Environmental DNA, digital PCR, and metabarcoding: New molecular tools in fisheries and wildlife research

Taal Levi, Jennifer Allen
Oregon State University, Dept. of Fisheries and Wildlife
What is eDNA?

Credit: Strickler, Goldberg, and Fremier
Forefront of eDNA

1. How reliably can we detect many species at once?
   - DNA metabarcoding

2. Can we quantify abundance in a management-relevant way?
   - Measure DNA concentration of a species in an environmental sample using qPCR or digital PCR
   - Possibly use number of sequences from DNA metabarcoding

3. Can we find novel sources of DNA for wildlife research?
DNA barcoding

• All individuals within a species share particular sequences

  *Thamnophis eques* (mtDNA):
  ...GAAAGGCCCTAACCTGGTAGGACCAATA...

  *Thamnophis cyrtopsis* (mtDNA):
  ...GAAAGGCCCTAACCTAGTTAGGACCAATA...

[Images of various species]
Mitochondrial Genome

- Control region or "d-loop"
- 12S rRNA
- Cytochrome b
- 16S rRNA
- NADH Dehydrogenase subunits
- 22 tRNA-encoding genes
- 13 protein-encoding regions
- Cytochrome Oxidase subunits
- ATP Synthase subunits
- NADH Dehydrogenase subunits
METABARCODING OF eDNA

Drawing by Lars Holm. Thomsen & Willerslev 2015 Biological Conservation.
DNA Metabarcoding

Total biodiversity survey
Amphibians and Fish

ATGACCACAGATAGACA
ATTATGACTACAGATAGACG
ATTAAAACTACAGATAGACG
ATTATGACTACAGATAGACA
ATTATGACTACAGATAGACA
CTTATGACTACAGGGGACA
DNA metabarcoding for diet analysis

With PhD student Aimee Massey

With MS student Charlotte Eriksson and Katie Moriarty (USFS)
Quantifying the diet of Alexander Archipelago Wolves

With PhD student Aimee Massey

Photo by Ian McAllister, Pacific Wild
PROJECT OBJECTIVES:
1. Compare DNA metabarcoding and mechanical sorting
2. Quantify diets of coastal wolves?
3. Use captive wolves fed known diets to test quantitation of species proportion in scats

Photo by Jeff Hyde
deer
beaver
black bear
fish
bald eagle
river otter
birds
marine mammals
mountain goat
moose
beaver

DNA metabarcoding for diet analysis

With PhD student Aimee Massey

With MS student Charlotte Eriksson and Katie Moriarty (USFS)

With PhD student Elissa Olimpi
Vertebrates in Diet of Coastal Martens (Fall sample session)

- **Bird (all species)**
- **Microtus**
- **Deer**
- **Peromyscus**
- **Myodes**
- **White-footed vole**
- **House mouse**
DNA metabarcoding of iDNA

[Image of a trap with a light bulb, cooler of dry ice, and collection cup.]
Number of sequences using a 16S Mammal Primer in upstate New York

<table>
<thead>
<tr>
<th>Herp</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chelydra serpentina</td>
<td>0</td>
</tr>
<tr>
<td>Hyla versicolor</td>
<td>75</td>
</tr>
<tr>
<td>Pseudacris crucifer</td>
<td>84,548</td>
</tr>
<tr>
<td>Rana sp.</td>
<td>0</td>
</tr>
<tr>
<td>Rana sylvatica</td>
<td>69</td>
</tr>
<tr>
<td>Terrapene carolina</td>
<td>25</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Mammal</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bos taurus</td>
<td>18,903</td>
</tr>
<tr>
<td>Canis latrans</td>
<td>15,557</td>
</tr>
<tr>
<td>Equus asinus</td>
<td>0</td>
</tr>
<tr>
<td>Felis catus</td>
<td>4</td>
</tr>
<tr>
<td>Odocoileus virginianus</td>
<td>280,388</td>
</tr>
<tr>
<td>Peromyscus leucopus</td>
<td>0</td>
</tr>
<tr>
<td>Procyon lotor</td>
<td>62,919</td>
</tr>
<tr>
<td>Sciurus carolinensis</td>
<td>45,809</td>
</tr>
<tr>
<td>Sciurus niger</td>
<td>299</td>
</tr>
<tr>
<td>Sciurus sp.</td>
<td>1,361</td>
</tr>
<tr>
<td>Sus scrofa</td>
<td>7,503</td>
</tr>
<tr>
<td>Tamias striatus</td>
<td>109,8115</td>
</tr>
<tr>
<td>Ursus americanus</td>
<td>7</td>
</tr>
</tbody>
</table>
BIODIVERSITY AND HOST-VECTOR-PATHOGEN RELATIONSHIPS ACROSS A FOREST LOSS GRADIENT IN AMAZONIA

With PhD student Aimee Massey
2. Getting quantitative results with eDNA
Absolute quantification with Droplet digital PCR
Absolute Quantification

1. High accuracy
2. High sensitivity
3. No standard curve
4. Overcomes PCR inhibition
Auke Creek Research Weir
Inbound Adult Sockeye 2015

Sockeye DNA/μL

Sockeye count

Sockeye Counts

Sockeye DNA/μL

Sockeye count

Sockeye Counts
Quantifying eulachon on the Chilkoot River
### Chilkoot River Eulachon

Table 1. Eulachon population estimates for the Chilkoot River using mark recapture techniques for a closed population.

<table>
<thead>
<tr>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>M = Marked Initially-adipose clipped</td>
<td>8,017</td>
<td>49,814</td>
<td>27,525</td>
<td>24,084</td>
<td>306</td>
</tr>
<tr>
<td>C = Total in second sample captured above weir</td>
<td>20,210</td>
<td>143,444</td>
<td>48,376</td>
<td>19,886</td>
<td>3,122</td>
</tr>
<tr>
<td>R = Marked recaptures above weir with clip</td>
<td>72</td>
<td>568</td>
<td>186</td>
<td>140</td>
<td>2</td>
</tr>
<tr>
<td>N^1 = Population Estimate</td>
<td>2.2 Million</td>
<td>12.6 Million</td>
<td>7.1 Million</td>
<td>3.4 Million</td>
<td>319,586</td>
</tr>
<tr>
<td>SE^2 = Standard Error</td>
<td>256,415</td>
<td>521,961</td>
<td>516,583</td>
<td>283,226</td>
<td>158,934</td>
</tr>
<tr>
<td>CI^3 = 95% Confidence Interval</td>
<td>1.7 to 2.7 Million</td>
<td>11.5 to 13.6 Million</td>
<td>6.1 to 8.1 Million</td>
<td>2.9 to 3.9 Million</td>
<td>8,074 to 631,098</td>
</tr>
</tbody>
</table>

1. Population Estimate
2. Standard Error
3. Confidence Interval
Mark-recapture to estimate run size
Cost of ~$15-20k/yr
Mobile eDNA sampling
2014 Eulachon ddPCR results

Biological replicates

Eulachon DNA / μL

Eulachon DNA / μL x Flow rate

Bridge

FlowCorrect

5/1/14 5/6/14 5/11/14 5/16/14 5/21/14 5/26/14
Large 2014 run compared with failed 2015 run


Eulachon DNA /uL x xFlow rate

2014 VS 2015

FlowCorrect2014
FlowCorrect2015

Large 2014 run compared with failed 2015 run
### Year Comparison of eDNA Peak (Flow Corrected) Population Estimates

<table>
<thead>
<tr>
<th>Year</th>
<th>eDNA Peak (Flow Corrected)</th>
<th>PopEst</th>
<th>Min95</th>
<th>Max95</th>
</tr>
</thead>
<tbody>
<tr>
<td>2014</td>
<td>1,607,536</td>
<td>3,397,009</td>
<td>2800000</td>
<td>3900000</td>
</tr>
<tr>
<td>2015</td>
<td>106,008</td>
<td>319,586</td>
<td>8074</td>
<td>631098</td>
</tr>
</tbody>
</table>

**Ratio**  
(2014 : 2015)  
15.2 : 10.6

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Given large confidence interval associated with statistical estimates with mark-recapture, are eDNA based estimates of abundance likely to be even MORE accurate?
This works on a small stream, but will this work on the Columbia River?
Cowlitz River and mainstem Columbia River monitoring
3. New sources of DNA for wildlife research
Bear genotyping from residual saliva on salmon vs. scats

Identifying seed disperser from residual saliva on berry stalks

With MS Student Laurie Harrer
Identifying seed disperser from residual saliva on berry stalks

64% of 105 swabbed Devil’s Club fruit were consumed by brown bears
36% black bears

With MS Student Laurie Harrer
Thank You