Exploring Microbial Indicators of Coastal Ecosystem Health

Across a Gradient of Human Development in Tutuila, American Samoa

On Pacific islands, subsistence agriculture and fishing remain essential for sustaining Indigenous populations, yet intensified agriculture and increased wastewater impacts have significantly altered the state of coastal ecosystems. Concomitant shifts in coastal microbial assemblages can alter nutrient cycling and create human health issues. Moreover, having informative microbial datasets available could support management decisions, but are often absent or rely on a single indicator species. We recognized that detailed microbial community assessments are important as long-term shifts in the microbial community structure could be pathological, are tied to health of a coastal region and thus have serious ecological and management implications.

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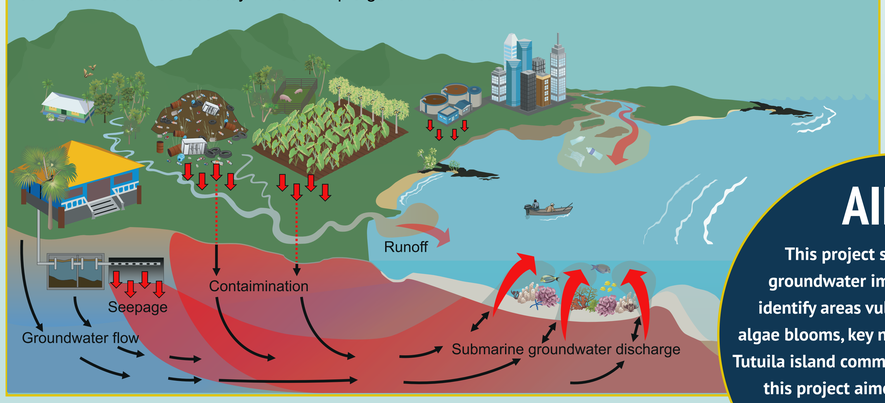
Introduction

Tainted submarine groundwater discharge (SGD) threatens nearshore enviornments by exporting plumes of nutrient-rich discharge, causing persistent harmful algae blooms (HABs), and introducing fecal and manure bacteria to the reef. Over the course of our decade-long study of algal blooms in Hawai'i, we have developed techniques enabling identification of coastal sites where tainted groundwater discharges, offering new tools to identify hot spots – sites that may be precipitously poised to suffer significant collateral impacts if ecosystem features are further exacerpated.

The objectives of our research was to examine the features of emerging indicatorsof ecosystem health: **1)** variations in δ 15N and % N in deployed noncalcified plant tissues, and **2)** modifications in microbial communities as assessed by whole sample genomic assessments.



AIMS

This project sought to assess groundwater impact on reefs and identify areas vulnerable to harmful algae blooms, key needs identified by the Tutuila island community. On a larger scale, this project aimed to describe how development in Pacific Islands impacts coastal reefs.

Site Description

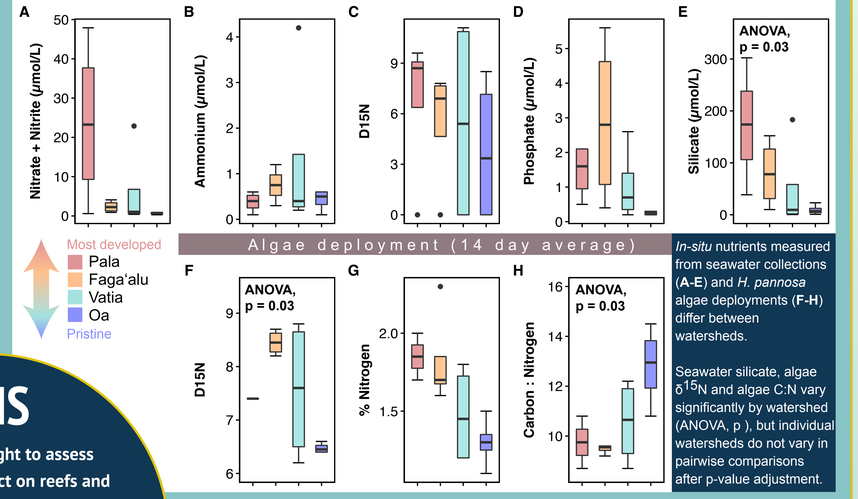
We utilized benthic habitat mapping of Tutuila to categorize finescale sites and established a gradient from impacted to pristine sites, as part of a previous Water Resources Research Center study in 2015.

Field Design

(A) *Coral*: *Porites cylindrica* and *Pocillopra damicornis* fragments were collected from Faga'alu, Vatia, and Oa (absent in Pala). Coral host and symbiont tissue was removed from the skeleton and homogenized. *Water:* 1L of seawater was collected and filtered on a 0.2 µm GF/F filter for four sites in each watershed. *Biofilm:* At each site, four horizontal and four vertical replicate glass slides were deployed on bricks at 1m depth and swabbed after 14 days. **(B)** *Algae*: *Hypnea pannosa* was collected from adjacent locations, starved onshore, and redeployed with the biofilm slides. Resident algae was also surveyed and collected. Assimilated algae nutrients, including $\delta^{15}N$, C:N ratios, and N%, were measured. **(C)** *Enviornment*: *In-situ* conditions and nutrient levels were measured with a YSI on-site and a Seal Analytical AA3 HR Nutrient Autoanalyzer at the SOEST Laboratory for Analytical Biogeochemistry at the University of Hawai'i at Mānoa. Total genomic DNA was extracted from coral blastate, water filters, and biofilm swabs. Water and biofilm libraries were amplified in PCR with the 16S V4 Earth Microbiome Primers (Caporaso et al., 2011). Coral libraries were amplified with 16S V3-4 primers (Kozich et al., 2013). Amplicons were normalized, pooled, and sequenced on an Ilumina GAIIX at the Evolutionary Genetics Core Facility at the Hawaii Institute for Marine Biology. Resulting sequences were processed through the C-MĀIKI (Center for Microbiome Analysis through Island Knowledge and Investigation) pipeline (Arisdakessian et al., 2020) and clustered to 100% identidy Amplicon Sequence Variants (ASVs) for statistical analysis.

Analysis

Environmental Parameters Vary

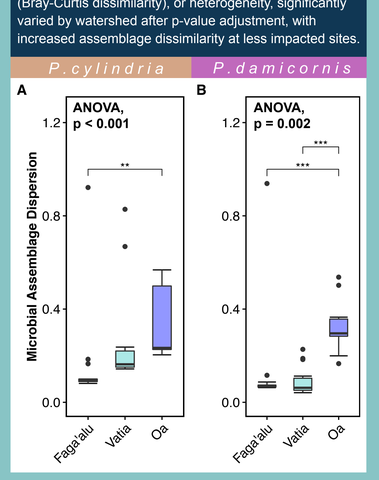


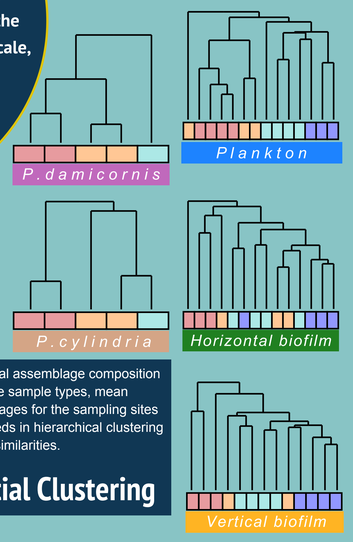
In-situ nutrients measured from seawater collections (**A-E**) and *H. pannosa* algae deployments (**F-H**) differ between watersheds.

Seawater silicate, algae $\delta^{15}N$ and algae C:N vary significantly by watershed (ANOVA, p), but individual watersheds do not vary in pairwise comparisons after p-value adjustment.

Dispersed Coral Microbiomes

P. cylindrica and P. damicornis microbiome dispersion (Bray-Curtis dissimilarity), or heterogeneity, significantly varied by watershed after p-value adjustment, with increased assemblage dissimilarity at less impacted sites.



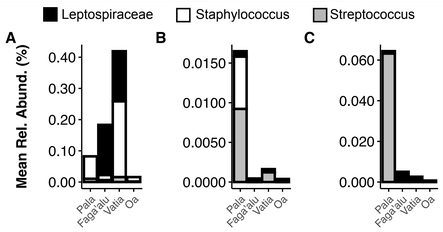


While reef microbial assemblage composition differs between the sample types, mean microbial assemblages for the sampling sites group by watersheds in hierarchical clustering of Bray-Curtis dissimilarities.

Differential Clustering

Traditional Microbial Indicators

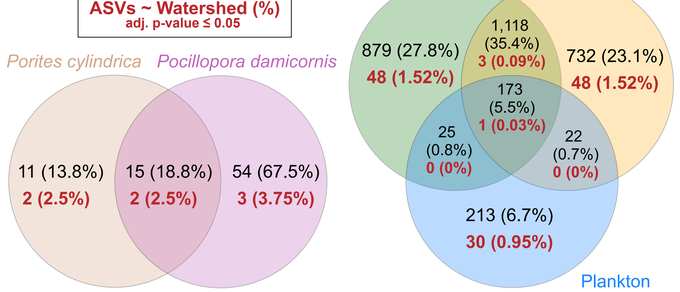
Groups containing traditional fecal idicators *Enterococcus* and *Streptococcus* and groups containing opportunistic zoonotic pathogencs *Staphylococcus* and *Leptospira* differ in mean relative abundace between watersheds.



Exploring New Microbial Indicators

We sought to characterize novel microbial indicators of coastal ecosystem health by modelling ASVs that vary by development level. Linear mixed-effects models quantified the correlation of individual ASVs to the watershed gradient. We found that while ASVs are shared between sample types, very few candidate microbial indicators are shared.

Total ASVs No. (%)
ASVs ~ Watershed (%)
adj. p-value ≤ 0.05



Conclusions

We applied water quality monitoring tools developed and implemented in Hawai'i to augment ongoing water quality monitoring on Tutuila, American Samoa, specifically to identify microbial indicators of ecosystem health in areas vulnerable to water quality degradation. Trends in nutrient loading followed levels of expected human development in each watershed. Different sample types exhibit different compositions but all group by development level to some degree. *In-situ* nutrient measurements and the presence of specifc microbial consituents have identified Vatia as a potential hot spot for tainted SGD and future HABs. We have conducted preliminary, more in-depth studies at the site as a result of this work. We continue to investigate novel microbial indicators of ecosystem health from the wealth of data this work has provided. And this ongoing collaboration continues to build better understanding of SGD and commensurate responses in coastal microbial communities.

We would like extend a big mahalo and fa'afetai to the villages of Afono, Vatia & Faga'alu, Lisa of Faga'alu Catholic Church, Afa and Lina of Vatia Village, American Samoa Community College, Eric Welch, Fa'asalasa Kitiona, Jean Anderson, Abe Voight, Craig Nelson, Krissy Remple, Helen Turano, and the Water Resources Research Center.