An underwater photograph showing a diver in the upper left, a school of blue fish in the middle, and a large coral reef in the foreground. The water is clear blue.

Censusing the Sea in the 21st Century

Nancy Knowlton
&
Matthieu Leray

Smithsonian's
National Museum
of Natural History

Photo: Ove Hoegh-Guldberg

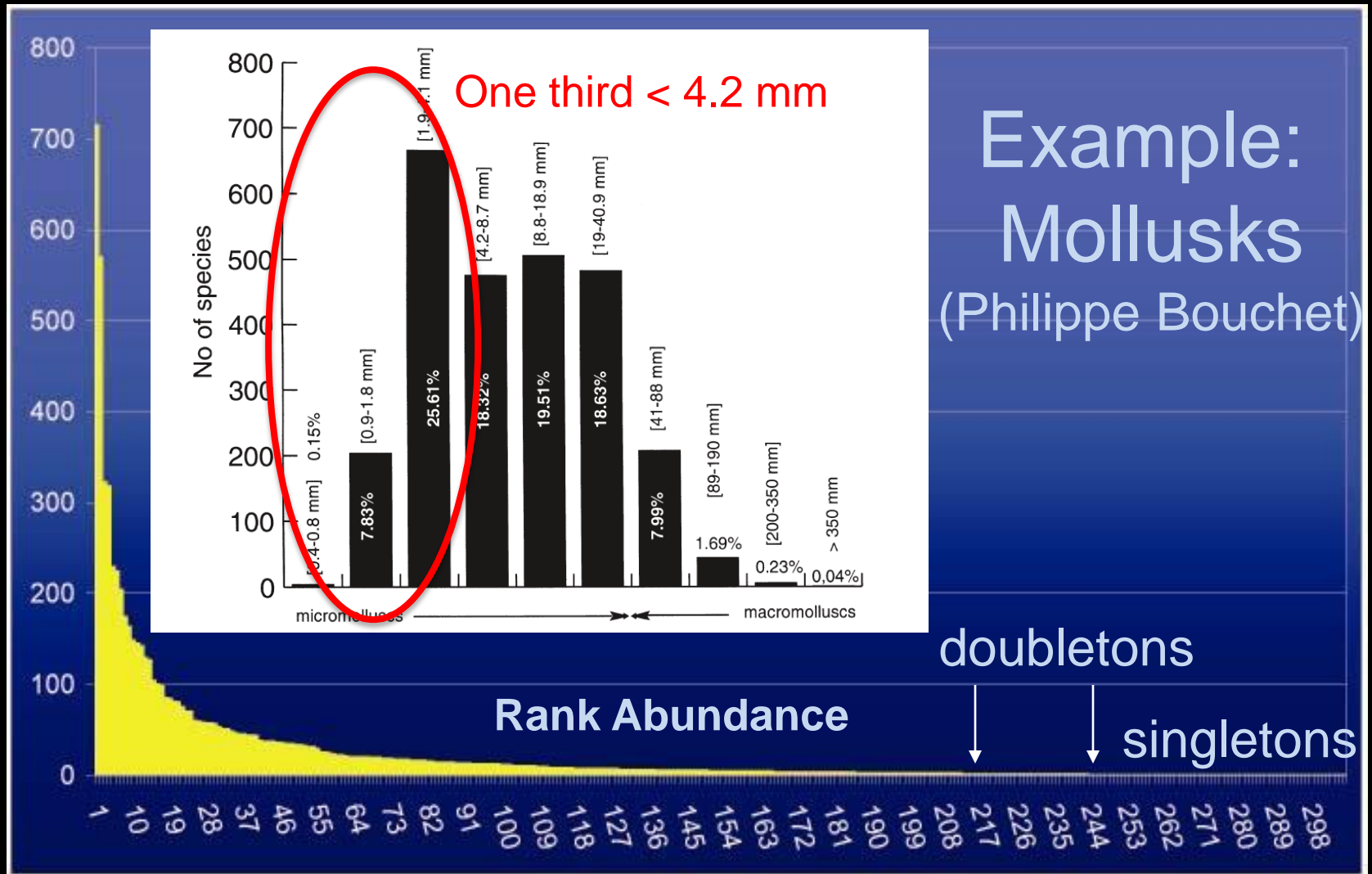
Estimates of Marine/Reef Species Numbers (Millions)

Marine Habitat	Global Ocean	Data Type	Author, Date
10 deep sea	—	box cores, 21 m ²	Grassle & Maciolek, 1992
0.6-9.5 coral reefs	1.9-30.6*	tropical forests, species-area	Reaka-Kudla, 1997
1.7-3.2 coral reefs	5.5-10.3*	5 m ² reef samples, species-area	Small et al., 1998
0.4-0.5* Coral reefs	1.4-1.6	European brachyuran crabs	Bouchet, 2006
0.7* coral reefs	2.2	ratios of taxa	Mora et al., 2011
0.2-0.3* coral reefs	0.7-1.0	expert opinion	Appeltans et al., 2012
0.5-1.3 coral reefs	1.6-2.4*	expert opinion	Fisher et al. 2015

*Assumes 31% of marine species live on reefs

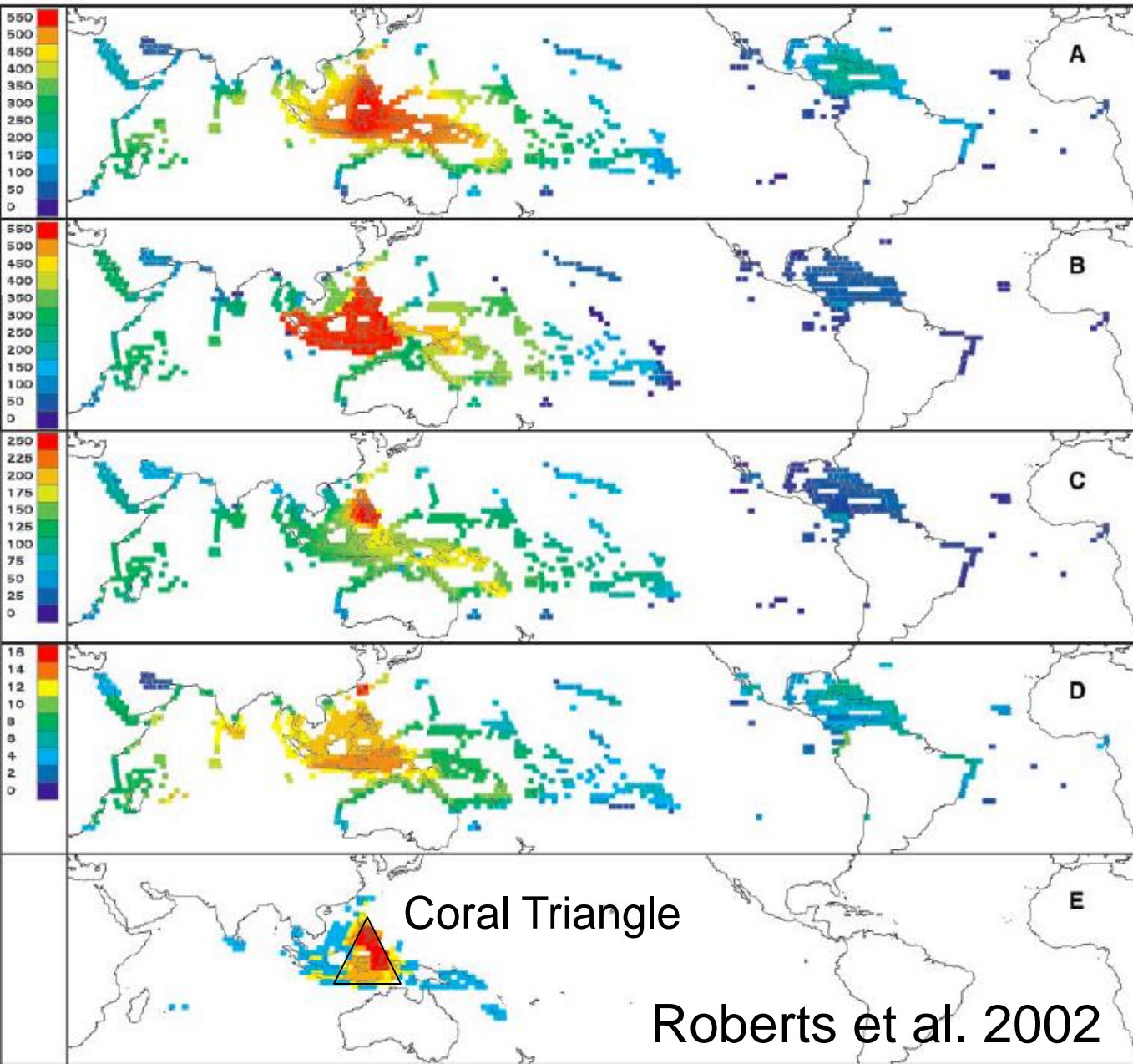
Why so hard?

Most marine species are small and rare



33-91% of all marine species still to be named

Global analyses ignore almost all biodiversity



Roberts et al. 2002

Some fish
1700 spp

Corals
804 spp

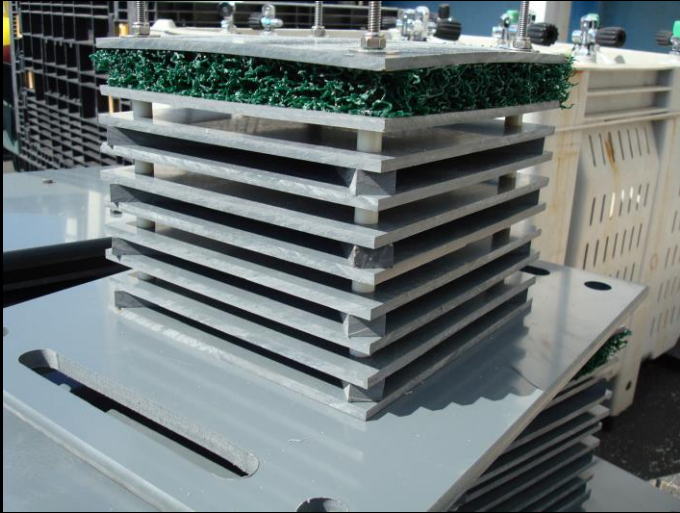
Some snails
662 spp

Lobsters
69 spp

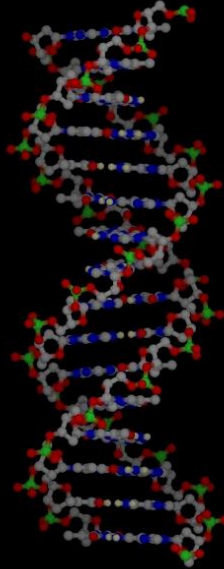
All???
3235 spp
(~250,000 descr.)

Census of Marine Life - CReefs

Artificial Surfaces (ARMS)



DNA



Natural Surfaces (dead heads)



Standardization -

Allows results of different studies to be compared

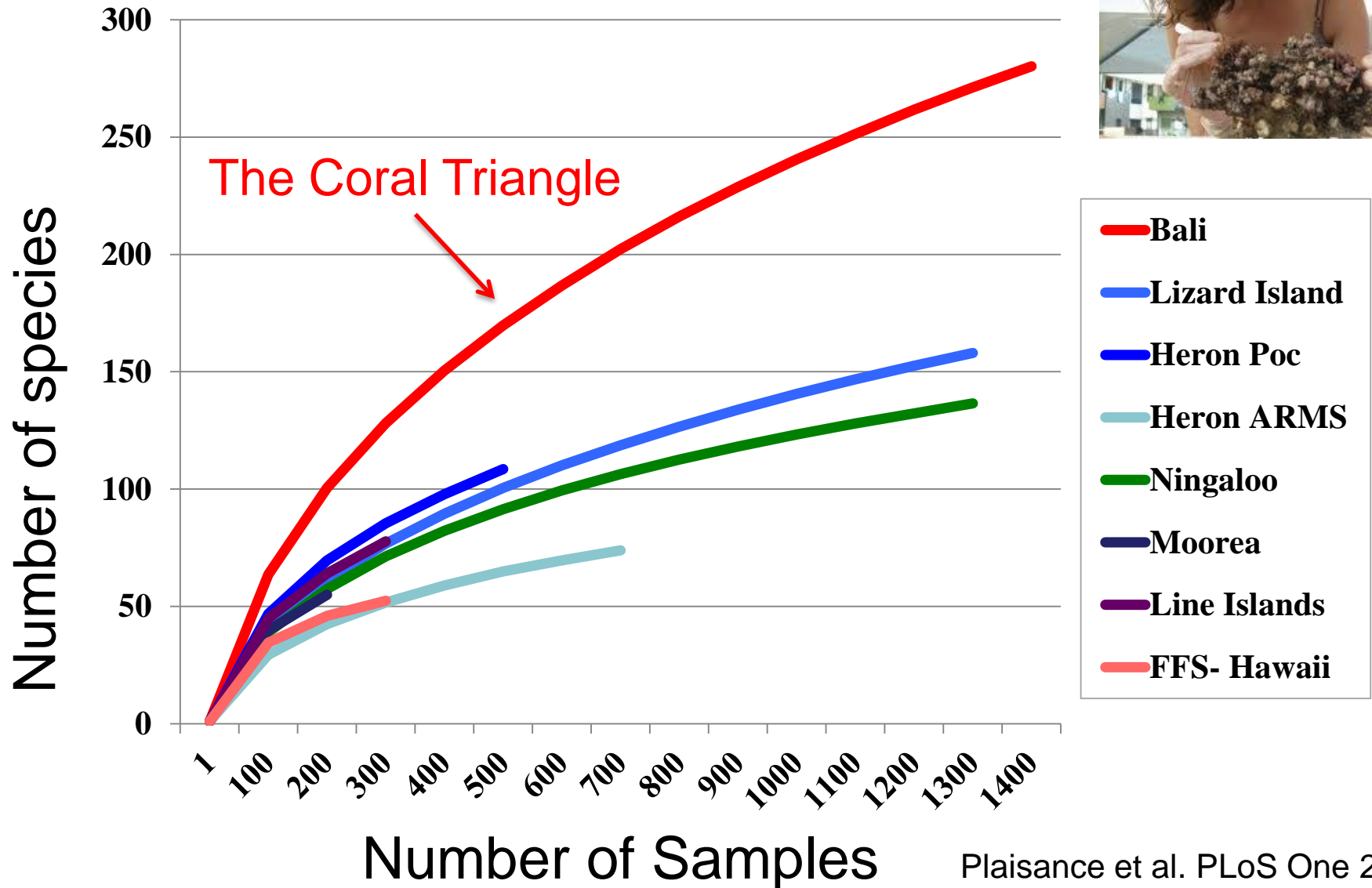
Automation -

Identify species using genetics rather than names

Scalability -

World-wide sampling strategy is feasible

First Studies: Barcoding Crustaceans



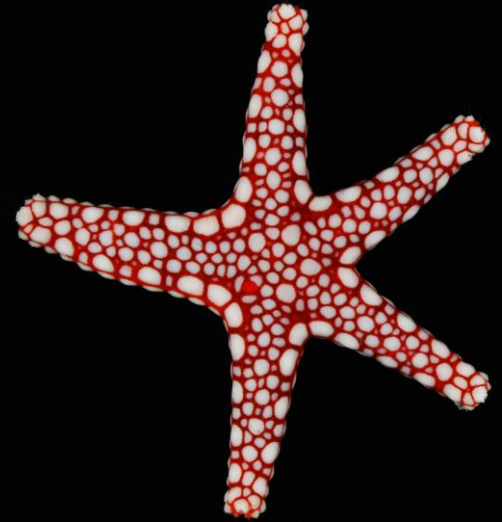
Plaisance et al. PLoS One 2011
Unpublished data

Put Another Way....



Just 6.3 m² contained the equivalent of 80% of the described crab diversity of European seas!

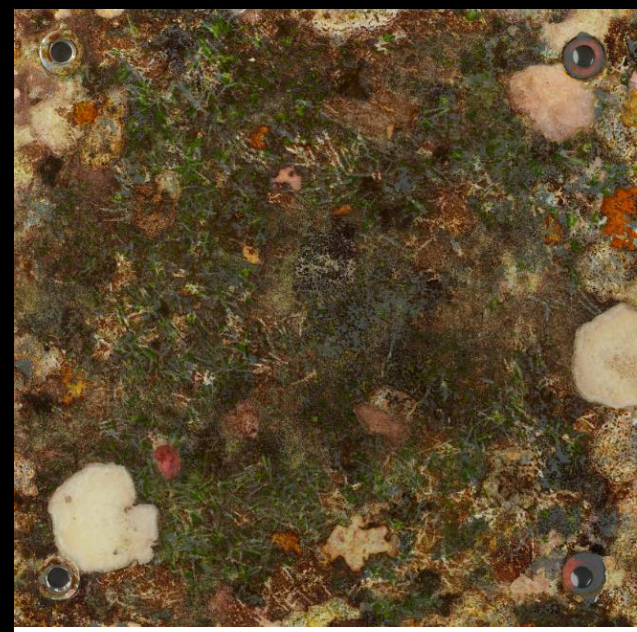
Barcoding works well for the charismatic microfauna



Photos: Matthieu Leray

What about the sessile stuff?

Indonesia (Photos: David Littschwager)



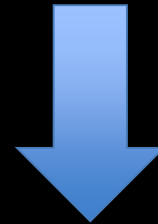
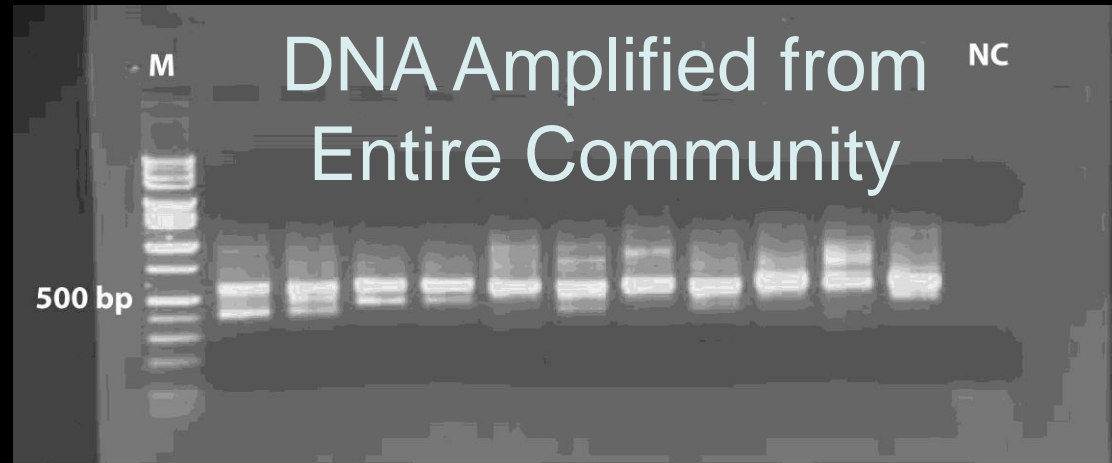
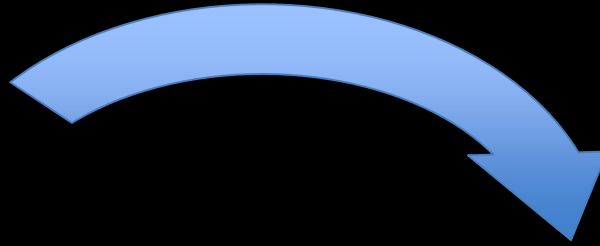


Or the fairly small stuff?
($<2\text{mm}$)

Photos: Matthieu Leray

From Barcoding to Metabarcoding

Bulk Sample



Next-Gen Metabarcodes
(easier said than done)

ARMS



4 Fractions
(+ vouchers)



1) sessile



2) > 2mm



COI barcode

COI Metabarcoding



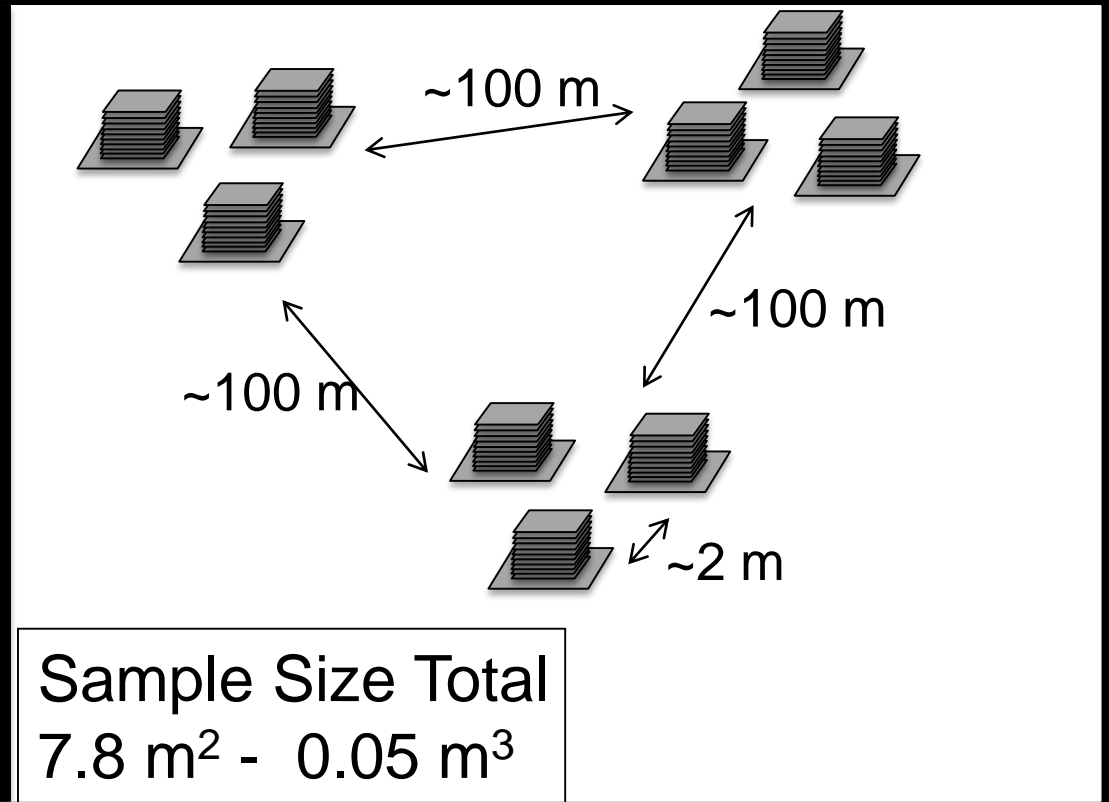
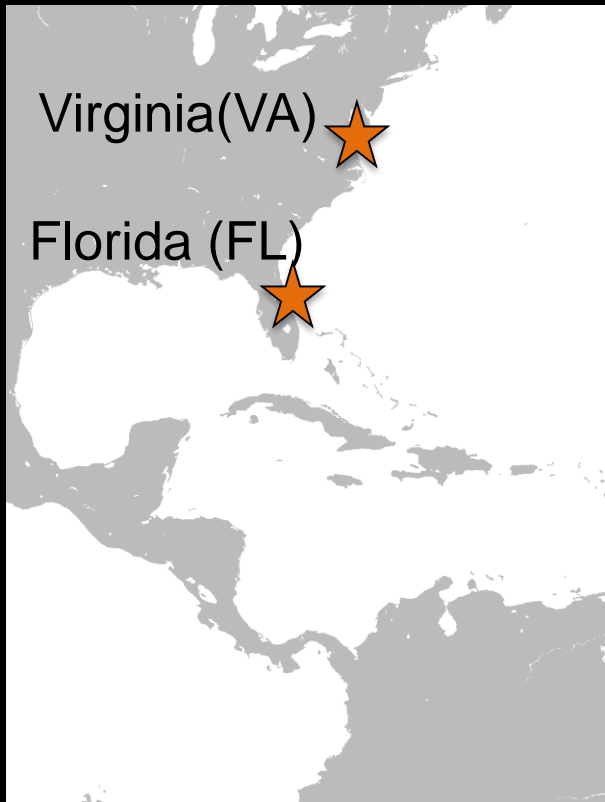
4) 500–106 µm



3) 2mm–500 µm

Oyster reefs (6-month deployment)

Leray & Knowlton, PNAS 2015



At what geographic scale
are communities different?

Chesapeake Bay, Virginia (9 ARMS)



Ft. Pierce, Florida (9 ARMS)



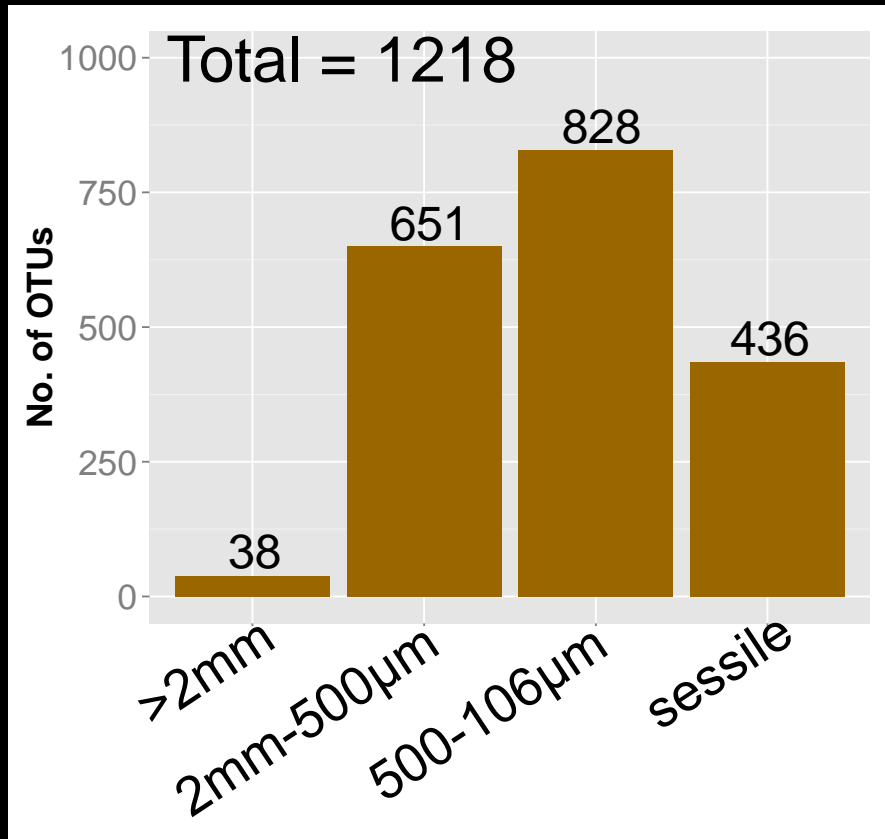
SHARED
4%
barcode
21%
meta
-barcode

#sequences: 572,290
% matched: 10.2
% unknown: 40.9
% singletons: 34.8

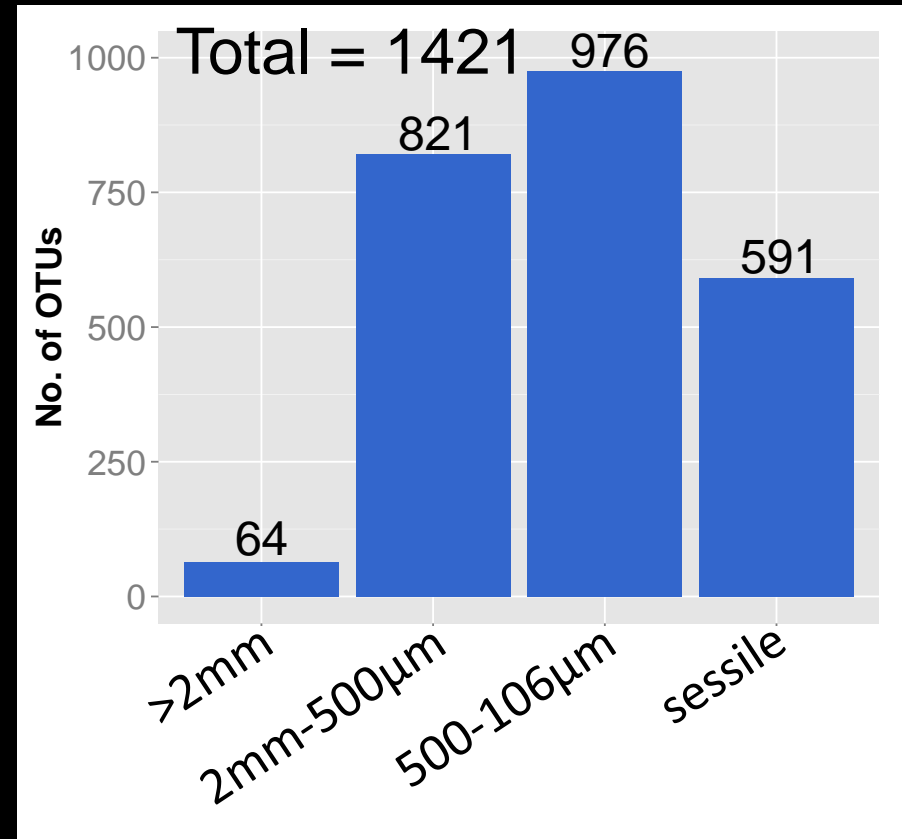
409,613
11.9
28.3
31.1

Diversity

Virginia



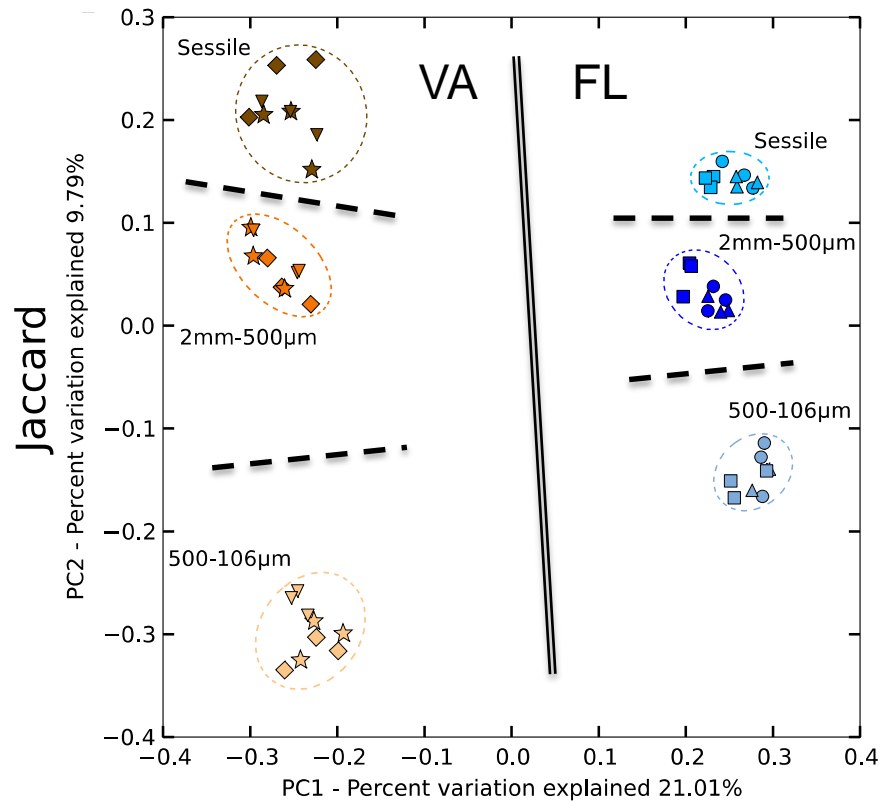
Florida



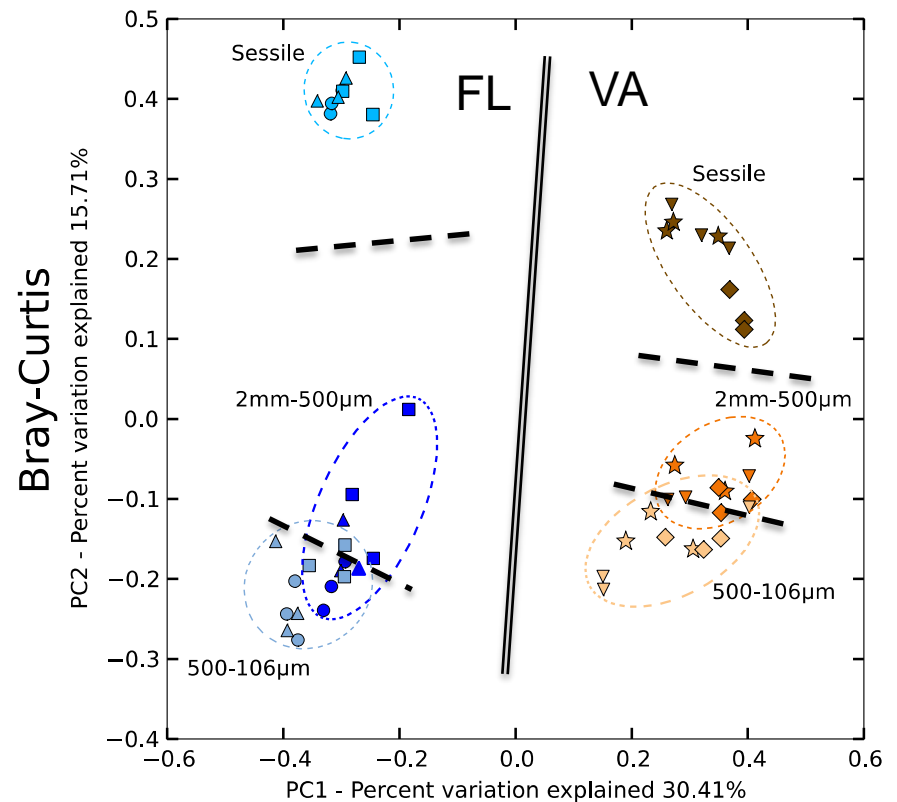
Sub-tropical Florida is more diverse
2/3 of diversity is < 500µm

Community similarity

Presence-absence



Relative abundance

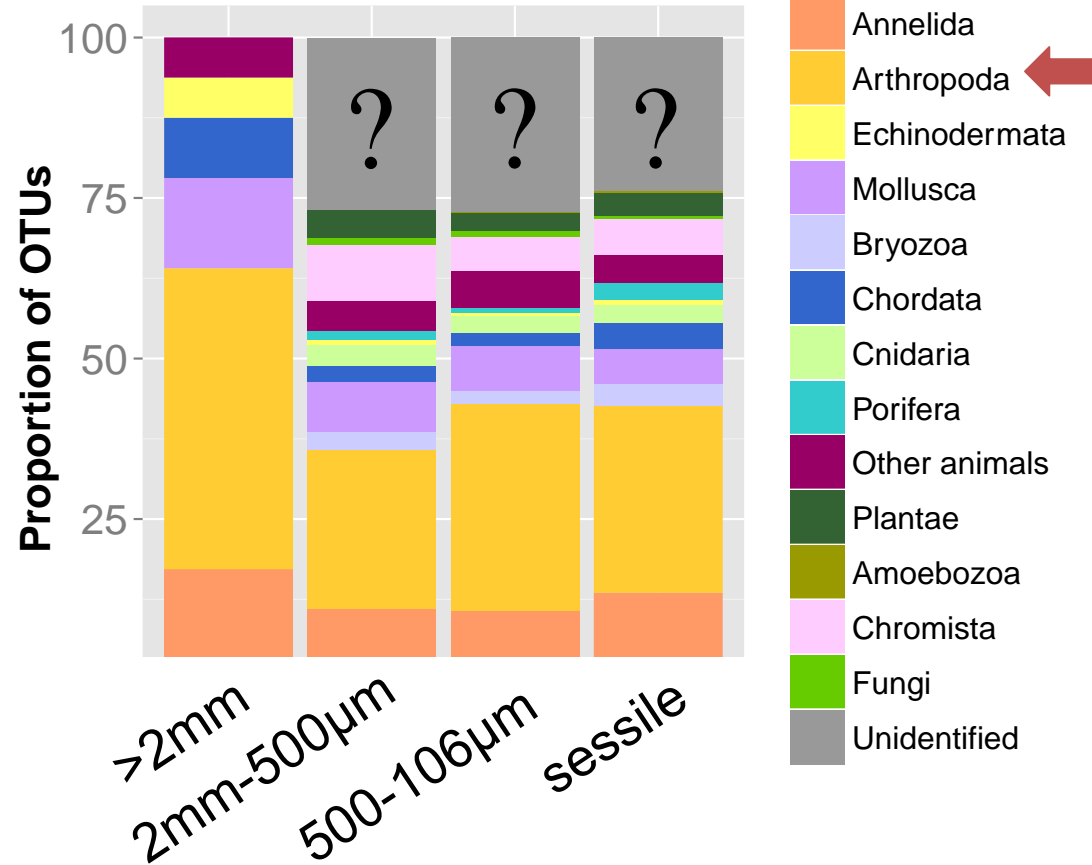
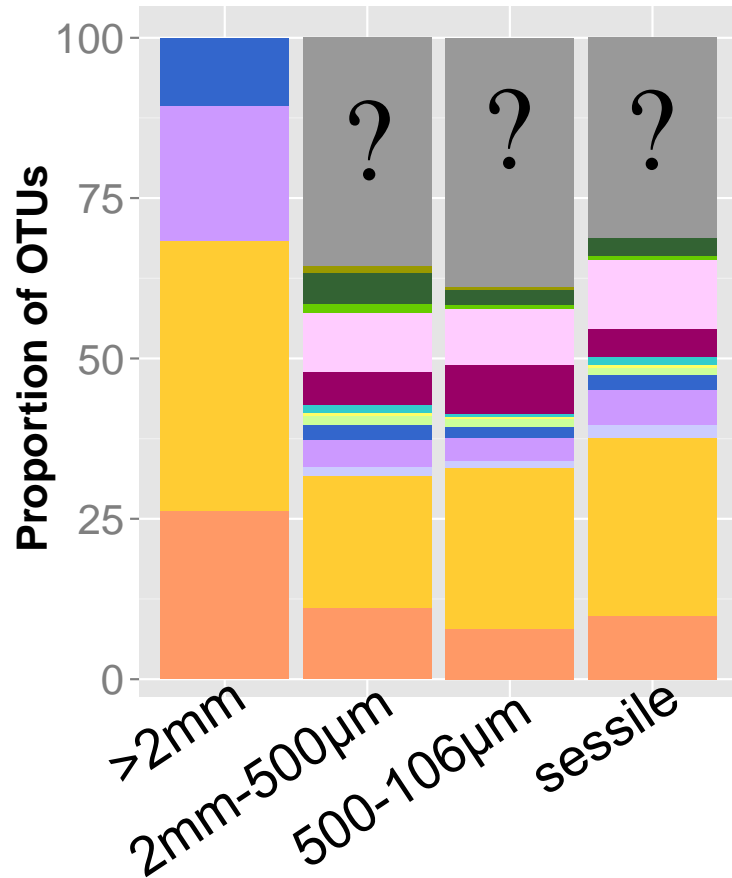


Different locations and metabarcoding communities are distinct

Taxonomic composition

Virginia

Florida



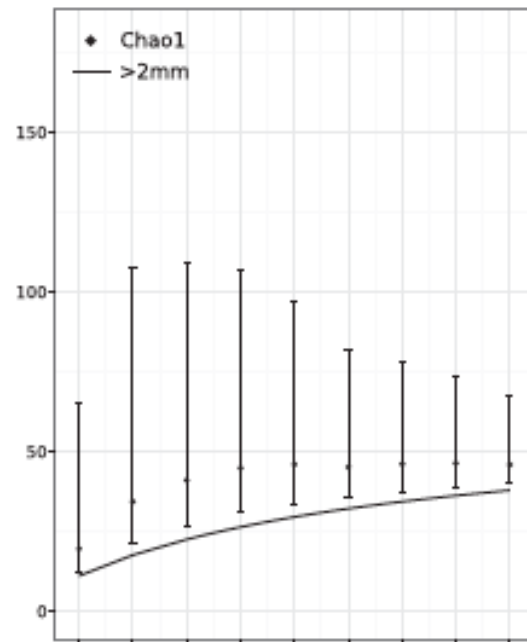
Leray & Knowlton, PNAS 2015

22 animal phyla - Arthropods most diverse

VA

DNA barcoding

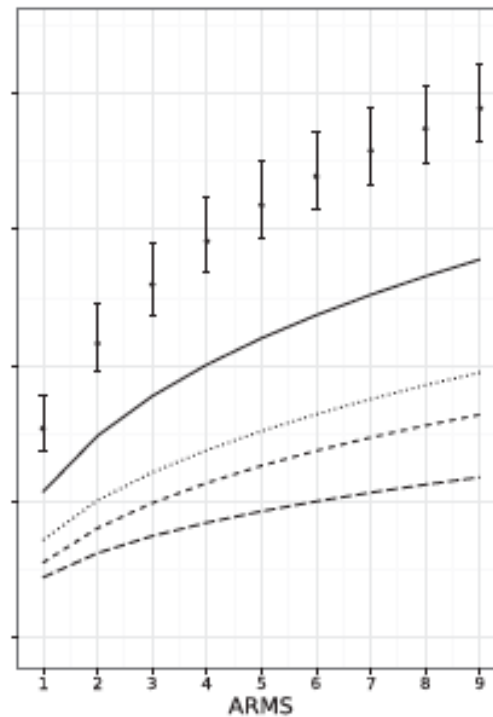
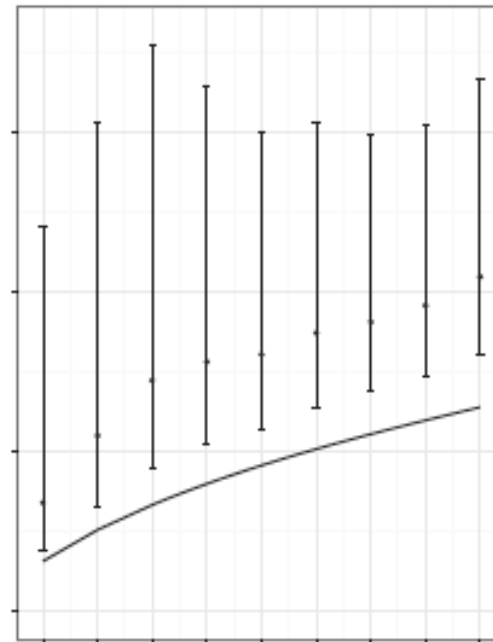
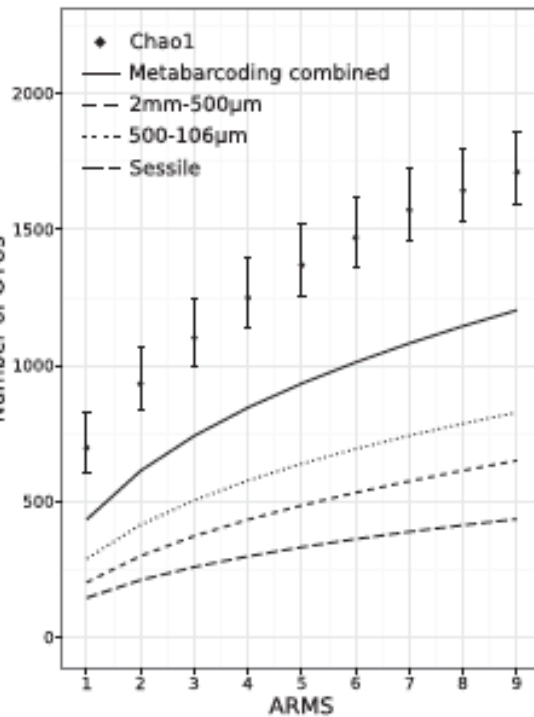
Number of OTUs



FL

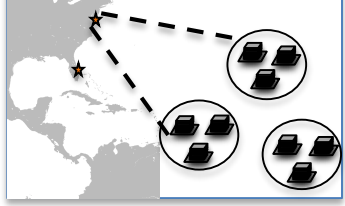
DNA metabarcoding

Number of OTUs



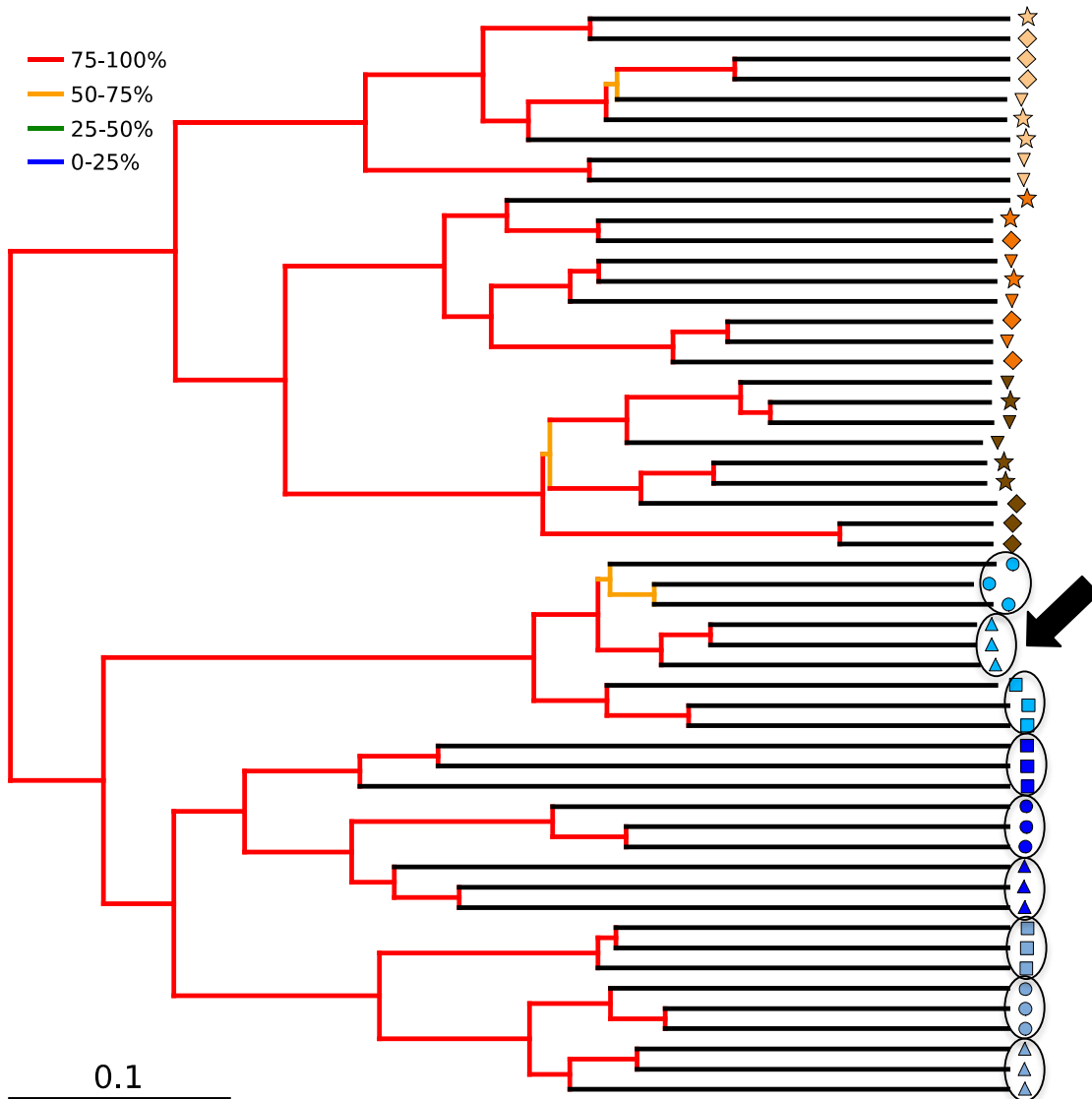
More Sampling Needed

(similar pattern for sampling effort measured by # sequences)



Jackknifed UPGMA
tree based on relative
abundance

- ☆ Site 1
 - ▽ Site 2
 - ◇ Site 3
 - Site 1
 - △ Site 2
 - Site 3
- Virginia
- Florida



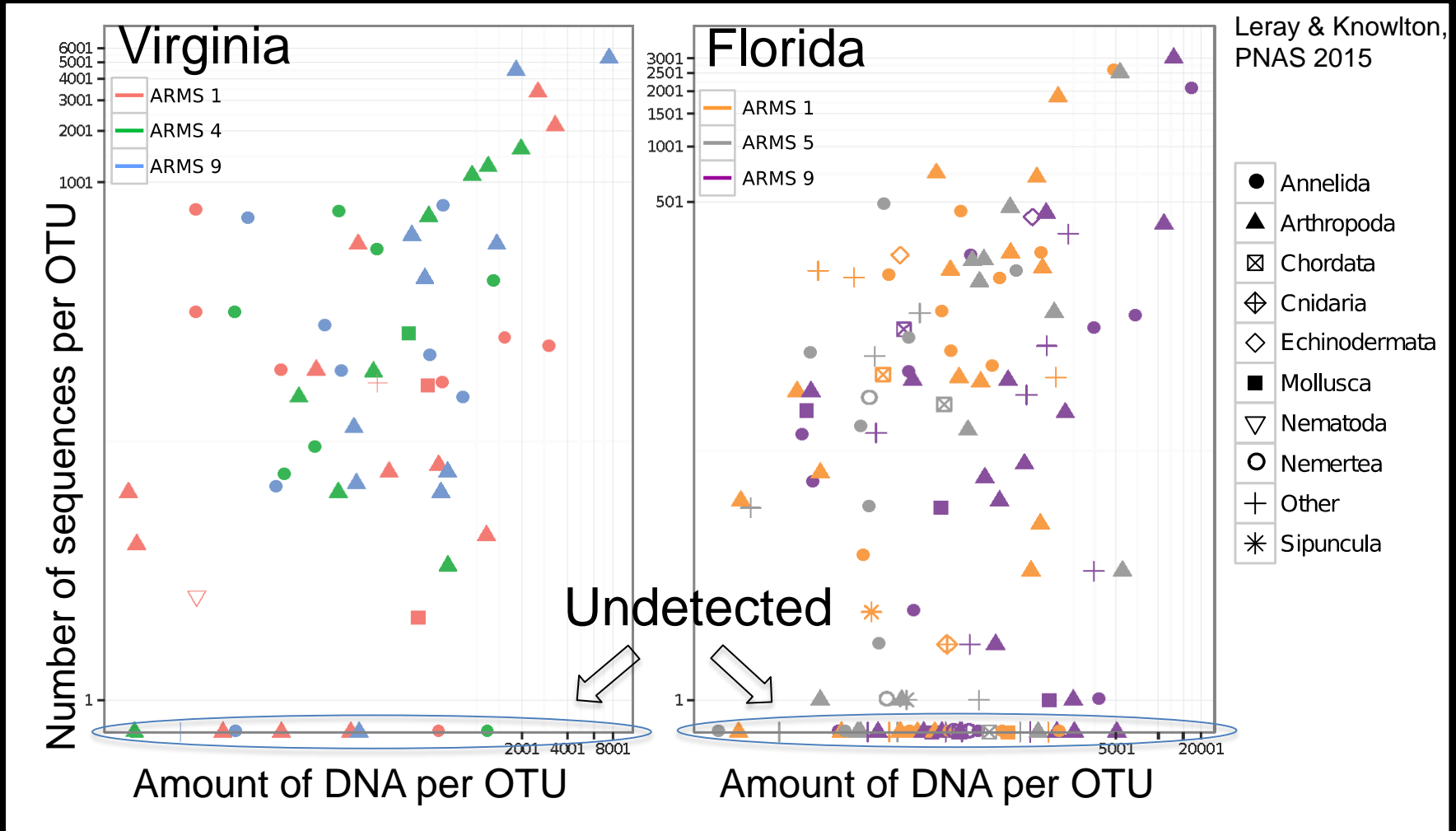
Fine-scale
geographic
structuring

VA

Adjacent
samples cluster
together at the
meter scale

FL

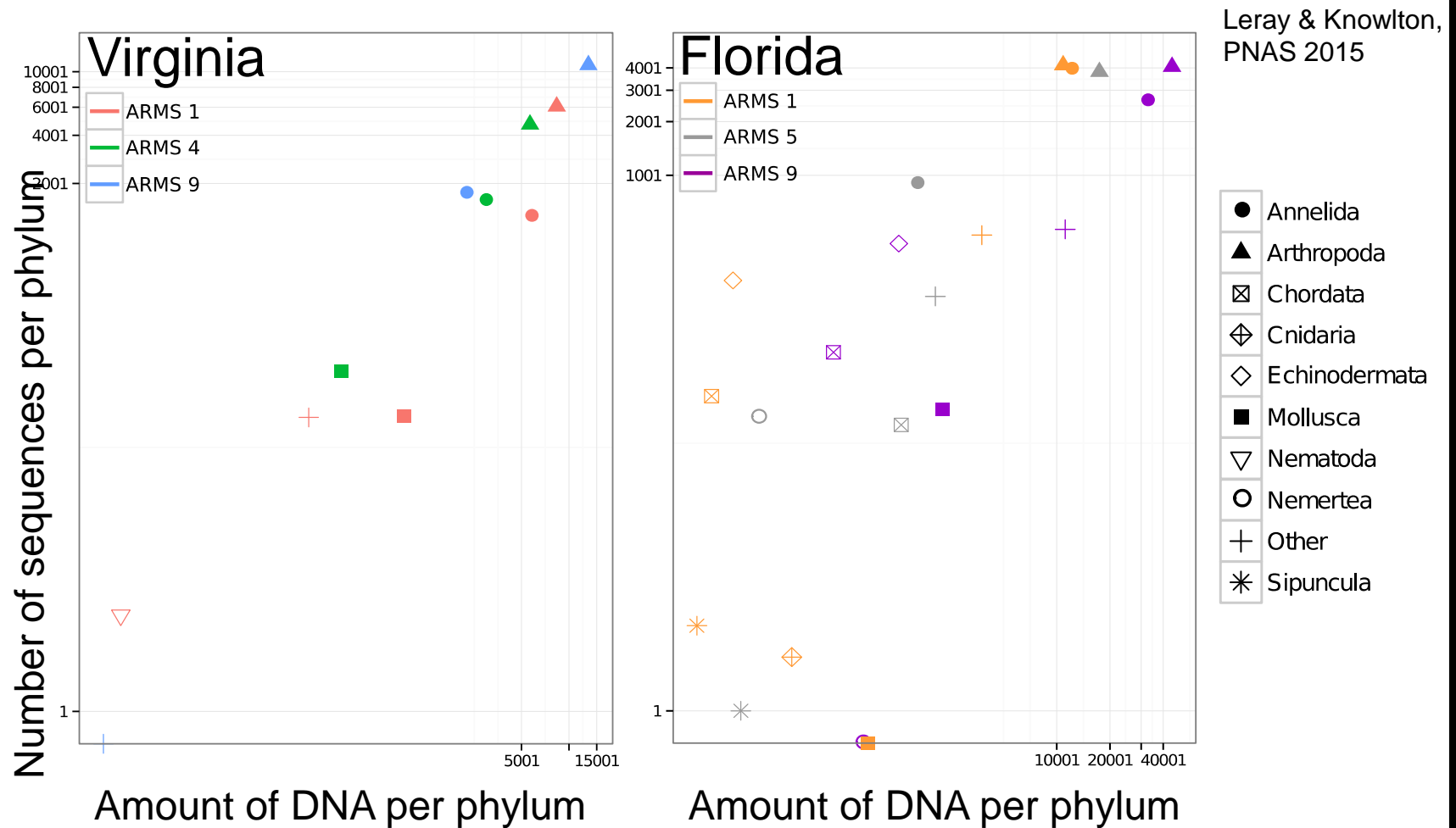
Abundance information as well as presence-absence



65.3% to 91.7% of OTUs successfully recovered per sample

Linear relationship between number of reads and amount of DNA

Even stronger relationship for functional groups



Similar results for sessile community
calibrated by % cover

Not Just an Abstract Exercise

Marine Biodiversity in the Anthropocene



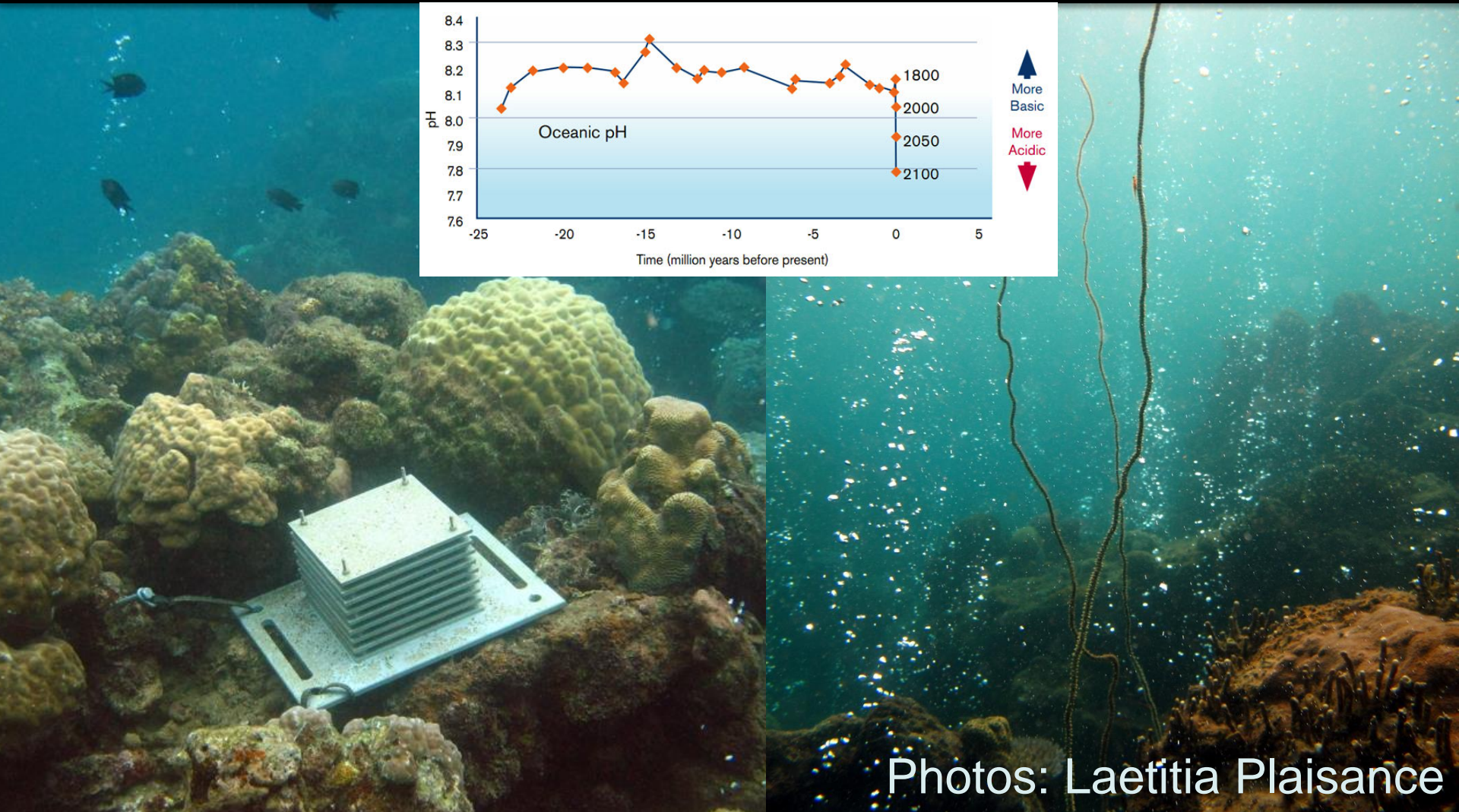
Marine defaunation: Animal loss in the global ocean

Douglas J. McCauley^{1,*}, Malin L. Pinsky², Stephen R. Palumbi³, James A. Estes⁴, Francis H. Joyce¹,
Robert R. Warner¹

Science 16 January 2015:
Vol. 347 no. 6219

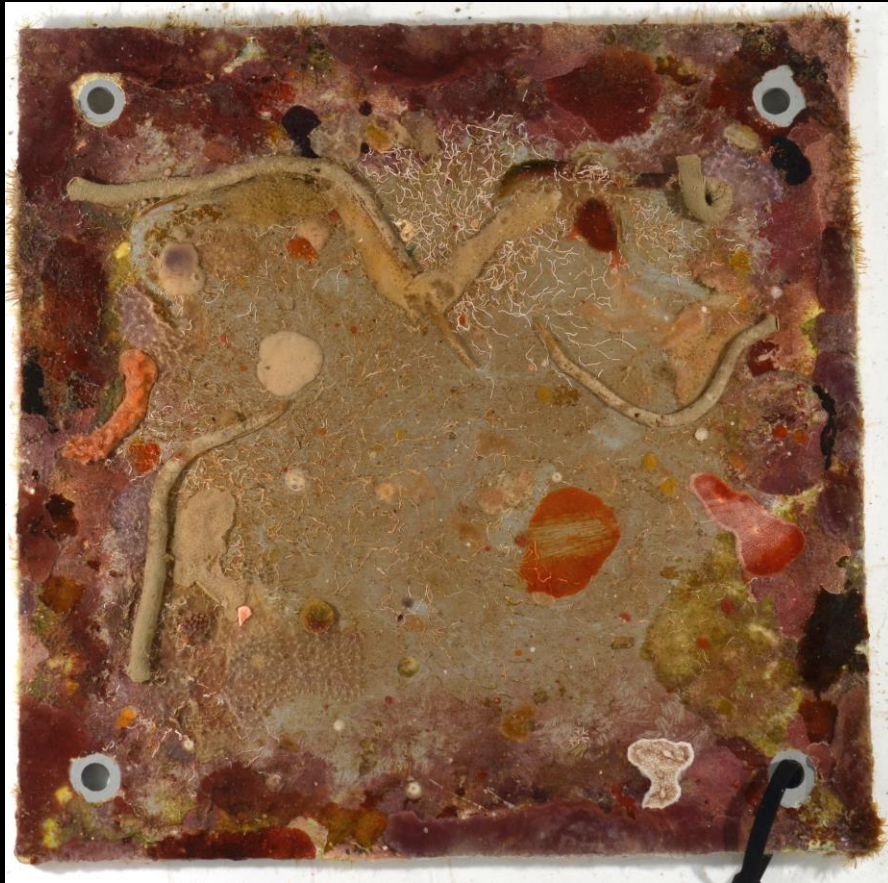
Example: Effects of Ocean Acidification

Biodiversity sampling from PNG CO₂ seeps



Example: Effects of Ocean Acidification

Normal (pH 8.0)



284 individuals >2mm
82 OTUs

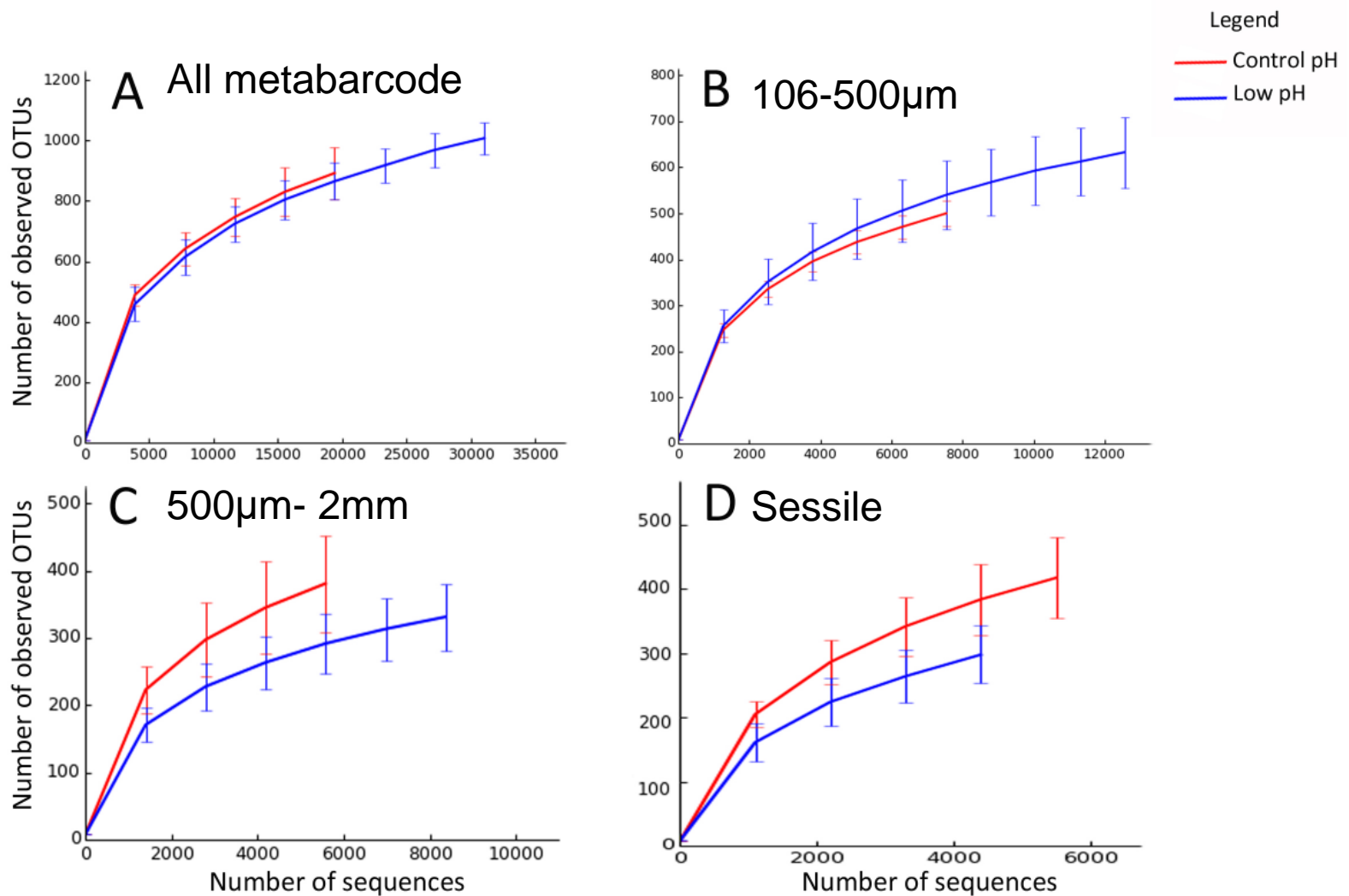
Low (pH 7.7)



Phoos: Laetitia Plaisance

114 individuals >2mm
43 OTUs

Smallest Fraction Less Sensitive to Acidification?



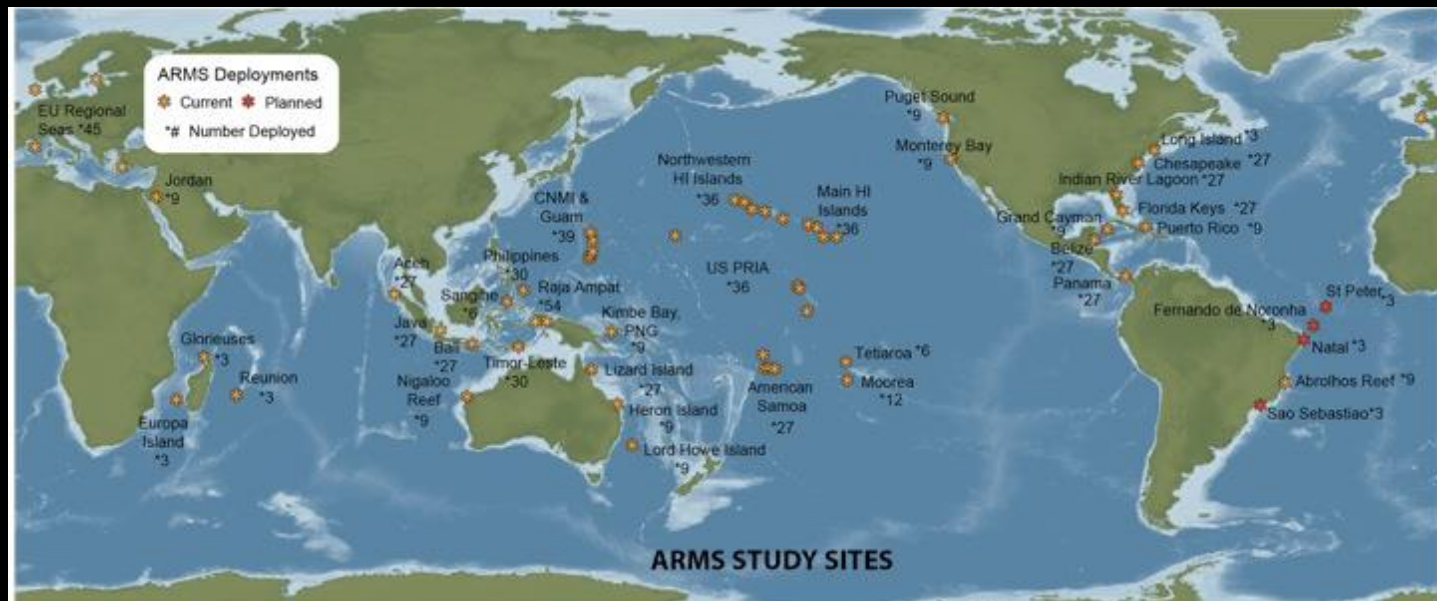
Looking Forward

Multi-gene PCR-based to increase taxonomic coverage

Shotgun metagenomics approach to

- avoid PCR bias
- capture metabolic genes and look at local adaptation

Global deployments and analyses: MarineGEO/PBM?



What are some big questions/challenges?

What are the really dark taxa?
(need branches, not twigs)

How does biodiversity
scale geographically
by taxonomic group?
by body size?
as a function of location?

What is the nature of rarity?

Are there biodiversity collapse
thresholds?

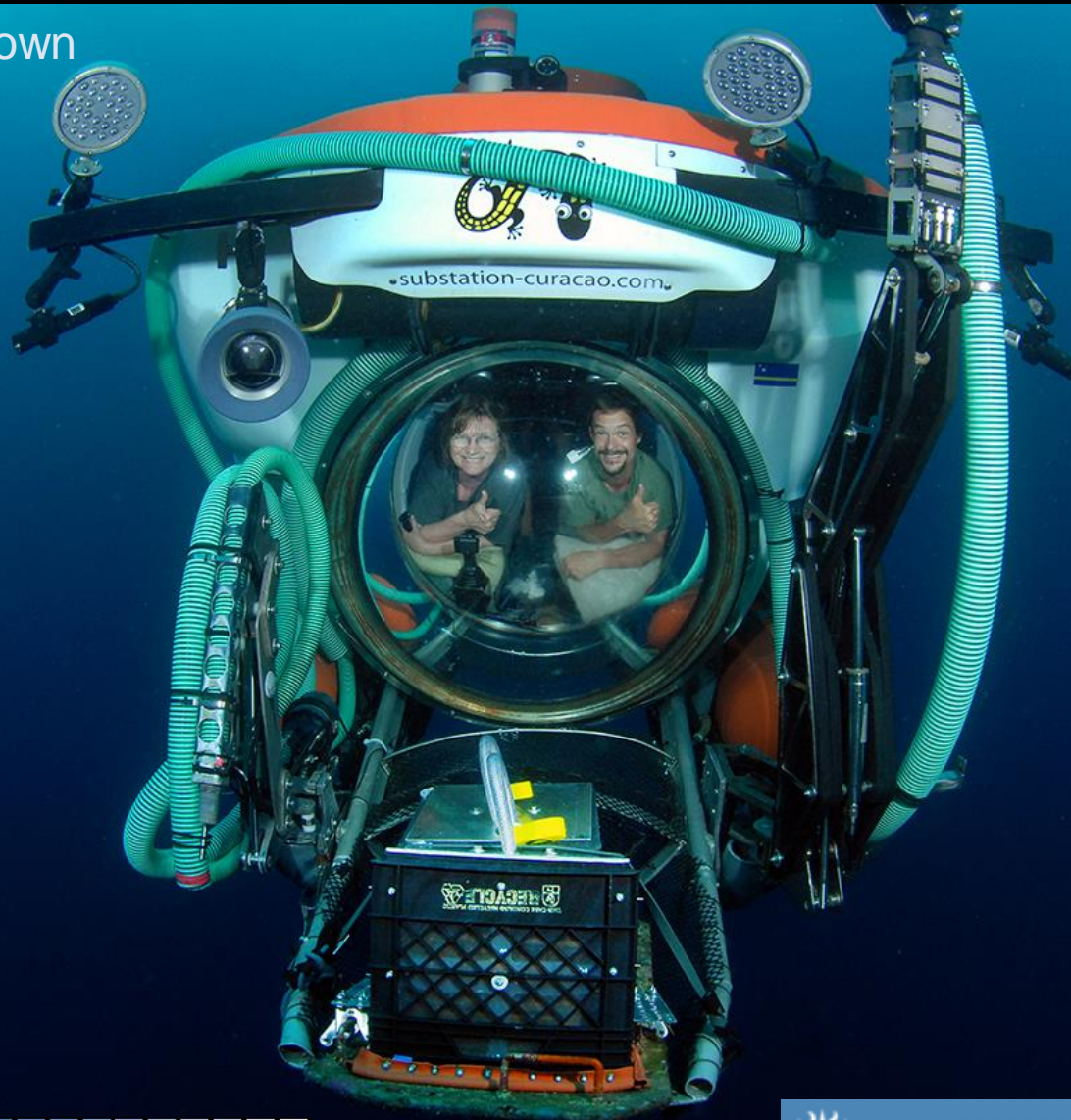
How is biodiversity changing?
(need community
vouchers)



What lives in the sea (or even a bay)?

Thanks – Questions?

Photo: Barry Brown



Bioinformatics pipeline

WORKFLOW

PROGRAMS

Initial quality filtering

----->

Mothur



Alignment to ref. barcode
&
Removal of non functional sequences
based on amino acid translations

----->

MACSE



Chimera removal

----->

UCHIME



OTU clustering

----->

CROP



Taxonomic assignments

----->

Blast & SAP



OTU table



Individual- & sample-based
rarefactions

PCoA and jackknifed
clustering analysis

-->

EstimateS
QIIME
R (Vegan)