From barcoding single individuals to metabarcoding biological communities:
Towards understanding and managing invasive species
Global biotic homogenization

Olden 2006 Journal of Biogeography
Global biotic homogenization

Image via the National Center for Ecological Analysis and Synthesis: shipping density (commercial)
Aquatic Invaders the ‘ecological villains’

- European green crab
- Zebra mussel
- Water lettuce
- Giant Salvinia
- Mnemiopsis leidyi
“Nowadays we live in a very explosive world, and while we may not know where or when the next outburst will be, we might hope to find ways of stopping it or at any rate damping down its force.”

The Ecology of Invasions by Animals and Plants, 1958

Charles S. Elton 1900 - 1991
Living in a Changing World

G. L. Stebbins (1906 – 2000)

H.G. Baker (1920-2001)

(1965)
Using Genetic Tools

- The identify of aquatic invaders
- The evolutionary and invasion history
- Vectors of introduction and secondary spread
- Number of independent introductions
- Colonization dynamics across invaded areas
- Causes and consequences of invasiveness
- Early detection and preventive measures

Cristescu 2015 *Mol. Ecol*
Invasive Ascidians

Golden star

Violet tunicate

Vase tunicate
Invasion genetics of *Botryllus schlosseri*

- Native to European coastal waters
- Global distribution
- Major biofouler
Invasion genetics of *Botryllus schlosseri*

Invasion genetics of *Botryllus schlosseri*

Invasion genetics of *Botryllus schlosseri*

Reproductive Isolation

Why do some species proliferate at an alarming rate while their closest relatives are facing extinction?
Early detection of high risk aquatic invaders
Early detection of high risk invaders

Barcoding + NGS = Metabarcoding

Cristescu 2014 TREE
Mock communities

Brown et al. 2015 *Ecol Evol*
Mock communities

13 species

Brown et al. 2015 *Ecol Evol*
The impact of intra- and inter-specific diversity

- Simple communities (single individuals per species) generated a high correspondence of 60-84% between OTU number and species richness.

- When complex community were examined, this correspondence dropped to 31-63%.

- Intra-specific (or intra-individual) variation in the 18S marker sometime exceeds 3%.

Brown et al. 2015 *Ecol Evol*
Evaluating different methods of clustering

• Stringent and relaxed filtering;
• Singleton sequences included and excluded;
• Three clustering algorithms (mothur, UCLUST, UPARSE);
• Three methods of treating alignment gaps.

✓ The number of OTUs varied by nearly three orders of magnitude;
✓ The use of relaxed filtering and the inclusion of singleton greatly inflated the number of OTUs.

Flynn et al. 2015 *Ecol Evol*
Spatiotemporal zooplankton biodiversity trends across 16 Canadian ports
Methodology

<table>
<thead>
<tr>
<th>The clustering approach</th>
<th>A taxonomy dependent approach</th>
</tr>
</thead>
<tbody>
<tr>
<td>OTU clustering</td>
<td>Blast searches of individual reads against local databases</td>
</tr>
<tr>
<td>Requires a reliable barcoding gap and accurate divergence threshold</td>
<td>Requires a well developed reference library and concerted global effort</td>
</tr>
<tr>
<td>Validated methodology based on mock communities</td>
<td>Requires customized pipelines specific to the marker and taxa of interest</td>
</tr>
<tr>
<td>Taxonomic extension is facultative</td>
<td>Taxonomic extension is at the core of the approach</td>
</tr>
</tbody>
</table>
Highlights

• Taxonomic classification and phylogenetic inferences of over seven million sequences;

• Identified species spanning 392 metazoan families and 105 orders, complementing previous surveys based on morphological identification;

• Revealed species from over 30 orders that were previously not reported in zooplankton surveys;

• Certain taxonomic groups were underrepresented due to incomplete reference databases and low interspecific variation;

• Zooplankton communities were distinct among coastlines and among habitat types;

• Biodiversity varied substantially across seasons particularly for the subarctic region;

• Revealed a large number of NIS, many of them in previously unreported regions.
Proportional abundance of phyla across samples

- Arthropoda: 90.7%
- Annelida: 2.2%
- Ctenophora: 1.6%
- Mollusca: 1.4%
- Chordata: 1.1%
- Rotifera: 0.7%
- Cnidaria: 0.6%
- Echinodermata: 0.6%
- Dinoflagellata: 0.5%
- Platyhelminthes: 0.2%
Proportional abundance of families across samples

Pacific
Arctic
Atlantic
Great Lakes

Port

Proportion

Temoridae
Clausocalanidae
Oithonidae
Paracalanidae
Diaptomidae
Calanidae
Daphniidae
Acartiidae
Cercopagidae
Metridinidae
Balanidae
Laophontidae
Podonidae
Bosminidae
Polynoidae
Oikopleuridae
Spionidae
Thalassocalycidae
Dreissenidae
Ophiacanthidae
Leptodoridae
Microstomidae
Canthocamptidae
Thalassocalyidae
Dreissenidae
Ophiacanthidae
Leptodoridae
Microstomidae
Synchaetidae
Callianassidae
Corycaeidae
PCA of sample (zooplankton composition) at the family-level (after rarefaction)

Axis 1 (21.3%)
Axis 2 (13.4%)

Great Lakes
Arctic
Pacific
Atlantic
Bayside
Identifying invasive species

Number of non-indigenous species (NIS)

Pacific  Arctic  Atlantic  Great Lakes  Total

Taxa:
- Copepoda
- Mollusca
- Malacostraca
- Branchiopoda
- Tunicata
- Thecostraca
- Rotifera
Conclusions

The metabarcoding approach is very sensitive if applied appropriately

We need to farther advance:

✓ Reference libraries that incorporate reliable taxonomic information;
✓ Comprehensive databases of invasive species;
✓ Methodological validations using mock communities;
✓ Multi-marker approaches;
✓ Continue to promote standardization and reproducibility of metabarcoding approaches;
✓ Global coordinated effort that integrates traditional approaches and effectively implements the emerging technologies.
Aknowledgements

Collaborators

- Hugh Maclsaac
- Teri Crease
- Cathryn Abbott
- Sally Adamowicz
- Paul Hebert