DNA Barcoding as Tool for Marine and Freshwater Bioassessment

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The Promise of Molecular Methods

- Faster answers
  - Weeks vs. months

- Less expensive

- Better data
  - Recognizing misidentifications
  - Improving taxonomic keys
  - Helping with difficult to ID taxa
  - Supports QA programs
  - Less dependent on availability of taxonomists
  - Gateway to new biological indicators
Barcoding as a New Tool

A DNA barcode is a short gene sequence taken from standardized portions of the genome, used to identify species.

Similar to the UPC, DNA barcodes provide a universal system of unique tags for each species.
How Does Barcoding Work?

Barcode of Life Database (BOLD)

Specimen → Collection Data → Tissue Sample → Photograph → Extract DNA → PCR Amplify → Sequence → Web-Accessible Data and DNA Barcodes
Transition to Routine Bioassessment

Issues that need to be addressed

Routine Implementation → Standardized Methods

- Develop sampling and preservation methods
- Develop a Reference Library
- Evaluate efficacy of molecular approaches
- Standardize species delimitation methods
- Test performance of indices
- Integrate into environmental monitoring programs
  - Quality control, data management, etc.
Preservation Methods: Study Approach

- Test preservatives
  - Volume of ethanol
  - Number of ethanol replacements
  - Addition of glycerin

- Test holding times of 1 week – 6 months

- Barcode all samples to determine effect of preservation method
Ethanol Preservation is OK

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Ratio</th>
<th>Solution</th>
<th>In matrix</th>
<th>DNA Extraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>5:1</td>
<td>95% ethanol</td>
<td>7 d</td>
<td>50 d</td>
</tr>
<tr>
<td>B</td>
<td>2:1</td>
<td>95% ethanol</td>
<td>7 d</td>
<td>50 d</td>
</tr>
<tr>
<td>C</td>
<td>2:1</td>
<td>95% ethanol + 5% glycerin</td>
<td>7 d</td>
<td>50 d</td>
</tr>
<tr>
<td>D</td>
<td>2:1</td>
<td>95% ethanol</td>
<td>30 d</td>
<td>50 d</td>
</tr>
<tr>
<td>E</td>
<td>2:1</td>
<td>70% ethanol</td>
<td>30 d</td>
<td>50 d</td>
</tr>
<tr>
<td>F</td>
<td>2:1</td>
<td>70% ethanol</td>
<td>6 m</td>
<td>174 d</td>
</tr>
</tbody>
</table>
Ethanol Preservation is OK

Plans to repeat study with marine organisms using formalin
Building the Reference Library

Freshwater ≈ 3,800 spp

Marine ≈ 4,400 spp

- Priority spp
  - No barcode
  - in BOLD

- Total Taxa
  - # of Taxa

- Tolerance Score
  - 0 to 200
Improved Taxonomic Identification

- Morphologic identification
- Genetic identification
- Cluster analysis
- Identify differences

Cluster analysis

- B. tricaudatus 1
- B. adonis 2
- B. adonis 3
- B. tricaudatus 4
Can Barcoding Aid in Marine Benthos Identification?

**Cosmopolitan spp**

<table>
<thead>
<tr>
<th>Species</th>
<th>Courser taxonomic level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampelisca agassizi</td>
<td>gammarid</td>
</tr>
<tr>
<td>Ampelisca careyi</td>
<td>gammarid</td>
</tr>
<tr>
<td>Euphilomedes carcharodonta</td>
<td>ostracod</td>
</tr>
<tr>
<td>Nephtys caecoides</td>
<td>phyllodocid</td>
</tr>
<tr>
<td>Nephtys ferruginea</td>
<td>phyllodocid</td>
</tr>
<tr>
<td>Spiophanes berkleyorum</td>
<td>spionid</td>
</tr>
<tr>
<td>Spiophanes norrisi (bombyx)</td>
<td>spionid</td>
</tr>
<tr>
<td>Tellina modesta</td>
<td>tellinid</td>
</tr>
</tbody>
</table>

**Cryptic spp**

<table>
<thead>
<tr>
<th>Taxon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aphelochaeta glandaria complex</td>
</tr>
<tr>
<td>Capitella capitata complex</td>
</tr>
<tr>
<td>Leptochelia dubia</td>
</tr>
<tr>
<td>Pholoe spp.</td>
</tr>
<tr>
<td>Protomedeia spp.</td>
</tr>
<tr>
<td>Scoloporus arminger complex</td>
</tr>
<tr>
<td>Spio filicornus</td>
</tr>
<tr>
<td>Tellina spp.</td>
</tr>
</tbody>
</table>
Does Barcoding Improve Indices?

- How does information on species and community composition vary between barcoding and traditional taxonomy?

- What effect might this have on “Biological Indices?”
Richness Measures

- Morphology: 101
- Barcoding: 184
Match Unidentified Specimens to Existing Libraries

- **Simulium bracteatum**
- **Simulium piperi**
- **Simulium vittatum**
**Simulium piperi**
- High quality sites
- Cool water
- Good vegetated margins

**Simulium vittatum**
- Tolerant species
- Extreme temperature
- Low oxygen
- Often associated with agriculture
Some Barcode Derived Metrics are More Sensitive
San Gabriel Watershed Demo

- Implement barcoding in context of a routine monitoring program

- High, medium, low quality sites
  - Test metric performance

- 7,200 total specimens
  - Barcoding in process

- Rigorously document relative effort, time, challenges, and costs
  - Field collection
  - Sample processing
  - Barcoding
**Standardized Species Delimitation**

Need Standardized Methods to Define OTU or species

<table>
<thead>
<tr>
<th>Delimitation method</th>
<th>Taxa</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Baetis</td>
<td>3</td>
<td>4</td>
<td>4</td>
<td>5</td>
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<tr>
<td></td>
<td>Eukiefferiella</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Simulium</td>
<td>8</td>
<td>7</td>
<td>7</td>
<td>10</td>
</tr>
</tbody>
</table>

658 base pairs

420 base pairs
Where are We Going Next?
Bulk Sampling Using Next-generation Methods
Evaluating Ability to Use Bulk Sampling

- Created virtual composites from archived specimens
- 3 “treatments” representing H, M, L conditions based on taxonomic composition
  - Approximately 50 individuals from 10 taxa in each treatment
- Bulk DNA extraction from each → PCR
- Blind DNA extract samples to labs to test next-generation sequencers
- Results in process
Bulk Sampling with Environmental DNA (eDNA)

- Nuclear or mitochondrial DNA released from an organism into the water column
- Persists for 7-21 days depending on conditions
- Initial applications for detecting invasive species
- Potential future application of broader community analysis
Distribution of New Zealand Mud Snail in California

Affected counties include: Del Norte, Shasta, Mendocino, Sonoma, Napa, Yolo, Solano, Sacramento, Marin, Solano, San Francisco, Contra Costa, Alameda, San Joaquin, Calaveras, San Mateo, Santa Cruz, Santa Clara, Stanislaus, Mono, Inyo, Los Angeles and Orange counties.

Updated March 2011
For the most updated maps, please visit: http://nawex.rsgis.gov/taxgroup/mollusks/newzealandmudsnaildistribution.aspx
Can Existing Indices Accommodate Next-generation Methods?

- Lack of abundance data should not be a limitation.
- Presence BRI and AMBI perform well.
- Able to discern both spatial and temporal gradients.
Are We There Yet?

**Molecular**
- Additional markers besides COI
- Improved primers
- Next generation sequencing
- Ability to process bulk samples

**Bioassessment**
- Species delimitation
  - Character based analysis
- Revised bioassessment scoring tools
- Additional taxonomic groups
  - algae, prokaryotes, meiofauna

**Bioinformatics**
- Adequate vouchering (specimens & DNA)
- Data management and analysis tools
THANK YOU

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