Nathan Greenslit Data Analysis Project

Nathan Greenslit

4/20/23

# Edits

1. I would do a lot of visual inspections to see how the different models fit and what they predict. You can try a linear model and a more complex ML one and compare to see if maybe something beyond a linear relationship improves results.
2. Given that you don’t have a lot of data, I’m not sure a PCA makes sense. I think in-depth analysis of the relation between the variables and the outcome and then in addition to the linear models trying a few more complex ones will probably be the better approach.
3. The figures in the manuscript look good, but some are too small to read. Needs breaking up and/or increasing font size

# Summary/Abstract

Each year, plumes of Saharan dust travel across the Atlantic to be deposited in the surface waters of the Caribbean and Gulf of Mexico. Dust aerosols serve as a significant source of nutrients that can elicit a fertilization effect on marine coastal waters, leading to rapid and potentially harmful blooms (e.g. red tides). Previous work has characterized the response of the marine bacteria *Vibrio* to dust input under the oligotrophic settings of the Florida Keys, but less is known regarding settings with higher baseline nutrient levels. This project aims to quantify *Vibrio* population dynamics in response to dust input in coastal sites with higher ambient nutrient levels. Daily sampling took place in Corpus Christi, TX to capture before, during, and after a Saharan dust event and quantitative PCR (qPCR) was used to estimate total counts of *Vibrio.* This study will provide an increased understanding of the conditions that can elicit potentially harmful blooms, highlighting the need for further research to understand the effects of dust deposition in non-oligotrophic waters.

# Introduction

## Saharan Dust

The Sahara Desert is a significant source of atmospheric dust, eliciting an estimated one billion metric tons each year Lenes et al. (2008).

## Microbial Blooms

Previous work conducted in the West Florida Shelf has shown that increased iron availability can stimulate the growth of the nitrogen-fixing cyanobacterium *Trichodesmium*. The high amount of nitrogen produced via fixation can be adequate enough to stimulate toxic red tides, caused by the dinoflagellate *Karenia brevis* (Lenes et al. 2008).

### Vibrio

While it is documented that these dust events can stimulate growth of harmful phytoplankton, emerging evidence shows that heterotrophic bacteria are responding to the episodic influx of limiting resources and substrate, resulting in blooms of bacteria that are associated with disease, which we refer to as harmful bacterial blooms. Among those that respond are bacterium belonging to the genus *Vibrio*, a group of ubiquitous marine opportunistic heterotrophs. While considered conditionally rare (comprising of <1% of the microbial community), under the proper conditions, this genus can rapidly bloom to make up a large percentage of the community over a short period of time (Westrich et al. 2016 ; Westrich et al. 2018). *Vibrio* species are found in brackish to marine waters with salinities ranging from 0-35 and will grow in waters with temperatures ranging from 5°C-40°C (Sampaio et al. 2022).

*Vibrio* species are excellent opportunists, having multiple advantages that allow them to quickly respond to any newly introduced substrate or nutrients. These advantages include multiple copies of rRNA genes, a rapid doubling time, efficient chemotactic motility, large genomic repertoire (consisting of two circular chromosomes), and multiple Fe-siderophore complexes that allow for rapid uptake of iron into the cell (Jensen, Frost, and Torsvik 2009; Ringgaard et al. 2018 ; Eagon 1962 ; Okada et al. 2005 ; Payne, Mey, and Wyckoff 2016).

Within the *Vibrionaceae* family, there are at least 12 species that are commonly known as human pathogens. Illness can be induced through either a foodborne route via the consumption of raw or uncooked seafood and contaminated drinking water, or through nonfoodborne routes such as wound exposure while swimming. The most common illnesses caused by *Vibrio* include self-limiting diarrhea and cholera (*V. cholerae*), shellfish-induced gastroenteritis (*V. parahaemolyticus, V. vulnificus*), extreme cases of necrotizing fasciitis and septicemia (*V. vulnificus*), and wound and ear infections  (*V. alginolyticus*, *V. vulnificus*) Tran et al. (2013) among others.

### Previous Work

Previous work has characterized *Vibrio* blooms in response to Saharan dust input in the oligotrophic setting of the Caribbean and subtropical Atlantic (Barbados and Florida Keys, respectively), with surface water concentrations of total *Vibrio* increasing by five to thirty times that found during non-dust conditions and returning to baseline levels within 24-48 hours. *Vibrio* composition within the larger microbial community also shifted following dust deposition, with initial levels of <1.4% to a peak of 19.8% of the bacterial community (Westrich et al. 2016). A similar phenomenon was also observed in the surface waters of the tropical and open ocean mid-Atlantic with *Vibrio* populations increasing 1.5-fold in the mid-Atlantic following deposition (Westrich et al. 2018) In a follow up study in the Florida Keys, episodic dust events during a daily time series promoted a succession of bacterial responses, with declines in *Prochlorococcus* coinciding with initial increases in bacteria belonging to the order *Vibrionales*, followed by subsequent shifts in response of different bacterial groups (Borchardt et al. 2020). To date, our understanding of *Vibrio* and other microbial response to dust input has focused on oligotrophic settings, where dust input is considered to be a critical source of limiting nutrients. However, the composition of desert dust can be complex and may have the potential to deliver critical resources that could be exploited by opportunistic microbes (like *Vibrio*) and elicit a growth response despite higher baseline nutrient levels. Given that human exposure is likely in these coastal waters, it is important to elucidate the relationship between dust input and microbial blooms in these higher nutrient environments.

## Present Research

This study aims to (1) quantify *Vibrio* population dynamics and composition, and (2) characterize potential microbial blooms and community shifts in response to dust input in coastal sites with higher ambient nutrient levels. The findings from this study will provide an increased understanding of the conditions that can elicit potentially harmful blooms (i.e., *Vibrio*), highlighting the need for further research to understand the effects of dust deposition in non-oligotrophic waters. We predict that (1) *Vibrio* will be among the first to respond to the influx of dust constituents and (2) that the fertilization effects of dust deposition (and possible successional changes) will be dampened at sites with higher baseline nutrient levels, as dust derived nutrients become less critical for growth.

With a heightened risk of *Vibrio* exposure in coastal waters, it is vital to understand microbial dynamics in nearshore environments. If dust is proven to elicit a microbial response, these events can be used to serve as an “early warning” of increased exposure risk to better protect public health.

## Questions/Hypotheses to be addressed

How do Saharan dust events influence Vibrio populations in high nutrient coastal waters?

I expect to see a more dramatic growth response in the Gulf (low nutrient), whereas the higher nutrient sites may exhibit a dampened growth response since they already have high baseline nutrient levels. If we still see a growth response despite already having supportive background nutrient levels at these sites, this may suggest that there are other constituents in the dust that can elicit a growth response. Nutrients, dust input, salinity, and temperature will be the primary factors of interest.

# Methods

## Description of Study Area

Sampling took place at three locations in Corpus Christi, TX: Blind Oso Bay, a residential canal system on Padre Island, and the Gulf. Sites were chosen based on differing baseline nutrient levels. Blind Oso Bay is a shallow tributary that is popular amongst wadefisherman and kayakers. The Bay is often impacted by fluctuating salinities and high levels of nutrients and chlorophyl due to its proximity to a wastewater treatment plant (Wetz, n.d.) Additionally, the Bay has persistent issues with high levels of fecal indicator bacteria, placing it on the U.S. impaired waters list ([Nicolau & Hill, 2014](https://www.tceq.texas.gov/downloads/water-quality/tmdl/oso-bay-oyster-harvesting-assessment-103/103-oso-bay-monitoring-report-2013.pdf), [Texas Commission on Environmental Quality, 2022](https://www.tceq.texas.gov/downloads/water-quality/assessment/integrated-report-2022/2022-303d.pdf)). The residential canal system is also impacted by high nutrients and chlorophyl from storm water runoff, and salinity is primarily driven by precipitation. The Gulf site is characterized by constant salinities (~36) and low nutrient levels.

## Sample Collection and Processing

#### Collection

A high resolution (daily) time series was conducted starting on July 7th and ending on July 19th, 2022 capturing before, during, and after a Saharan dust event. Samples were collected in 1L autoclaved Polypropylene Bottles and immediately placed in a cooler on ice. Following transport to the laboratory, the samples were concentrated onto 0.2µm pore size, hydrophobic polycarbonate membranes (25mm in diameter) and stored at -80°C until DNA Extraction using a ZymoBIOMICS DNA Miniprep Kit (Cat#:4300). To evalulate contamination in the lab, negative controls (DI and MilliQ Water) were introduced at sample collection, filtration, DNA Extraction, and downstream quantitative polymerase chain reaction (qPCR).

Vertical profiles of salinity, temperature, pH, and dissolved oxygen were obtained using a YSI ProPlus sonde. Samples were collected for chlorophyl *a* analysis, inorganic nutrients, dissolved and particulate organic matter, and microbial analysis.

#### Vibrio Enumeration

Total *Vibrio* concentrations were quantified using a SYBR Green qPCR method. Estimates were made using genus-specific quantitative polymerase chain reaction (qPCR) PCR product using Vibrio group specific primers targeting a variable region of the 16S rRNA gene, 567F, 5′GGCGTAAAGCGCATGCAGGT3′ and 680R, 5′-GAAATTCTACCCCCCTCTACAG-3. Master mix and PCR conditions were derived from (Westrich et al. 2016). Briefly, 5µL of 2X SYBR PowerUp Master Mix (Applied Biosystems, Foster City, CA) were added to primers with a final concentration of 0.16µM and PCR water for a final volume of 10µL. Reactions were run in triplicate on a CFX96 Touch Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA) with the following cycling conditions: 2 minutes at 50◦C for UDG activation and 95◦C for 2 min to activate AmpliTaq polymerase and UP, followed by 40 cycles of 95◦C for 3 seconds for denaturation and 60◦C for 30 seconds for annealing and extension. Each run was followed by a dissociation step (60C to 95C by 0.5C increments) to determine a melt curve for analysis of specificity. Each test was run with positive and negative controls in triplicate. Cycle threshold values for each qPCR test was compared to a standard curve representing 10^1 to 10^6 gene copies per reaction volume.

## Data import and cleaning

This project works with three primary data sets. (1) Enumeration of Vibrio bacteria as copies/mL from quantitative PCR (copies\_master.csv), (2) Dust concentrations as aerosol optical thickness (AOT) derived from the Naval Research Lab (nrl\_conc.csv), and (3) Temperature and Salinity across the daily time series (ysi\_2.csv). Below is a brief summary of how each data set was cleaned. More details can be found in the supplementary files, containing the code and comments describing what each line does. Please refer to the project\_README.md file located in the NathanGreenslit-MADA-Project folder for details on the repository contents and instructions on reproducibility

#### Cleaning of Total Vibrio Enumeration data set

Quantitative PCR provides enumeration results as “cycle threshold or Cq” values. This depicts the cycle number in which enough of the DNA target was present to be amplified and thus detected. Lower Cq values correspond to higher target concentrations (as it takes less cycles to amplify), and vice verse for higher Cq values. The cleaning of this data set consisted of converting these Cq values into something that can be used to quantify Vibrio (copies of target per mL of seawater). To do so, I needed to take into account the amount of water sample that was concentrated on a filter, the total amount of DNA that was eluted following a DNA extraction, and the amount of DNA template added to each mix for qPCR (to name a few). Taking these into account, we are able to calculate our way from Cq values to copies of Vibrio per mL of seawater. qPCR was conducted in triplicate, so the last step was to take the average of the three replicates to have a final value per sample. A date column was also added for each sample.

#### Cleaning of Dust Concentration (AOT) Data set

Dust concentration (AOT) was collected at time points 0 hour, 6 hour, 12 hour, and 18 hour. In this script, I converted timpoints from UTC to CST/CDT and created different dust permutations (individual time points, sums by day, and average by day). Because samples were collected between 0700 and 1100, a 24h composite permutation was made. This permutation is what was used for all downstream analyses.

#### Cleaning of Environment data set

This data set did not require any cleaning.

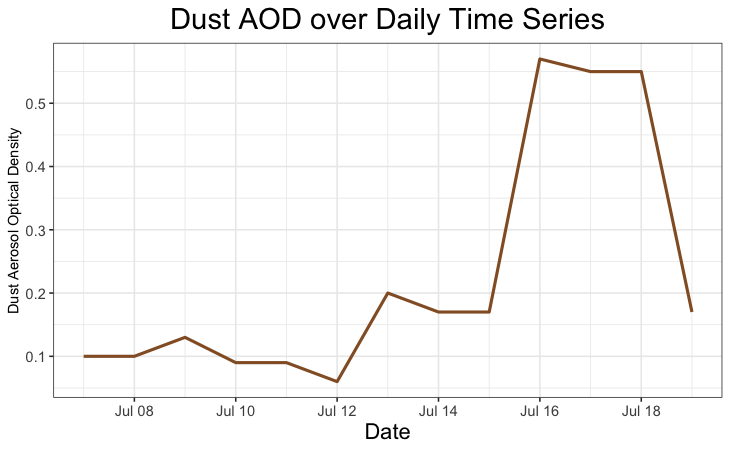
# Results

## Exploratory Analysis

### Visualization

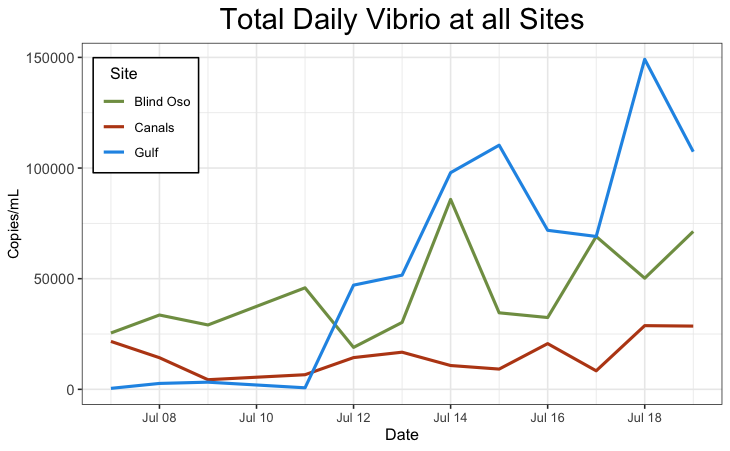
#### Dust AOD

This figure depicts the summed dust concentration 24hr prior to sample collection (time points 13hr–> 7hr the next day) across the daily time series. The daily time series captured two dust events. One light event starting on the 13th of July, where AOD reached 0.2, and a much heavier dust event from the 16th to the 18th of July with AOD = 55-57. Dust AOD returned to lower concentrations following the time series.



#### Vibrio Enumeration

This figure depicts total enumerated Vibrio as copies per mL, with color by site. There is a noticeable shift in *Vibrio* growth at the Gulf site, a less noticeable but still present shift in Blind Oso Bay, and practically no changes in the Canal site.



#### Environmental Parameters

Environmental parameters were site-specific. Temperature and salinities were consistent and high at the Canals ( ). While salinity remained relatively constant at the Gulf , temperature was observed to drop starting on the 12th of July, possibly due to a coastal upwelling event. Blind Oso exhibited fluctuations in both temperature and salinity within the daily time series with a drop in salinity from 42 to 20 within an eight day period.

Nutrients (orthophosphate and nitrate+nitrite) were observed to increase following dust deposition at both Blind Oso and the Canals site, with the former having more dramatic peaks. Nutrient levels at the Gulf remained relatively constant throughout the time series. DON and DOC had distinct peaks at Blind Oso on days following dust deposition, where DON exhibited smaller peaks at the Canals.

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| Figure 2: |

### Statistical analysis

#### Check Distributions:

Shapiro-Wilk’s tests were conducted to assess distribution of the data. Any data that did not have a normal distribution was log-transformed and the test was run again. Variables that continue to fail normality will have non-parametric tests run on them.

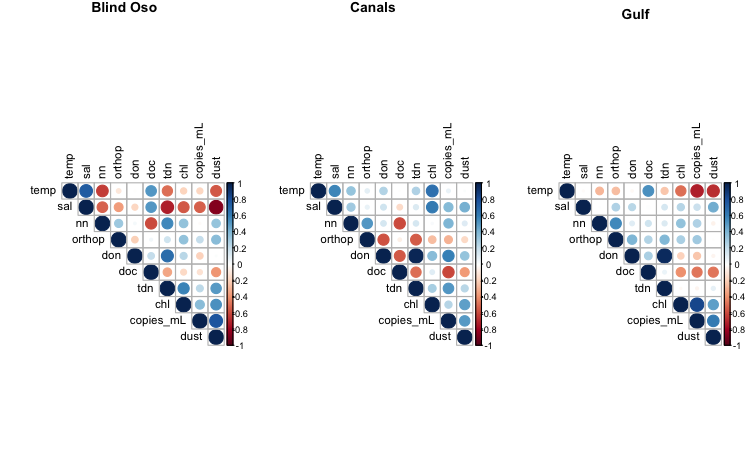
#### Cross-correlation Analysis:

Previous literature has shown that Vibrio respond 12-24 hours following dust deposition events. Based on this, we need to look at lags between the two times series for dust data and growth data. To do this, we can set up a cross-correlation analysis, which allows us to examine the lag/lead relationship between the two variables.

CCF plots showed that BO has a significant lag relationship a value of -1. In other words, dust occurs, and one day later, we see a response in copies\_mL. C2 has a lag at -2, and RD at -1.

#### Correlation Matrices

Correlation matrices were run on each variable (in order of left to right: temperature, salinity, nitrate+nitrite, orthophosphate, dissolved organic nitrogen, dissolved organic carbon, total dissolved nitrogen, chlorophyll, *Vibrio* gene copies/mL, and dust). A majority of the environmental variables had a non-normal distribution, thus Spearman’s Correlation (indicated by the color gradient) was used to examine the relationship between the variables.



## Full analysis

### Linear Regression Models

Linear regression models between dust AOD and copies per mL of Vibrio. R2 values indicates the strength of the linear model, and the Pearson Correlation Coefficient indicates the strength of the linear relationship between the two variables.

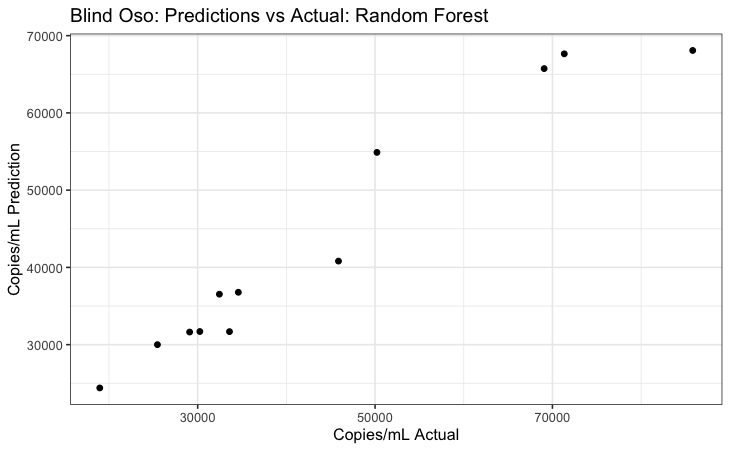
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| Figure 4 |

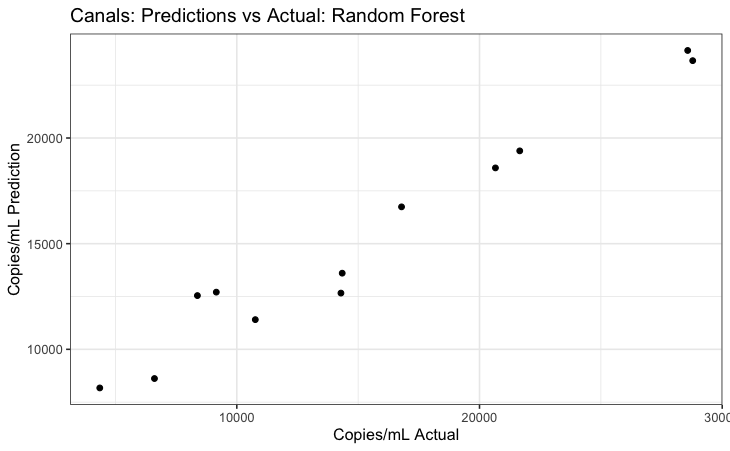
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| Table 1: Uni-variate Linear Regression Model Metrics |

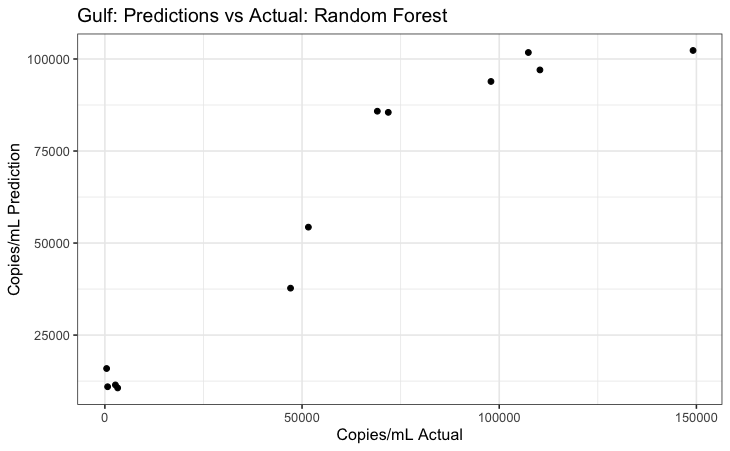
### Multi-variate Models

#### Random Forest

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| Table 2: Random Forest Model Metrics |







#### Linear Regressions

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| Table 3: Blind Oso: Comparative linear regression analysis of Vibrio growth between six operational models. |

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| Table 4: Canals: Comparative linear regression analysis of Vibrio growth between six operational models. |

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| Table 5: Gulf: Comparative linear regression analysis of Vibrio growth between six operational models. |

Multi-variate models that included dust, water chemistry, nutrients, and DOM performed the best, with mean RMSE values much lower than the Null Model.

# Discussion

## Summary and Interpretation

This study aimed to examine microbial response to Saharan dust deposition in high nutrient coastal waters, specifically focusing on bacteria belong to the genus *Vibrio*, a bacterium that is important for marine processes such as carbon cycling, as well as human and marine health.

## Methods

For this analysis, we examined *Vibrio* growth dynamics in response to Saharan Dust deposition in three sites in Corpus Christi, TX that had differing baseline nutrient levels. The Gulf, with relatively constant salinities and low nutrient levels, Blind Oso Bay with high nutrients and fluctuating salinities, and a residential canal system with consistent temperature and salinities and nutrient levels. Samples were collected before, during, and after a dust event and *Vibrio* were enumerated using qPCR. Data on nutrient levels and other environmental parameters (e.g. salinity and temperature) were also collected during this period. The three data sets (Vibrio Enumeration, Environmental parameters, and Dust Concentration) were then processed and cleaned for analysis.

## Findings/Explanations

*Vibrio* growth was found to significantly correlate with dust aerosol optical density at Blind Oso (r = 0.7, p = 0.01), Canals (r = 0.75, p = 0.005), and the Gulf (r = 0.67, p = 0.02). The introduction of dust constituents have the potential to serve as a temporary alleviation from the limitation of nutrients that are essential for growth. *Vibrio* species have one of the fastest doubling times among bacteria Ringgaard et al. (2018). These advantageous traits have resulted in *Vibrio* being classified as ‘oppurtunitrophs’. The following declines in *Vibrio* gene copies/mL may be attributed to topdown control, such as viral lysis , or bottom-up controls due to an exhaustion of the resources provided by the dust.

Similar to previous findings, peaks in growth were observed 24-48 hours following dust deposition, with both the Gulf and Blind Oso having a peak response 24hr following peak dust deposition, and the Canals 48hr after peak deposition (Westrich et al. 2018, 2016). .

Multivariate models were tested using specific environmental parameters that have been associated with *Vibrio* population dynamics (**bullington2022?**). Ultimately, a model that included water chemistry, dust, nutrient, and dissolved organic matter parameters provided the best performance (Table 3, Table 4, Table5), suggesting that while dust may contribute to a growth response in *Vibrio*, the site specific nutrient and chemistry dynamics play an important role in dictating the degree of response observed.

*Vibrio* gene copies/mL at the Gulf experienced the greatest change in growth as compared to the other two sites. This response in a lower nutrient marine environment lines up with previous work that was conducted in the Florida Keys and North Atlantic, where a 30-fold and 1.6-fold population increase was observed within a 24hr period following dust deposition.

While *Vibrio* growth exhibited a strong correlation at the Canals, the degree of growth was lesser in comparison to the other sites (Figure 1). Field studies have suggested that salinities for optimal growth lie between 5-25, depending on the species (Sullivan and Neigel 2018). The higher salinities at this site (40-42) may have attributed to this observed weaker growth response.

*Vibrio* growth was observed at Blind Oso Bay, but slightly dampened in comparison to the Gulf. This site is an enclosed shallow tributary that frequently experiences high levels of nutrients (e.g.total dissolved nitrogen) and chlorophyll due to its proximity to the Oso Wastewater Plant (OSP) (Wetz, n.d.). The Bay experienced a dramatic drop in salinity from July 11th to July 19th that could be attributed to discharge from the OSP. While growth was significantly correlated with dust deposition, there are many environmental factors at play at this site, making it difficult to identify the specific mechanisms that are eliciting the growth response.

Additionally, the Gulf exhibited a sharper increase during the second, heavier dust event, while Blind Oso exhibited a smaller increase during the heavy dust event. This difference in response may be attributed to the nutrient dynamics of the sites. In marine waters, nutrients are typically limiting due to tight microbial recycling . Therefore the introduction on nutrients at such a site may result in rapid uptake and utilization. At Blind Oso, a priming effect may be occurring, where these dust constituents may not be as essential for growth following an initial deposition, reaching a maximum in how much it can support growth.

## Limitations

One primary limitation of this project was the small samples size (n = 12 per site). One sample per day limits the interpretation of the results of this project and changes the scope from an explanatory report to exploratory.

Additionally, while qPCR can reliably detect the concentration of DNA of the target, the results do not differentiate between living and dead cells, and therefore provides an estimate of quantification.

While it can be proposed that the degree of microbial response is contingent on the environmental settings of the site, further work is needed to look at each specific environmental parameter and how it may impact *Vibrio* abundance. At Blind Oso for example, the drastic drop in salinity during an eight day period coincided with dust input. It then becomes difficult to parse these two variables out as either may have contributed to growth at this site.

## Conclusions

Ultimately, *Vibrio* growth was observed following dust deposition in each of these sites. However, the degree of response varied by site. This may be explained by each site having a specific and complex environment with varying water chemistry and nutrient levels.

Sites like the Gulf, that have lower nutrient levels, may have a more responsive microbial population, as they are temporarily relieved form nutrient limitation. Inland sites similar to the Canals or Blind Oso may exhibit dampened *Vibrio* growth following dust input, as the dust derived nutrients may be less critical for growth, as there is already a supporting nutrient foundation, or the environmental conditions may not be favorable for growth initially. The growth response at Blind Oso, despite having higher nutrients, suggests that there may be other constiuents in the dust that are not present in the site that elicit this response. More work will be needed to fully understand the dynamics, as there were many other attributes that may have also contributed to growth in the Bay.

To date, our understanding of *Vibrio* and other microbial response to dust input has focused on oligotrophic settings, where dust input is considered to be a critical source of limiting nutrients, and iron in particular. This study aided in filling the gap in how comparable previous findings in oligotrophic settings are to coastal areas with higher ambient nutrient levels. The composition of desert dust can be complex and may have the potential to deliver critical resources that could be more readily exploited by opportunistic bacteria (like *Vibrio*) and elicit a population bloom despite higher baseline nutrient levels. During these bloom periods, human exposure through recreation or consumption of raw seafood may be more likely, presenting a risk to the health of the public.

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