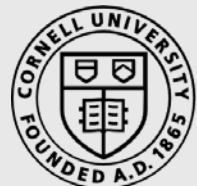


# Testing Global Ship-borne Species Spread Models with Metabarcoding

\*Erin K. Grey<sup>1</sup>, Paul Czechowski<sup>2,3</sup>, Kara Andres<sup>2</sup>, Jose Andres<sup>2</sup>, Kristy Deiner<sup>4</sup>,  
Yiyuan Li<sup>5</sup>, Michael Pfrender<sup>6</sup>, Mandana Saebi<sup>6</sup>, Nitesh Chawla<sup>6</sup>, David Lodge<sup>2</sup>

<sup>1</sup>Governors State University, <sup>2</sup>Cornell University, <sup>3</sup>University of Otago, <sup>3</sup>London Museum of Natural History, <sup>4</sup>University of Texas at Austin, <sup>5</sup>University of Notre Dame



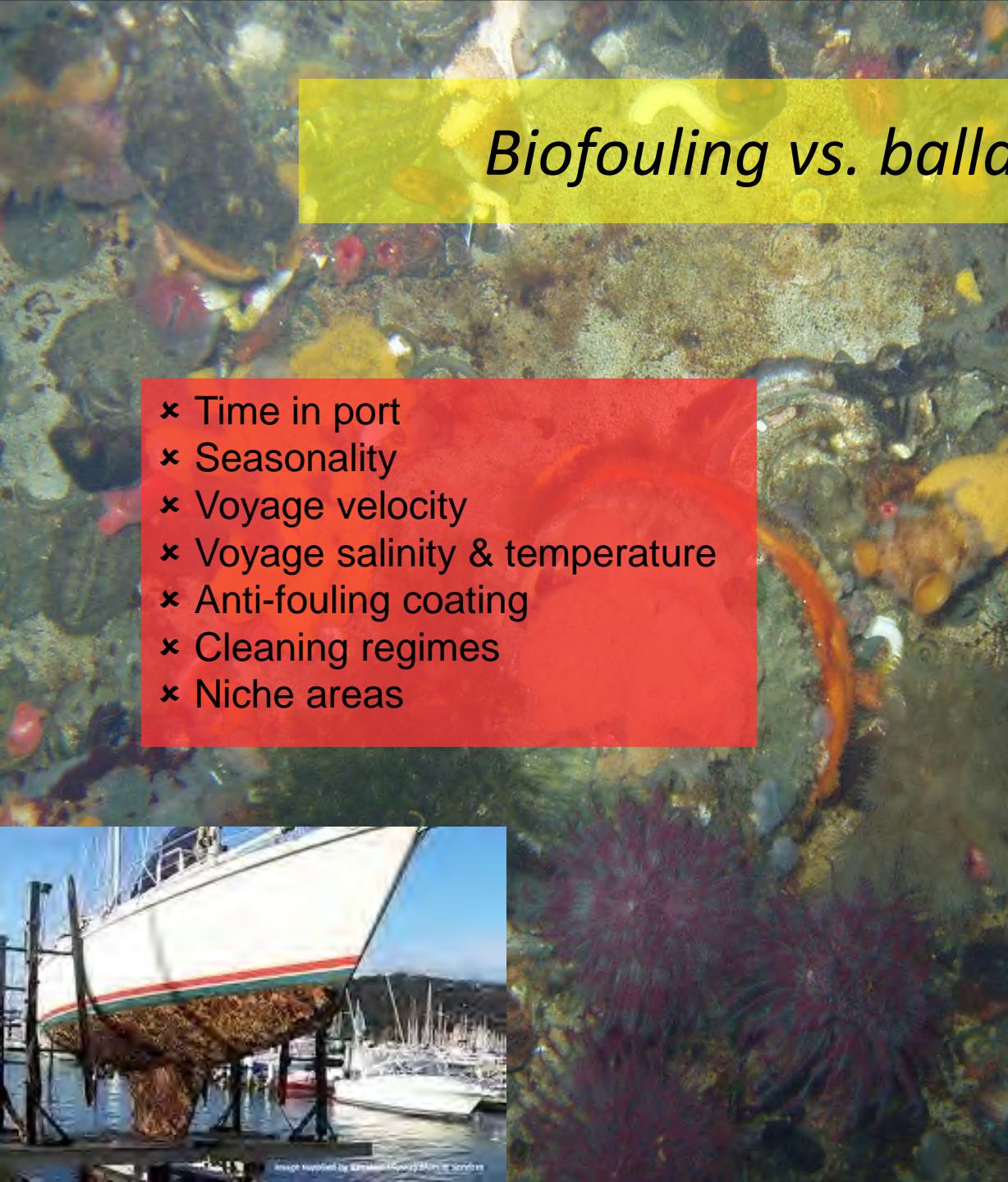
Cornell University



Ports are hotspots for aquatic nonindigenous species (NIS). But...

- ✓ *Ballast and biofouling transport*
- ✓ *Complex and dynamic global shipping patterns*
- ✓ *Challenging to capture and identify organisms*

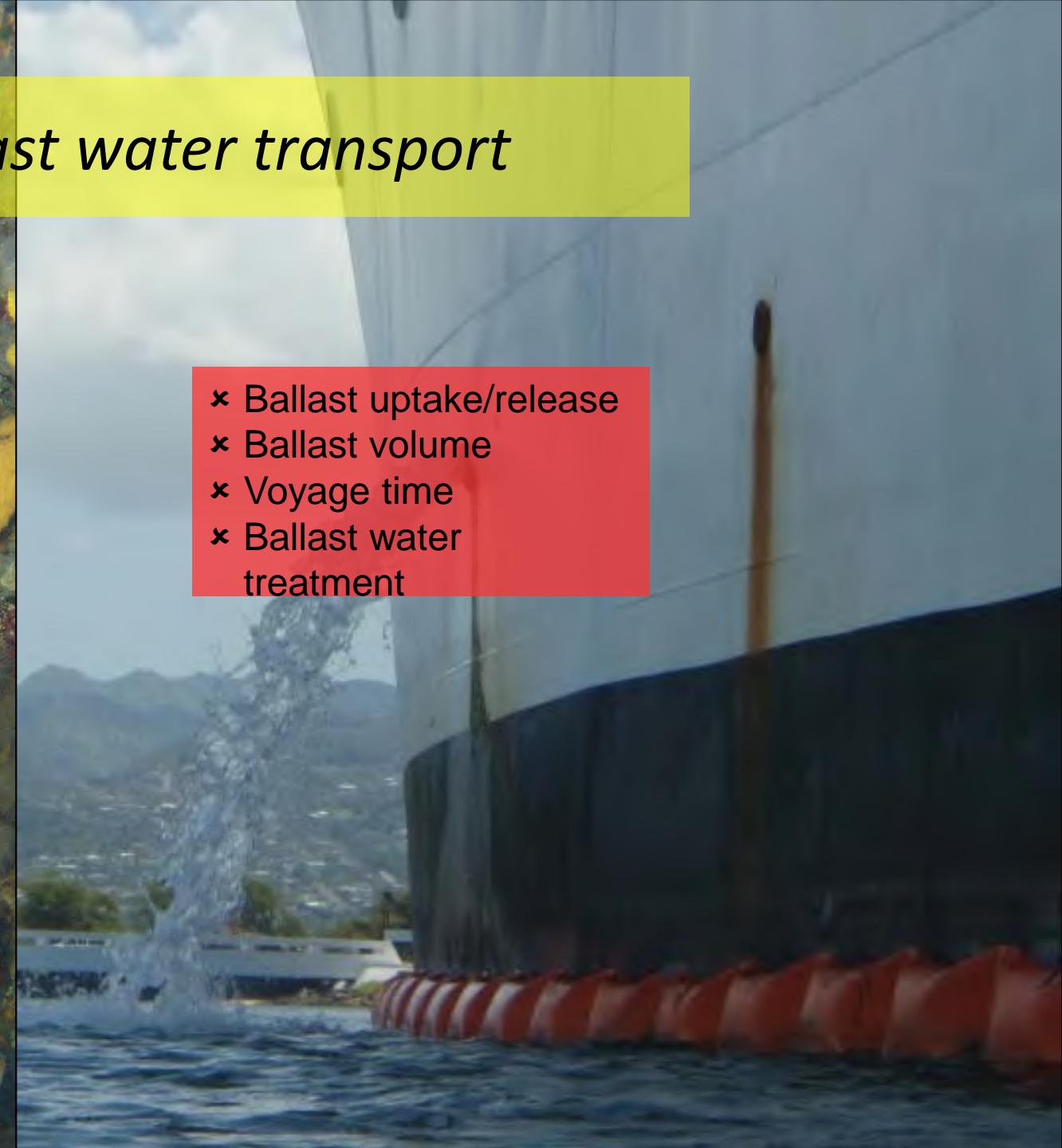




## *Biofouling vs. ballast water transport*

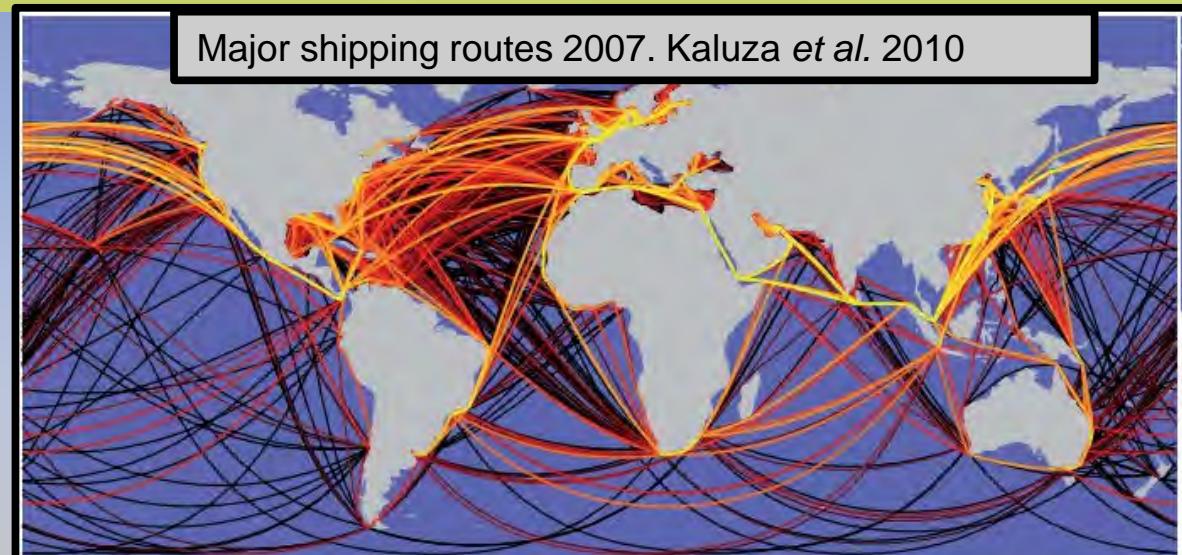
- ✗ Time in port
- ✗ Seasonality
- ✗ Voyage velocity
- ✗ Voyage salinity & temperature
- ✗ Anti-fouling coating
- ✗ Cleaning regimes
- ✗ Niche areas



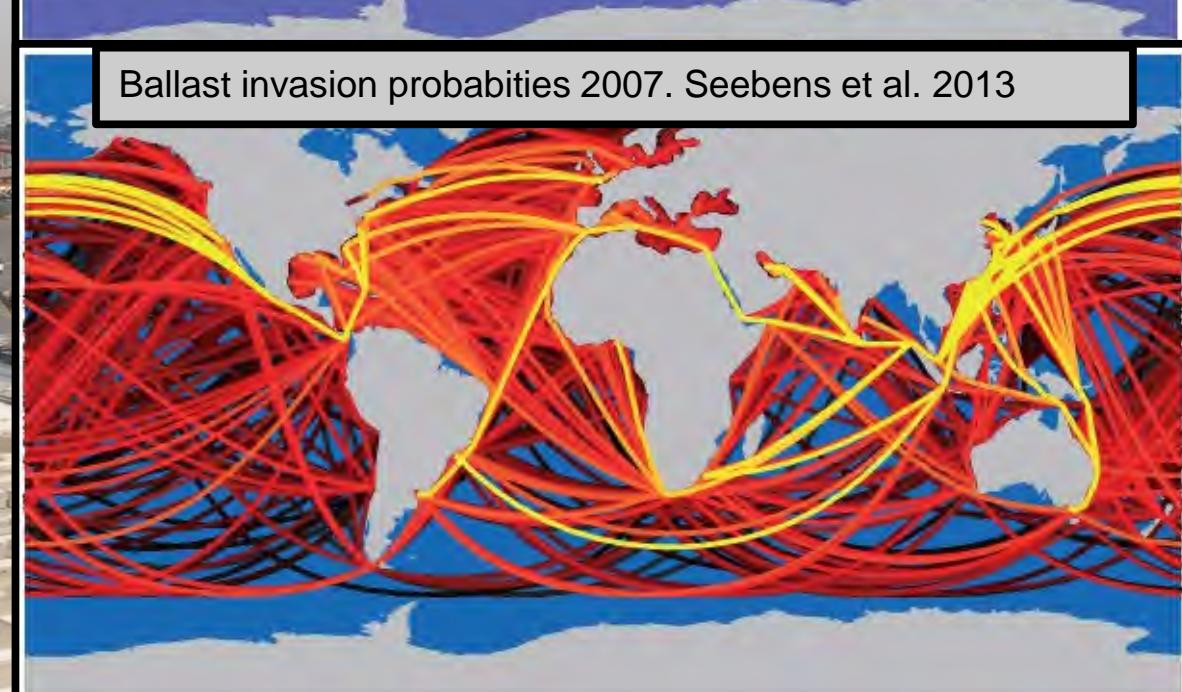
- 
- ✗ Ballast uptake/release
  - ✗ Ballast volume
  - ✗ Voyage time
  - ✗ Ballast water treatment

# Complex and dynamic global shipping patterns

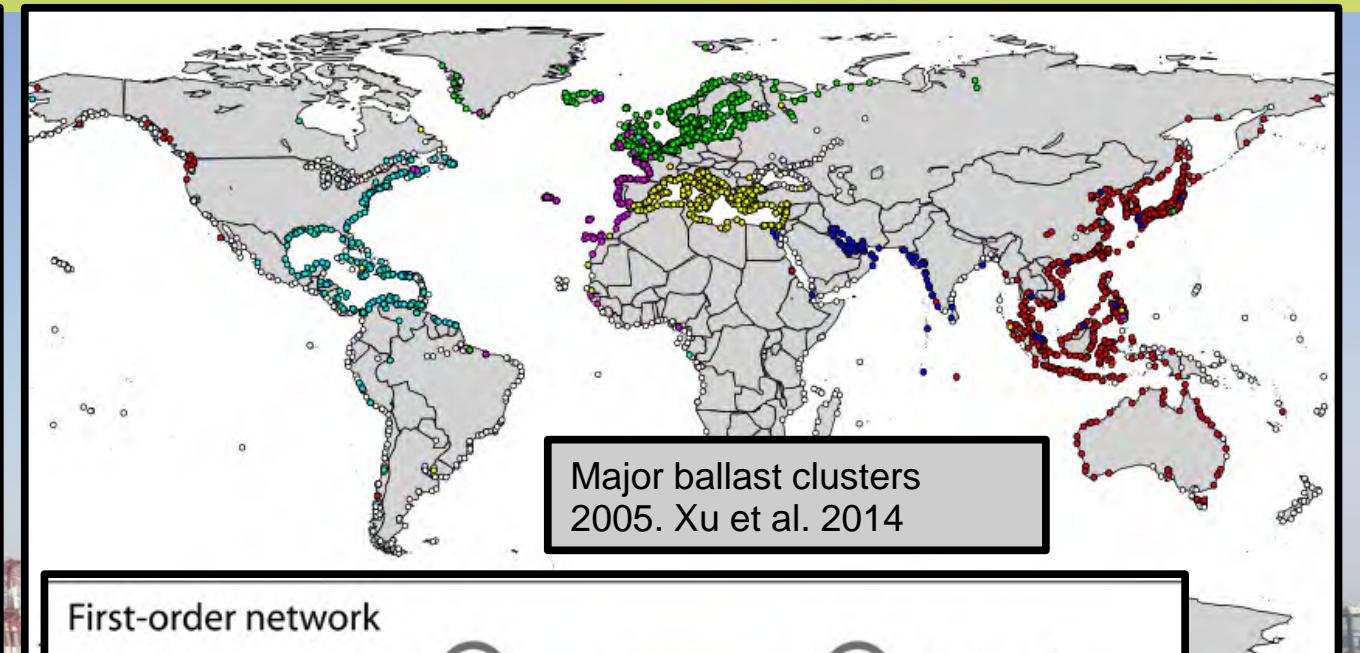
Major shipping routes 2007. Kaluza et al. 2010



Ballast invasion probabilities 2007. Seebens et al. 2013

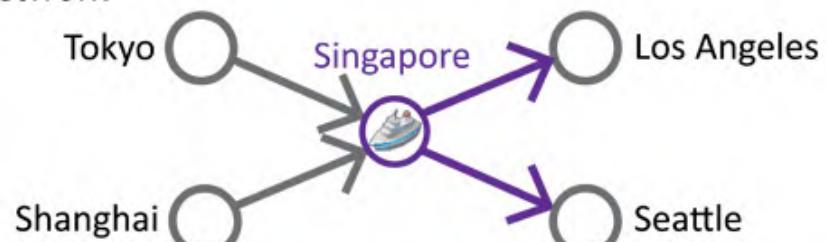


Major ballast clusters  
2005. Xu et al. 2014



First-order network

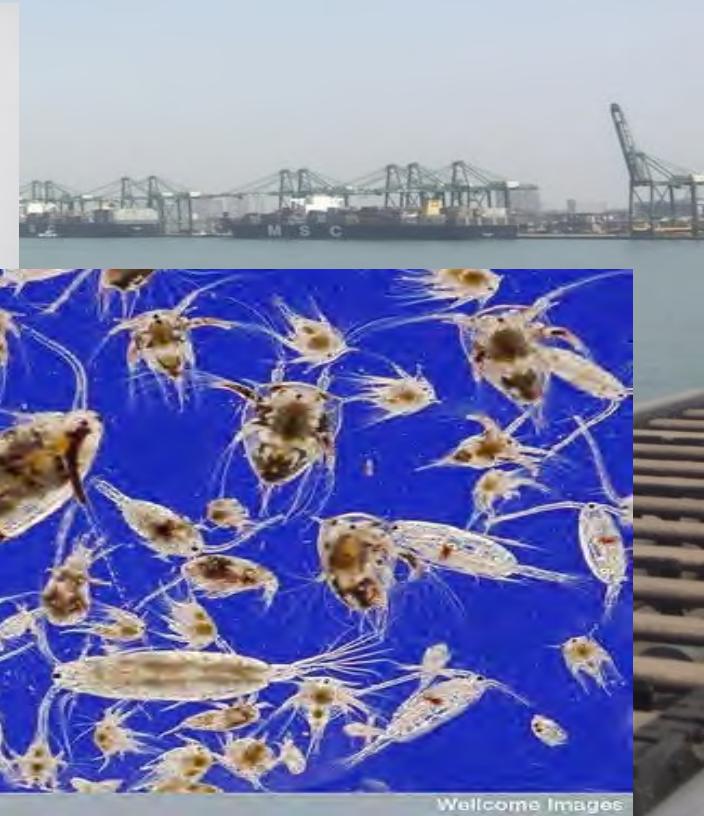
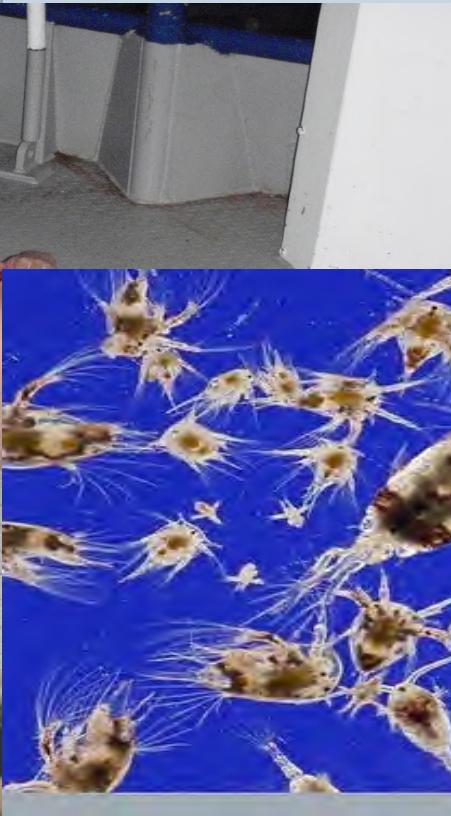
Xu et al.  
2016



Higher-order network (HON)

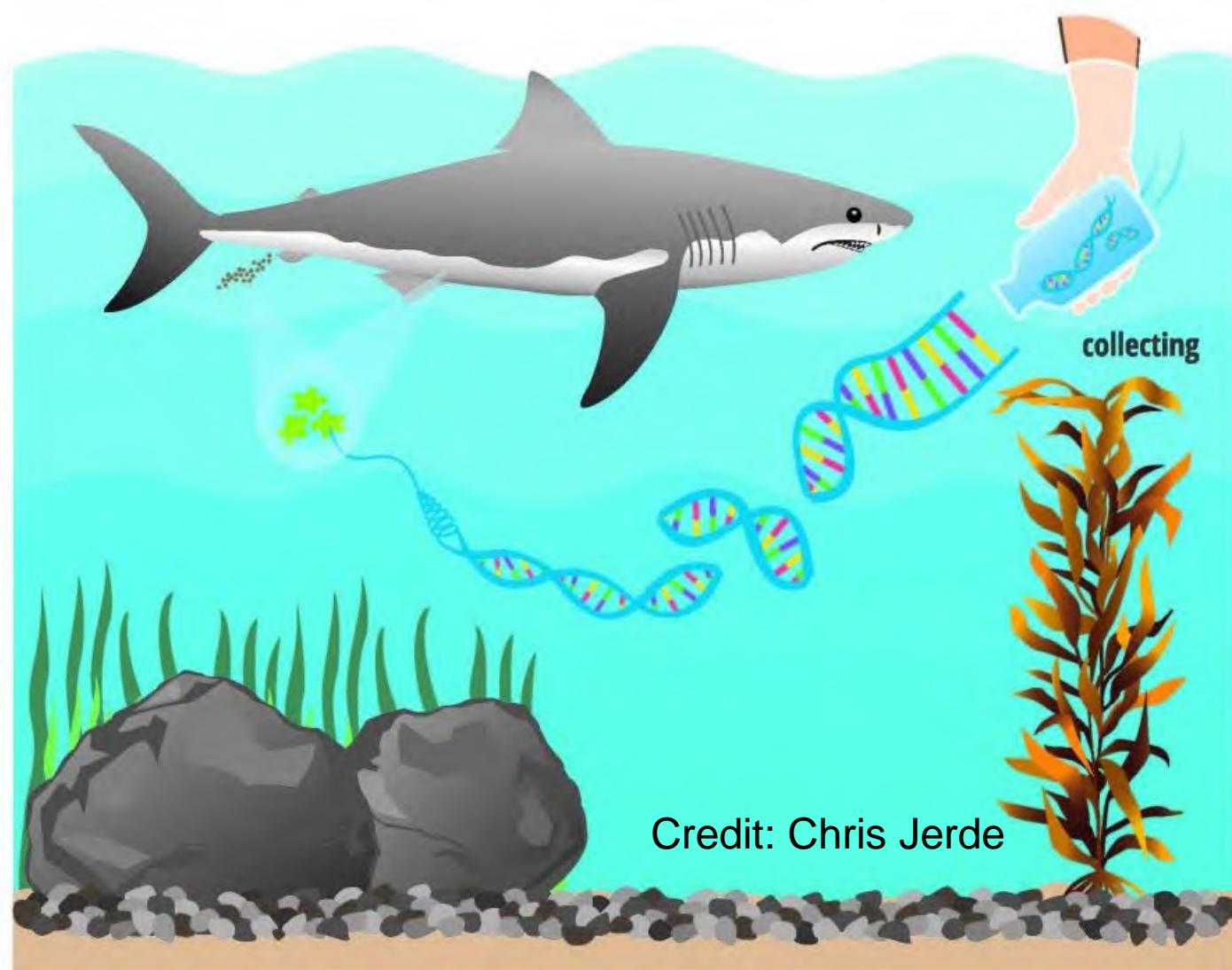


# Challenging to capture and identify organisms



Wellcome Images

# Can eDNA surveys help test global NIS transport models?



## Benefits

- ✓ Easier fieldwork
- ✓ Easier standardization
- ✓ Taxonomic expertise not required

## Drawbacks

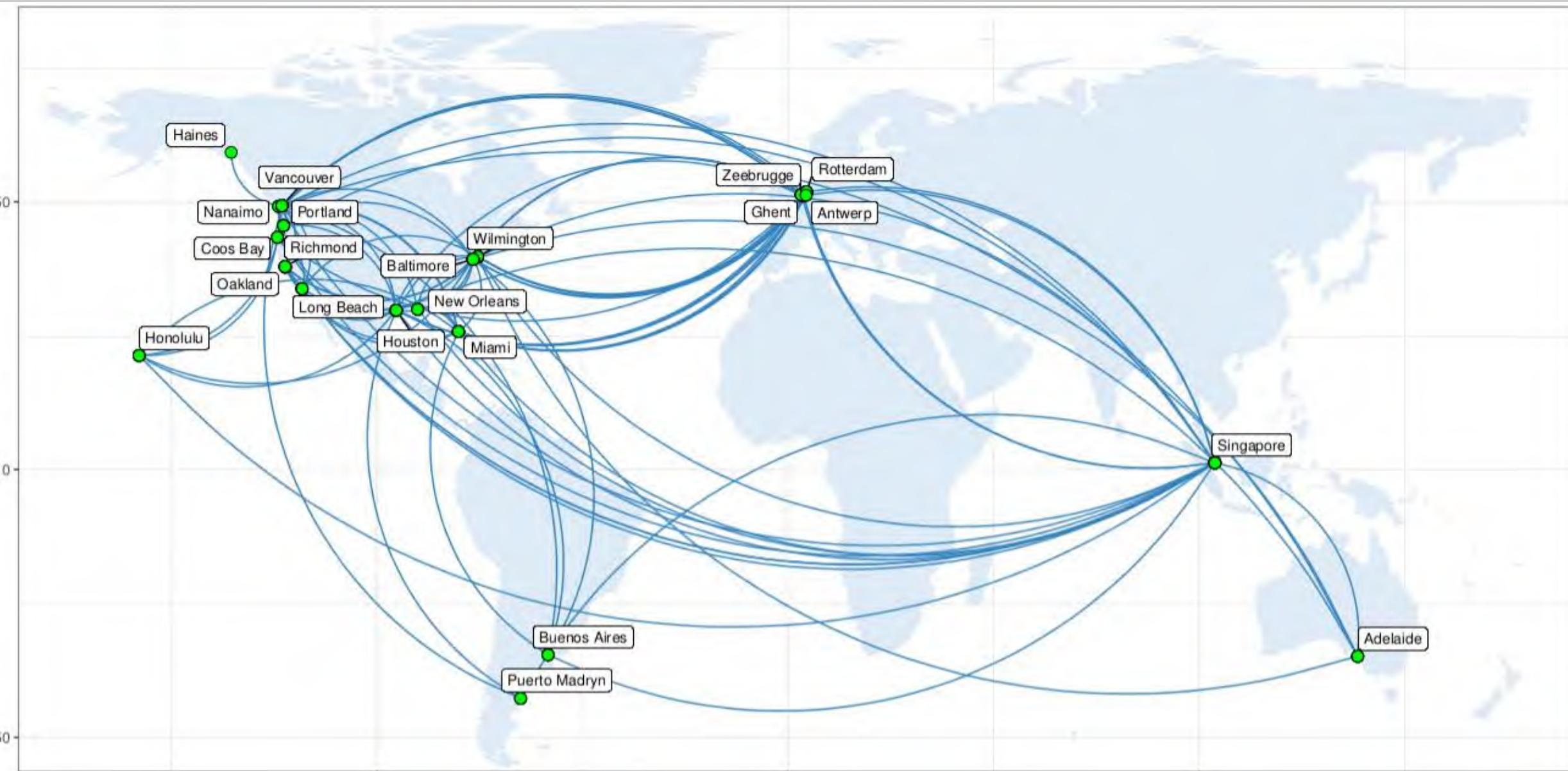
- ✗ Cannot identify all species
- ✗ No size/stage/condition info
- ✗ False positives
- ✗ False negatives



## METABARCODING DATA

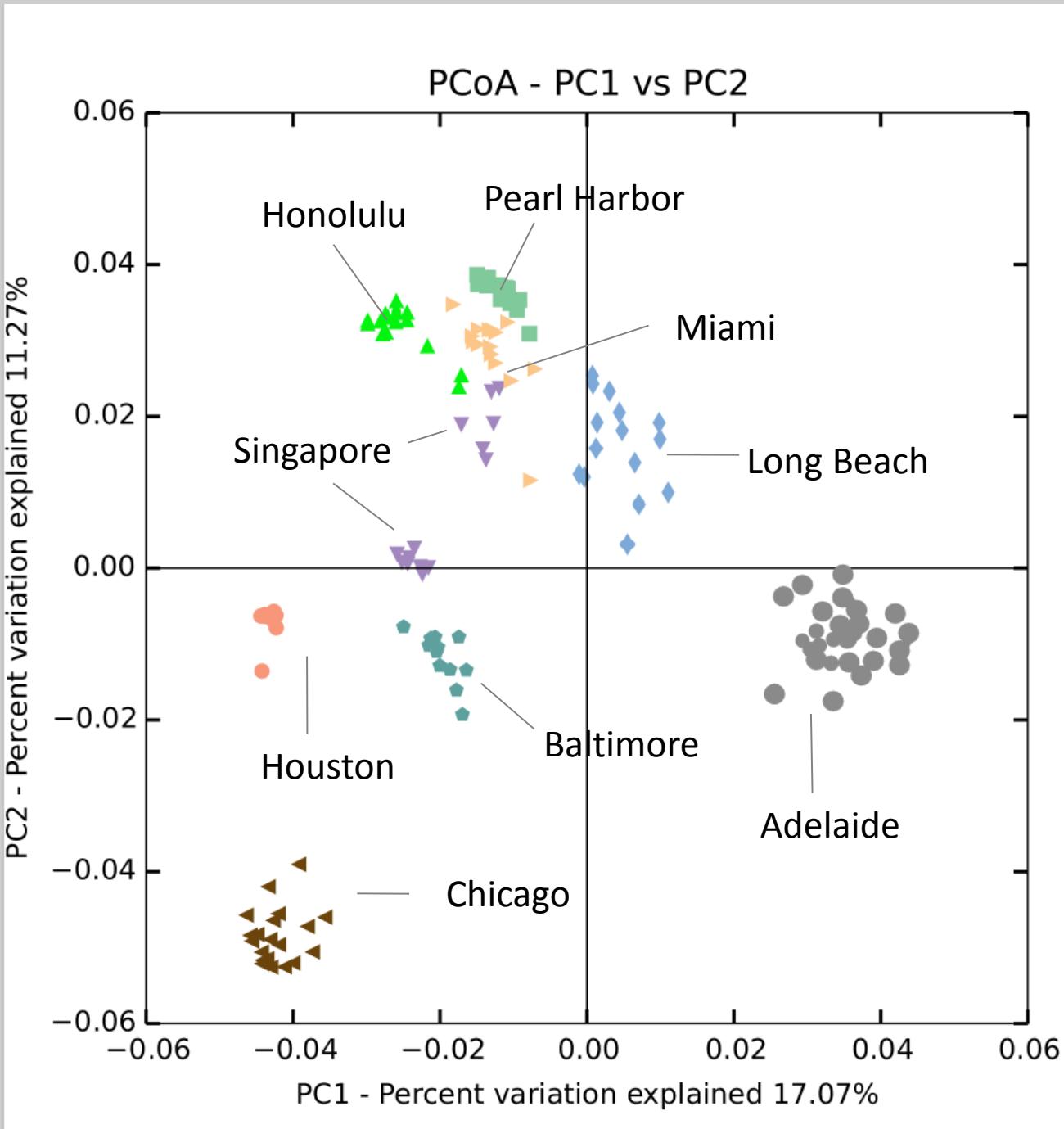
amplicon sequence variant **ASV 1** ATGCGGATGCATCGCTGACTAGCTATTGGACATGTA...  
**ASV 2** ATGCGGATGCATCGCTGACTAGCTATTGGACATGTG...  
**ASV 3** ATGTAGCAGCATCGCTGACCCCCCTAGTTGGACATGTA...  
**ASV 4** ATAAGCTTGCATCGCTGACTAGCTATTGGACATGTC...  
**ASV 5** ATGCAGATGCATCGCTGACCCGTCAAATTGGACATGAA...  
**ASV 6** ATTGCGATTATGCATCGCTGACTAGCTATTGTCCCCATGTT...  
ASV 7 ACGCGGATGCATCGCTGACTAGCTATTGGACATGTA...  
**ASV 8** ATGCAGATTTCAGGGGACTAGCTATTATGGACATGG...  
**ASV 9** ACGCGTATGCGATCGCTGACTAGCTATTCTGGACATGTA...

# 22 port survey



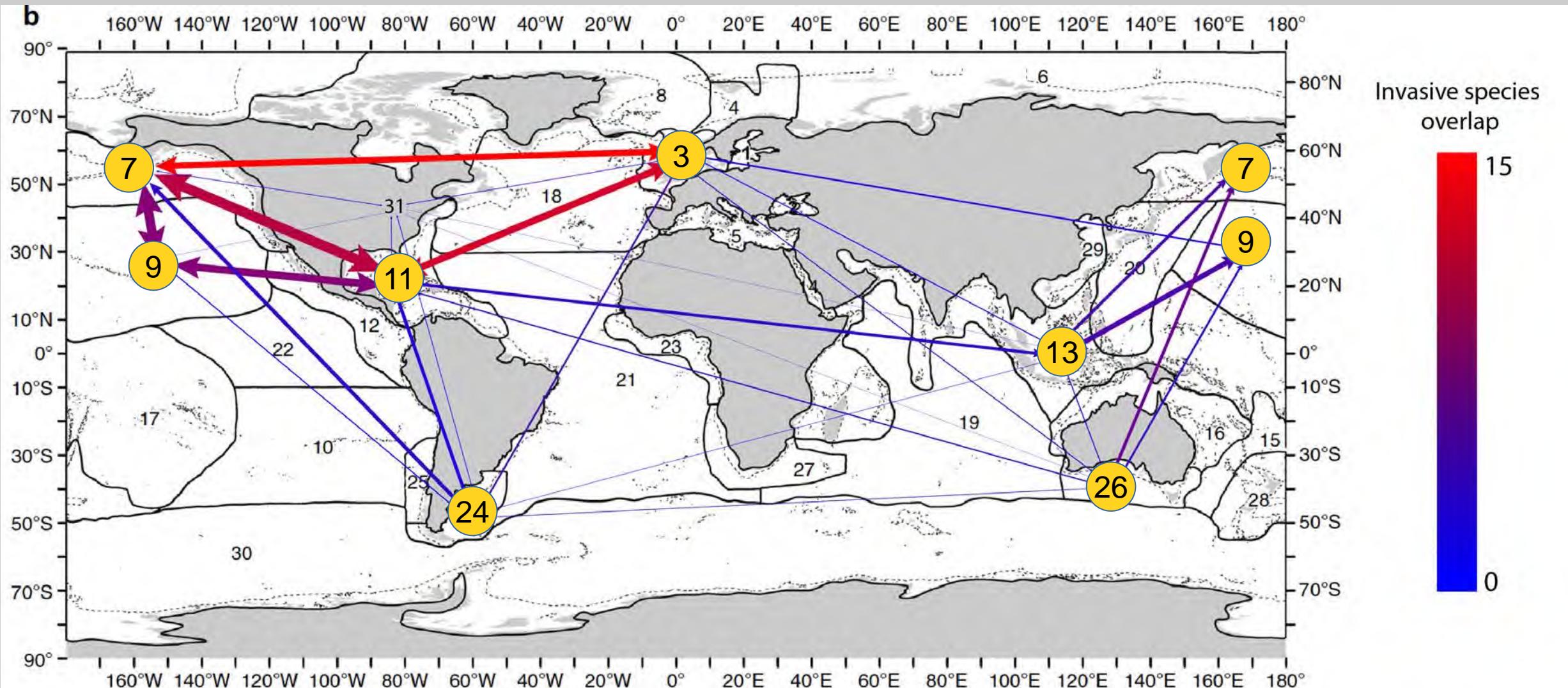
# Survey Results

- 22 ports/173 samples
- General eukaryote 18S barcode
- ~90 million raw sequences
- 17,586 clean eukaryote ASV's
- Distinct  $\beta$ -diversity separates ports
- $\beta$ -diversity pattern very robust to sampling effort and dissimilarity index



# Species assignments must be treated w caution, ***but....***

- ✓ The most common taxa make sense: *Bestiolina similis* (copepod), *Skeletonema menzeli* (diatom), *Synchaeta pectinata* (rotifer)
- ✓ eDNA-based overlap of all species (line width) & NIS (color) btwn realms looks about right.



# Testing a simple hypothesis: Shipping decreases genetic dissimilarity between ports.

$$Unifrac.Dist_{AB} \sim Env.Dist_{AB} + Biogeо.Dist_{AB} + Voyages_{AB} + (1|PortA) + (1|PortB)$$

Genetic dissimilarity between ports

*depends on*

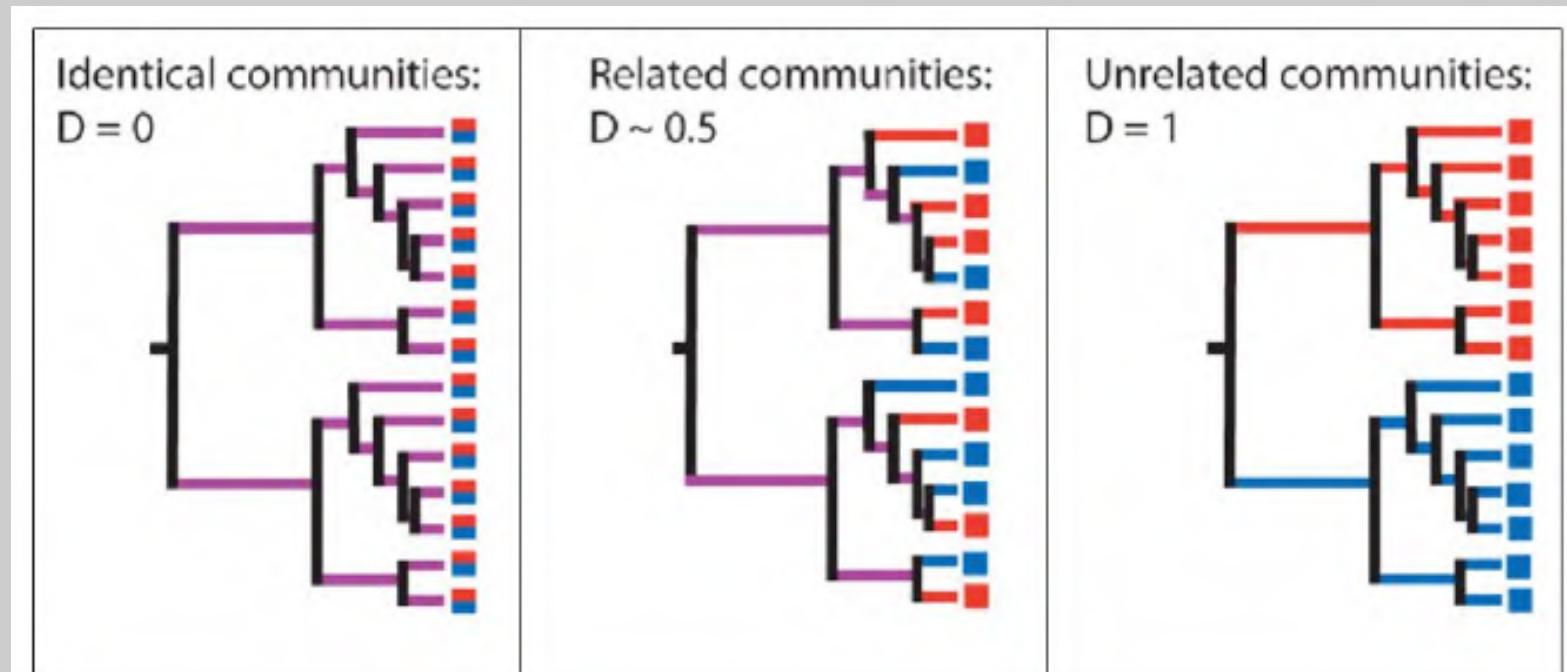
Environmental similarity between ports

Are ports in same realm?

# of voyages between ports

$$\text{Unifrac.Dist}_{AB} \sim \text{Env.Dist}_{AB} + \text{Biogeо.Dist}_{AB} + \text{Voyages}_{AB} + (1/\text{PortA}) + (1/\text{PortB})$$

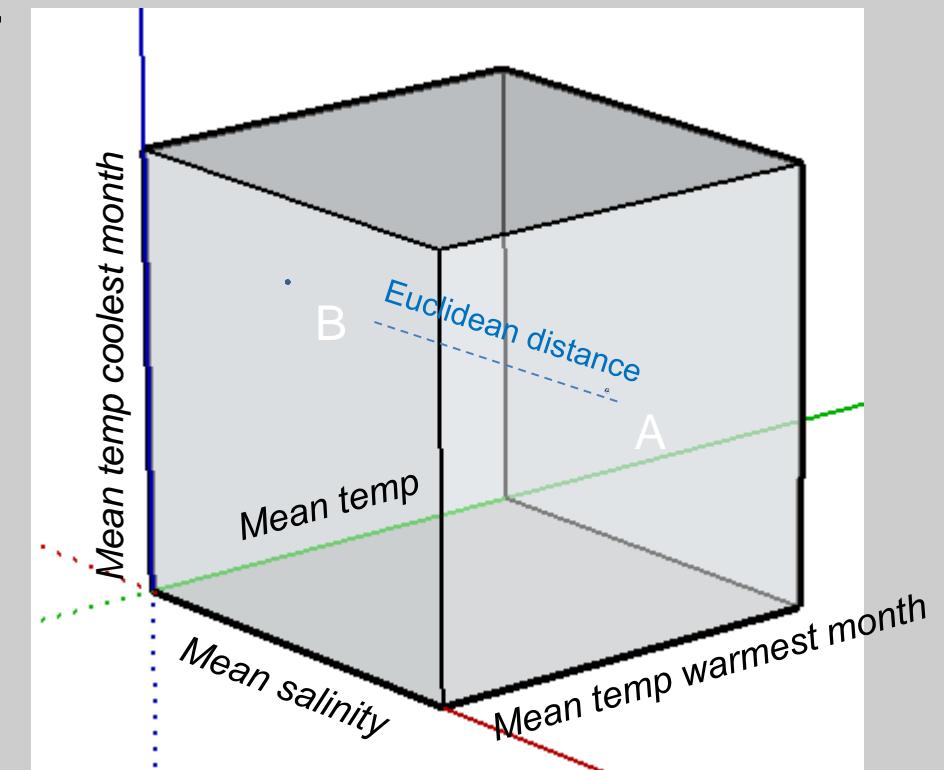
**UniFrac** is community dissimilarity metric that incorporates phylogenetic distances between organisms in each community.



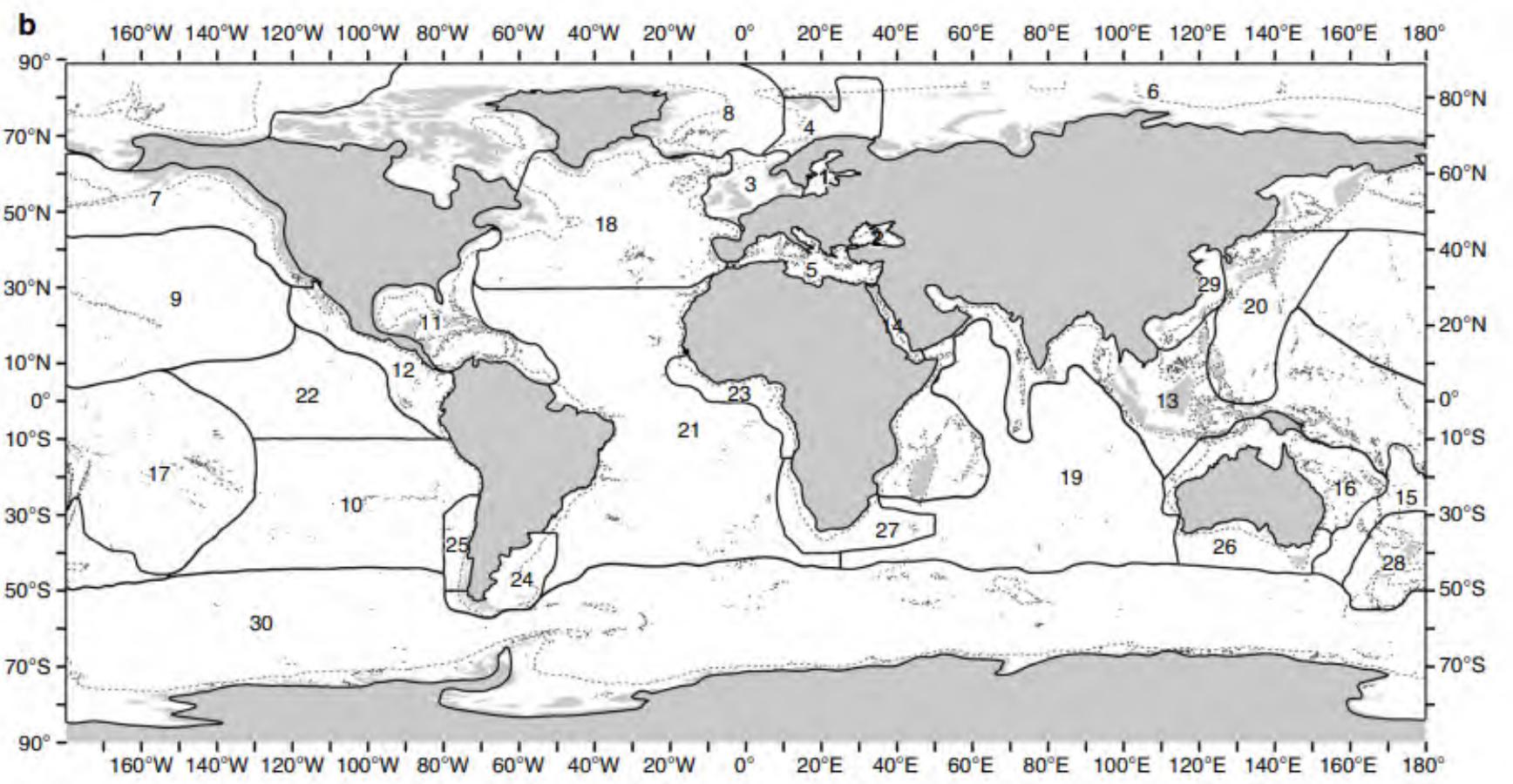
Lozupone and Knight, 2005, Appl Environ Microbiol 71:8228

$$Unifrac.Dist_{AB} \sim Env.Dist_{AB} + Biogeo.Dist_{AB} + Voyages_{AB} + (1|PortA) + (1|PortB)$$

- Scaled Euclidean distance between 4 port environmental variables  
(Keller et al 2011 – World Ocean Atlas 2013):
  - mean annual temp
  - mean temp warmest month
  - mean temp coolest month
  - mean salinity



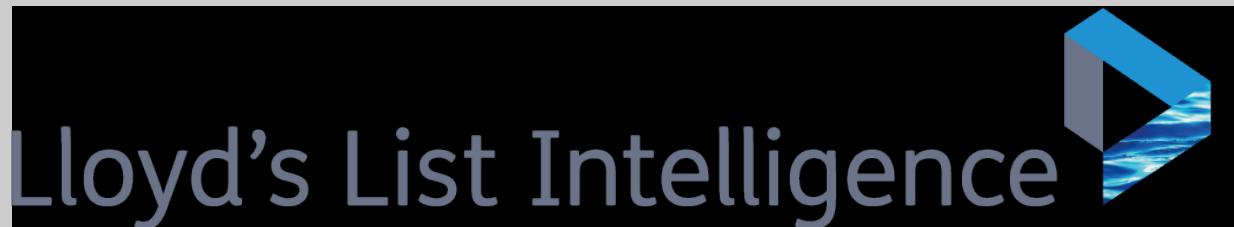
$$Unifrac.Dist_{AB} \sim Env.Dist_{AB} + Biogeog.Dist_{AB} + Voyages_{AB} + (1|PortA) + (1|PortB)$$



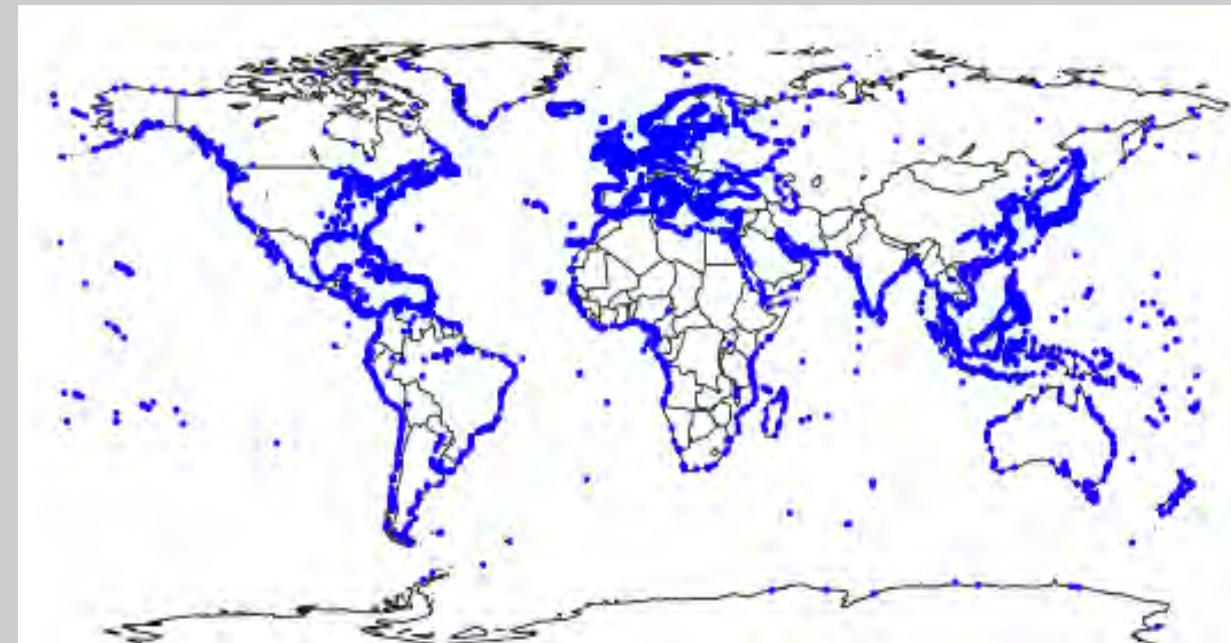
Marine realms from Costello et al.

Same Realm = 0  
Different Realm = 1

$$UnifracDist_{AB} \sim Env.Dist_{AB} + Biogeо.Dist_{AB} + Voyages_{AB} + (1|PortA) + (1|PortB)$$

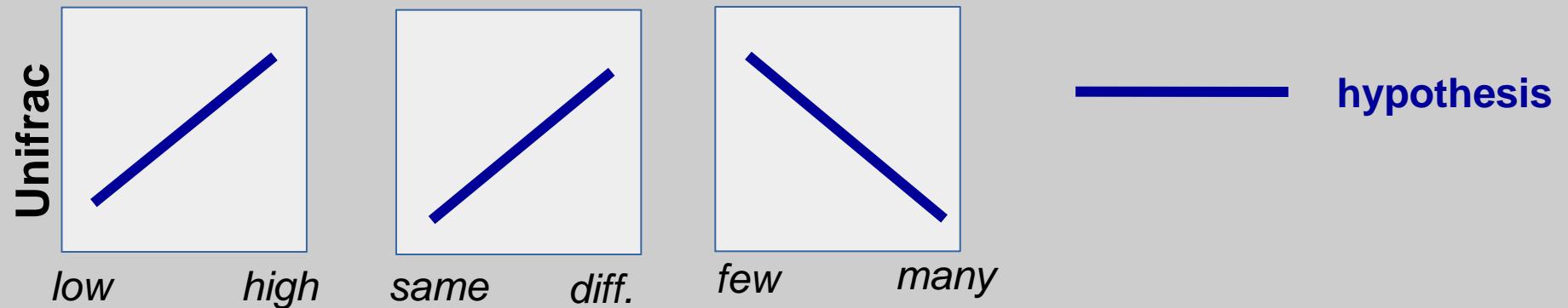


- Ship traffic data from Lloyds Maritime Intelligence unit
- Sum all voyages between port pairs from years 1997, 2002, 2008, 2012



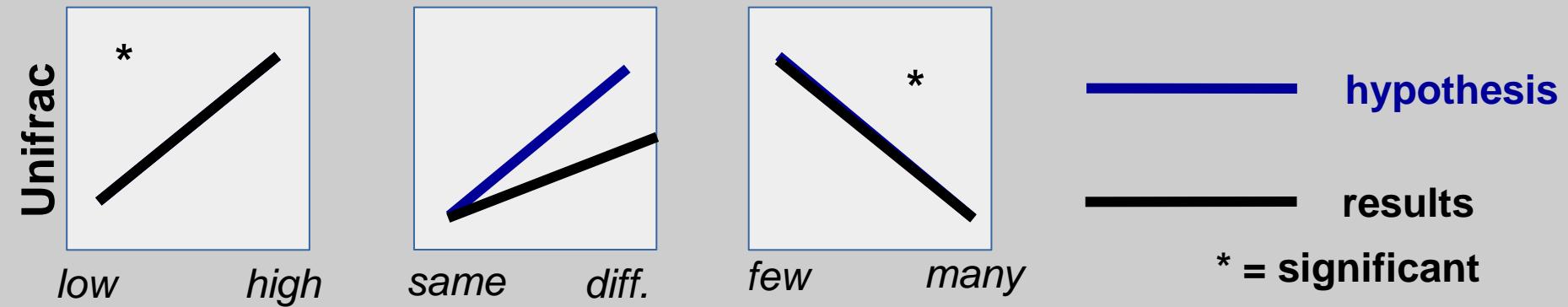
# Statistical Model - Hypotheses

$$UnifracDist_{AB} \sim Env.Dist_{AB} + Biogeо.Dist_{AB} + Voyages_{AB} + (1|PortA) + (1|PortB)$$



# Statistical Model - Results

$$UnifracDist_{AB} \sim Env.Dist_{AB} + Biogeо.Dist_{AB} + Voyages_{AB} + (1|PortA) + (1|PortB)$$



- This model was better than a null model (not shown).
- The slopes were going in predicted direction for all 3 factors.
- Environmental similarity and voyages significant, realm not significant.

# Future Work

1. More ports

- Canadian Arctic!
- China!

2. Refine Env.dist  
& Biogeo.dist  
variables

3. Test more  
complex  
hypotheses



# Remember this:

- ✓ eDNA metabarcoding resolves port biodiversity patterns nicely
- ✓ Preliminary support for hypothesis that shipping homogenizes port communities

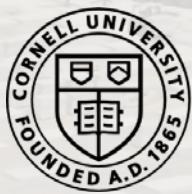


# Thank You!

- NSF Coastal Sees Grant #1427157
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- Undergrads Yuri Lopez, Reni Truhtcheva-Owikoti, Clarice Gumm, Xiara Moreno, Andrea Fuentes
- 20+ port sampling volunteers!
- NIS-RAPS collaborators
- Giles Hooker, Cornell University Statistics Consulting Unit



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