

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



Living in a Changing World

"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

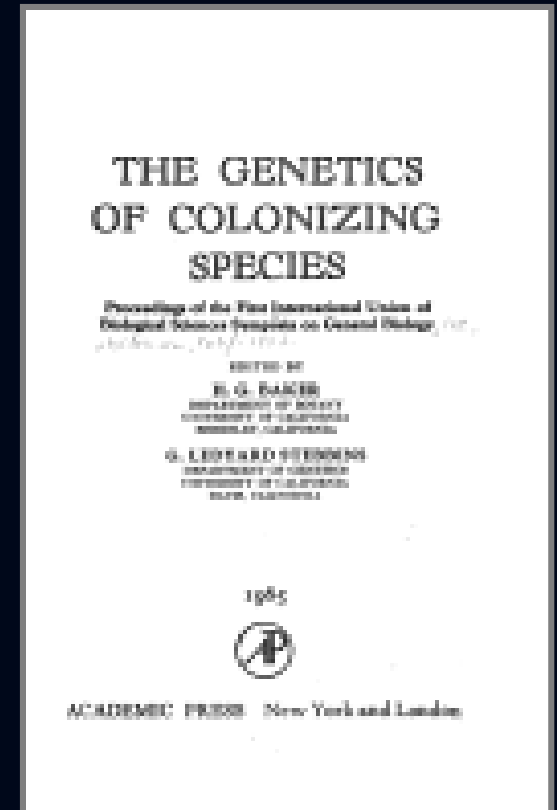


G. Ledyard Stebbins

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

Invasive Ascidians



Golden star

Violet tunicate

Vase tunicate

Invasion genetics of *Botryllus schlosseri*

- ✓ Native to European coastal waters
- ✓ Global distribution
- ✓ Major biofouler



Geographic distribution of *B. schlosseri*



Native range



Invasive range

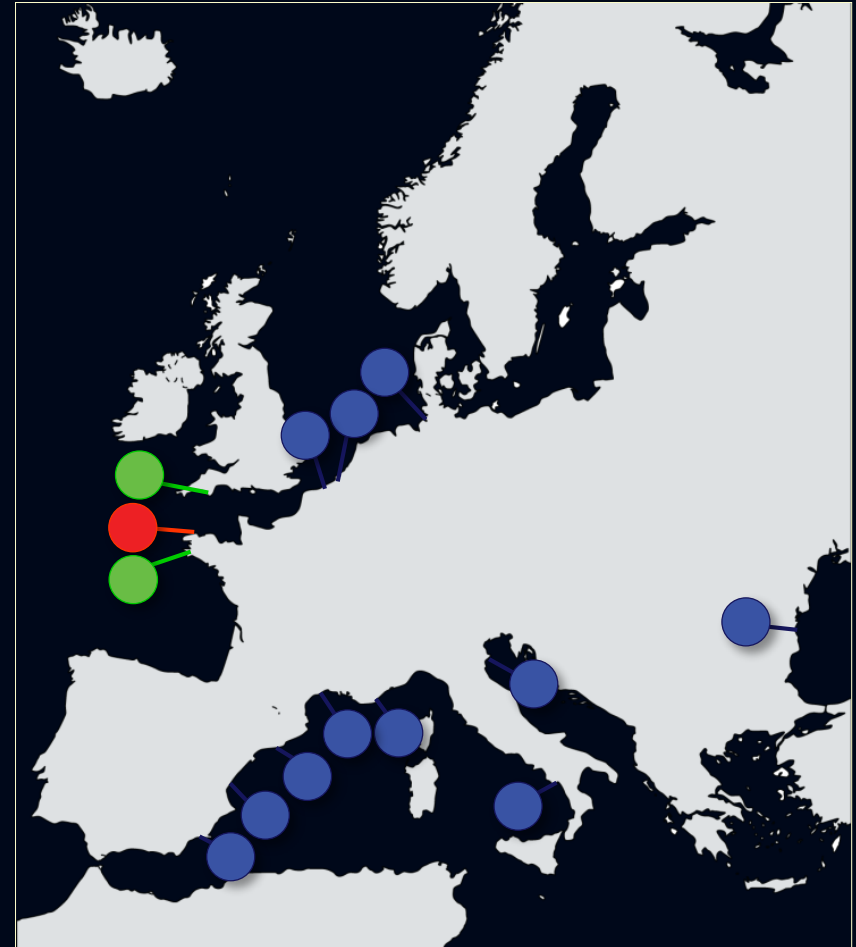
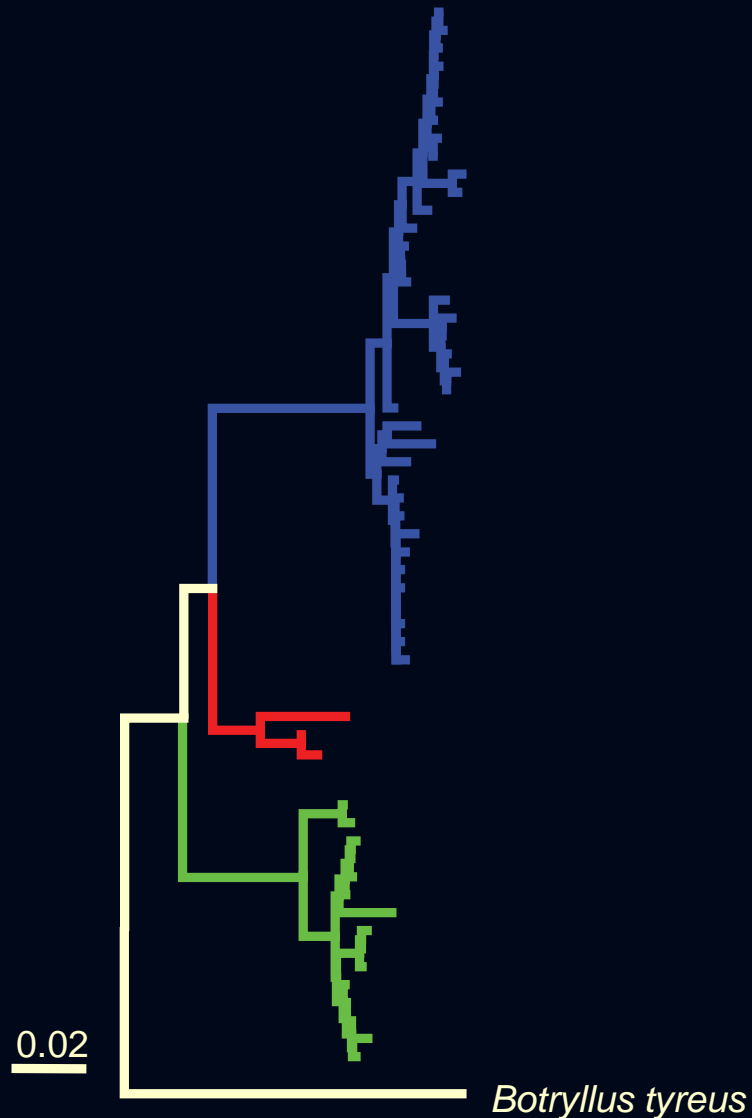
Geographic distribution of *B. schlosseri*



■ Native range

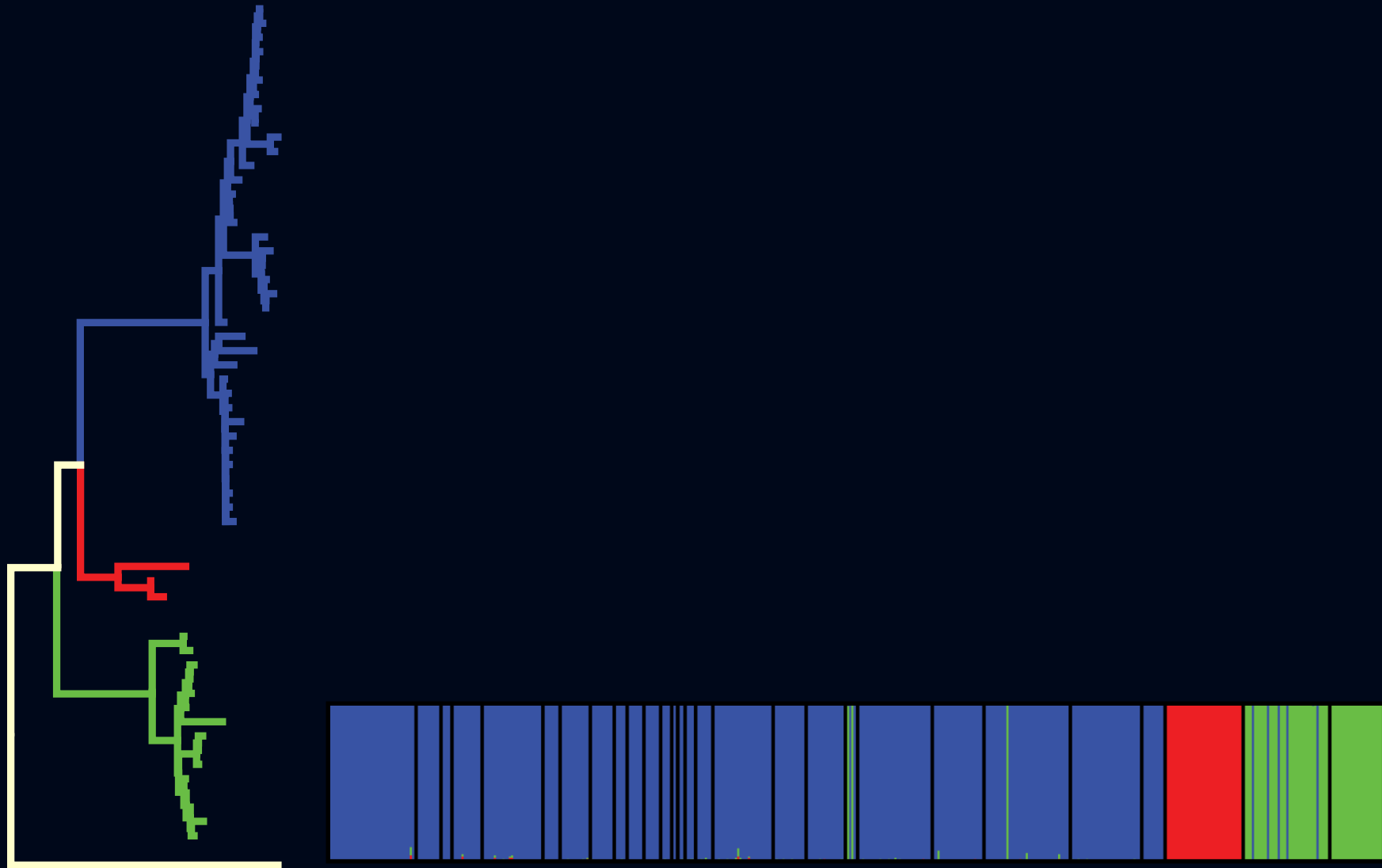
■ Invasive range

Invasion genetics of *Botryllus schlosseri*

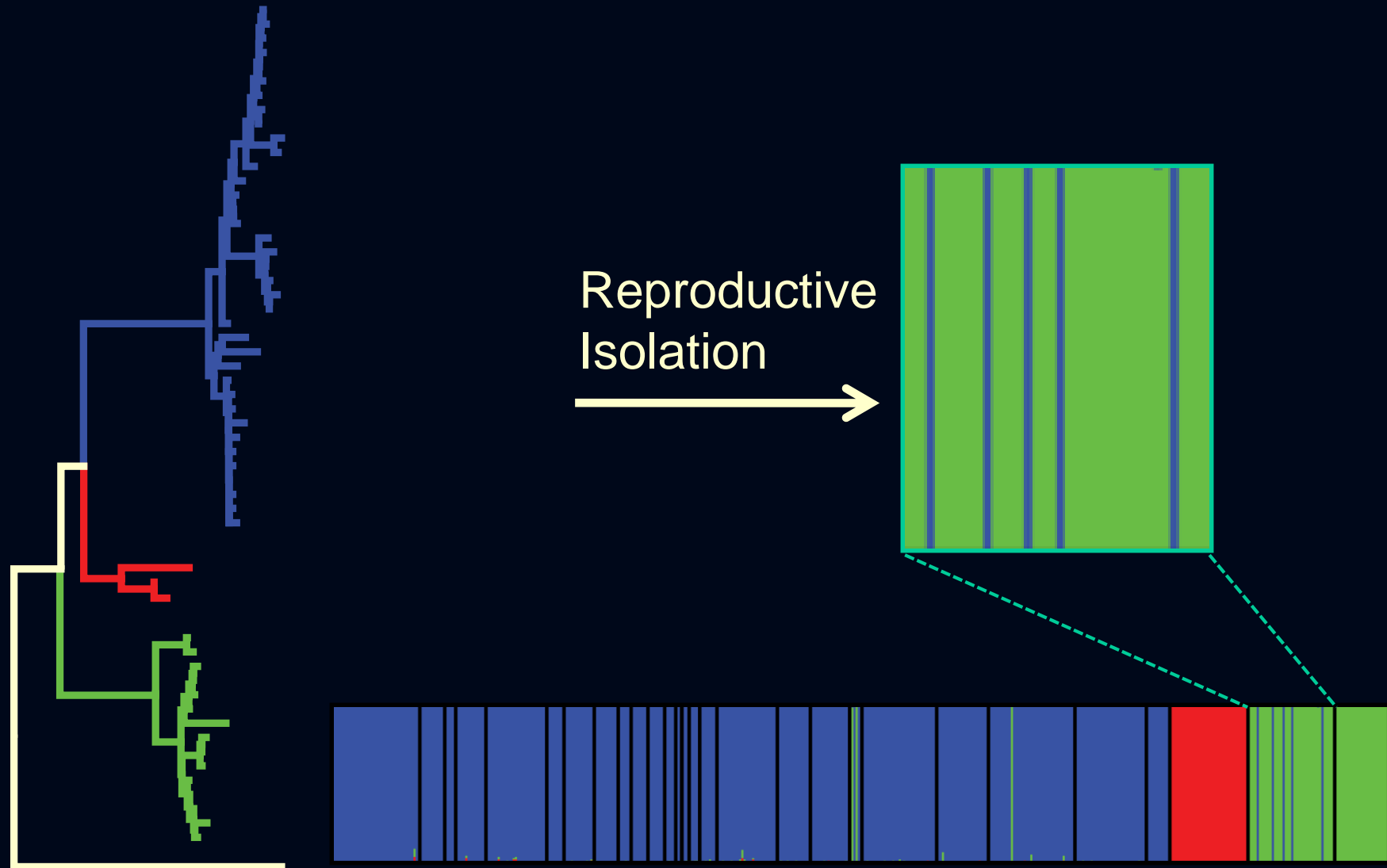


Bock et al. 2012, *Proc. R. Soc. B*

Invasion genetics of *Botryllus schlosseri*



Invasion genetics of *Botryllus schlosseri*

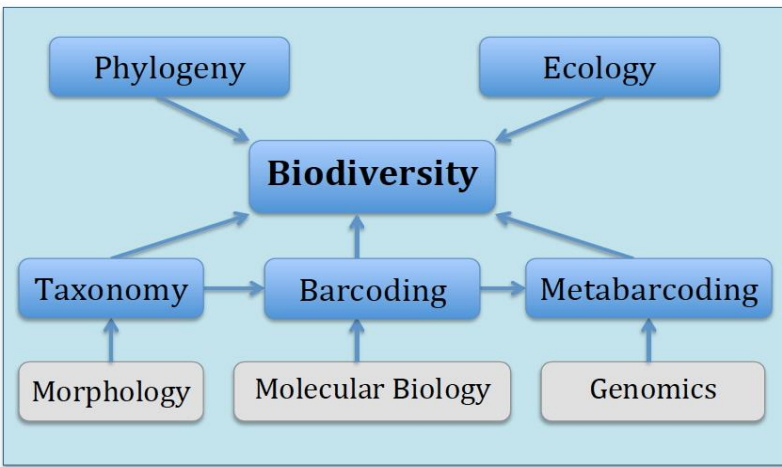


Why do some species proliferate at an alarming rate while their closest relatives are facing extinction?

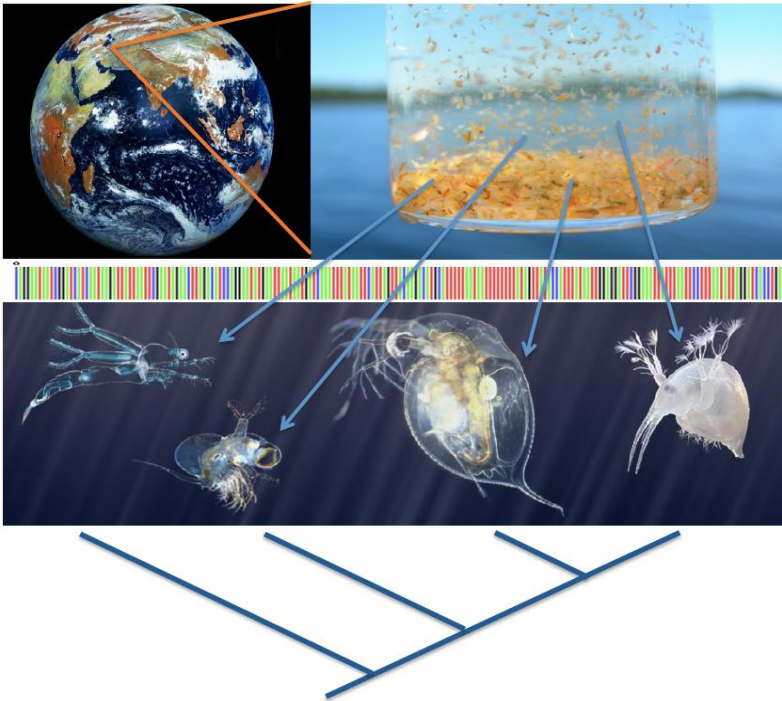
Early detection of high risk aquatic invaders



Early detection of high risk invaders



Barcoding
+
NGS
=
Metabarcoding



Cristescu 2014 *TREE*

Mock communities

20 species



Brown et al. 2015 *Ecol Evol*

Mock communities

13 species

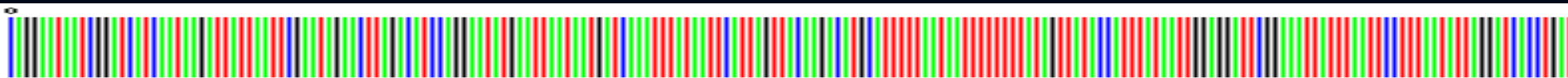


Brown et al. 2015 *Ecol Evol*

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*



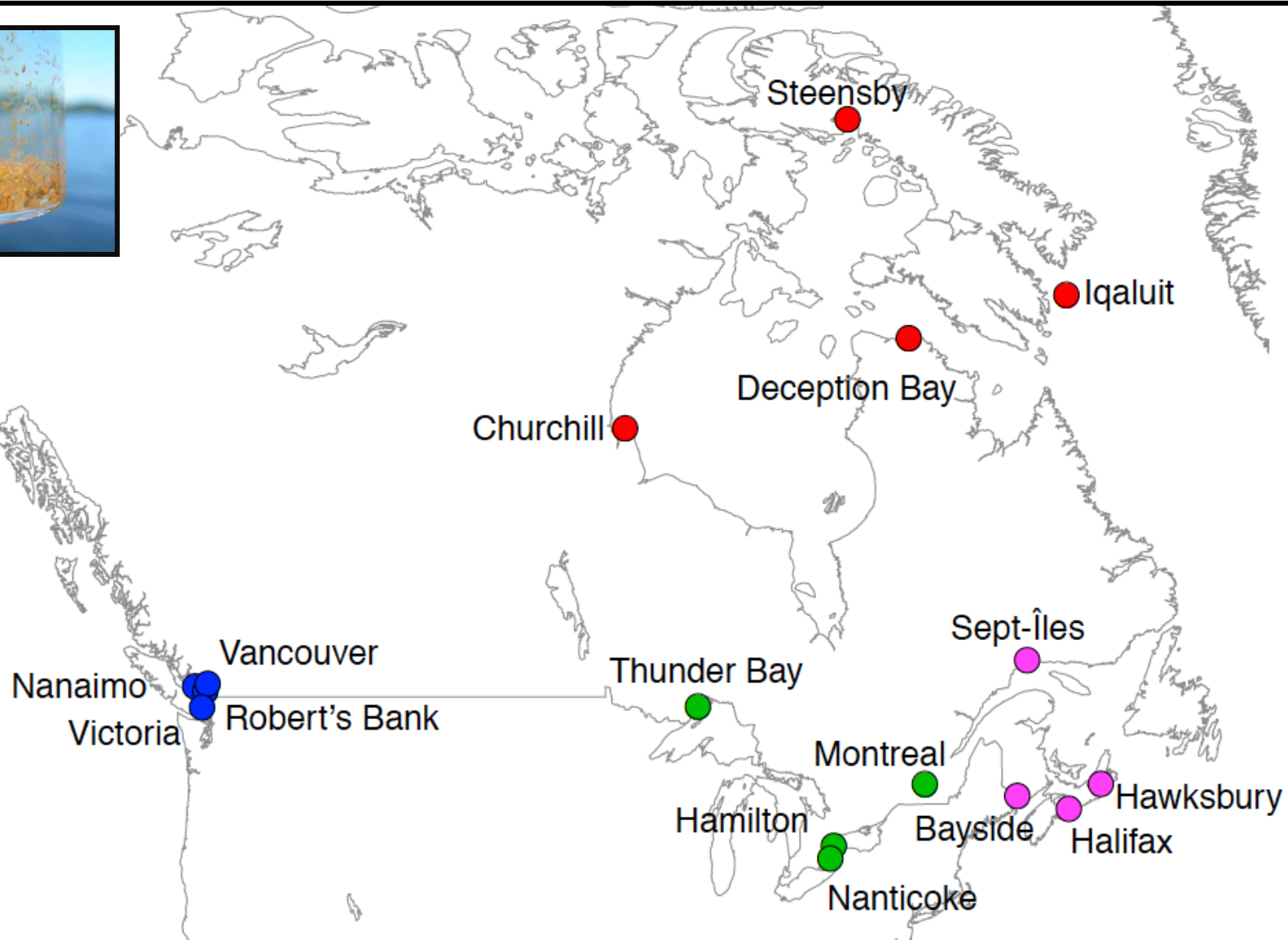
Evaluating different methods of clustering

- 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



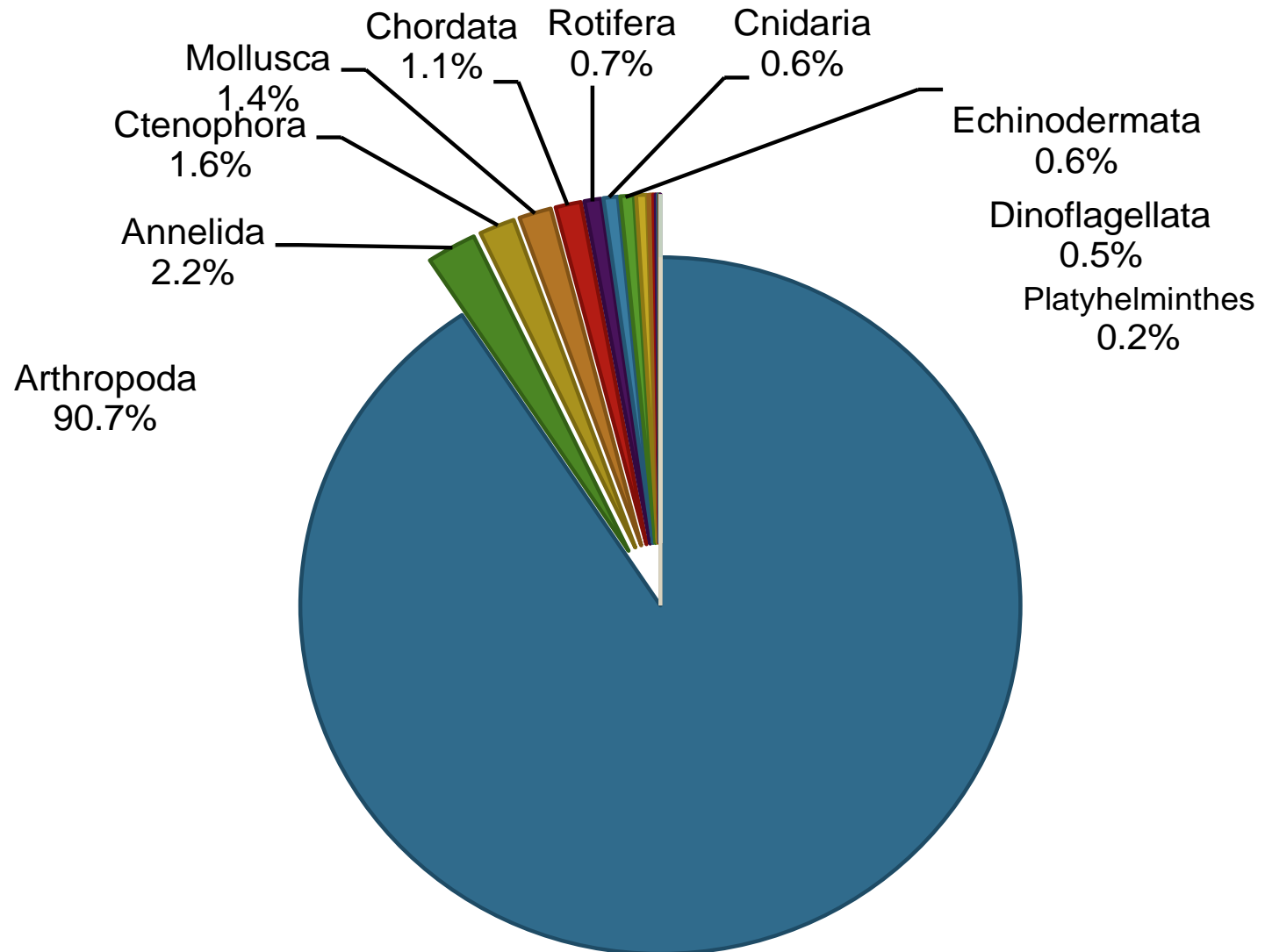
Methodology

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

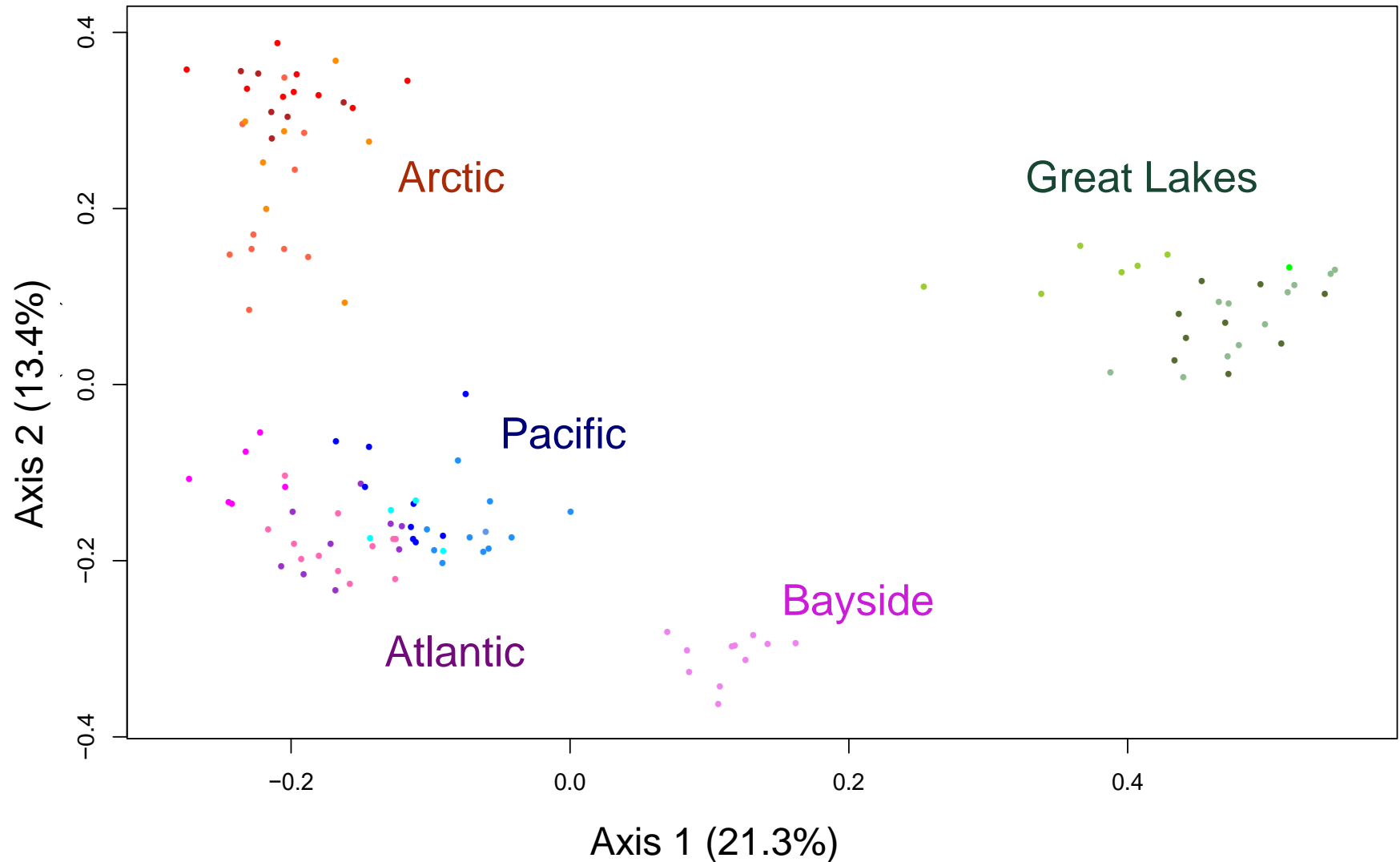
Highlights

- 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.

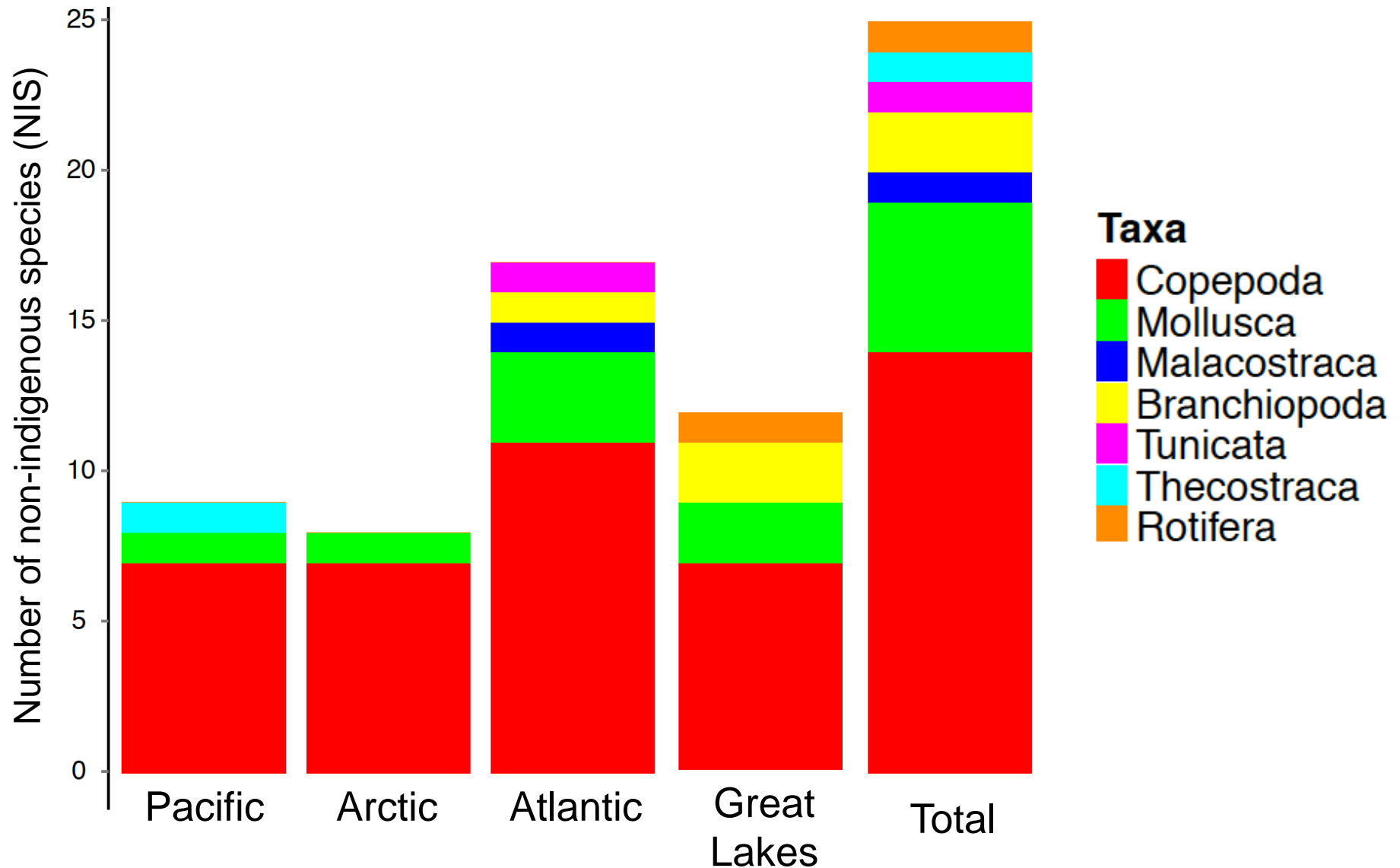
Proportional abundance of phyla across samples



PCA of sample (zooplankton composition) at the family-level (after rarefaction)



Identifying invasive species



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.



Acknowledgements

Lab members

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NETWORK



Barcoding as a Canadian Icon



An NRC Research Press Journal

