DNA QA" methods

APPLICATIONS:

- Detection and identification of larval invertebrates in ballast and recipient waters (see Poster LTG 3-2)
- Differentiation of morphologically indistinct species of benthic invertebrates
- Validation of taxonomic identifications based on field morphological analysis
- Objective measurements of the incidence and extent of hybridization

How accurate is field identification of stream fishes in regional assessments (MAIA)?

- A minimum of 96% of white suckers were morphologically identified correctly
- All creek chub were morphologically identified correctly, but the taxon is composed of two morphologically similar but genetically distinct groups in the MAIA region.
- A minimum of 85% of fallfish were morphologically identified correctly.
- All central stonerollers were morphologically identified correctly, but the taxon is composed of four morphologically similar but genetically distinct groups in the MAIA region.



Analysis of mitochondrial DNA sequences for a sample of white suckers (*Catostomus commersoni*) indicates that 4 individuals were misidentified (approximately 2% error rate). Comparison of DNA sequences to those in an international DNA sequence database (Genbank) reveal the most likely taxonomic origin of each sample.

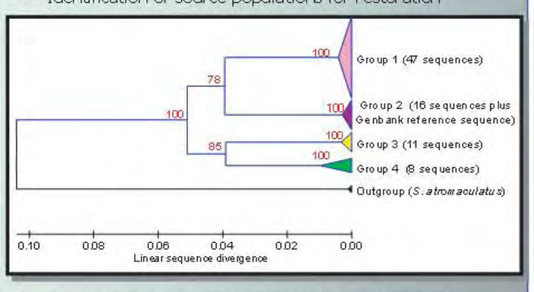
2. DELINEATION OF ECOLOGICAL ASSESSMENT UNITS

RELEVANCE:

- Measurement and evaluation of ecological condition must be performed at the correct environmental scale
- Many assessments incorporate the watershed or ecoregion as the fundamental assessment unit
- For biological resources, the fundamental unit that responds to and adapts to the environment is the biological population.
- It is not clear how adequately ecoregions or watersheds represent biological structure for specific taxa.

APPLICATIONS:

- Strengthening of EMAP and REMAP assessment protocols
- State and Tribe biological resource assessments
- Identification of source populations for restoration



Genetic divergence between central stonerollers (*Campostoma anomalum)* is revealed by mitochondrial DNA sequence analysis. Four different genetic groups are identified with high statistical (bootstrap) confidence. Creek chub (*Semotilus atromaculatus)* is included as an outgroup for comparison

Geographic distribution of genetic groups of central stonerollers (*Campostoma anomalum)* sampled during the 1997-1998 MAIA assessment. The four genetic groups are colored as in the genetic distance analysis.

3. CHANGES IN GENETIC DIVERSITY AS AN INDICATOR OF ECOLOGICAL CONDITION

RELEVANCE:

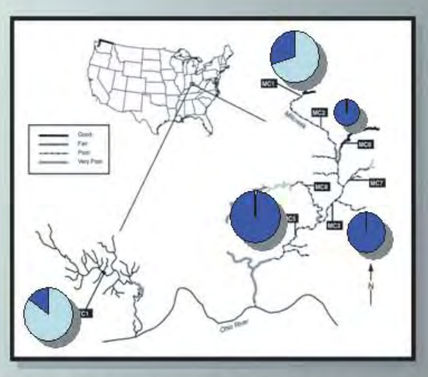
- Environmental stressors that alter the genetics of populations have lasting effects
- Genetic change is brought about by environmental alterations that affect the breeding population size, mutation rate, population connectivity, or selective forces.
- Genetic change is an indicator of population and species-level effects, scales at which we have few good indicators.

APPLICATIONS:

- Strengthening EMAP and REMAP assessment protocols
- State and Tribe biological resource assessments
- Watershed and sub-watershed assessments

Multi-species assessment of fish genetic diversity in the MAIA region

- Microsatellite diversity of white sucker was strongly associated with agricultural impacts and human population density.
- Creek chub diversity was associated with stream substrate condition and geochemistry.
- Central stoneroller diversity was associated with agriculture, human population density, runoff, pH, stream substrate and geochemistry.
- Different species and genetic groups within recognized species appeared to respond to different environmental dimensions.



Genetic diversity was measured over 8 years in a single watershed in Cincinnati Ohio (Mill Creek). This temporal genetic assessment allowed estimation of effective population size (essentially, the effective number of breeders, represented by the area of pie diagram) and the effective immigration rate (green = proportion of breeders that are immigrants, orange = proportion of breeders that are residents). The pattern of immigration relative to assessment of habitat quality suggests that genetic assessment can determine whether different habitats support sustainable populations

4. GENETIC DIVERSITY AS AN INDICATOR OF VULNERABILITY

RELEVANCE:

- Vulnerability is a function of anticipated physical habitat changes *and* the ability of populations to respond to those changes
- The ability of populations to respond (adapt) to altered habitats is directly proportional to standing genetic diversity

APPLICATIONS:

- Genomic diversity as an indicator of 'biotic buffering capacity' in regional and local ecological assessments
- Genetic diversity at diagnostic loci to predict multigenerational responses to specific stressors

Genetic diversity of stoneroller populations in the Eastern Cornbelt region of Ohio. Green populations had higher genetic diversity than predicted, yellow had average genetic diversity, and red populations had lower genetic diversity than predicted

New EPA research

- Develop experimental systems to define the predictive relationship between different genetic diversity measures and risk of extinction (amphipods, mysids, fish)
- Assess variation among natural populations in ability to respond to specific environmental insults (amphipods)
- Identify and assess variation in genetic pathways responsible for determining tolerance/sensitivity to contaminants (zebrafish)

5. LINK LANDSCAPE-LEVEL STRESSORS TO POPULATION-LEVEL OUTCOMES

RELEVANCE:

- Most stressors of concern derive from multiple points in the landscape
- Increasingly, assessment of condition and vulnerability are dependent on sophisticated models of biological response
- Genetics can provide the link between landscape stressors and population response models.

APPLICATIONS:

- Integrated, model-based risk assessment of long-term, multigenerational effects on biological populations at watershed and regional scales.

Incorporating landscape processes into fish population models with population genetic analyses.

New EPA research

- "Landscape genetics" analysis of the interaction between landscape level changes (impervious surfaces, agricultural development, nutrient loadings) and fish population responses in the Little Miami and Great Miami watersheds
- Assessment of the influences of urbanization on the propensity and ultimate extent of hybridization between invading red shiners and native cyprinid fishes in the Southeastern USA. (see Poster LTG 3-2)

6. ASSESS BASELINE ECOLOGICAL CONDITION VIA GENETIC ANALYSIS OF ARCHIVAL SAMPLES

RELEVANCE:

- Currently reference conditions are assessed using paired geographic samples
- It is unknown whether differences in biological response at these sites are due to stressors or to historical factors.
- Archival specimens provide the best measure of baseline conditions for sites under evaluation.

APPLICATIONS:

- Archival specimens provide baseline data on long-term effects on biological populations at watershed and regional scales.

Museum collections from Lake Tahoe are being analyzed for comparison with modern samples

New EPA research

- Identify and assess baseline conditions as a temporal reference using archival material from museums and sediments
- Develop experimental methods to recover genetic and contaminant data from archival specimens
- Assess variation over time in natural populations in response to environmental stressors

SIGNIFICANCE

Existing indicators and tools do not perfectly reflect ecosystem integrity and sustainability; therefore the EPA is striving to develop new methods that incorporate the best available science into ecological assessments.

Incorporation of modern molecular genetic methods into ecological assessments and development of genetic indicators to supplement other ecological indicators advances this goal.

Genetic information is interpretable at a variety of geographic and temporal scales and provides meaningful information about the long-term vitality of biological populations.

Ultimately, this work will produce highly effective tools that the States, Tribes and EPA regions will be able to implement for assessing and protecting the aquatic environment

Although this work was reviewed by EPA and approved for publication it may not necessarily reflect official Agency policy

ACKNOWLEDGING OUR PARTNERS:
- ORD labs and centers: NERL, NHEERL, NCEA
- EPA Regions 5, 9, 10
- Ohio DNR
- Washington DFW
- California DFG
- USGS, USDA
- DFO Canada

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