



Community ecology, genomics, and eDNA

Elaine Shen
BIO 594
April 18, 2019



Outline

- Community ecology
 - Bottom-up & top-down controls
 - Resistance, resilience, and succession
 - Biodiversity
 - Traditional approaches
- (Meta)genomics
 - Sequencing technologies
- Environmental DNA
 - Methods overview
 - Analysis and visualization
 - Challenges, opportunities, & applications

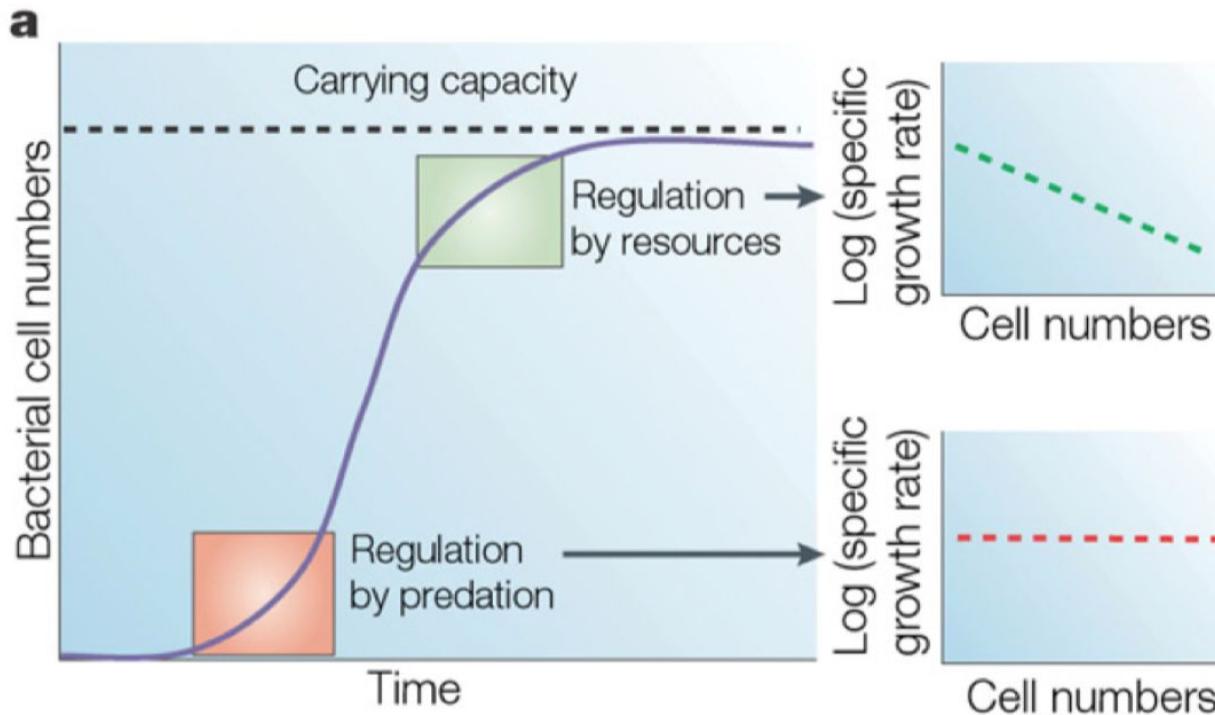


1.

Community ecology

From populations to ecosystems

Ecosystem population dynamics



BOTTOM UP CONTROL - RESOURCES

- Negative correlation between growth rate and cell numbers

TOP DOWN CONTROL - PREDATORS

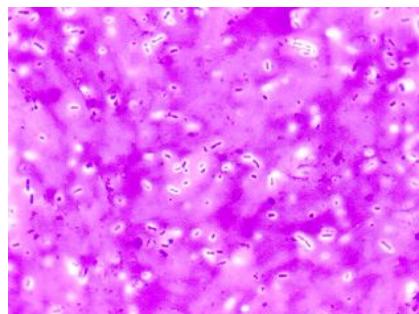
- No correlation between growth rate and cell numbers



A model system for predator-prey dynamics

Food:

Aerobacter aerogenes



Prey:

Paramecium aurelia

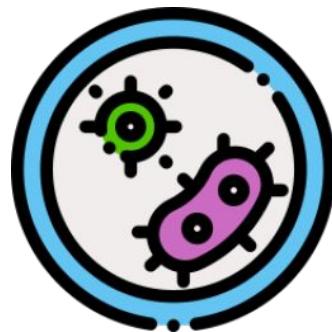


Predator:

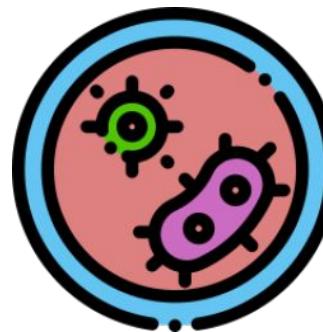
Didinium nasutum



Non-viscous media



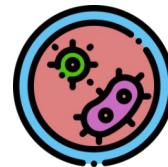
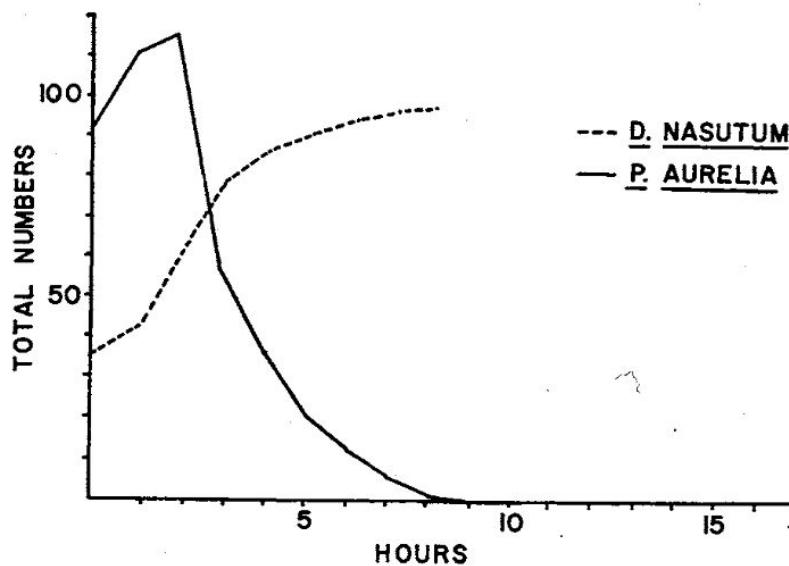
Viscous media



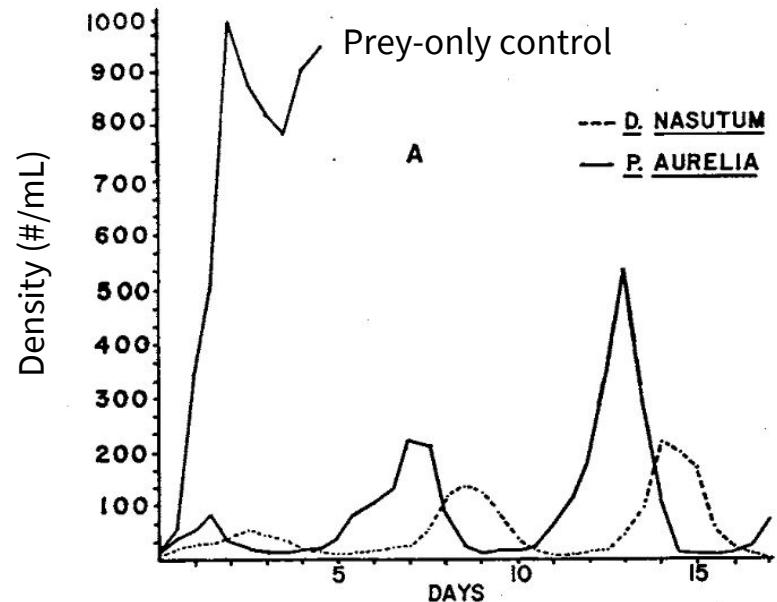
A model system for predator-prey dynamics



Non-viscous media



Viscous media

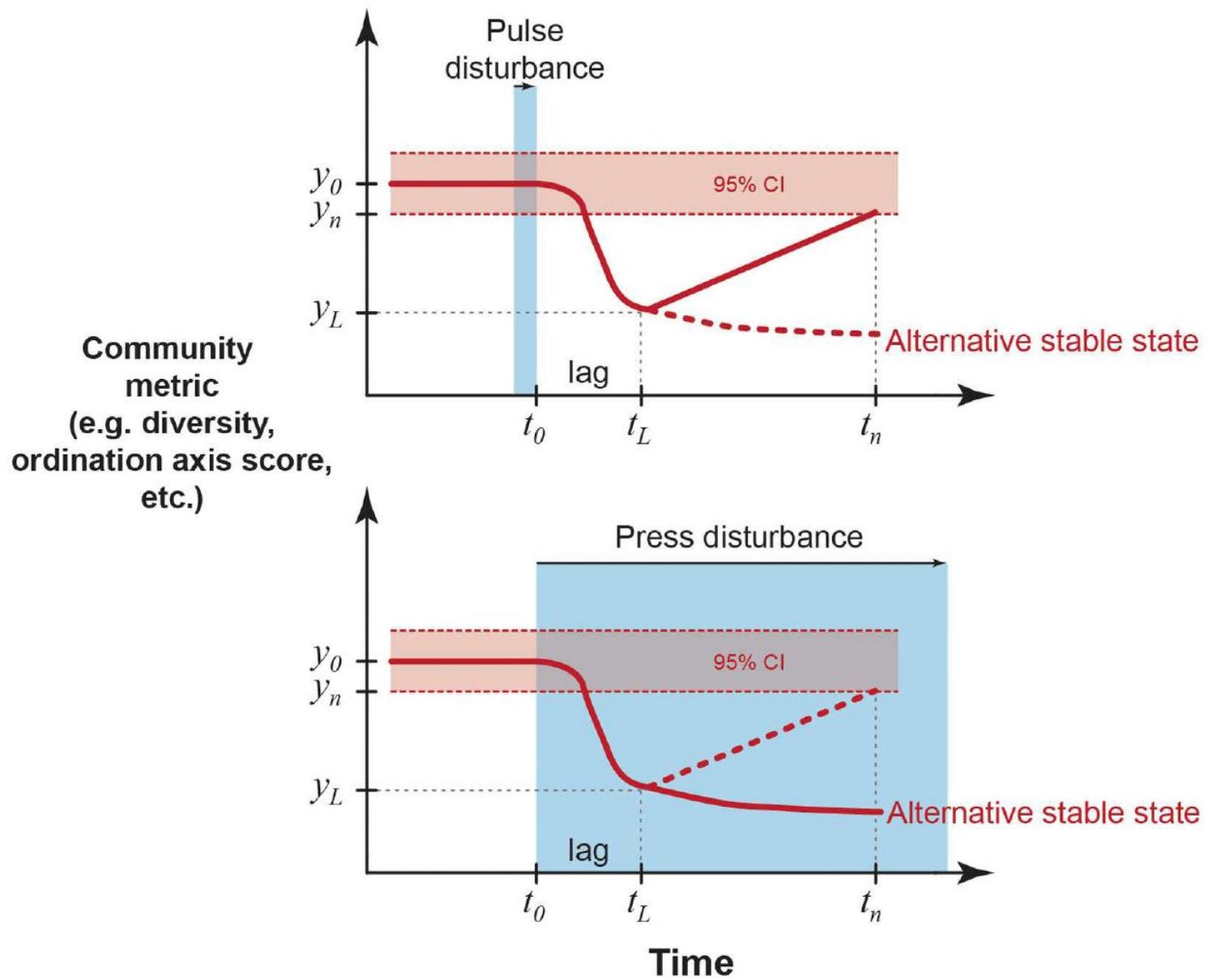


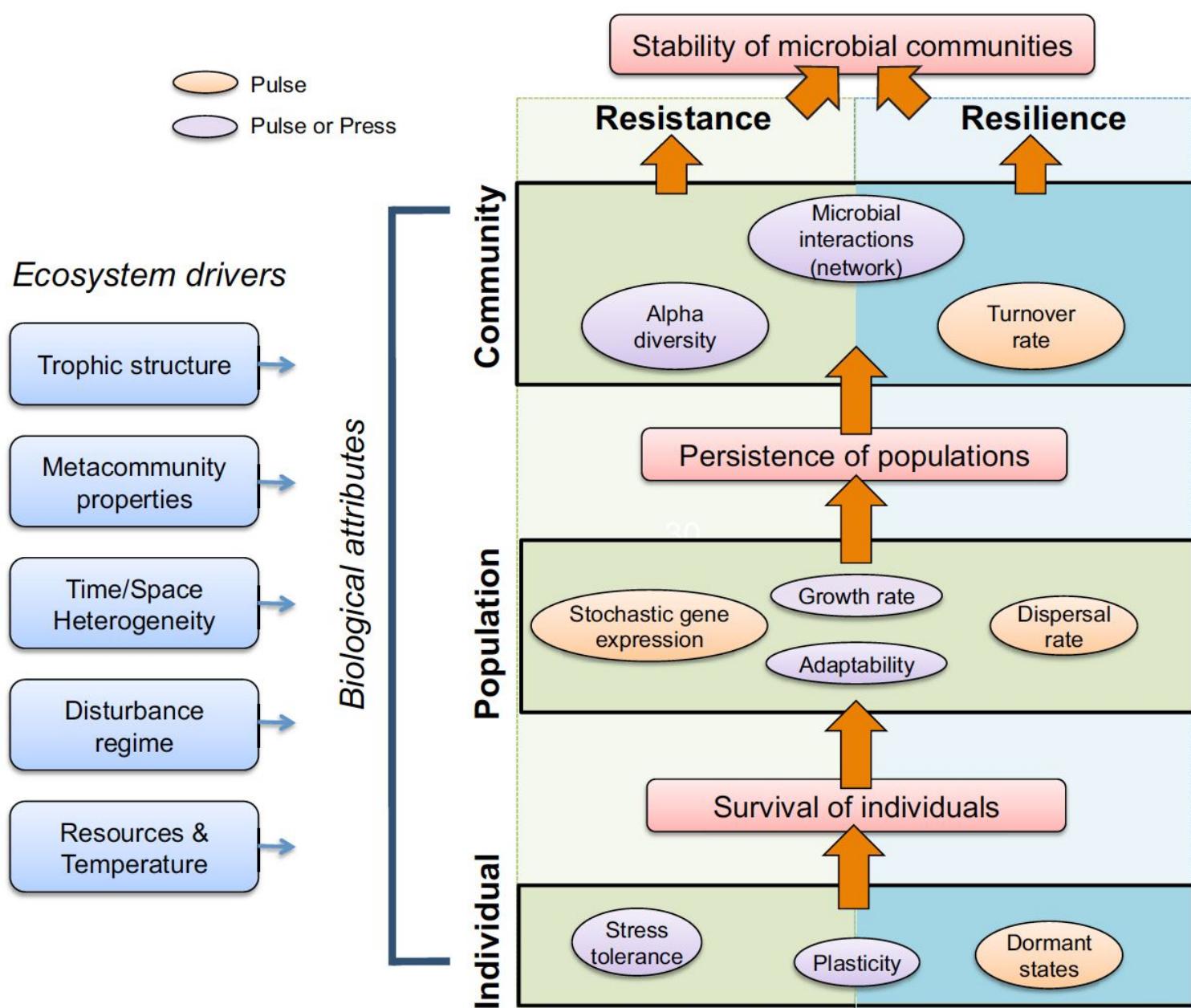
- High predator-prey encounter frequency
- No cyclical relationship observed - prey was driven to extinction

- Low predator-prey encounter frequency
- Viscous medium allowed prey population to regrow + bounce back

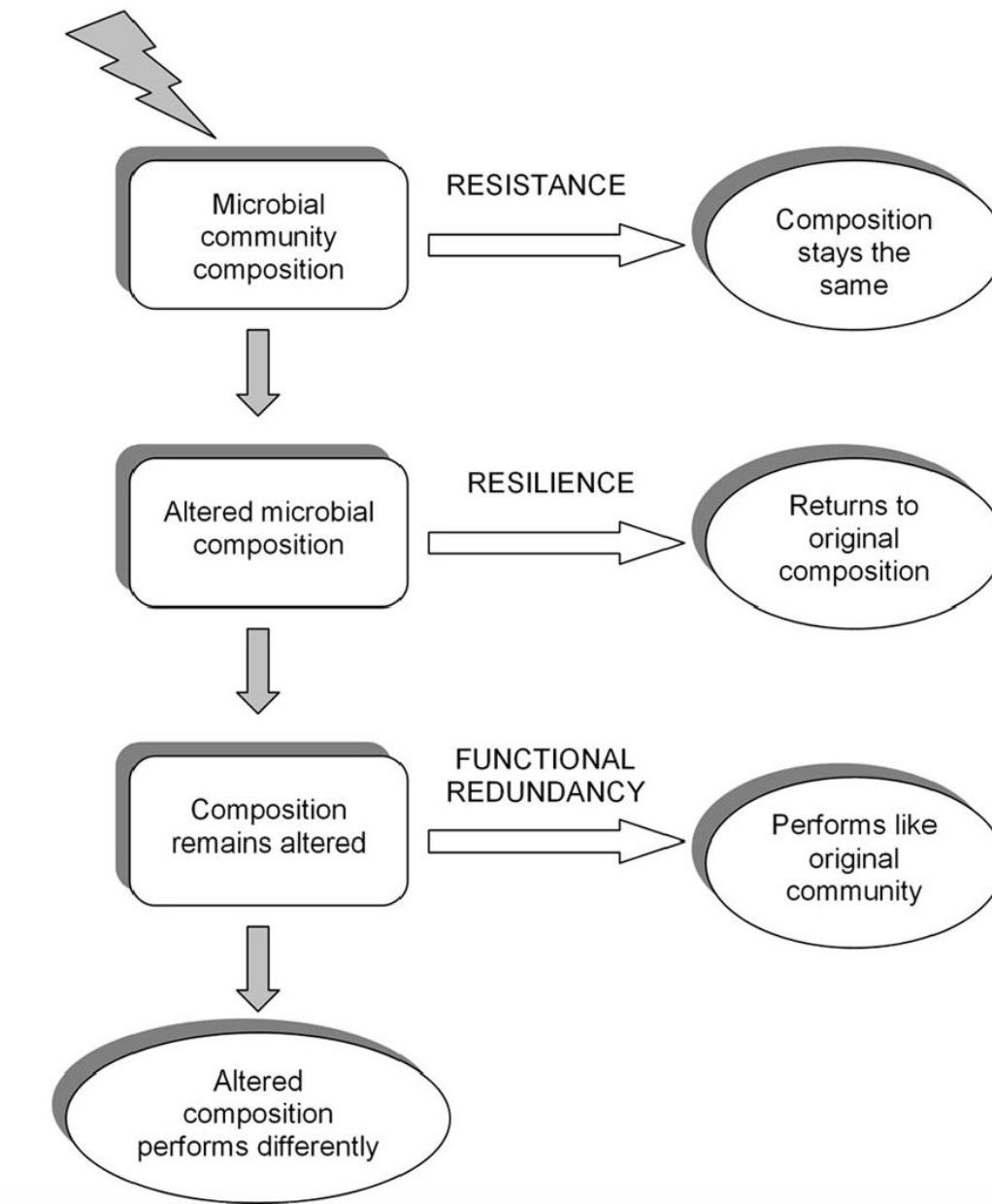
Table 1 | Common terms for disturbances, community responses, and community outcomes.

DISTURBANCE TERMS	
Disturbance	A causal event that causes a discrete change in the physical or chemical environment that has anticipated effects on a community (Rykiel, 1985; Glasby and Underwood, 1996)
Press disturbance	A continuous disturbance that may arise sharply but reaches a constant level that is maintained over a long period of time (Lake, 2000)
Pulse disturbance	A short-term, often intense disturbance that rapidly decreases in severity over a short period of time (Lake, 2000)
COMMUNITY TERMS	
Community	An assemblage of microorganisms that live in the same locality and potentially interact with each other or with the environment (Konopka, 2009)
Metacommunity	Within a regional landscape, a set of local communities whose members are linked by dispersal (Wilson, 1992; Logue et al., 2011)
COMMUNITY RESPONSE TERMS	
Stability	The tendency of a community to return to a mean condition after a disturbance (Pimm, 1984); includes the components of resistance and resilience Ecological stability can be measured in many ways, including the persistence of populations through time, constancy of ecological attributes through time, resistance to a disturbance, or resilience after a disturbance (Worm and Duffy, 2003)
Resistance	The degree to which a community withstands change in the face of disturbance (Pimm, 1984; Allison and Martiny, 2008). Inverse of sensitivity
Sensitivity	The degree to which a community changes in response to disturbance, the inverse of resistance
Resilience	The rate at which a microbial community returns to its original composition after being disturbed (Allison and Martiny, 2008). Commonly referred to as community recovery. Inverse of return time
COMMUNITY OUTCOMES	
Stable state	A condition where a community returns to its original composition or function following a disturbance (Beisner et al., 2003). Also known as community equilibrium or an attractor
Alternative stable state	A condition where a community moves to a different but stable composition or function following a disturbance. One of multiple, non-transitory stable states in which a community can exist (Beisner et al., 2003)
Regime shift	A large change in community composition arising from a shift between alternative stable states (Scheffer and Carpenter, 2003)



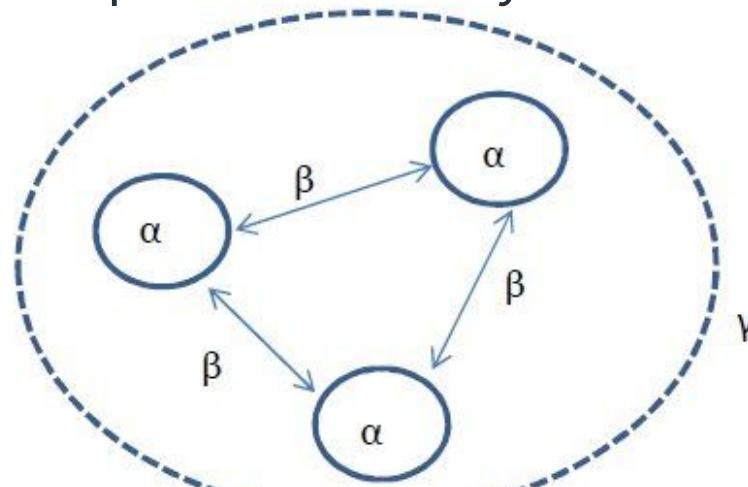


DISTURBANCE

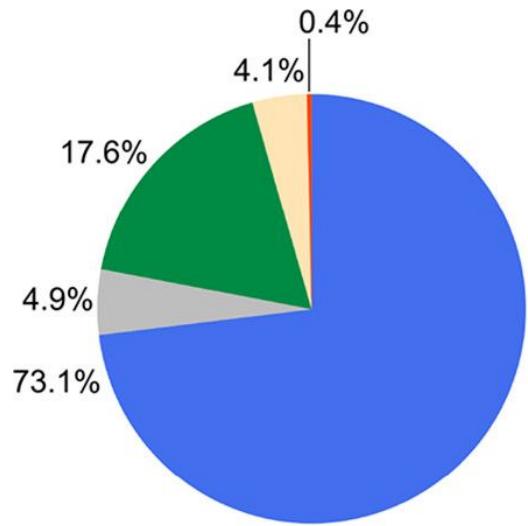


Biodiversity

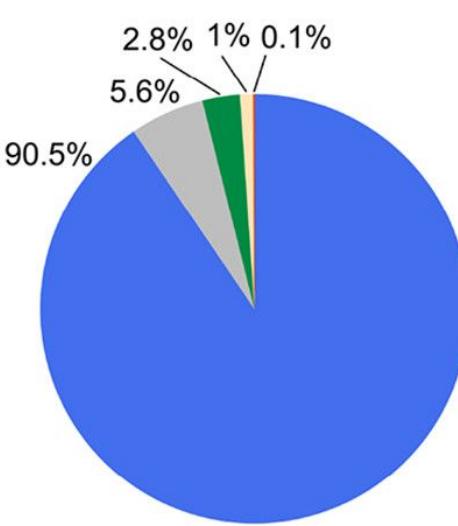
- ◎ **α (alpha)** - Feature diversity within individual samples; can be compared across sample groups
 - Chao1 - true species diversity (richness)
 - Shannon - richness (# sp.) and evenness (how equal taxa abundances are in a community)
- ◎ **β (beta)** - Feature dissimilarity between each pair of samples; distance matrix
 - binary-Jaccard & unweighted UniFrac - presence and absence only (qualitative)
 - Bray-Curtis, Canberra, weighted Unifrac - feature abundance (quantitative)
- ◎ **γ (gamma)** - landscape-level diversity



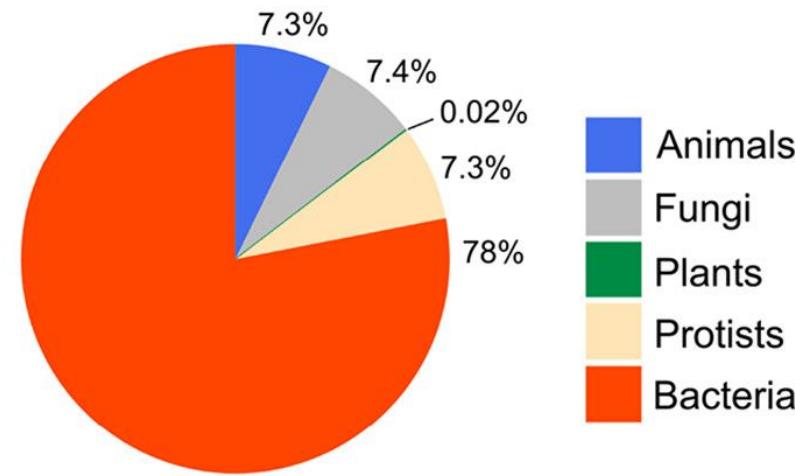
How many species are there?



Wilson (1992)

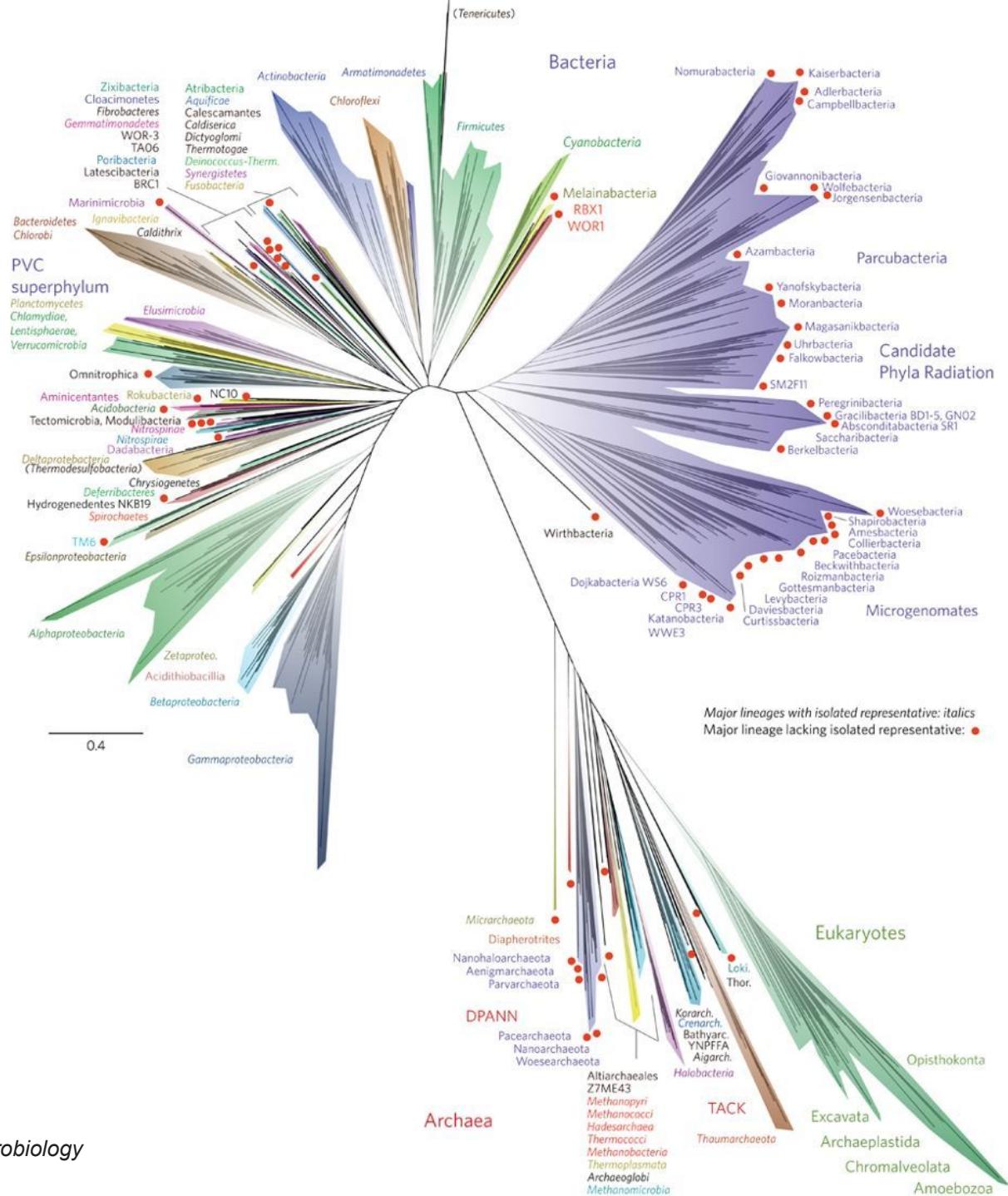


Mora et al. (2011)



this study

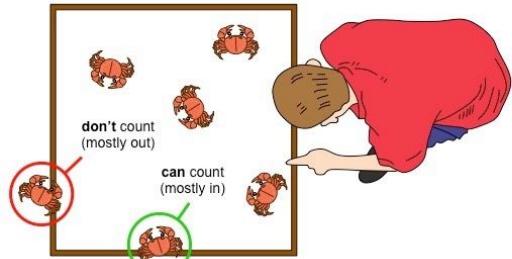
- ◎ ~1.5 million described species (Roskov et al. 2014)
- ◎ Projections of global biodiversity:
 - ~2 million species (Costello et al. 2012)
 - ~100 million (Ehrlich and Wilson 1991; May 1992; Lambshead 1993)
 - ~1 trillion (Locey and Lennon 2016)
 - **This paper: 1-6 billion species, dominated by bacteria**



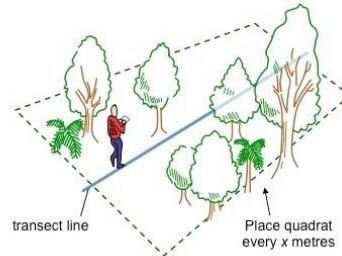
How do we collect biodiversity data?

Direct

Quadrat Counting Method



Line Transects

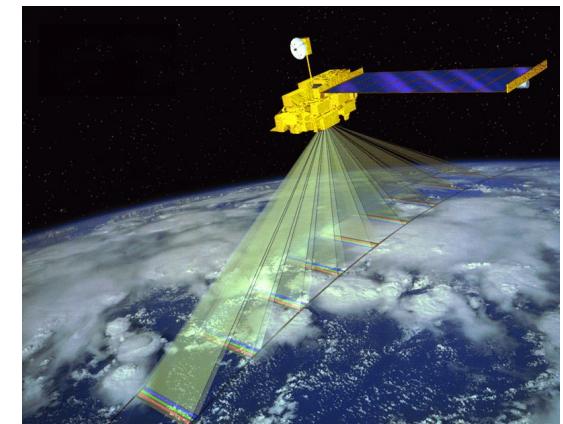
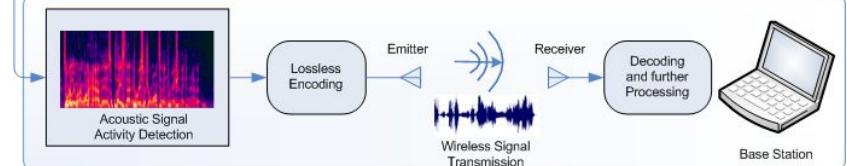


Indirect

Animal sound sources



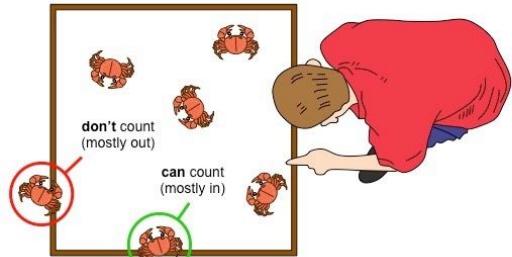
Hardware components of autonomous monitoring station



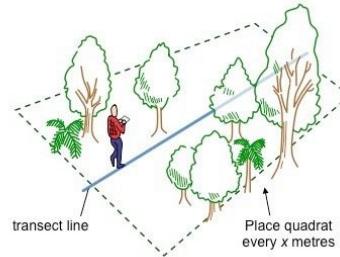
How do we collect biodiversity data?

Direct

Quadrat Counting Method



Line Transects



What are the strengths & weaknesses of these methods?

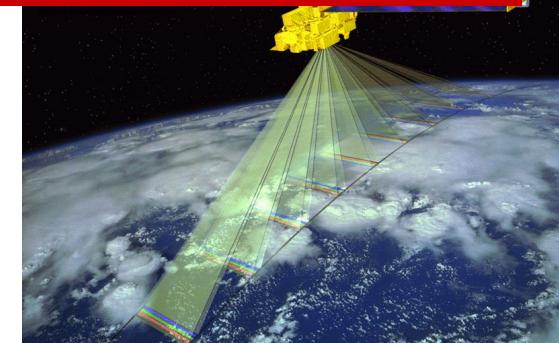
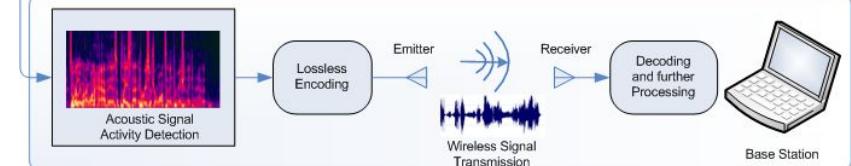


Indirect

Animal sound sources



Hardware components of autonomous monitoring station

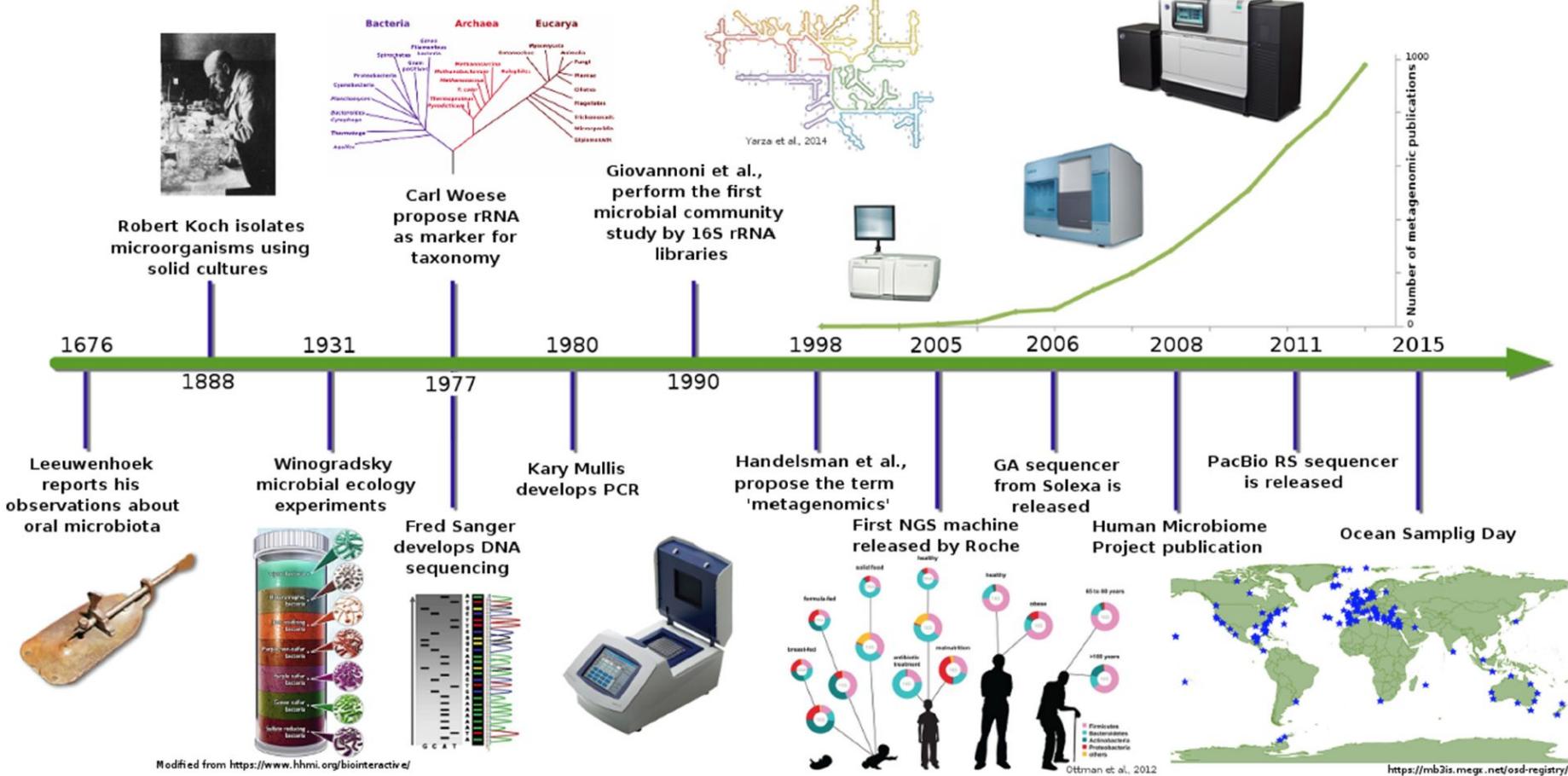




2. **(Meta)genomics**

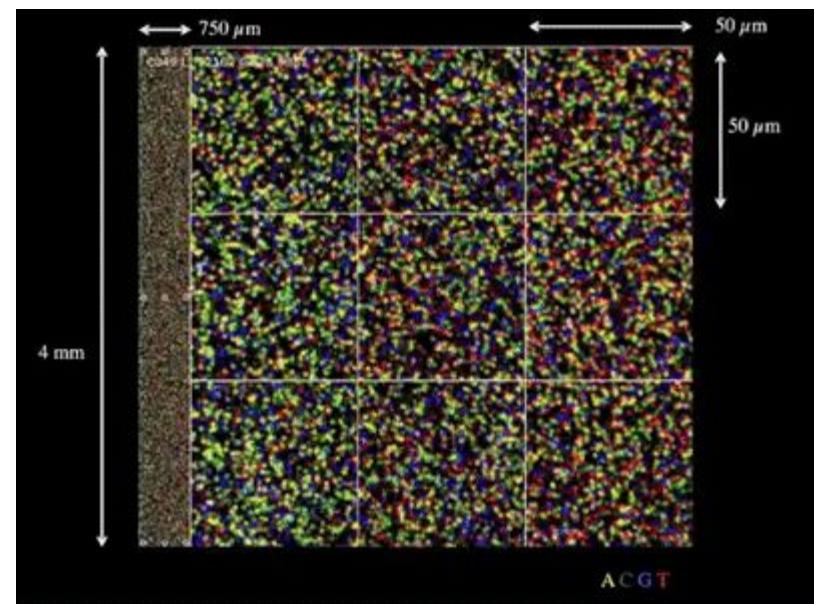
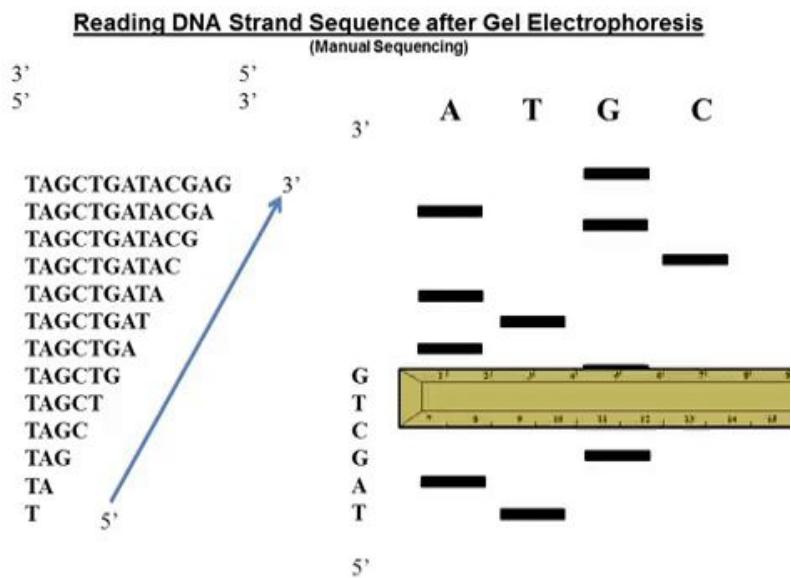
From marker genes to metabarcoding

History of sequencing technologies



Escobar-Zepeda et al. (2015). *Frontiers in Genetics*

How far we've come:



Sanger

Illumina
Sequence-by-Synthesis

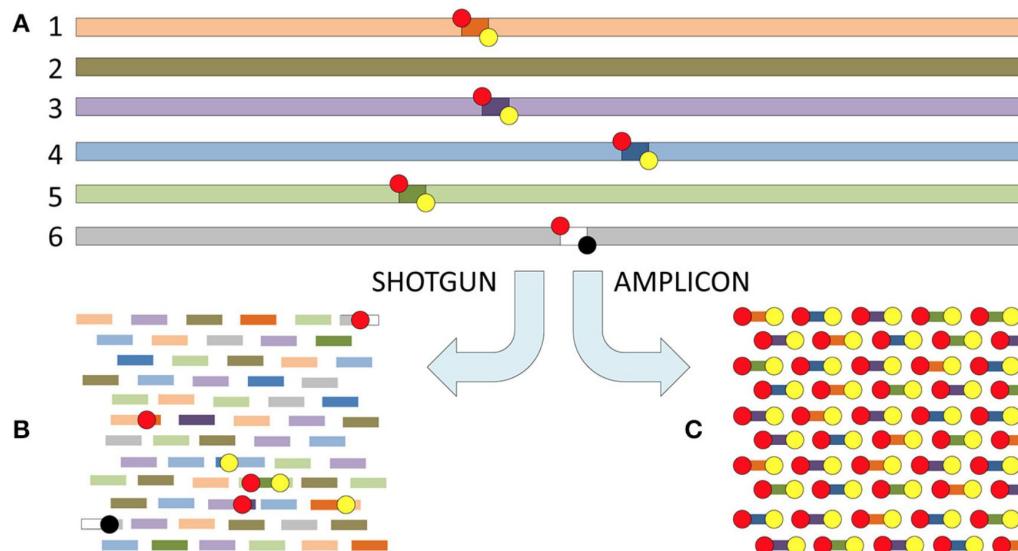
Microbial Genomics using Next-Generation Sequencing (NGS)

Shotgun metagenomics

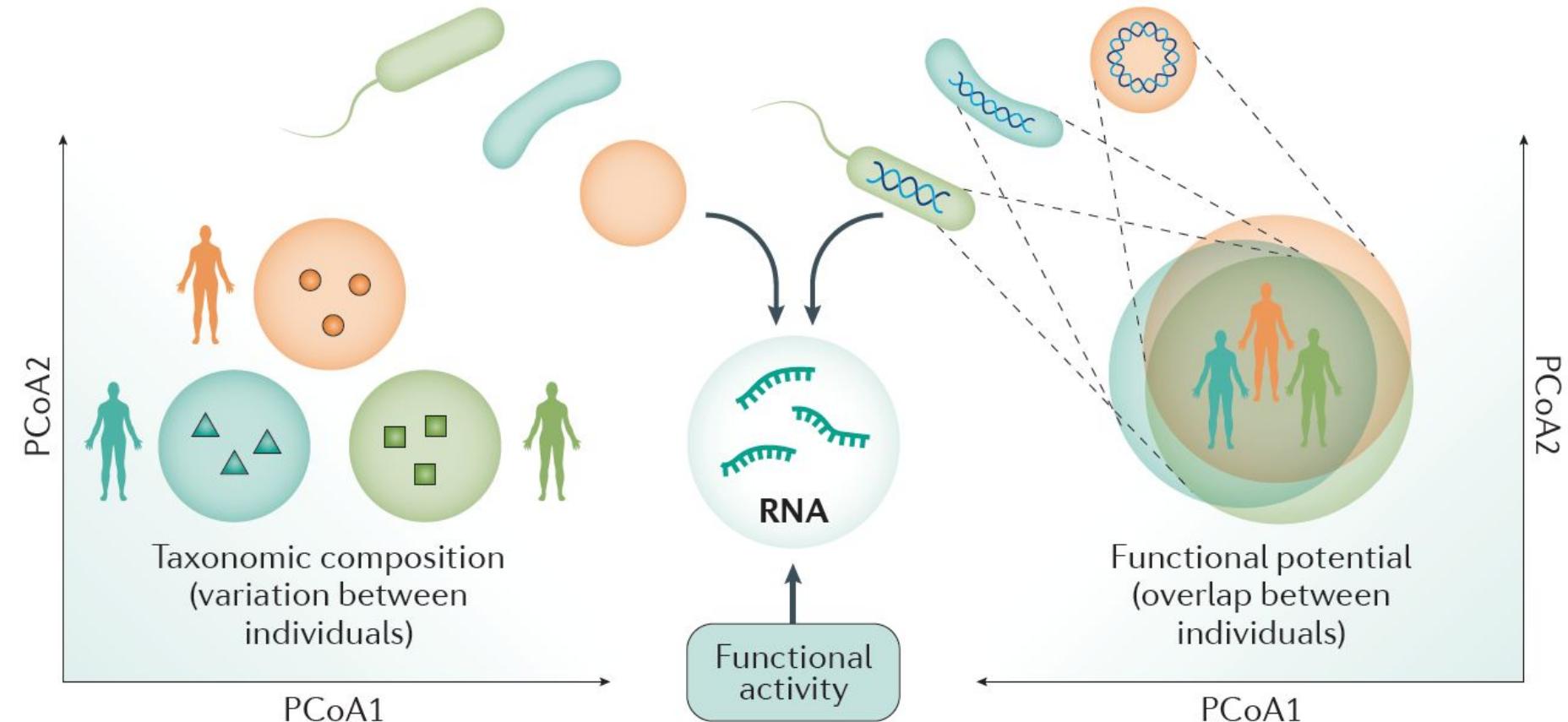
- ◎ *What are they doing?*
- ◎ Whole metagenome approach:
 - Total DNA/RNA obtained to prepare whole shotgun libraries
- ◎ Functional assignment

Amplicon sequencing

- ◎ *Who is there?*
- ◎ Specific region(s), or marker genes, of DNA/RNA
 - 16S rRNA for prokaryotes
 - ITS or LSU for eukaryotes
- ◎ Taxonomic assignment



Microbial Genomics using Next-Generation Sequencing (NGS)



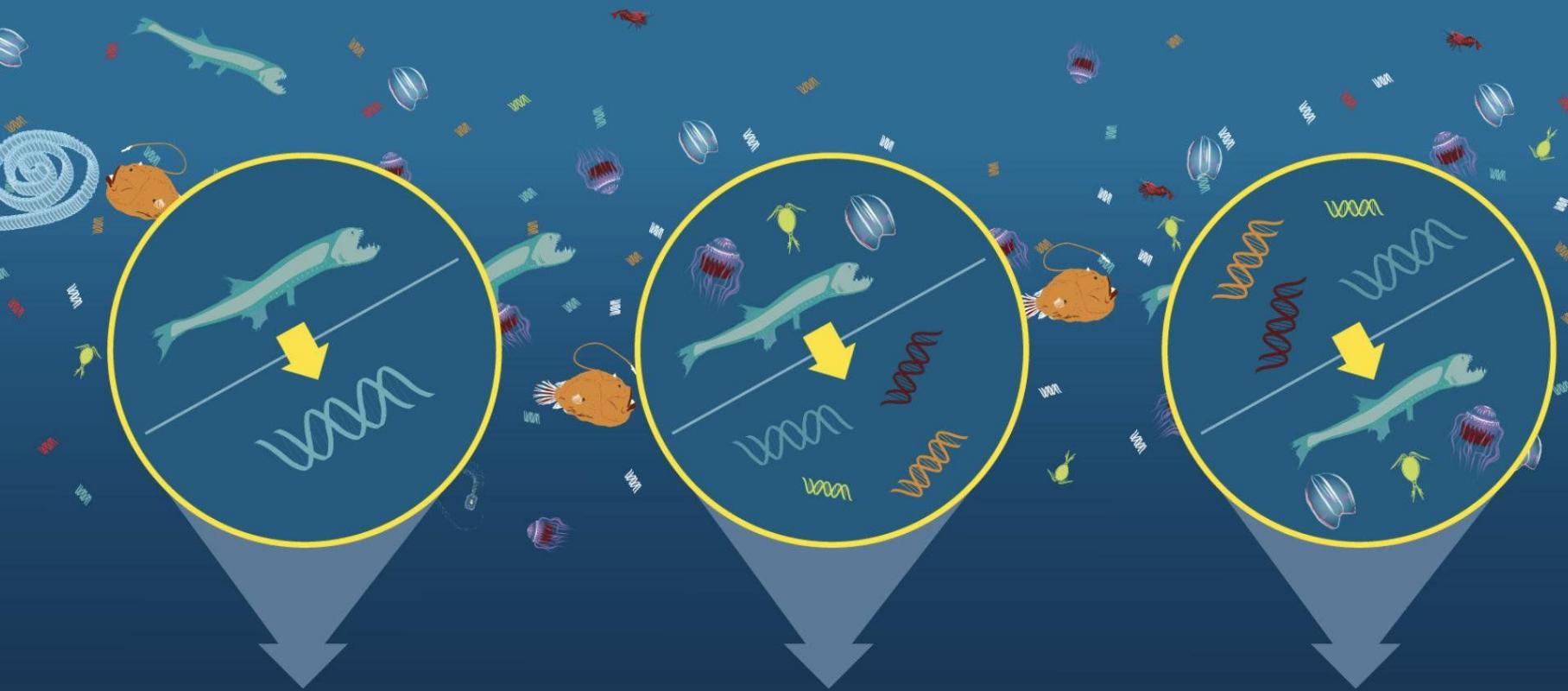
Lavelle and Sokol (2018). *Nature Reviews Gastroenterology & Hepatology*



3.

Environmental DNA (eDNA)

(Meta)barcoding for ecosystem
monitoring



BARCODING

Scientists find diagnostic DNA sequences called barcodes that they use like fingerprints to identify individual species.

METABARCODING

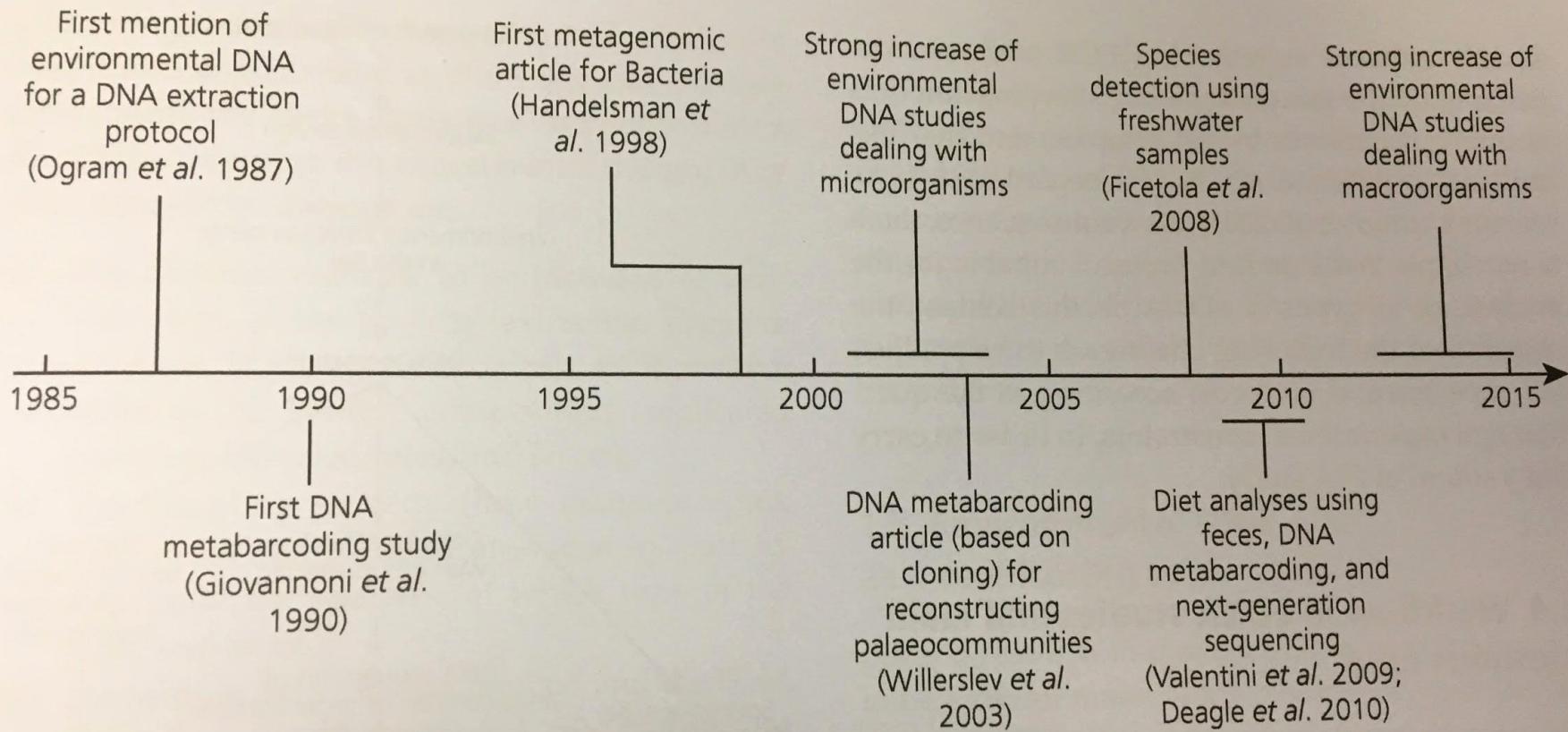
Metabarcoding is a technique to efficiently find many barcodes from many different organisms at one time.

eDNA

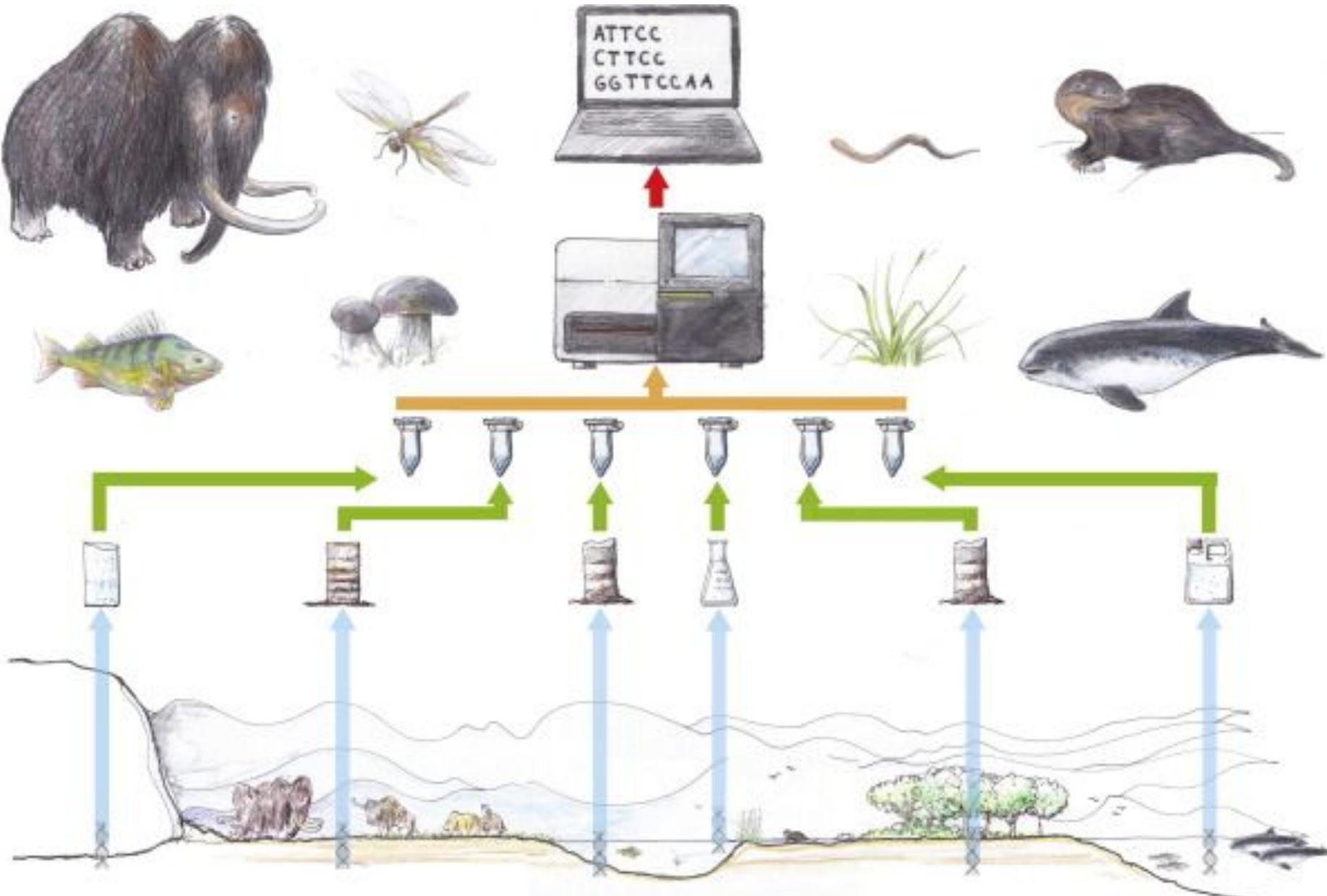
Scientists collect “environmental DNA” in water samples and use metabarcoding to identify what animals have been there.

Illustration by Natalie Renier and Eric S. Taylor, WHOI Creative

History of eDNA



Taberlet, P., Bonin, A., Zinger, L., & Coissac, E. (2018). *Environmental DNA: For biodiversity research and monitoring*. Oxford University Press.



eDNA Methods Overview

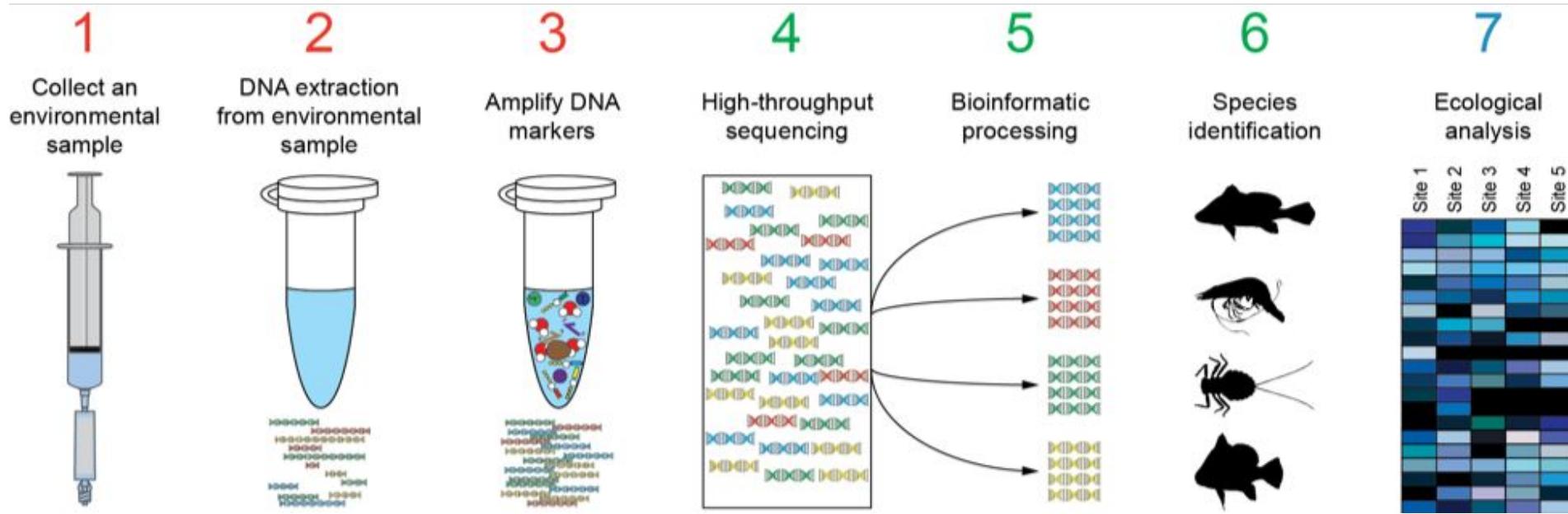


Photo: <http://www.naturemetrics.co.uk>

eDNA Methods Overview

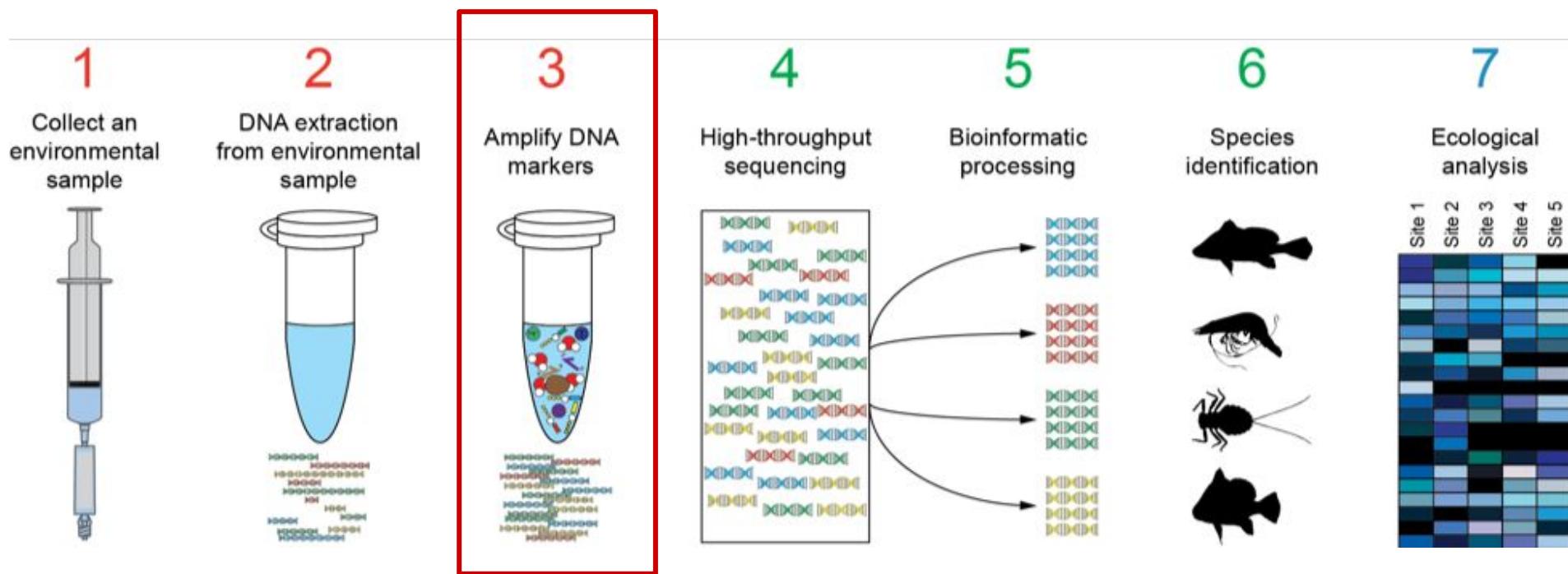


Photo: <http://www.naturemetrics.co.uk>

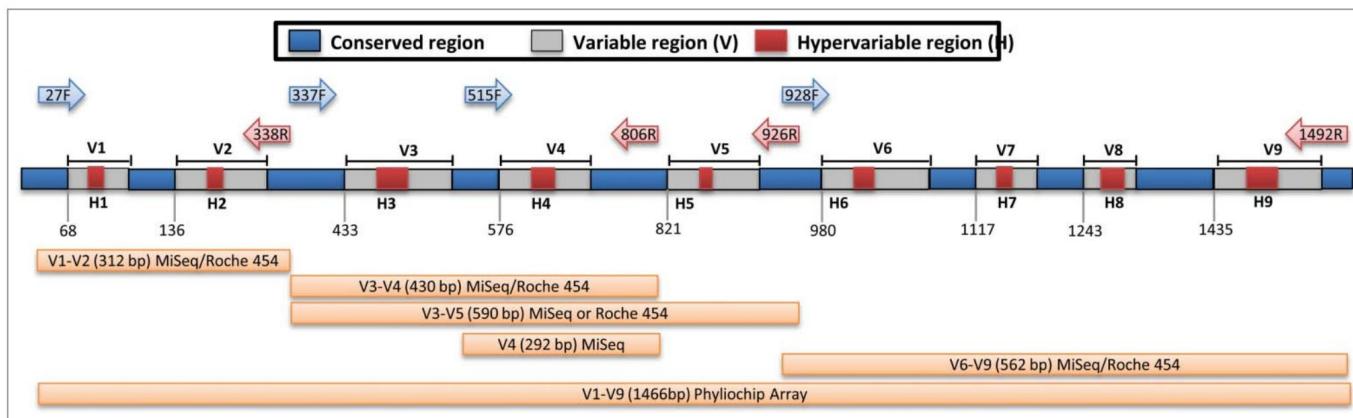
Primer design

For barcoding

- Amplifies DNA from a single specimen
- Can be species-specific
- Amplification success high-low
- Amplicon sequence must be very variable between closely-related species
- Short to long amplicon size
- Length between 18-30 bp

For metabarcoding

- Amplifies DNA from many specimens
- Must be universal (within target group)
- Amplification success equally high for all taxa
- Amplicon sequence must be very variable between closely-related species, but flanked by conserved regions
- Amplicon length limited by resolution capacity (> 100 bp) and sequencing technologies + eDNA degradation (<400)
- The longer the primer, the lower probability of being conserved (18-22 bp)



Slide info from Daniel Marquina Shahi et al. (2017) *Gut microbes*

eDNA Methods Overview

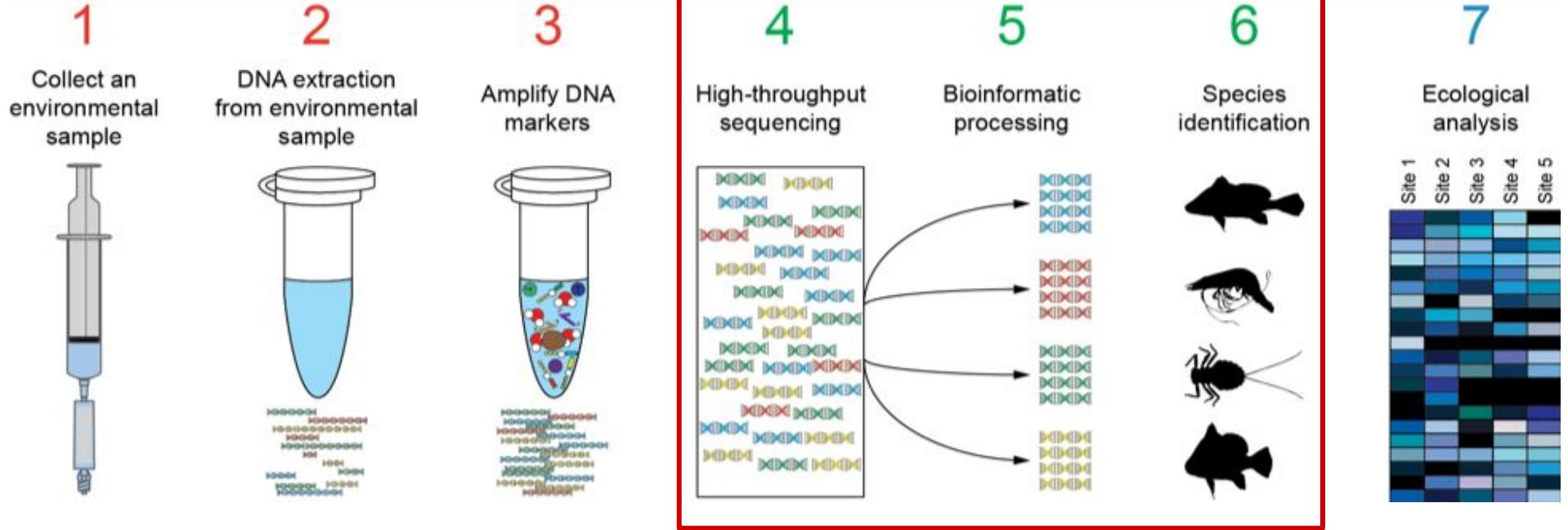
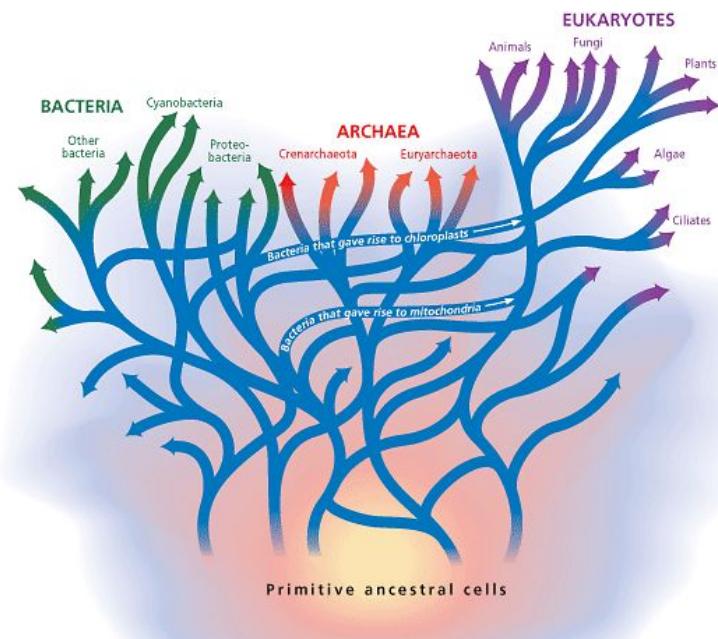
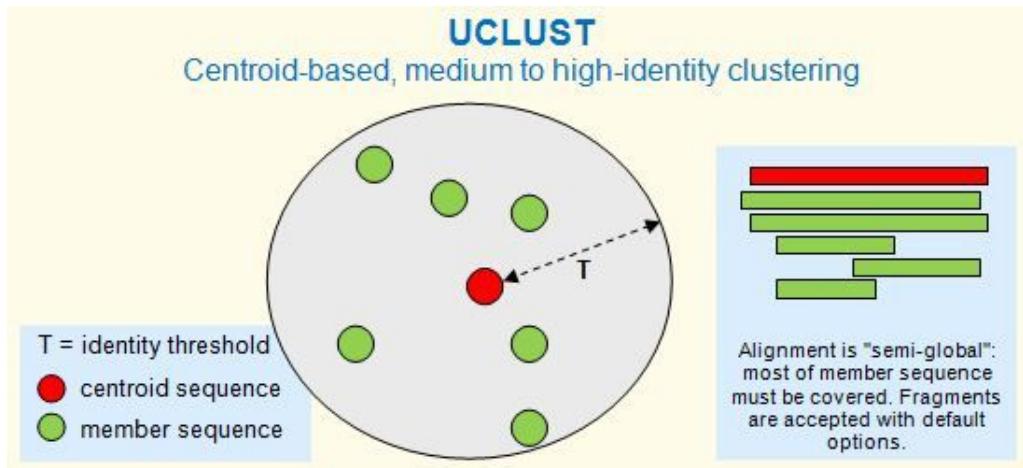


Photo: <http://www.naturemetrics.co.uk>

Operational Taxonomic Units

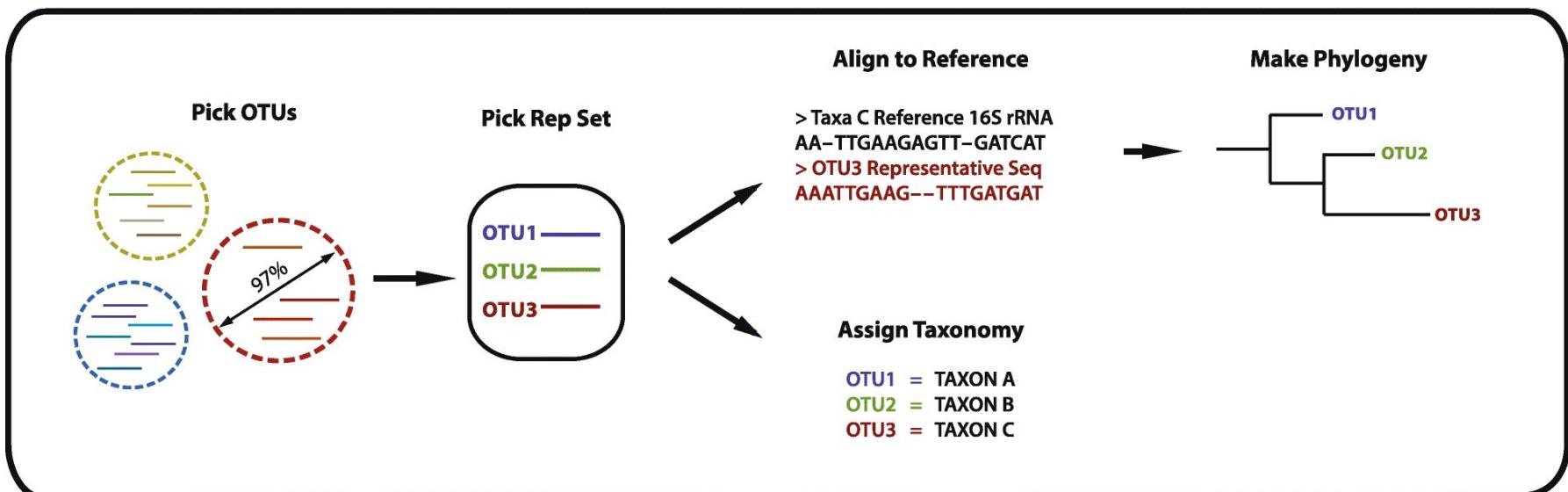
- Groups of organisms identified through use of cluster algorithms and a predefined percentage sequence similarity (97-99%)
- Proxies for microbial “species”



Operational Taxonomic Units

- Groups of organisms identified through use of cluster algorithms and a predefined percentage sequence similarity (97-99%)
- Proxies for microbial “species”

a Standard QIIME Workflow



eDNA Methods Overview

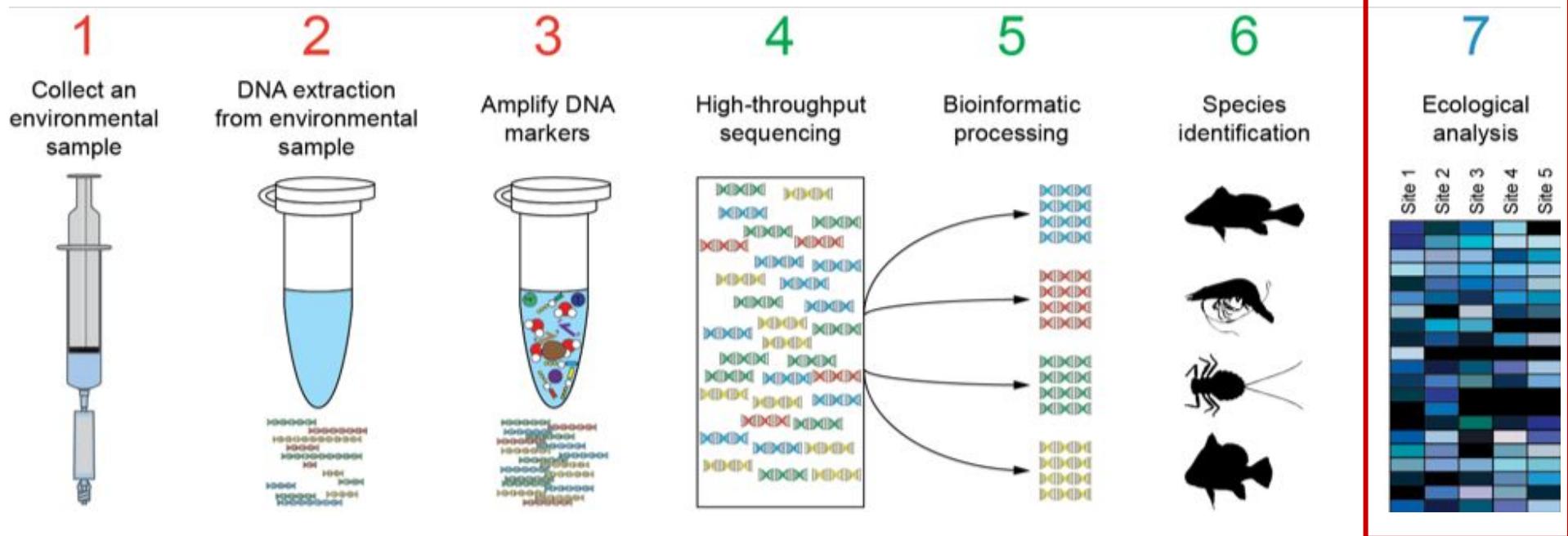
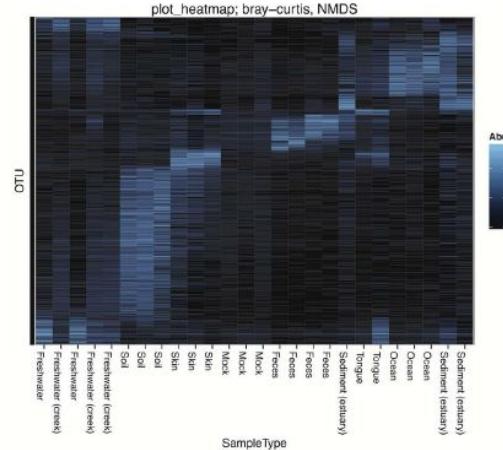
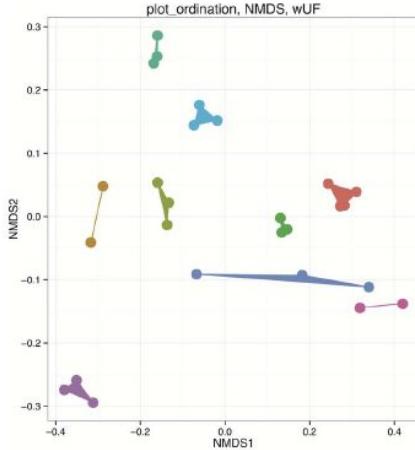
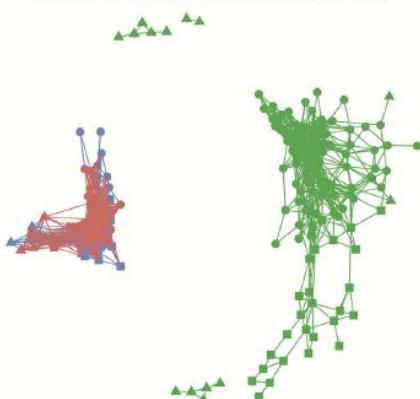


Photo: <http://www.naturemetrics.co.uk>

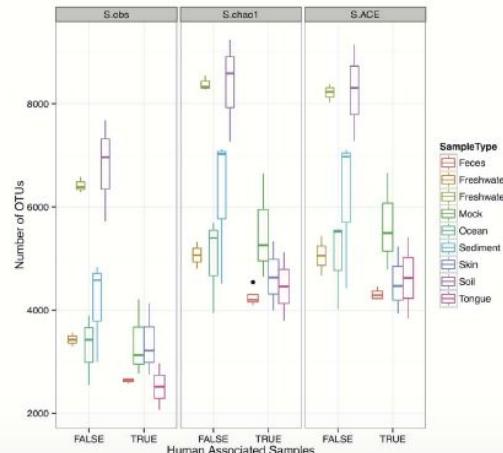
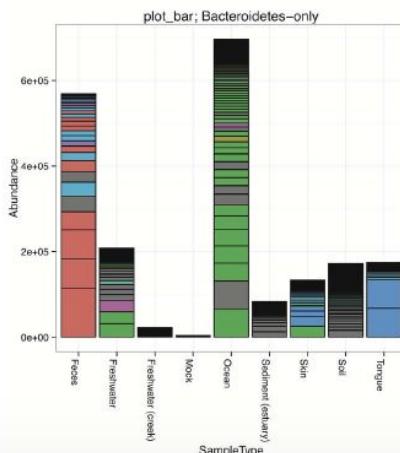
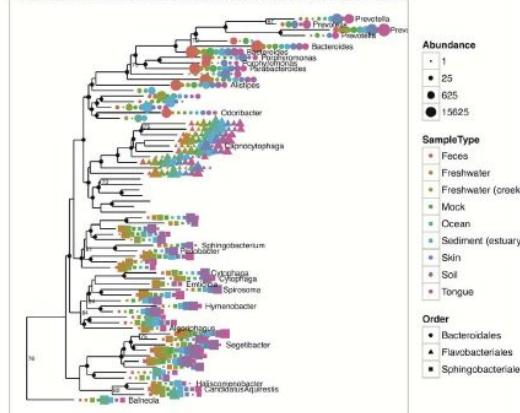
eDNA analysis & visualization



plot_network; Enterotype data, bray-curtis, max.dist=0.25



plot_tree; Bacteroidetes-only. Merged samples, tip_glm=0.1



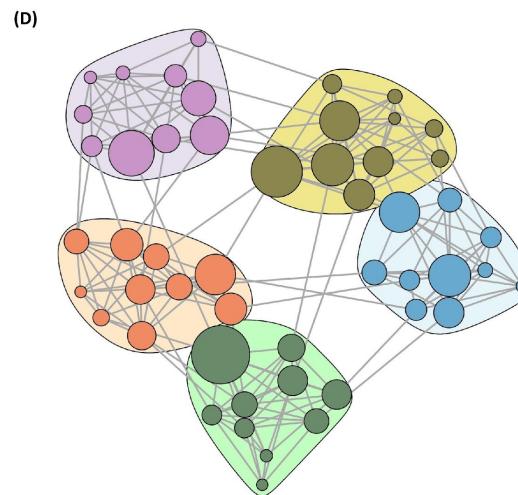
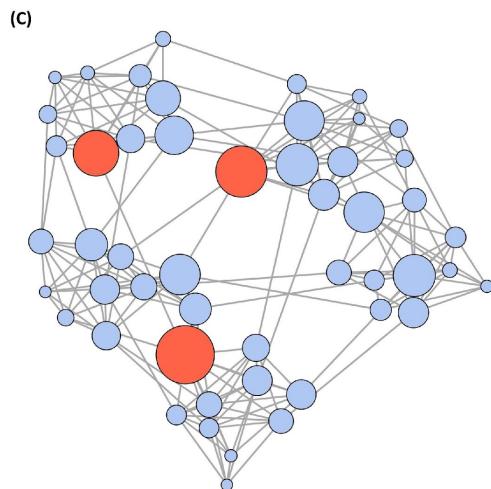
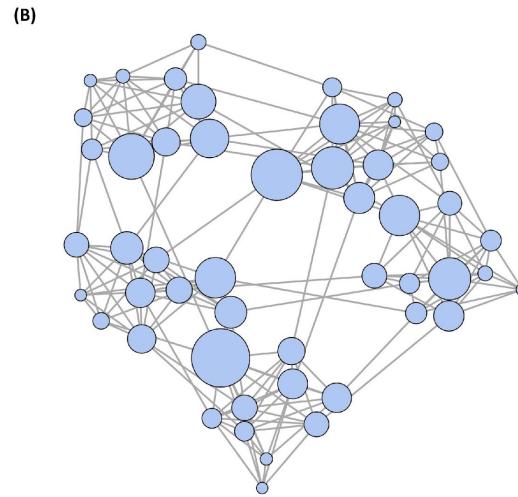
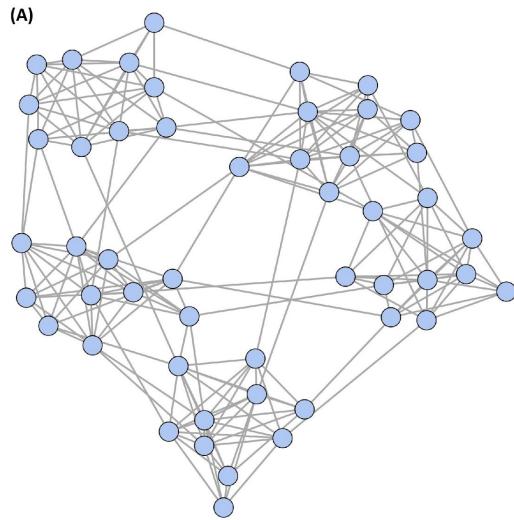
phyloseq

<https://joey711.github.io/phyloseq/>

PHINCH

<http://phinch.org/index.html>

eDNA analysis and visualization (cont.)



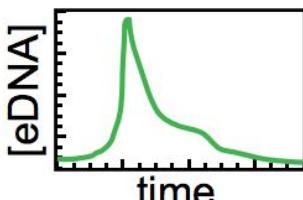
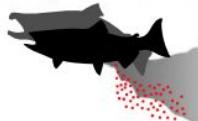
Trends in Microbiology

Layeghifard et al. (2017). *Trends in microbiology*

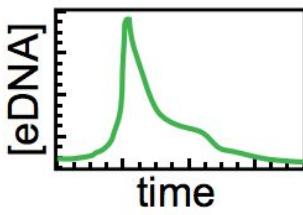
THE ECOLOGY of eDNA

A ORIGIN

reproduction



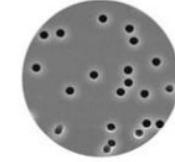
decomposition



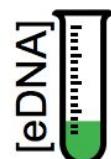
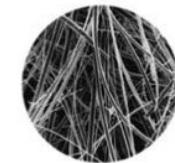
B STATE



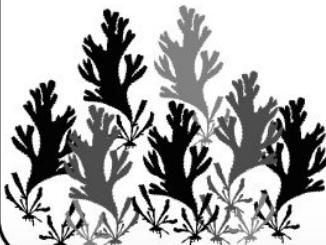
filter type A



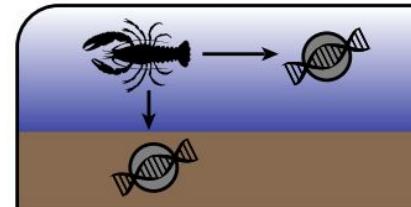
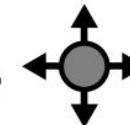
filter type B



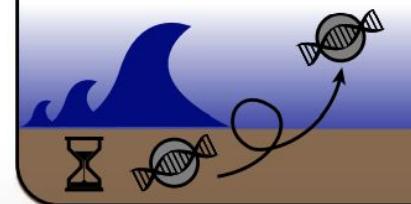
D FATE



C TRANSPORT



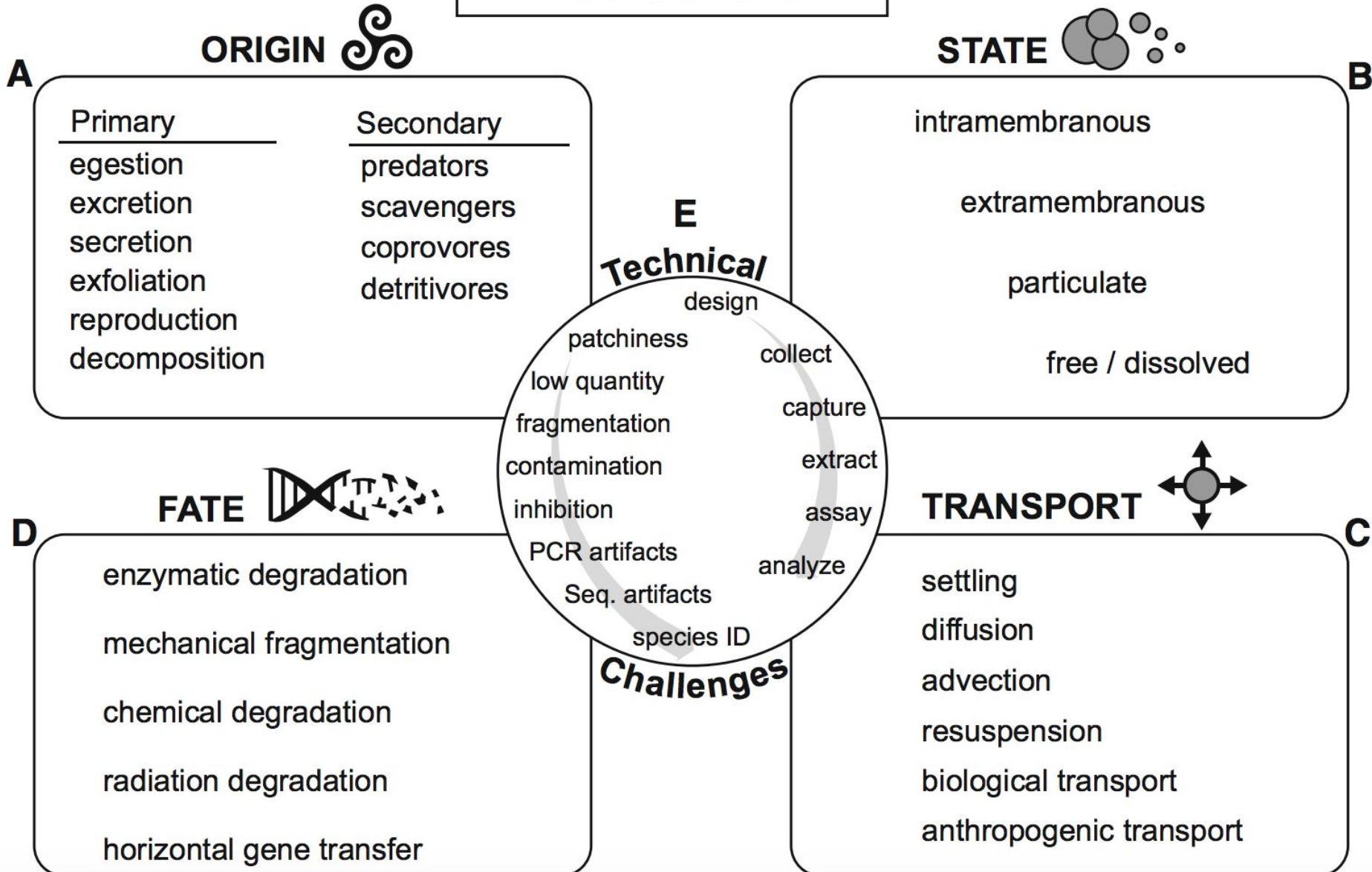
water sample →



water sample →

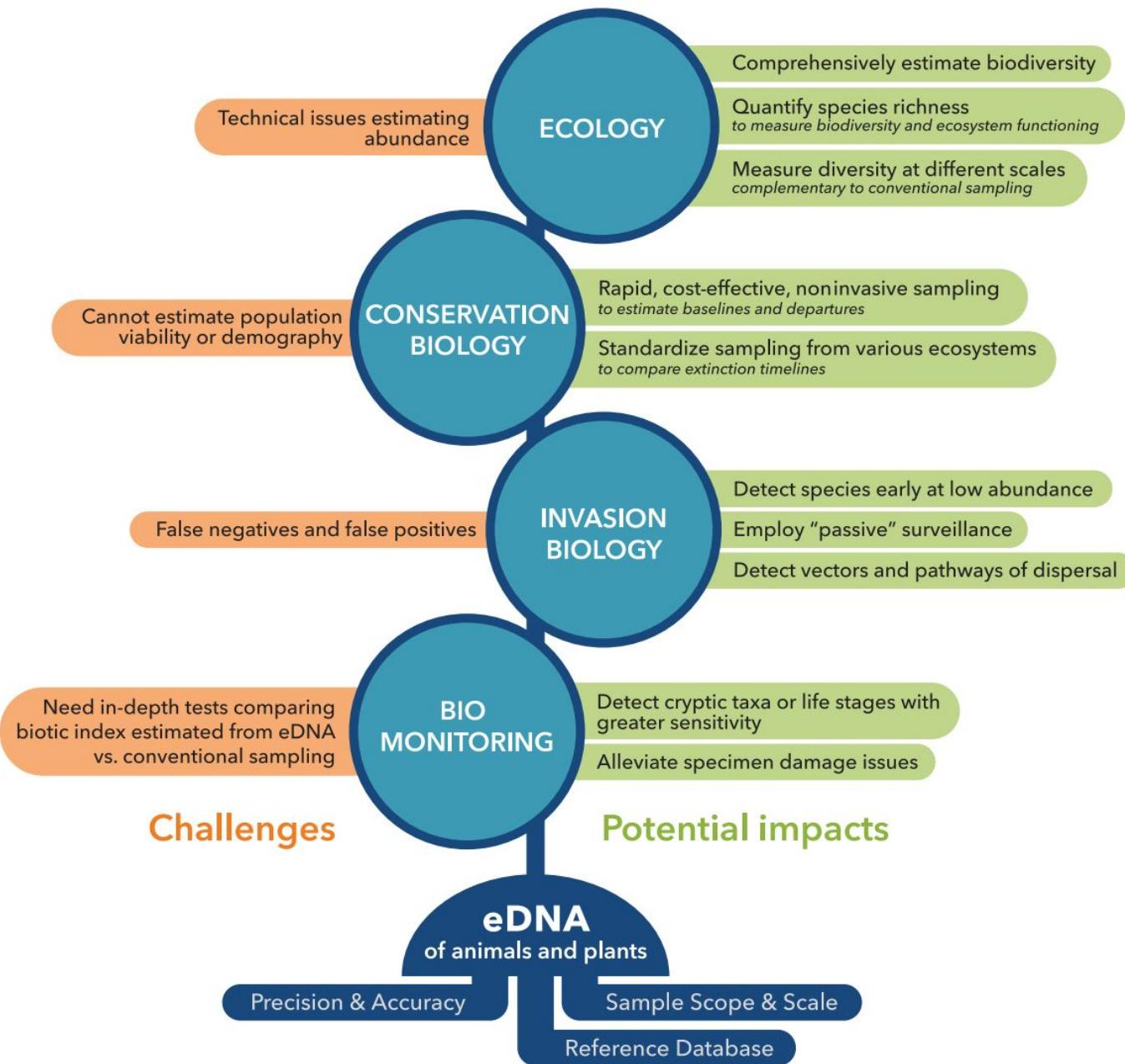


THE ECOLOGY of eDNA



WORKFLOW

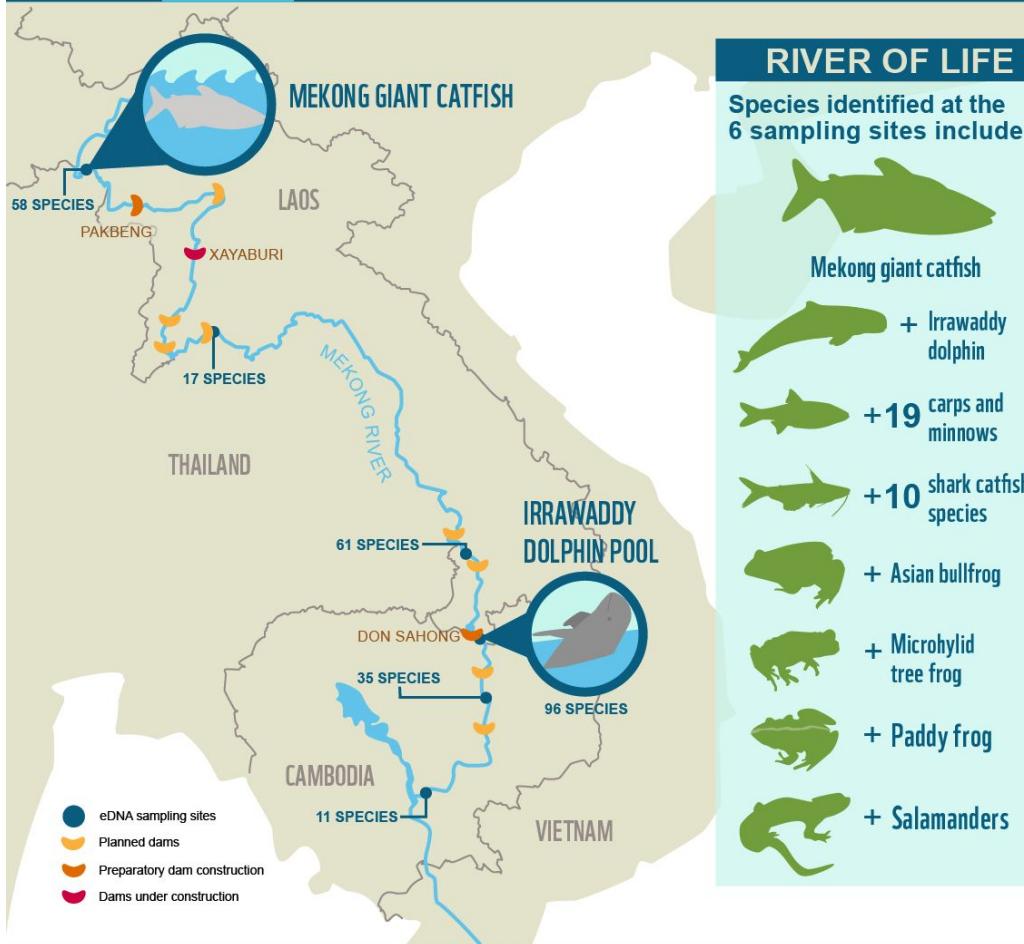
Study design	In the field	In the laboratory	At the keyboard
 <p>Basic science or applied? (e.g., environmental biomonitoring)</p> <p>What is your study goal?</p> <ul style="list-style-type: none">• presence/absence• diversity assessment• absolute quantification <p>What taxa will you target?</p> <p>Is the scale of inference for your sample type appropriate to your question?</p> <p>Can you compare complementary data types? (e.g. traditional vs. eDNA)</p> <p>Does your sampling/replication scheme provide good statistical power?</p>	 <p>What type of sample is needed? (water, soil, air)</p> <p>What metadata should you collect?</p> <p>How many replicates will you collect?</p> <p>Does your sampling protocol minimize/control for :</p> <ul style="list-style-type: none">• contamination (e.g., positive and negative controls)• any known biases (e.g., inhibitors, sample volume)	  <p>Sample Handling Phase</p> <p>What extraction method? (physical vs. chemical)</p> <p>How much sample?</p> <p>What locus and primers?</p> <p>Do you need to generate reference sequence data?</p> <p>Are technical replicates needed?</p> <p>What library preparation method will you use?</p> <p>How many samples will you index and pool?</p> <p>What sequence depth is needed per sample ?</p> <p>What read length will you use?</p>	 <p>DNA Processing Phase</p> <p>What sequencing platform will you use?</p> <p>Do you need paired-end sequencing?</p> <p>Have you included appropriate quality assurances? (e.g., mock community, qPCR, bioanalyser traces)</p> <p>Does your laboratory protocol minimize/control for:</p> <ul style="list-style-type: none">• contamination (e.g., positive and negative controls)• any known biases (e.g., primer bias, coverage, taxonomic resolution) <p>Are you including appropriate quality filtering of your data? (see Box 2)</p>



DIVERSITY BEFORE THE DAMS



Cutting-edge environmental DNA (eDNA) sampling shows strong promise for surveying the Mekong River's biodiversity. A first test found over 175 species at just 6 sampling sites. But time is running out to study the Mekong's incredible biodiversity, with many species endangered by the 11 hydropower dams planned or under construction on the river's mainstream.



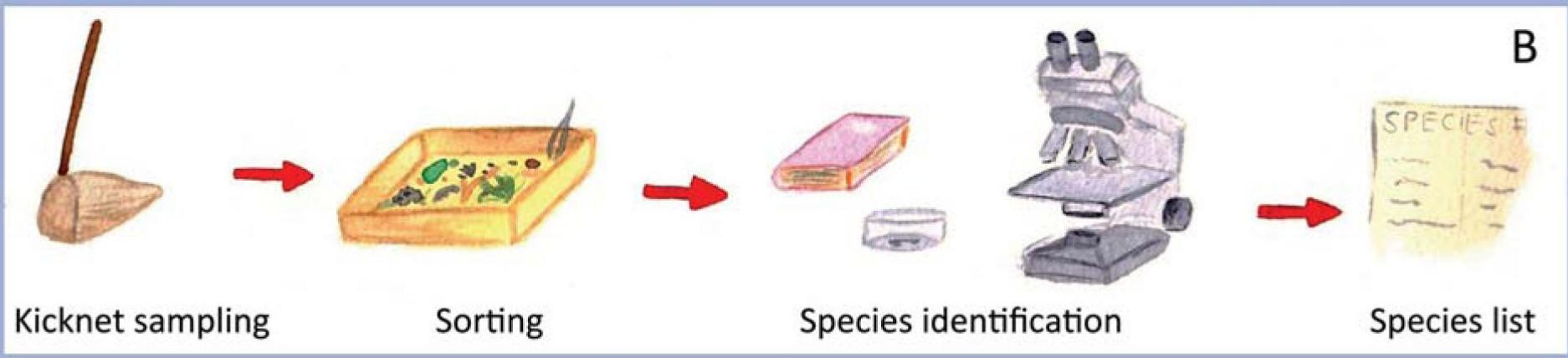
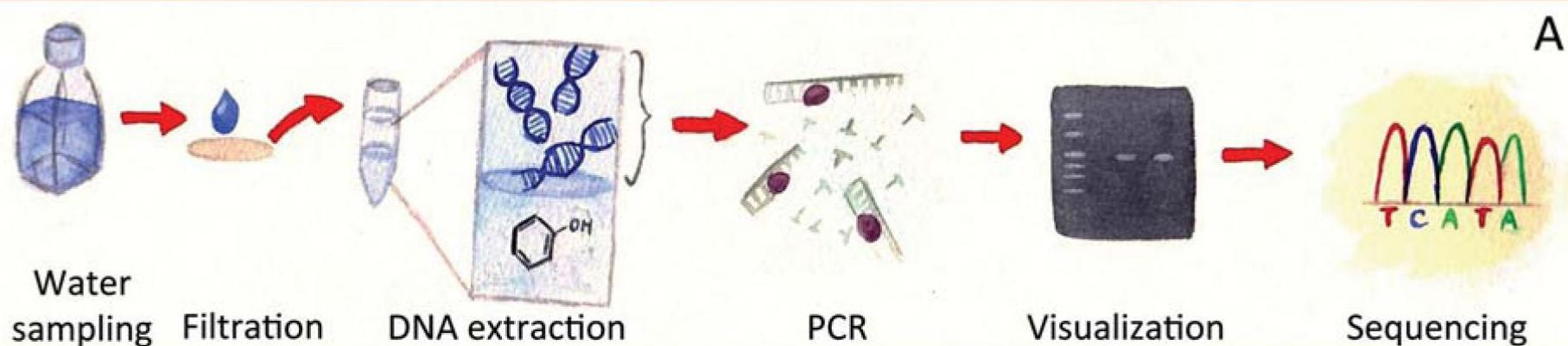
eDNA: A NEW WAY TO STUDY BIODIVERSITY

Environmental DNA (eDNA) tells the story of the life in a given area. Every species leaves traces of its presence, including skin cells, urine and feces. In a freshwater environment like the Mekong, scientists can use cutting-edge methods to filter the water and detect these trace particles of eDNA. By comparing the eDNA in a sample to genetic databases, it is possible to create a snapshot of life in the river at the time and place of sampling, including rare and difficult to detect species like the Mekong Giant Catfish.



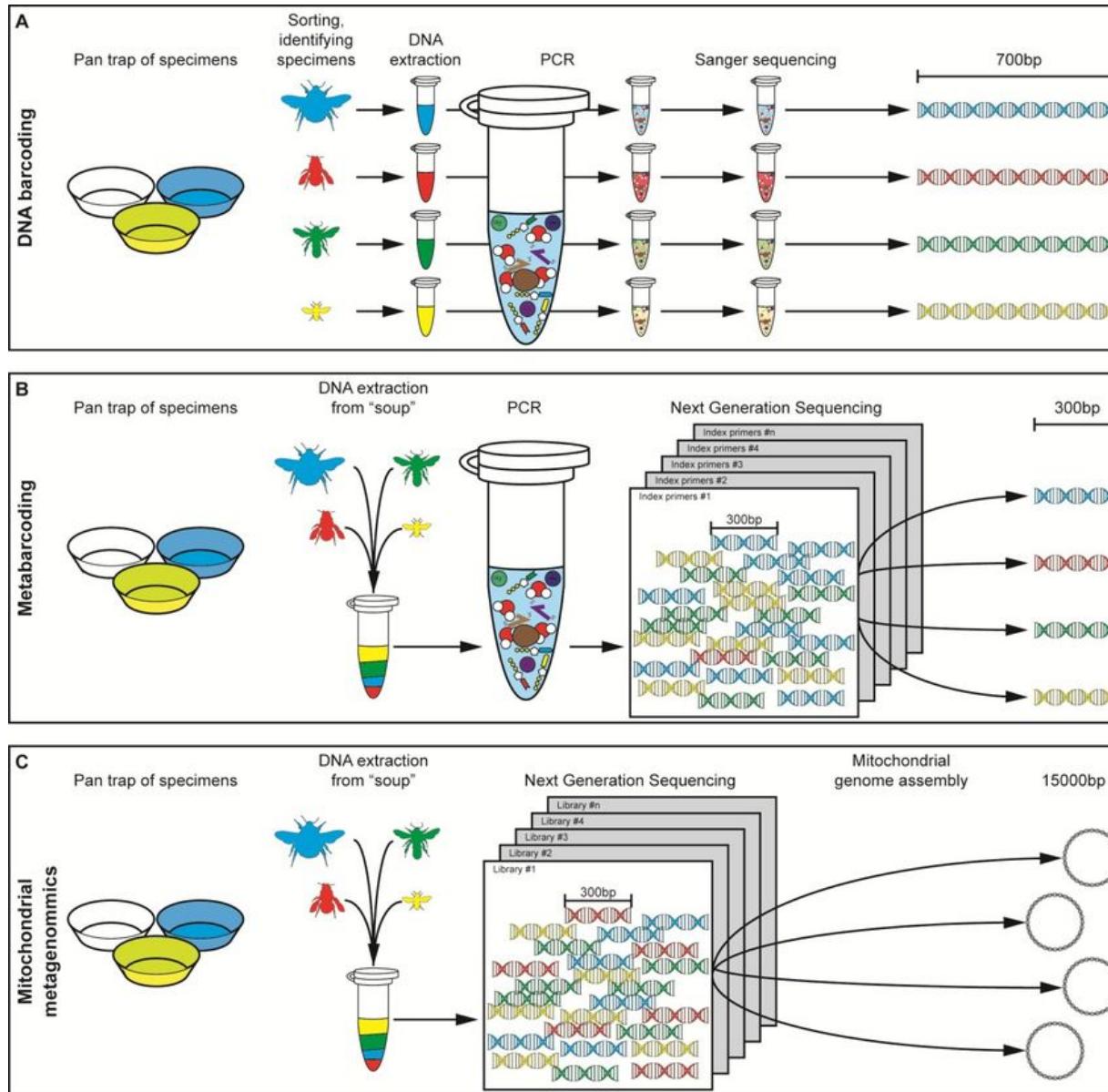
© 2015

Are you convinced?



Mächler et al. (2014) *Freshwater Science*

Review: Genomics, metabarcoding, or metagenomics?



Readings:



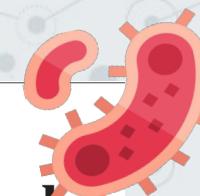
SCIENCE ADVANCES | RESEARCH ARTICLE

ECOLOGY

Environmental DNA illuminates the dark diversity of sharks

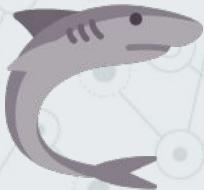
Germain Boussarie,^{1,2*} Judith Bakker,^{3*} Owen S. Wangensteen,^{3,4} Stefano Mariani,³ Lucas Bonnin,^{1,2} Jean-Baptiste Juhel,^{1,2} Jeremy J. Kiszka,⁵ Michel Kulbicki,⁶ Stephanie Manel,⁷ William D. Robbins,^{8,9,10} Laurent Vigliola,^{1†} David Mouillot^{2,11†‡}

OCEAN PLANKTON

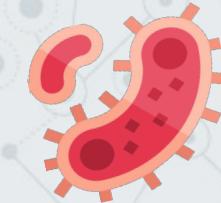


Structure and function of the global ocean microbiome

Shinichi Sunagawa,^{1*†} Luis Pedro Coelho,^{1*} Samuel Chaffron,^{2,3,4*} Jens Roat Kultima,¹ Karine Labadie,⁵ Guillem Salazar,⁶ Bardya Djahanschiri,¹ Georg Zeller,¹ Daniel R. Mende,¹ Adriana Alberti,⁵ Francisco M. Cornejo-Castillo,⁶ Paul I. Costea,¹ Corinne Cruaud,⁵ Francesco d'Ovidio,⁷ Stefan Engelen,⁵ Isabel Ferrera,⁶ Josep M. Gasol,⁶ Lionel Guidi,^{8,9} Falk Hildebrand,¹ Florian Kokoszka,^{10,11} Cyrille Lepoivre,¹² Gipsi Lima-Mendez,^{2,3,4} Julie Poulain,⁵ Bonnie T. Poulos,¹³ Marta Royo-Llonch,⁶ Hugo Sarmento,^{6,14} Sara Vieira-Silva,^{2,3,4} Céline Dimier,^{10,15,16} Marc Picheral,^{8,9} Sarah Searson,^{8,9} Stefanie Kandels-Lewis,^{1,17} Tara Oceans coordinators‡ Chris Bowler,¹⁰ Colombar de Vargas,^{15,16} Gabriel Gorsky,^{8,9} Nigel Grimsley,^{18,19} Pascal Hingamp,¹² Daniele Iudicone,²⁰ Olivier Jaillon,^{5,21,22} Fabrice Not,^{15,16} Hiroyuki Ogata,²³ Stephane Pesant,^{24,25} Sabrina Speich,^{26,27} Lars Stemmann,^{8,9} Matthew B. Sullivan,^{13§} Jean Weissenbach,^{5,21,22} Patrick Wincker,^{5,21,22} Eric Karsenti,^{10,17†} Jeroen Raes,^{2,3,4†} Silvia G. Acinas,^{6,†} Peer Bork^{1,28†}



Discussion Questions:



1. How were genomics, metagenomics and/or metabarcoding used in each paper? Do you buy their results/eDNA?
2. What is the importance of taxonomic resolution (i.e., species vs. phylum) between the two papers?
3. What (feasible) future studies and ecological questions would you ask based on the results of both papers?
4. What aspects of community ecology, metagenomics, and/or metabarcoding could be used in your study system or lecture topic from this class?

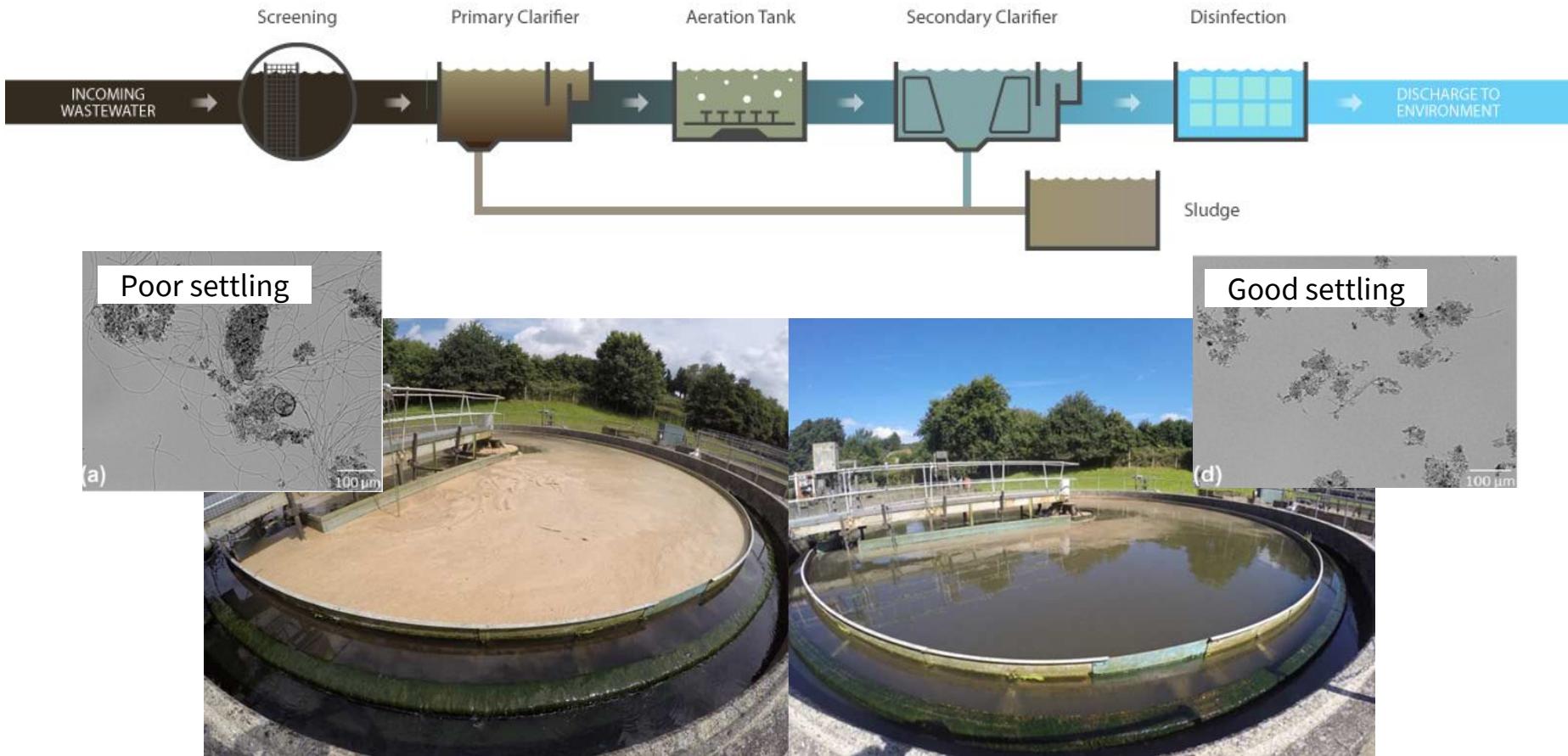
Dr. Lauren Stadler

Assistant professor at Rice University in Civil and Environmental Engineering

"We combine microbial ecology, environmental chemistry, and sustainability assessment to study used water treatment processes, resource recovery, and their impact on the environment and human health."



Microbes in an engineering context



Mesquita, D. P., Amaral, A. L., & Ferreira, E. C. (2011). *Chemosphere*