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Ecological Patterns of Marine Bacteria, Archaea, Protists and Viruses on Small to Large Temporal and Spatial Scales

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Abstract: We used metagenomes and mock communities to validate the use of universal 3-domain rRNA primers which simultaneously amplify bacteria, archaea, and protist sequences with excellent overall coverage. Amplicons and metagenomes were used to study patterns in distributions of microbes and viruses on a wide range of scales. At the San Pedro Ocean Time Series site, the prokaryotic and viral communities have had a remarkably stable and resilient average community composition for more than a decade, with superimposed seasonality at the "species"-like level; however, viruses show Red Queen dynamics, constantly changing strains, suggestive of an "arms race." In contrast to prokaryotes and viruses, eukaryotes were surprisingly unstable in their community composition, and showed an opposite pattern with depth in Shannon Diversity. Large-scale ocean transects of "whole seawater" samples (>0.2  $\mu$ m) analyzed with universal primers were able to discern global patterns in the distribution of organisms present, relative to each other and the community as a whole, providing ground-truth data for improvement of global biological-physical models, and showing that prokaryotic rRNA genes dominate quantitatively in most of the world's oceans.

PRIMER VALIDATION: Universal V4-V5 515Y/926R amplifies from all three domains in a single reaction.

San Pedro Ocean Time-series (SPOT): Ecological patterns of whole microbial community composition



Advantages: Excellent coverage, remarkable quantitative accuracy vs other tested primers, and abundances reported relative to the total community, allowing everything to be compared to each other



(BioGEOTRACES/HOT/BATS + Malaspina + MBARI + SPOT + Tara)

Prokaryotes unexpectedly dominate rRNA genes of both size fractions at all depths at SPOT (see also global biogeographies)

## Prokaryotic 16S 🚔 Chloroplast 16S 🚔 18S



Prokaryotes and protists have similar Shannon Diversity index in the euphotic zone, but opposite patterns with increasing depth at SPOT: prokaryotes increase and protists decrease. May relate to low oxygen at depth. Note also higher prokaryote diversity in the larger size fraction, suggesting more diversity in attached prokaryotes.



Virus Community at SPOT: Steady Average, Seasonality, Red Queen Strain Dynamics Viral Community Composition at SPOT

Rows

J Cesar Ignacio-Espinoza, Nathan Ahlgren, Jed Fuhrman Nature Microbiology 2020

Monthly DNA (0.02-0.2 µm size fraction, 5m depth) metagenomes. For assembled contigs determined to be viral, abundance analyzed by metagenomic read recruitment, 98% ID.



Metagenomic abundance comparisons verify 515Y/926R amplicons are highly quantitative



We used the same DNA as from metagenomes, did PCR with 515Y/926R Universal Primers, compared taxon proportions in MG and amplicons (stations with open circles). 16S linear (r<sup>2</sup> ~0.95), close to 1:1 proportion, i.e. minimal bias.



Global Marine Microbial Biogeographies, All Taxa Simultaneously **Non-size-fractionated** > 0.2  $\mu$ m samples, Universal V4-V5 515Y/926R amplicons Jesse McNichol, Yubin Raut

V4-V5 Amplicon ASVs are Highly Resolving Phylogenetically E.g. Can distinguish cyanobacterial *Prochlorococcus* ecotypes at a level previously thought to require targeted sequencing





Compare all 16S and 18S together Fraction of 18S amplicons / (16S + 18S)



When analyzed at 98% identity:

rity 8'0

-9.0 Sin

Curtis 0.4

Bray 0.2

1. Surprisingly steady composition over 5 years – like prokaryotes 2. Seasonality also like prokaryotes, similarity peaks at 12,24,36, 48 month intervals

3. Contigs recruit (@98% ID) a majority of reads in viromes



12 24 30 36 54 18 42 48 Months between samples

Each trace essentially follows combinations of SNVs, i.e. strains



time with 7populations decade range of sorted from relative most (TOP) to least abundance. abundant Almost all types (BOTTOM) present almost all the time. In general, abundant ones stay abundant, rare ones stay rare.  $0^{1} 10^{2} 10^{3} 10^{4} 10^{5} 10^{6} 10^{6}$ ormalized relative abundance 60 However, when examined at 100% identity, characterizing Single Nucleotide Variants, each combination of microvariants (making up the SNV profile) within a contig population is abundant only for months Time Shift (months) For all viral *contigs* with enough data to i.e. Strain level Red confidently call SNVs (4002 of them, **Queen** –like dynamics. 10X coverage over 90% of length). The "species" stay the The typical detectable lifetime of an same, with constantly SNV profile (strain) is 1 - 1.5 years,

changing strains.

Almost all variations are

