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Taxonomic and antibiotic resistance changes to coastal microbiomes in response to rainstorm runoff

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Abstract

Antibiotic resistance (AR) is a global healthcare issue driven by the overuse of antibiotics in clinical, agriculture, and aquaculture applications. Urban and agricultural runoff introduce antibiotic-resistant bacteria and antibiotic contamination to recipient environments. Antibiotics change microbial community compositions in favor of resistant species and can trigger the exchange of DNA carrying antibiotic resistance within a given community. However, mapping the changes in microbial community compositions and AR gene abundance is yet to be elucidated in response to rainstorm runoff. We therefore analyzed the taxonomic and antibiotic resistance gene abundance changes to coastal microbiomes in response to rainstorm runoff. Sampling at the Batiquitos lagoon outlet in Carlsbad, California occurred over 14 days; before, during, and after the first two rainstorms of the 2019-2020 season. Coastal water was captured on-site on a 0.22 µm mixed cellulose ester (MCE) membrane filter. We performed total DNA isolation and shotgun library preparation on the isolated microbiomes followed by 2 x 150 base paired-end sequencing. Microbial composition and antibiotic resistance gene identification was performed on the resulting metagenomes, to determine a time course profile of relative microbial abundance and antibiotic resistance profiles. Additionally, we performed meta-SourceTracker analysis on the time course metagenomes to investigate proportions of exogenous and endogenous microbial community members throughout and following rainstorms, as well as to explore possible sources of exogenous taxa. We observed an overall bimodal increase in alpha diversity and AR gene counts in the 24-72-hour period following each rainstorm. Taxonomic changes are reflected by a relative depletion of Cyanobacteria and relative increase in Proteobacteria and Bacteroidetes. Increases in Proteobacteria appear to be predominantly marine eutrophication-associated microbes while Bacteroidetes increases were predominantly freshwater- and soil-associated microbes commonly implicated in fish and human disease. The microbial community profile returned to a pre-storm composition after approximately six days contrary to the three-day recovery time commonly referenced.

Background & Hypothesis

Antibiotics and antibiotic resistant (AR) bacteria are flushed into aquatic ecosystems via storm runoff

Antibiotics alter the microbial community by
- Selecting for resistant strains
- Triggering gene transfer between microbes

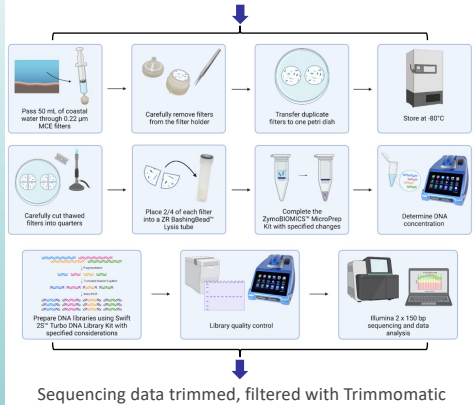
Recipient environments with stressing conditions may select for higher resistant bacteria

Microbial community compositions and AR gene abundance mapping have yet to be elucidated

Rainstorm runoff results in:
- **Increase in AR gene abundance**
- **Alter microbiome taxonomic profiles**

Methods

50 mL coastal water filter captured over 14 days: before, during, and after first 2 seasonal rainstorms



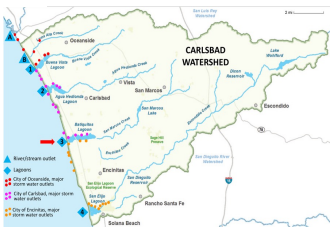
Sequencing data trimmed, filtered with Trimmomatic

Microbial composition determined with Centrifuge

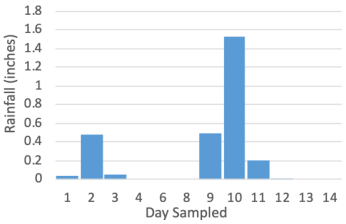
ARG calling with RGI bwt plus wildcard database

Results

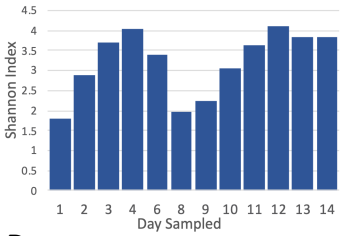
A Carlsbad Watershed Map



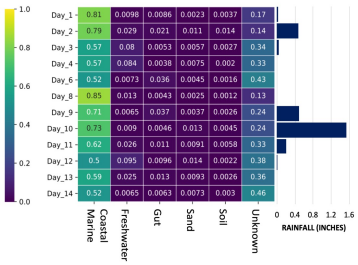
B Rainfall throughout Sampling



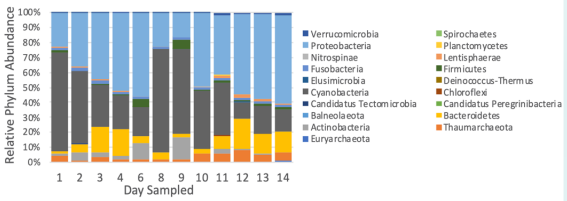
C Species-Level Alpha Diversity



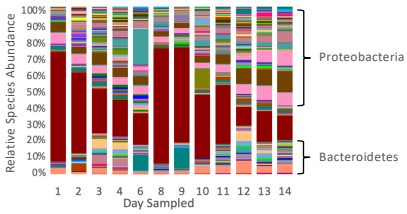
D Tracking Environmental Microbial Sources



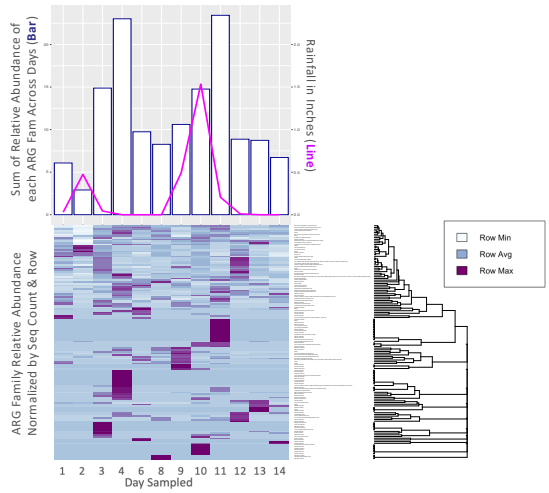
E Prokaryotic Relative Phylum Composition



F Prokaryotic Relative Species Composition



G Relative ARG Changes throughout Sampling



Conclusions

- A bimodal increase in alpha diversity and AR gene counts within a 72-hour period following rain.
- A relative increase in Proteobacteria and Bacteroidetes with a relative depletion of Cyanobacteria
- After >5 days, the microbial community showed recovery to a pre-storm state
- Marine and freshwater associated microbes show relative abundance changes
- Relative increase in antibiotic resistance genes two days following the highest amount of rainfall

Future Directions

- Longitudinal statistical analyses on observed changes
- Reanalyze data through meta-SourceTracker after addition of relevant databases to decrease the amount of unknown reads
- Repeat studies in 2020/2021 storm season
- MetaHiC studies to map AR genes to specific hosts

Acknowledgments:

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Pilot Attempt of MetaHiC Sequencing on Coastal Microbiomes to Map Mobile Genetic Elements

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Abstract

Coastal microbial communities are altered in response to rainstorms due to exogenous factors such as the runoff of antibiotics, and the introduction of foreign nutrients and microbes to the coast. These factors all have the potential to influence the composition and diversity of the coastal microbiome, as well as the rate of transfer of mobile genetic elements between microbial community members. Current sequencing technologies don't provide long-range linkages to generate complete taxonomic and mobile genetic element profiles. Therefore, we implemented a novel metagenomic chromatin conformation capture (MetaHiC) assay to perform more comprehensive binning of metagenome-associated genomes and mapping of mobile genetic elements to specific host organisms. Sampling was done at the Batiquitos Lagoon in Carlsbad, CA over 14 days; before, during, and after a seasonal rainstorm in February 2022. Samples were taken by passing 50 mL of coastal water through a 0.22 µm mixed cellulose ester (MCE) filter. As a pilot test, we performed, proximity ligation, and library preparation of on of the filter-captured microbiomes. The library QCed and underwent Illumina 2 x 150 base-pair sequencing. Results of the 1.7 million reads generated indicated that we were capturing long-range genomic interactions and revealed 11 binned metagenomes ranging from 0.86% to 97% completion. Following these data, the remaining samples will undergo MetaHiC and deeper sequencing for metagenome and mobilome assembly.

In this pilot attempt of a chromatin conformation capture (MetaHiC) assay, we established protocols to crosslink and proximity-ligate coastal metagenome samples for metagenome and mobilome assembly.

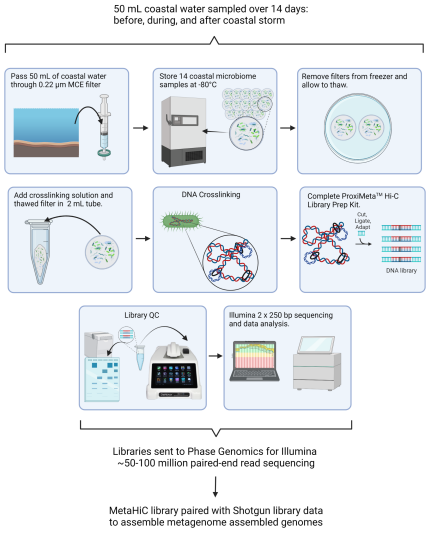
Background & Hypothesis

Rainstorms cause runoff of antibiotics, nutrients, and exogenous organisms into coastal ecosystems.

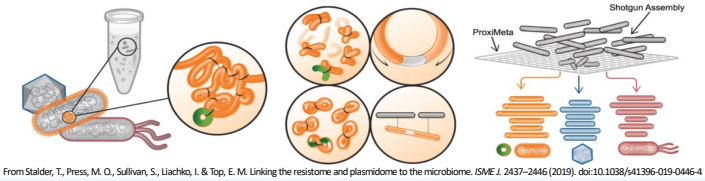
Rainstorms affect coastal microbiomes by:
- Selecting more resistant organisms
- Introducing mobile genetic elements

Perturbations resulting from rainfall may cause a cyclic shift in coastal microbiome species composition.

Methods



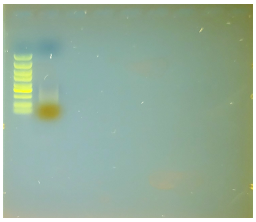
ProximMeta™ Hi-C Workflow



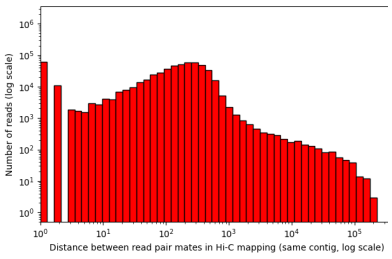
From Stalder, T., Press, M. O., Sullivan, S., Liachko, I. & Top, E. M. Linking the resistome and plasmidome to the microbiome. *ISME J.* 2437–2446 (2019). doi:10.1038/s41396-019-0446-4

Results

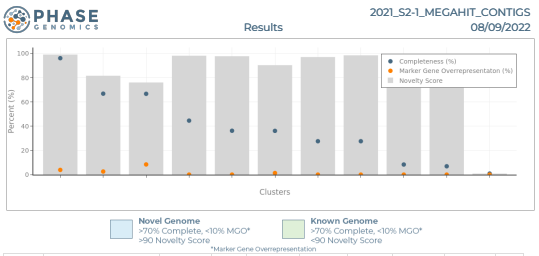
Gel Electrophoresis of Test Sample MetaHiC Library to visualize library size distribution



Mate distance distribution for first 1797886 read pairs for test MetaHiC library

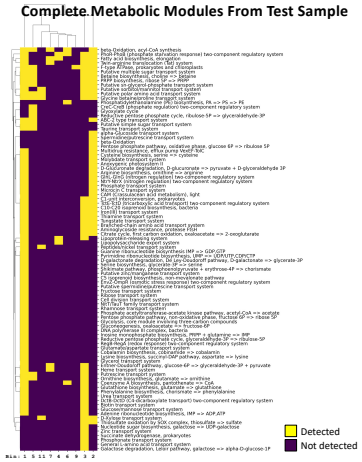


MetaHiC Bins and Metagenome Assembled Genomes from Test Sample



Cluster ID	Top Reference	Complete (%)	MGO (%)	Novelty Score	Abundance[1]	Contig N50	Genome Size	Num Contigs	GC (%)
bin_1	*f__Rhodobacteraceae (UID3340)*	96.19	3.89	99.17	4.74	48,710	2,971,624	79	51.03
bin_3	*f__Rhodobacteraceae (UID3356)*	66.88	2.56	81.68	5.34	5,908	1,610,997	191	56.53
bin_2	*f__Rhodobacteraceae (UID3356)*	66.76	8.41	76.13	3.26	6,648	2,152,706	345	54.01
bin_4	*p__Bacteroidetes (UID2605)*	44.60	0.00	98.22	0.18	272,605	791,317	5	32.23
bin_6	*k__algicola (UID2846)*	36.25	0.00	97.81	0.56	43,258	565,498	13	28.76
bin_5	*p__Proteobacteria (UID3580)*	36.16	1.32	90.41	1.43	9,154	791,206	83	58.55
bin_7	*k__Bacteria (UID203)*	27.59	0.00	97.13	0.78	36,556	480,489	8	40.42
bin_8	*k__Bacteria (UID203)*	27.59	0.00	98.56	0.24	66,269	360,544	7	36.70
bin_9	*root (UID1)*	8.33	0.00	90.74	0.05	140,421	206,295	2	35.72
bin_10	*k__Bacteria (UID203)*	6.90	0.00	94.33	0.41	32,970	114,919	7	44.45
bin_11	*k__Bacteria (UID203)*	0.86	0.00	0.84	0.12	6,526	110,707	15	45.13

Complete Metabolic Modules From Test Sample



Conclusions

- MetaHiC enables deep sequencing of metagenomes, allowing for more accurate MAG/MGE assembly and taxa identification
- Low pass sequencing of test MetaHiC library 1) captured long-range interaction and 2) resulted in 11 metagenome bins, with Rhodobacteracaea being the most prevalent taxa present.
- QC of sequencing data from 1.8 million pair-end reads resulted in 8 binned metagenomes with >90% novelty score.

Future Directions

- Perform MetaHiC assay on remaining 13 filter samples
- Map MetaHiC libraries with respective shotgun sequencing data
- Bin metagenomes to classify taxonomic classes within samples
- Identify mobile genetic elements present in specific hosts
- Identify changes in species diversity and taxonomy throughout 14-day rainstorm

Acknowledgments:

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Engineering an incubation environment that mimics *in situ* conditions for *in vitro* coastal microbiome studies

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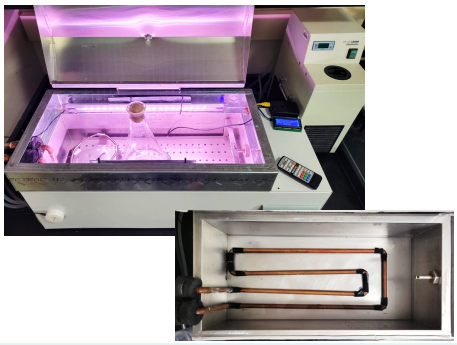
Abstract

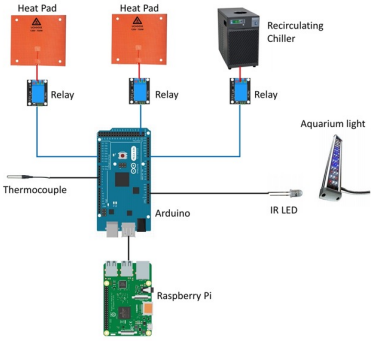
Coastal environments are dynamic and can vary widely on short- or long-term scales depending on location and weather. Incubation equipment that reflects these changes through programmable gradient light and temperature cycles would permit more precise *in vitro* coastal microbiome studies. Here, we present an open-source incubation environment that mimics *in situ* conditions for *in vitro* coastal microbiome studies using a modified shaking water bath that has fully customizable temperature and light gradients that can also mimic real-time field conditions. We compared coastal microbial community profiles incubated *in situ* and in our build mimicking field conditions over 48 hours. Analyses of congruence indicated significant overlap ($p > 0.2$) between microbial communities incubated *in situ* and *in vitro* at each time point.

Background

- Coastal waters experience temperature and light fluctuations on time scales ranging from seconds to seasons
- Temperature and light are major contributing factors to coastal microbiome taxonomic and functional composition
- Longitudinal coastal microbiome studies often require on-site field work, but can be logistically challenging
- *In vitro* methods can be more convenient, but are often limited to isothermal or strict on/off lighting conditions
- An incubation coastal environment that mimics *in situ* conditions would allow implementation of *in vitro* coastal microbiome assays with dynamic conditions

Build Design





Live Temperature Test

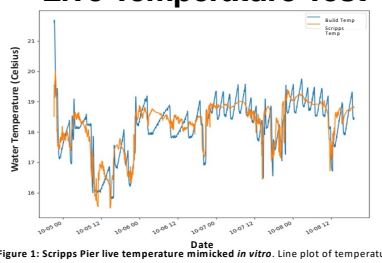


Figure 1: Scripps Pier live temperature mimicked *in vitro*. Line plot of temperatures recorded over 96 hours at the Scripps Pier (orange) and mimicked within our build (blue) to be within 0.5 C of the Scripps Pier temperature.

Lighting Test

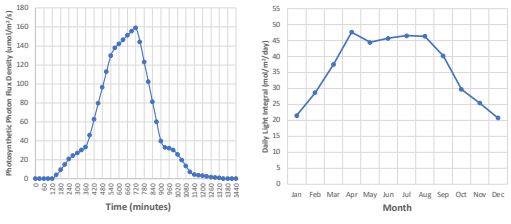
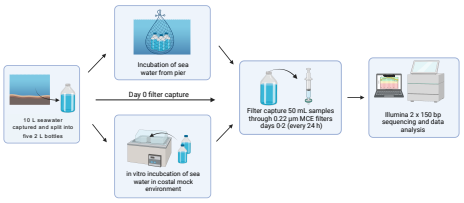


Figure 2. Characterization of Growth Lights and Daily Light Integrals of sample site
a) PPDF measurement of a single Fennix light fixture across the entire 24-hour diel cycle, in units of micromoles of PAR photons per square meter per second. b) Daily light integrals at Scripps Pier across a calendar year, in units of moles of PAR photons per square meter per day.

Microbial Community Composition Test



Conditions Analyzed	p-values	
	ANOSIM	perMANOVA
All time points	0.01	0.03
24h v. 48h	0.01	0.0077
24h *(in situ v. in vitro)*	0.2	0.21
48h *(in situ v. in vitro)*	1	1

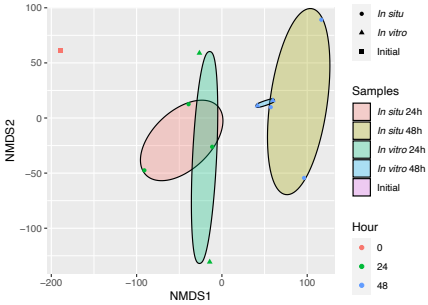


Figure 2: Species-level NMDS plot from clr-transformed taxonomic profiles of each sample/timepoint

Materials

- 27 L isotemp shaking water bath
- Benchtop recirculating chiller
- 2x heating mats for 3D printers
- 3x solid state relays
- Arduino Mega microcontroller
- Raspberry Pi 4
- IR LED and aquarium light
- C channel metal studs
- Copper pipe and fittings

Conclusions

- A dynamic system mimicking *in situ* conditions was constructed using laboratory equipment and hobbyist electronics
- Coastal microbial community profiles incubated *in situ* and in our build over 48 hours. Analyses of congruence indicated significant overlap ($p > 0.2$) between communities incubated *in situ* and *in vitro* at each time point

Future Directions

- Refine setpoint accuracy with PID algorithm
- Test against isothermal *in vitro* setup
- Open vs closed systems
- Further field vs *in vitro* longitudinal comparison testing
- Longitudinal horizontal gene transfer assay
- Longitudinal microplastic biofilm assay

Acknowledgments:

We would like to thank Dr. Justine Debelius and Dr. Arun Sethuraman for their edits and suggestions in developing this manuscript and the analyses herein. Additional thanks to Laura Sisk-Hackworth and Dr. Scott Kelley for providing code and guidance for the compositional data analysis. MLC-A was supported by the Department of Education TRIO McNair Postbaccalaureate Achievement Program under Award Number P217A170281. MLC-A and JMH were supported by the National Institute of General Medical Sciences of the National Institutes of Health under Award Number 3R25GM066341. BTH was supported by the Fenstermaker Foundation, and JDF and JMH were supported by Hologic, Inc.

Date: 08-12-2022

RNA sequencing of coastal microbial communities to investigate transposon expression response to tetracycline

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Abstract

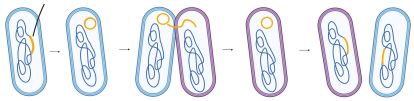
Tetracycline (Tet) is a commonly used antibiotic, and runoff into waterways can alter the stability of the bacterial communities in the ecosystem and select against bacterial species sensitive to Tet. In response to Tet treatment, bacteria often share antibiotic resistance genes (ARGs), on mobile genetic elements such as transposons, with nearby bacteria. **This pilot study was completed to determine if transposase expression levels increase in response to Tet treatment in Southern California nearshore surface waters.**

Seawater was collected from Oceanside Pier and incubated submerged in bottles for different time periods (0, 1, 3, and 6 hours) with varying tetracycline concentrations (0, 1, 10, and 100 ng/mL). RNA from the filter-captured bacterial communities was isolated and underwent low-pass Illumina 2 x 150bp sequencing. Preliminary analyses revealed no immediate differences in transposase expression between conditions, however ARG expression differences were observed. While more samples and replicates are needed to fully establish expression changes in response to Tet, these pilot data serve as proof of concept for our methodological approach.

Background

Tetracycline (Tet) can alter bacterial communities by:

- Selecting against bacterial populations that are sensitive to antibiotics
- Inducing the sharing of antibiotic resistance genes (ARGs) between bacteria



Transposases can assist in mobilizing genes in a "copy-and-paste" mechanism between bacteria.

Messenger RNA levels reflect transposase expression. To assess this, total RNA was extracted, and ribosomal RNA was depleted, and remaining RNA was sequenced in samples treated with/without Tet

Broader Implications

High Tet levels in nearshore waters can pose environmental and human health risks. Microbial community dysbiosis from Tet exposure can imbalance nutrient cycles, cause a build-up of organic matter, and disrupt the overall ecosystem.

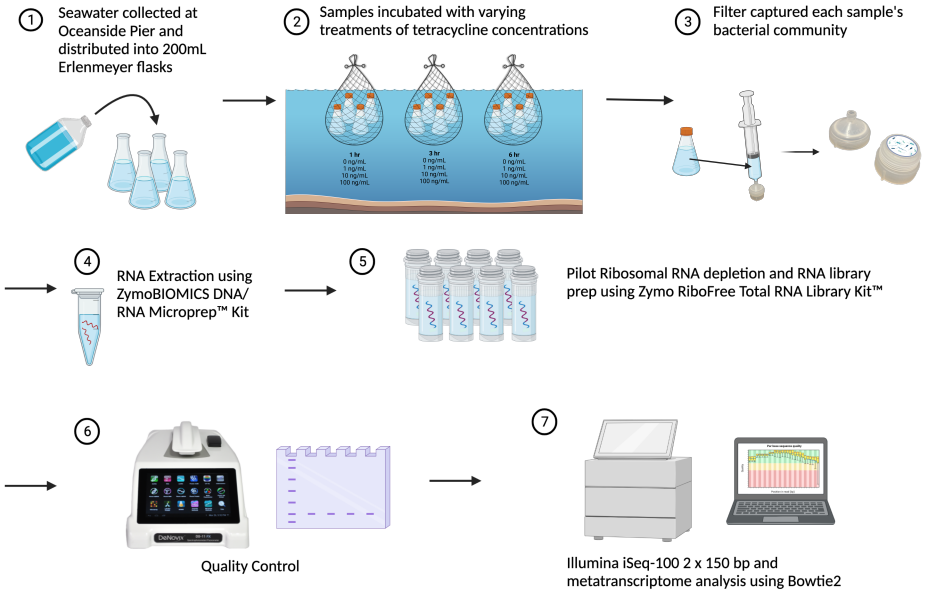
Hypothesis

Tetracycline exposure in nearshore microbial communities results in:

• Increase in transposase expression

• Increase in antibiotic resistance gene expression

Methods



Results

A

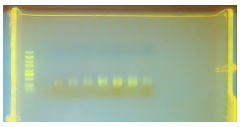


Figure A. Image of gel used to confirm library size (in bps) and check for contamination

C

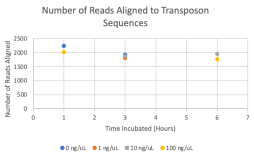


Figure C. Plot showing the number of reads aligned to TNCentral database of transposon sequences across conditions

E

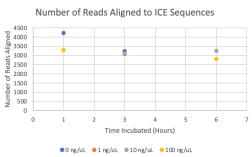


Figure E. Plot showing the number of reads aligned to ICEberg database of ICE sequences across conditions

B

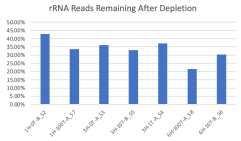


Figure B. Plot showing the percent of reads removed from each sample with the rRNA removal step using BBDuk

D

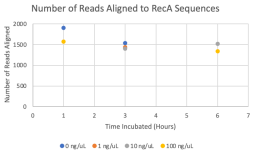


Figure D. Plot showing the number of reads aligned to RecA sequence homologs across conditions

F

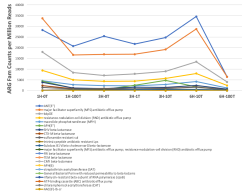


Figure F. Plot showing ARG family transcript counts per million reads for each sample, clustered by 1) incubation time then 2) Tet conc

Conclusions

- rRNA depletion and library preparation on complex metagenomic RNA was successful
- No immediate differences in transposase expression were seen between conditions
- ARGs expression differences were observed but more samples and replicates are needed

Future Directions

- Repeat rRNA depletion and sequencing for remaining 18 samples
- Investigate alternative databases to compare sequences against
- Perform full RNA-seq analysis pipelines for differentially expressed genes

Acknowledgments:

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Investigating the Effect of Tetracycline Treatment on the Mobility of Integrative Conjugative Elements

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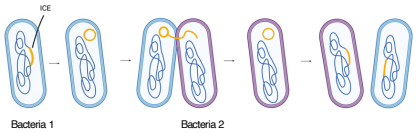
Background



- Integrative Conjugative Elements (ICEs) are mobile genetic elements (MGEs) the integrate and excise from host genomes
- ICEs can "copy and paste" into other bacteria through conjugation
- ICEs often carry antibiotic resistance genes (ARGs)
- Environmental variables impact how often ICEs are shared
- Tetracycline (antibiotic) is present in coastal waters and triggers ICE transfer

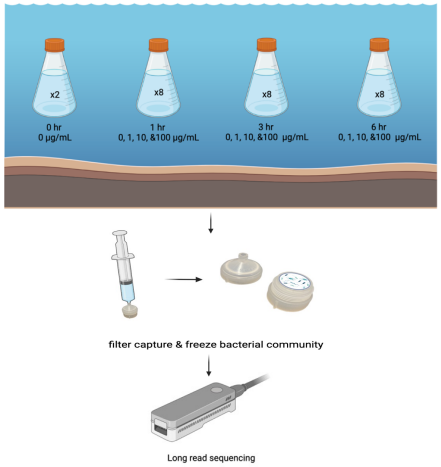
Hypothesis

Hyp. 1: Tetracycline has significant effects on coastal microbial communities

Hyp. 2: Exposure to tetracycline (antibiotic) increases the transfer of ICEs in coastal microbiomes in time and conc-deo manner

Methods

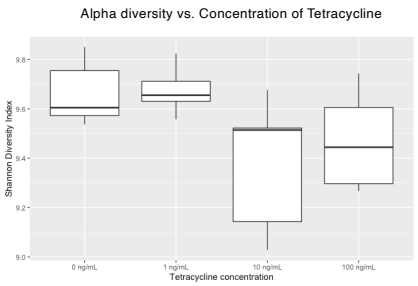
Time	Tetracycline concentration (ng/mL)			
0 hours	0	-	-	-
1 hours	0	1	10	100
3 hours	0	1	10	100
6 hours	0	1	10	100

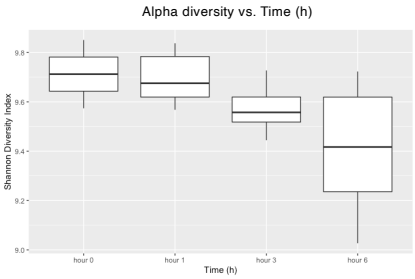


Known ICE sequences present in our samples

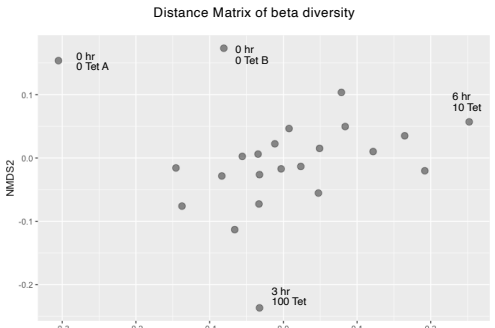
ICE name	ICE class	Strain
ICESe4	NA	*Salmonella enterica* subsp. *enterica serovar* Hadar 18
ICEPmiCHN2407	SXT/R391	*Proteus mirabilis* 09MAS2407
ICEValHN437	SXT/R391	*Vibrio alginolyticus* HN437

Time and tetracycline concentration significantly affect microbial diversity





Pr(>F)
Time 0.01666 *
Tet 0.01521 *



Pr(>r)
Time 0.003 **
Tet 0.035 *

Conclusions & Future Directions

- Known ICE sequences identified in our Nanopore sequence data
- Time and the concentration of tetracycline significantly change the alpha diversity and beta diversity of microbial communities
- Compare the amount of excised vs integrated ICEs between samples
- RNA-seq of RNA isolated from the same samples to infer how Tetracycline affects the excision and transfer of ICEs through transposase expression
- Deep sequencing of the same samples for better community and ICE representation

Acknowledgments:

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Investigating wound microbiome composition in Type 2 Diabetic mice

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Abstract

Chronic, nonhealing wounds affect many patients with Type 2 diabetes. Microorganisms are believed to play a significant role in the process of wound healing, as more diverse microbiomes induce faster healing. Various commensal and pathogenic bacteria including *Staphylococcus*, *Corynebacterium*, and *Pseudomonas* tend to colonize wounds once they are established on the host. However, how the microbiome influences chronic wounding is not fully understood. To better understand the role of microbes in diabetic wound healing, we performed 16S rRNA sequencing to compare the wound microbiomes of diabetic and nondiabetic mice. Each mouse was anesthetized and wounded with a double 2 mm biopsy punch, and those wounds were swabbed pre-wounding and days 2 and 7 post-wounding. The swabs then underwent DNA extraction, V3-V4 16S rRNA library preparation, and Illumina-based sequencing. ANOVA analyses on the change in wound diameter between the diabetic and nondiabetic mice groups revealed that the diabetic mice wounds healed significantly slower ($p=0.021$) than the control group. NMDS analyses on microbial community compositions revealed distinct community profile shifts between sampling days in nondiabetic mice wound ($p=0.01$) that were not observed in diabetic mice ($p=0.22$). Additionally, the taxonomic charts revealed unique profiles between the control and diabetic mice pre- and post-wounding. Collectively, those data suggest a correlation between wound healing and microbial composition.

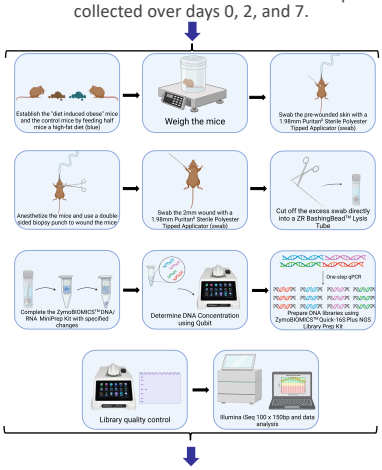
Background & Hypothesis

- Patients with Type 2 diabetes have a wound healing impairment that can lead to chronic, nonhealing wounds
- Microorganisms are believed to play a significant role in wound healing processes
- How microbiomes influence chronic wound healing is not understood
- More dynamic microbial communities are correlated to faster wound healing processes

Nondiabetic and diabetic wound microbiomes will have distinct microbial compositions pre- and post-wounding

Methods

12 diabetic and 12 nondiabetic wound samples collected over days 0, 2, and 7.

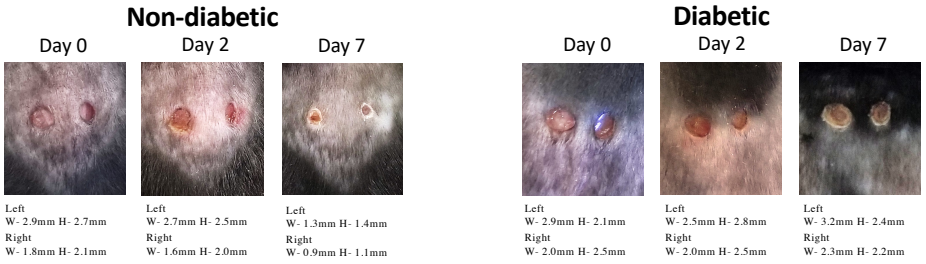


Sequencing data trimmed, filtered with Trimmomatic

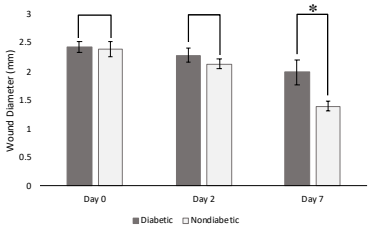
Microbial classification with Silva Database

Results

Chronic Wound Healing



A



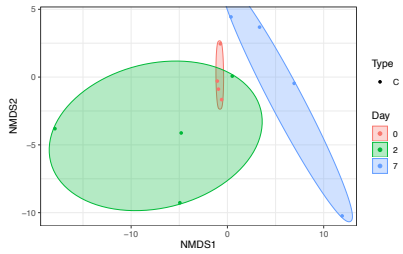
B

Analyses of congruence of clr-transformed taxonomic abundance profiles across experimental conditions

Conditions Analyzed		p-values ANOSIM	p-values perMANOVA
Control v Diabetic Mice	Day 0	0.03*	0.05*
	Day 2	0.23	0.2
	Day 7	0.33	0.58
Control Mice	Days 0 v 2	0.05*	0.03*
	Days 0 v 7	0.01**	0.03*
	Days 2 v 7	0.11	0.08
Diabetic Mice	Days 0 v 2	0.64	0.6
	Days 0 v 7	0.22	0.25
	Days 2 v 7	1	1

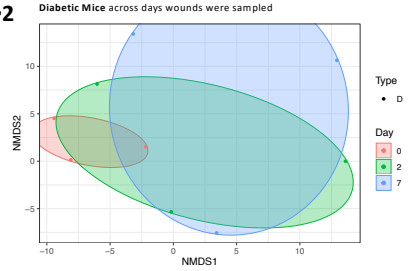
C_1

Species-level NMDS plot from clr-transformed taxonomic profiles of **Control Mice** across days wounds were sampled



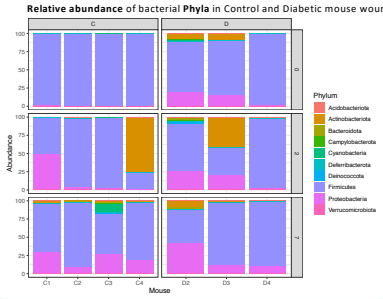
C_2

Species-level NMDS plot from clr-transformed taxonomic profiles of **Diabetic Mice** across days wounds were sampled



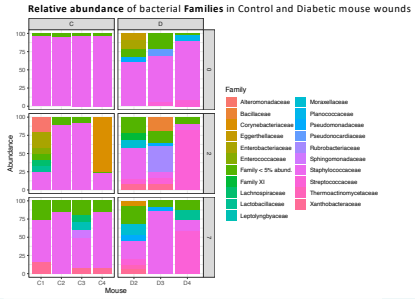
D

Relative abundance of bacterial **Phyla** in Control and Diabetic mouse wounds



E

Relative abundance of bacterial **Families** in Control and Diabetic mouse wounds



Conclusions

- The diabetic mice had a significantly smaller change in wound size over the experimental period
- Skin microbiome beta diversity was significantly different pre-wounding between diabetic and control mice
- Skin microbiome beta diversity was significantly different between days sampled within the control group, but NOT within the diabetic group

Future Directions

- Re-run 16S rRNA sequencing for deeper throughput and coverage
- Optimize 2 mm wound swabbing methods to increase biomass
- Repeat 16S rRNA sequencing on biological replicates of diabetic and nondiabetic mice
- Perform metatranscriptomics on the extracted RNA samples

Acknowledgments:

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Investigating Bacterial Biomass and Communities on Plastics

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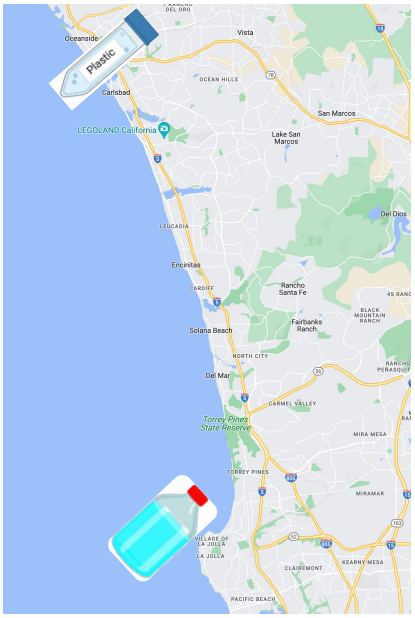
Abstract

Plastic pollution is a growing global threat due to consistent increase demand in production. These contaminants enter our environment via urban run-off, mismanaged waste, litter, and waste from maritime vessels. Once in the ocean environment, microbes establish communities on them. To establish methods to study these communities, we collected plastic directly from the ocean, and ocean water for incubating a plastic in lab. We then extracted the DNA from our samples and measured how much DNA we collected. Next, we attempted to amplify our DNA using different PCR methods. The DNA concentrations from our extractions were enough for PCR amplification, however, our DNA amplification failed using several protocols. To verify our amplification results. we ran a gel to visualize our DNA. These data indicate that plastic samples do have enough bacteria living on it for analysis of what bacteria are present, but the failed amplification attempts suggest PCR inhibitors such as plasticizers that must be removed prior to analyzing the bacteria in microplastic communities.

Methods

***In Situ* Plastic**

1. Gather plastics from Oceanside beach for samples P
2. Transport plastics to lab on ice bucket
3. Cut plastics and extract DNA
4. Measure extracted DNA concentration from samples



***In Vitro* Plastic**

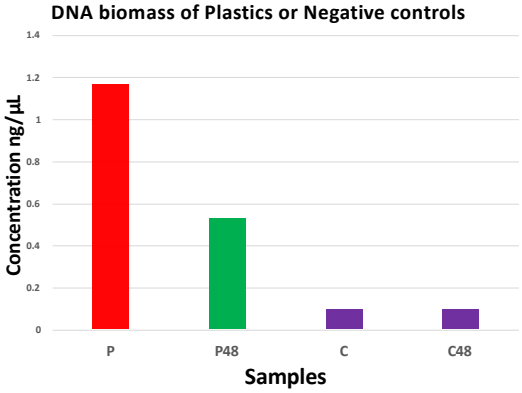
1. Sterilize a 1"x1" piece of plastic under UV light for samples P48
2. Gather sea water from Scripp's beach
3. 48 hour incubation of plastic from Step 1 into sea water from Step 2
4. Measure extracted DNA concentration from samples

Background

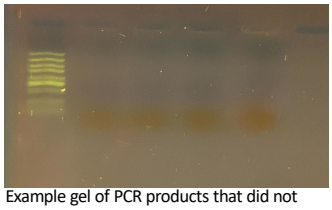
- Plastics are long lasting pollutants
- Plastics are found in rainfall, soil, Antarctic snow, deep ocean sediments, and the atmosphere
- They are also within organisms in the digestive tract and incorporated into tissue, plants included
- Plastics can serve as niches for pathogenic microbes
- Plastics often have very low biomass
- Plastics contain chemicals that interfere with PCR amplification

Goal: establish DNA isolation and amplification methods to analyze coastal microbial community profile, establishment, and stability on microplastics

Results



- **P**: DNA extraction of beach plastic
- **P48**: DNA extraction of plastic incubated for 48h in seawater
- **C**: DNA extraction negative control 1
- **C48**: DNA extraction negative control 2



Example gel of PCR products that did not amplify due to PCR inhibitor in DNA extractions

Hypothesis

We expect to reliably extract enough DNA from samples for sequencing while maintaining contamination negligible/undetected.

Conclusions

- Sample P expectedly had the highest concentration due to it being captured in the field and appearing weathered
- Sample P48 suggests that a 48 hours incubation period is sufficient for partial colony establishment
- Control biomass levels indicate minimal contamination but still useful to run alongside samples in future assays

Future Directions

- Modify DNA extraction methods to remove plasticizers
- Develop PCR protocol to reliably amplify microbial DNA from microplastics
- Sequence DNA to analyze coastal microbial community profile, establishment, and stability on microplastics

Acknowledgments:
I give gratitude to Dr. Elinne Becket for allowing me to train and perform the research in her lab. She encourages me to build my analytical and technical skills while providing the necessary resources and motivation. A special thank you to Hologic for the for supporting me and my team's research training as a CSTEM Summer Scholar