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Conference Abstract

Rewards and Challenges of eDNA Sequencing with Multiple Genetic Markers for Marine Observation Programs

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Abstract

Metabarcoding of environmental DNA (eDNA) samples holds new promise to increase our ability to measure changes in biodiversity and community composition over time. It can allow the characterization of large groups of organisms where traditional sampling may be impractical or not cost-effective. However, it is still unclear how best to compare and combine this information with morphological counts in order to inform policies and biodiversity metrics that are based on traditional sampling results. Under the Marine Biodiversity Observation Network (MBON) initiative, multiple taxonomic marker genes (16S rRNA, 18S rRNA, mitochondrial cytochrome c oxidase subunit I (COI), and 12S rRNA) have been used concurrently to examine the phylogenetic diversity of samples across trophic levels from microbes to vertebrates. Marker genes and their amplification primers target a different (and sometimes overlapping) group of organisms. Just as with traditional sampling methods, each have biases towards detecting certain organisms over others. Though eDNA metabarcoding often detects many more species than can be identified through microscopic or macroscopic net tow counts, processing and relating sequence

data to traditional counts and biodiversity measures is an ongoing challenge. For samples collected within the MBON project, an analysis pipeline has been adapted to standardize sequence analysis of each marker gene. The pipeline processes reads from quality control and trimming through clustering of sequences into Operational Taxonomic Units (OTUs). Taxonomic identification of OTUs uses publically available sequence databases. Finally, the results of the analysis pipeline are combined into a Biological Observation Matrix (BIOM) file with metadata pertaining to the biological sample, PCR processing, and bioinformatic analysis. BIOM files can be used in downstream analysis to analyze biodiversity patterns within the samples.

Monterey Bay in California, USA, is a hot spot of biodiversity and productivity fed by nutrient-rich upwelling water along the coast. A local time-series of samples has been collected by the Monterey Bay Aquarium Research Institute at coastal stations within the bay, providing several decades of contextual environmental data. Samples taken from this time series are ideal for testing the ability of eDNA sequencing to show variability in taxonomic groups over time. For metabarcoding analysis, samples were chosen representing different seasons corresponding to spring (early) upwelling, summer (late) upwelling, fall oceanic regime, and a winter (Davidson) regime from the years 2013-2016. Samples were analyzed across four taxonomic marker genes: two small-subunit ribosomal RNA genes targeting prokaryotic (16S rRNA) and eukaryotic (18S rRNA) organisms and two mitochondrial genes targeting eukaryotes (cytochrome c oxidase subunit I gene (COI)) and vertebrates (mitochondrial small-subunit ribosomal RNA gene (12S)). In order to combine data from multiple markers, species occupancy modeling was used to determine the probability that an OTU is truly present in a sample (as described in Kelly et al. 2017 and Lahoz-Monfort et al. 2015). Many taxonomic groups show seasonal trends in species abundance and diversity in Monterey Bay. Together this work illustrates the rewards and challenges of applying multiple genetic markers to eDNA sequencing analysis of an environmental time series.

Keywords

environmental DNA (eDNA), metabarcoding

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