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)

<table>
<thead>
<tr>
<th>Marine Habitat</th>
<th>Global Ocean</th>
<th>Data Type</th>
<th>Author, Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 deep sea</td>
<td>—</td>
<td>box cores, 21 m²</td>
<td>Grassle &amp; Maciolek, 1992</td>
</tr>
<tr>
<td>0.6-9.5 coral reefs</td>
<td>1.9-30.6*</td>
<td>tropical forests, species-area</td>
<td>Reaka-Kudla, 1997</td>
</tr>
<tr>
<td>1.7-3.2 coral reefs</td>
<td>5.5-10.3*</td>
<td>5 m² reef samples, species-area</td>
<td>Small et al., 1998</td>
</tr>
<tr>
<td>0.4-0.5* Coral reefs</td>
<td>1.4-1.6</td>
<td>European brachyuran crabs</td>
<td>Bouchet, 2006</td>
</tr>
<tr>
<td>0.7* coral reefs</td>
<td>2.2</td>
<td>ratios of taxa</td>
<td>Mora et al., 2011</td>
</tr>
<tr>
<td>0.2-0.3* coral reefs</td>
<td>0.7-1.0</td>
<td>expert opinion</td>
<td>Appeltans et al., 2012</td>
</tr>
<tr>
<td>0.5-1.3 coral reefs</td>
<td>1.6-2.4*</td>
<td>expert opinion</td>
<td>Fisher et al. 2015</td>
</tr>
</tbody>
</table>

*Assumes 31% of marine species live on reefs
Why so hard?
Most marine species are small and rare

Example: Mollusks
(Philippe Bouchet)

33-91% of all marine species still to be named
Global analyses ignore almost all biodiversity

Some fish
1700 spp

Corals
804 spp

Some snails
662 spp

Lobsters
69 spp

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

Roberts et al. 2002
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

The Coral Triangle

Number of species

Number of Samples

- Bali
- Lizard Island
- Heron Poc
- Heron ARMS
- Ningaloo
- Moorea
- Line Islands
- FFS- Hawaii

Unpublished data
Put Another Way…..

Just 6.3 m$^2$ 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?
(<2mm)

Photos: Matthieu Leray
From Barcoding to Metabarcoding

Bulk Sample

DNA Amplified from Entire Community

Next-Gen Metabarcodes

(easier said than done)
4 Fractions (± vouchers)

1) sessile

2) > 2mm

3) 2mm–500 μm

4) 500–106 μm

COI Metabarcoding

COI barcode
Oyster reefs (6-month deployment)
Leray & Knowlton, PNAS 2015

At what geographic scale are communities different?

Sample Size Total
7.8 m² - 0.05 m³
Chesapeake Bay, Virginia (9 ARMS)

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

Ft. Pierce, Florida (9 ARMS)

#sequences: 409,613
% matched: 11.9
% unknown: 28.3
% singletons: 31.1

SHARED

4% barcode

21% meta-barcode

Leray & Knowlton, PNAS 2015
Sub-tropical Florida is more diverse

2/3 of diversity is < 500μm
Presence-absence and relative abundance

Different locations and metabarcoding communities are distinct
Taxonomic composition

22 animal phyla - Arthropods most diverse

Leray & Knowlton, PNAS 2015
More Sampling Needed

(similar pattern for sampling effort measured by # sequences)
Adjacent samples cluster together at the meter scale.

Leray & Knowlton, PNAS 2015
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 defaunaion: Animal loss in the global ocean
Douglas J. McCauley¹, Malin L. Pinsky², Stephen R. Palumbi³, James A. Estes⁴, Francis H. Joyce¹, Robert R. Warner¹
Example: Effects of Ocean Acidification

Biodiversity sampling from PNG CO$_2$ seeps
Example: Effects of Ocean Acidification

**Normal (pH 8.0)**
- 284 individuals >2mm
- 82 OTUs

**Low (pH 7.7)**
- 114 individuals >2mm
- 43 OTUs
Smallest Fraction Less Sensitive to Acidification?

A. All metabarcodes

B. 106-500μm

C. 500μm - 2mm

D. Sessile

Legend
- Control pH
- Low pH

Plaisance, unpubl.
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)?
**Bioinformatics pipeline**

**WORKFLOW**

- **Initial quality filtering**
  - Alignment to ref. barcode &
  - Removal of non functional sequences based on amino acid translations
  - Chimera removal
  - OTU clustering
  - Taxonomic assignments

**PROGRAMS**

- Mothur
- MACSE
- UCHIME
- CROP
- Blast & SAP
- EstimateS
- QIIME
- R (Vegan)

**Analysis**

- OTU table
- Individual- & sample-based rarefactions
- PCoA and jacknifed clustering analysis