Chesapeake Community Research Symposium 2026

Session 23: Molecular Approaches for Chesapeake Bay Ecology and Biogeochemical Functions: from Genes to Insights

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Chesapeake Bay metagenomes across broad organisms and spatio-temporal scales

Chesapeake Bay is the largest estuary in North America and one of the most intensively studied estuarine ecosystems. Strong seasonal and spatial variability create a highly dynamic environment for organisms inhabiting the Bay. Molecular approaches such as 16S and 18S rRNA gene analyses have revealed substantial diversity and distribution patterns of prokaryotic and eukaryotic communities in the Bay. Metagenomics has been used to investigate microbial communities in the Bay and all organisms in specific sites of the Bay. However, a comprehensive, ecosystem-wide metagenomic survey encompassing all organismal groups and across the Bay has not previously been conducted. In this study, we collected water samples from the upper, middle, and lower Bay across four seasons associated with the Chesapeake Bay Program monitoring sampling regime and performed metagenomic analyses on three size fractions (0.2–1 μm, 1–3 μm, and >3 μm). In phase 1, 18 metagenomes (surface water) were sequenced at a depth of ~100 Gb per sample, yielding a total of 1.6 Tb of sequences. We identified 624 archaeal species, 22,508 bacterial species, 7,215 fungal species, 16,110 plant species (including microalgae), and 43,561 animal species (including zooplankton and eggs or larvae of crabs, shrimp, shellfish, and fish). Principal component analysis revealed distinct genomic compositions among size fractions, as well as pronounced spatial and seasonal variability in specific taxonomic groups. This extensive metagenomic dataset provides a powerful resource for investigating genomic traits and functional potential across Chesapeake Bay biota, tracking changes in microbes, flora, and fauna, and enabling comparisons with other estuarine ecosystems worldwide. We invite collaborations at all levels to leverage this dataset to advance understanding of the ecological health and economic sustainability of the Chesapeake Bay.

Clara A. Fuchsman (University of Maryland Center for Environmental Science Horn Point Laboratory), Michael E. Kalinowski, Jacob A. Cram, Sairah Y. Malkin

Examining Metagenomics Across Particle Size and Redox Gradients in Chesapeake Bay

Oxygen depletion or anoxia is common in estuaries with high anthropogenic impact. The mesohaline Chesapeake Bay main channel has eutrophication-induced sulfidic waters in the summer months. We examined both biogeochemical parameters and 45 standard-enabled metagenomes and organic matter C and N of size fractionated particles (0.2-3, 3-30, 30-180, >180 µm) in a water-column depth profiles across the redox gradient at two stations in the mesohaline region from August 2021. One station included an intrusion of salty oxic waters into the sulfidic zone. Particles were dominated by bacteria. Extraction standards in our metagenomes combined with quantification of single copy core genes using a phylogenetic read placement technique, allowed calculation of numbers of total bacteria and bacteria of known function for each particle size as counts per liter and counts per µM C. The concentrations of particulate carbon and nitrogen in the 30-180 and >180 µm fractions were much lower than in the 3-30 µm size fractions. Similarly, the number of bacteria were highest in the free-living and 3-30 µm size fractions. Metagenome Assembled Genomes were used to link biogeochemical functions to identity and phylogenetic read placement was used to obtain quantitative abundances. In large (30-180 and >180 µm) particles, nitrous oxide reductase, which mediates the final step of denitrification, was dominated by autotrophic S-oxidizing denitrifiers. Small particles were dominated by heterotrophic denitrifiers. Despite this, there were actually quantitatively more S-oxidizing denitrifiers on small particles due to the much higher number of small particles. Heterotrophic denitrifiers included novel groups such as Planctomycetota, Delongbacteria, and Krumholtzibacteria. Bacteria mediating DNRA and sulfate reduction, were present in particles but at much lower abundances than denitrifiers. S oxidation was the dominant metabolism in the free-living community. Quantitative metagenomics helps acquire less biased views of microbial ecology and will help modeling efforts.

Isabel Baker (Johns Hopkins University), Kalev Hantsoo, Anna Hildebrand, Alexandria Flynn, Sairah Malkin, Laura Lapham, and Maya Gomes

Microbial methane sinks are insufficient under continued eutrophication in the Chesapeake Bay

Coastal sediments account for over half of marine methane emissions, despite hosting microbial consortia that consume much of the methane produced in situ. Under oxygen-depleted conditions, anaerobic oxidation of methane (AOM) is traditionally mediated by archaea operating in syntrophy with sulfate-reducing bacteria, producing the canonical sulfate–methane transition zone observed in many aquatic sediments. More recently, some AOM archaea have been shown to independently couple methane oxidation to alternative electron acceptors, such as iron, suggesting a potential pathway for methane capture under sulfate-limiting conditions. How robust this microbial buffering capacity is under changing environmental conditions, especially in dynamic coastal settings where anthropogenic activity alters sulfate and methanogenic substrate supply, remains uncertain. Here, we combine metagenomics, porewater geochemistry, and stable isotope analyses from two sites in the seasonally dysoxic Chesapeake Bay to assess controls on methane containment. At the site with lower water-column sulfate (<3 mM), methane was completely oxidized before reaching the sediment–water interface. In contrast, methane escaped from sediments at the higher-sulfate

site (>10 mM), despite clear geochemical evidence for active AOM coupled to both sulfate and iron reduction. Paradoxically, methane-oxidizing archaea were more abundant at the site of methane release, with species-level niche partitioning consistent with multiple, vertically distributed AOM zones. We attribute methane escape at this site to high inputs of labile organic matter, which stimulate organotrophic sulfate reduction, depleting sulfate available for AOM while simultaneously fueling methanogenesis. We propose that periodic delivery of more refractory organic matter during storm events, combined with higher sedimentation rates, further exacerbates this effect, leading to multiple depth horizons where AOM and methanogenesis are vertically stacked or co-occur. Together, these results demonstrate that eutrophication-driven increases in organic matter reactivity can weaken biological methane containment, amplifying coastal methane emissions under future global change.

Michael E. Kalinowski (UMCES - Horn Point Laboratory), Clara A. Fuchsman, Carol Kim, Sairah Y. Malkin

The Bottom Water Oxygen Impacts on Downcore Sulfur Cycling in Chesapeake Bay Sediments Inferred using Metagenomics

Seasonal eutrophication-induced hypoxia and anoxia in the Chesapeake Bay main stem alters organic matter remineralization to favor sulfate reduction in surface sediments. Without an aerobic sediment layer, sulfide can efflux to surface sediments and the water column decimating benthic fauna diversity and abundance. Bottom water oxygen impacts on sediment microbial communities and their oxidative metabolic pathways can improve understanding of how eutrophication influences sediment biogeochemistry. We collected sediment cores with a gravity core per station during the 2019 summer aboard the R/V Rachel Carson. Eight stations (6 main channel, 2 western shoals) ranging in bottom water oxygen concentrations were sampled along the Chesapeake Bay. One core was allocated for porewater analyses including ammonia, DIC, ferrous iron, methane, and sulfur speciation. A second core collected sediment for metagenomes (n=72). After sequencing all samples were processed to create metagenome-assembled genomes and ran through a phylogenetic read placement pipeline which required a genetic marker. The chosen genes included microbial identity (rpoB), dark carbon fixation (aclAB/cbbLM), nitrification (amoABC/nxrAB), denitrification (nosZ), DNRA (nrfA), iron-oxide reduction (mtrB/omcBSZ/pilA), sulfur oxidation (r-dsrA/soxB), and sulfate reduction (dsrA). Main-channel stations (CB3.3C, CB4.3C) observed sulfidic bottom water coupled with a rapid depletion of sulfate:chloride ending at 8.5 and 4.5 cm, respectively. Western shoal (CB3.3W), and upper bay (CB3.1, CB3.2) stations contained bottom water oxygen and experienced deeper depletion of sulfate:chloride that ranged from 11-22.5 cm, which indicates deepening of the sulfate to methane transition zone. Most stations displayed greater microbial genetic potential for sulfur oxidation (r-dsrA/soxB) at surface sediments, then decreased with depth. Sulfate reduction genetic potential (dsrA) was limited at the surface but then increased as sulfate:chloride decreased. Our preliminary data suggests that surface sediments under measurable bottom water oxygen contain elevated oxidative potential.

Anand Gnanadesikan (Johns Hopkins University), Rui Jin

How does including heterotrophic bacteria in a biogeochemical model change the simulation of biogeochemical cycling?

Although heterotrophic microbial processes play a critical role in driving seasonal changes in water quality in the Bay, their effect is often implicitly parameterized in terms of linear decay rates. In this presentation we examine a series of simulations in which we relax this assumption. In the first set of cases, we direct different fractions of the remineralization of particulate and semilabile organic matter (which in the current model goes to ammonia) to a heterotrophic bacterial pool. Because this pool replaces a fraction of the reservoir of nutrients available to phytoplankton, it changes the spatial distribution of productivity, allowing nutrients to penetrate further down the bay and causing changes in hypoxia. We then consider what happens if we allow the remineralization rate to depend on bacterial biomass. We find that the results are very sensitive to how we represent bacterial mortality.

Anne Baldino (Institute of Marine and Environmental Technology, University of Maryland Center for Environmental Science), Dr. Tsvetan Bachvaroff

Deciphering the Functional Capacity of Chesapeake Bay Microbes through Long-Read Sequencing

Algal blooms in the Chesapeake Bay facilitate a complex of microbes rapidly growing in boom-and-bust cycles and act as major hotspots of productivity. Individuals within this diverse microbial community produce a unique suite of molecules which facilitate allelopathic interactions and can promote self growth, enhance nearby organisms, or inhibit competitors. Thus, comprehensive resolution of the metabolic potential of these microbial communities is key for understanding how algal blooms are biologically regulated. Advanced sequencing technologies have unlocked insights into the metabolic potential of microbial interactions within a controlled laboratory environment. As 1% of bacterial species are estimated to be culturable, we need novel methods to investigate understudied environmental microbes. Therefore, I propose the use of in situ long read sequencing for environmental harmful algal blooms in the Chesapeake Bay. Preliminary data confirms a high level of microbial diversity within active blooms, particularly within the bacteria and SAR supergroups (genera richness = 1400, shannon-weaver index = 5.505, simpson's-reciprocal index = 15.142). A wide variety of secondary metabolites were encoded within the metagenome of these microbes, revealing a rich metabolic suite. The sequencing and analysis pipeline developed here can bypass traditional culturing methods and be used to illuminate the potential metabolic pathways and species interactions encoded within diverse environmental genomes. Ultimately, this work aims to grant researchers and other stakeholders unprecedented observational detail into the driving biological forces behind an environmental bloom.

Jenna Lee (Princeton University)

Drivers of temporal co-occurrence patterns and microeukaryote community dynamics in a multispecies diatom bloom

Phytoplankton blooms are responsible for a large fraction of marine carbon fixation and their species composition and succession are modulated by complex abiotic and biotic interactions. Understanding the drivers and mechanisms of phytoplankton blooms is critical to assessing the resilience and stability of these communities. Co-occurrence networks of steady state marine communities have identified core microbial associations, including ubiquitous and diverse eukaryotic parasites. However, the highly dynamic and transient community interactions present in blooms are uniquely difficult to capture and reported associations among taxa vary between studies. Here, we utilized daily 18S rDNA amplicon sequencing and biogeochemical data from 24-L microcosm bloom experiments in Chesapeake Bay to investigate potential eukaryotic interactions during the progression of a diatom bloom. Co-occurrence network analysis resulted in a sparse network, which separated into temporal modules of primarily early- or late-bloom assemblages. Most blooming phytoplankton did not exhibit meaningful taxa-taxa associations, including 78% of diatom diversity (OTU richness), which either had no associations or only displayed associations that were likely caused by niche similarity (i.e., abiotically driven). Modeled grazing rates and additional cross-domain network analysis suggested that heterotrophic organisms dominated microbial interactions. Time-lagged successional patterns and non-specific grazing also contributed to network sparsity, highlighting the complexity of capturing transient community dynamics. Results revealed that biotic interactions were present, but environmental drivers ultimately underpinned the assemblages, interactions, and network topology of the dynamic diatom bloom.

Alex Flynn (Johns Hopkins University), Dr. Isabel Baker, Dr. William Schroer, Dr. Maya Gomes, Dr. Sarah Preheim

Characterizing Microbial Communities of Baltimore Harbor’s Pistachio Tide

Baltimore, Maryland’s ‘Pistachio Tide’ is a phenomenon that happens in the fall of some years, but not others, where large swaths of the Inner Harbor’s water appear to turn a pale, green color and emit a rotten egg odor. While the green color is often assumed to be the result of an algal bloom, heightened levels of chlorophyll that would indicate such a bloom are not detected. These changes in water color and odor have been attributed to green sulfur bacteria (GSB), microorganisms that are green in color and thrive in the presence of sulfide, which emits rotten egg odor. GSB use bacteriochlorophyll, which is not detected by chlorophyll sensors, to perform anoxygenic photosynthesis, using reduced sulfur compounds as electron donors. A key question remains about the relative importance of sulfide accumulated in bottom waters during the summer versus sulfide produced by sulfate-reducing microorganisms in the water column

during Pistachio Tide events in sustaining the microbial bloom, with potential influence over the extent and duration of Pistachio Tide events. This project will characterize the microbial communities of the Pistachio Tide through 16S rRNA sequencing before, during, and after a Pistachio Tide event in October 2025 from sampling areas throughout Baltimore's Inner Harbor. National Aquarium water quality data, USGS hydrological data, and Baltimore Social-Environmental Collaborative weather data will be used to contextualize microbial dynamics. This project investigates how microbial community dynamics and geochemical cycling lead to Pistachio Tide events, which will help to predict future Pistachio Tide events and other bacterial blooms in other densely populated coastal areas.

William F. Schroer (Johns Hopkins University), Shaochen Fan, Sarah P. Preheim

Quantitative sequencing coupled with dilution experiments reveals taxa specific growth and mortality rates in aquatic microbial communities

The structure of aquatic microbial communities is determined by the balance of growth and mortality, however quantifying these rates for specific taxa is a challenge. Disturbances to growth-mortality equilibria can trigger the onset of blooms, such as sulfidic "pistachio tides" that impact water quality in urban waterbodies including the Inner Harbor of Baltimore, MD. Here we pair modified Landry-style dilution experiments with quantitative 16S rRNA gene sequencing to quantify growth and mortality rates of individual taxa in the Inner Harbor prior to and during a "pistachio tide" bloom of green sulfur bacteria and sulfate reducing bacteria. Dilution experiments using whole seawater and grazer/virus free fractions were incubated under environmental conditions (oxic pre-bloom, anoxic mid-bloom). Bulk cellular populations were enumerated by flow cytometry based on size and pigmentation. Quantitative 16S rRNA gene sequencing, performed using an internal standard of Thermus thermophilus genomic DNA, predicted bulk cell counts (R^2= 0.62, p=1E-6), demonstrating the method's efficacy. Additionally, the quantitative sequencing provided taxonomic resolution to the amplicon sequence variant (ASV) level, enabling the elucidation of growth and mortality patterns masked by bulk measurements. For example, the bulk net growth rate of bacteria in undiluted mid-bloom water samples was a modest 0.08 (+/-0.04) per day, however several ASVs of dominant bloom members, Desulfobacterales and Chlorobiales, demonstrated net growth rates of >0.85 per day, indicating continuation of the bloom. Synchrony between sulfate reducers and sulfide oxidizers suggests sulfur cycling within the community may perpetuate the bloom. Similarly, though bulk combined grazer and virus mortality was 0.59 (+/-0.06) per day, ASV level mortality ranged from 4.8 to 0 per day, demonstrating that mortality pressure is not evenly distributed. This work helps elucidate the complex patterns of growth and mortality that shape microbial communities.

Sairah Malkin (Horn Point Laboratory, UMCES), Emily Brownlee, Alex Burns, Jacob Cram, Clara Fuchsman, Xiaoxu Guo, Jamie Pierson, Louis Plough, Greg Silsbe

Weekly eDNA Monitoring Captures Multi-Trophic Seasonal Dynamics and Emerging Interannual Variability: 125 Weeks from the PhytoChop Observatory

The PhytoChop Observatory was recently established on the Choptank River to advance an understanding of microbial ecology and its trophic and biogeochemical relevance. We are collecting weekly size-fractionated filters for DNA, targeting 16S/18S rRNA and COI amplicon analyses. Here we present the first ~125 weeks of amplicon data analysis (June 2023 to December 2025). Strong seasonal patterns emerge at all organizational levels. Among eukaryotes, well-known robust seasonal patterns are confirmed, and the weekly resolution of the dataset provides greater insight into their dynamics. Cold-season taxa include the copepod Eurytemora and dinoflagellate Heterocapsa, while warm seasons are enriched in cnidarians (Chrysaora, Diadumene), certain fish (Morone, Leiostomus), and green algae (Micromonas). Eukaryotes displayed high interannual variability, putatively associated with threshold winter minimum temperatures. As the dataset expands, examination of coordinated phytoplankton-zooplankton shifts may assist with illuminating previously unrecognized trophic connections. Among prokaryotes, stronger seasonal patterns emerged. Warm-season prokaryotes are enriched in Synechococcus (Cyanobacteria), Planctomycetota, and Nitrososphaeria (archaeal ammonia oxidizers), with the latter showing abrupt fall increases. Cold seasons favor Bacteroidota, Verrucomicrobia, and Pseudomonadota. Free-living fractions favor Actinomycetota, while particle-associated fractions are enriched in Planctomycetota. Unexpectedly, Synechococcus were also enriched in the larger size fraction, suggesting possible associations with chitinous surfaces and greater contributions to benthic fluxes than previously assumed. Such high-resolution dynamics have been previously unreported, and we expect to be useful for guiding biogeochemical modelling, such as nitrogen cycling parameterizations. This molecular approach also enables assessment of niche partitioning among ASVs within genera. While many genera exhibit single modes (e.g., Eurytemora prefers <10°C, Micromonas >25°C), others display multimodal distributions. Skeletonema, for example, exhibits three temperature modes (9°C, 19°C, 26°C), suggesting three co-dominant taxa with distinct thermal niches. Such analyses may reveal cryptic species and help inform predictions of community shifts under environmental change.

Katrina M. Pagenkopp Lohan (Smithsonian Environmental Research Center), Emma M. Palmer, Calli Wise, Hannah Brunelle, Robert Aguilar, Andrew Davinack, Patricia Santos-Ciminera, Ruth DiMaria, Matthew Ogburn

Hidden Connections: Uncovering Complex Trophic Networks Through DNA Metabarcoding

Coastal aquatic communities are increasingly subjected to stressors such as habitat fragmentation and climate-driven range shifts, altering species composition and interactions. Our research has demonstrated that amplicon based high throughput sequencing (“DNA metabarcoding”) provides a high-resolution snapshot of trophic connectivity, providing critical insights to changes in diet across age, space, and time. We present our findings on using multi-marker DNA metabarcode assessments on the diet of striped bass (Morone saxatilis) and the North American river otter (Lontra canadensis), taking a top-down assessment of trophic interactions. Our results demonstrate that DNA metabarcoding provides substantial improvements in taxonomic resolution, with many more prey items being assigned to the species level with genetics as compared to morphological methods. We demonstrate how these methods are comparable when assessing the occurrence of prey items with hard bodies, but genetic methods are superior for detecting soft-bodied organisms (e.g., worms). Additionally, this sensitive methodology allows for the detection and subsequent integration of parasites into the food webs, which are increasingly recognized as critical components of food web architecture. Our results demonstrate that these methods can detect parasites that are traditionally included in food webs (i.e., trophically transmitted parasites) and those that have not been considered in food webs (i.e., directly transmitted parasites). This method also allows for the increased detection and integration of concomitant prey into food webs. Finally, we provide a brief overview of the challenges associated with this methodology, including the lack of reference sequences for many taxa and the complications with the high sensitivity of the method. Despite these challenges, mapping trophic connections via DNA metabarcoding allows us to further elucidate multi-species networks, from microbes to top predators, and examine how these interactions respond to anthropogenic pressures.