



# Effects of Sample Size on Microbial Community Diversity in Marine Sediments

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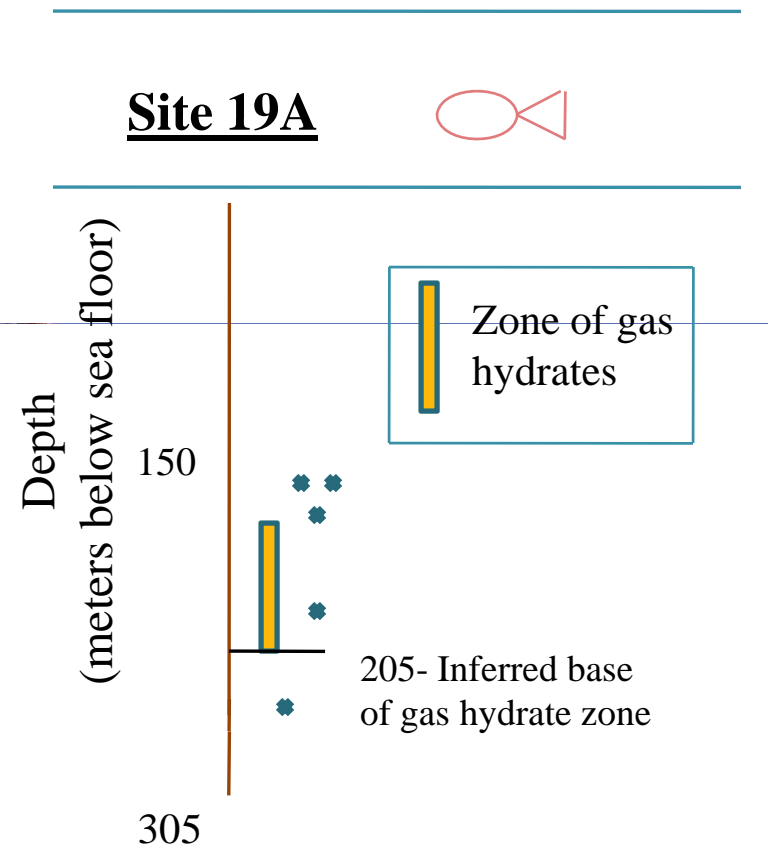


# Objective and Motivation

- Objective: To determine how sample size (ranging from 5g to 15g) impacts the diversity of microbes found in marine sediments taken from the Mahanadi Basin.
- Motivation: Microbial community diversity is dependent on sample size and can vary in different environments.
  - Can a larger sample size in actuality provide greater microbial diversity?
  - How does depth, methane presence, and other environmental factors play a role in microbial diversity?

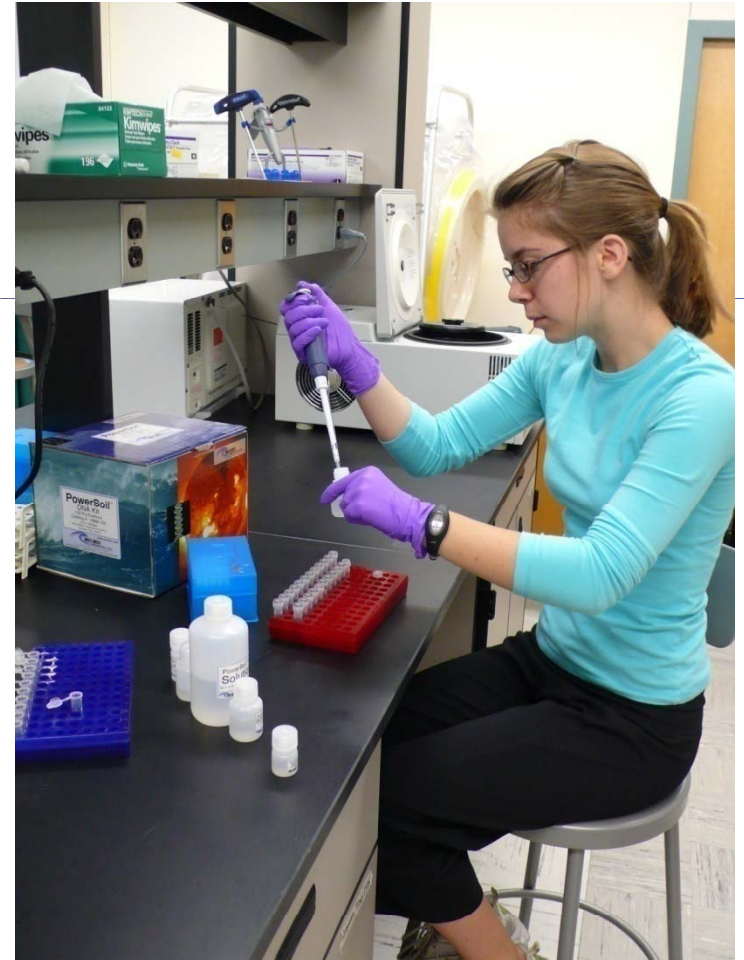
# Background

- 5 subsurface sediment cores were taken off the coast of India and studied.
- 1 sediment core was taken from a methane hydrate zone.
- Depths ranged from 170.3m to 207.6m below the sea floor.
- Geological Characteristics: 88 – 100% clay grain size



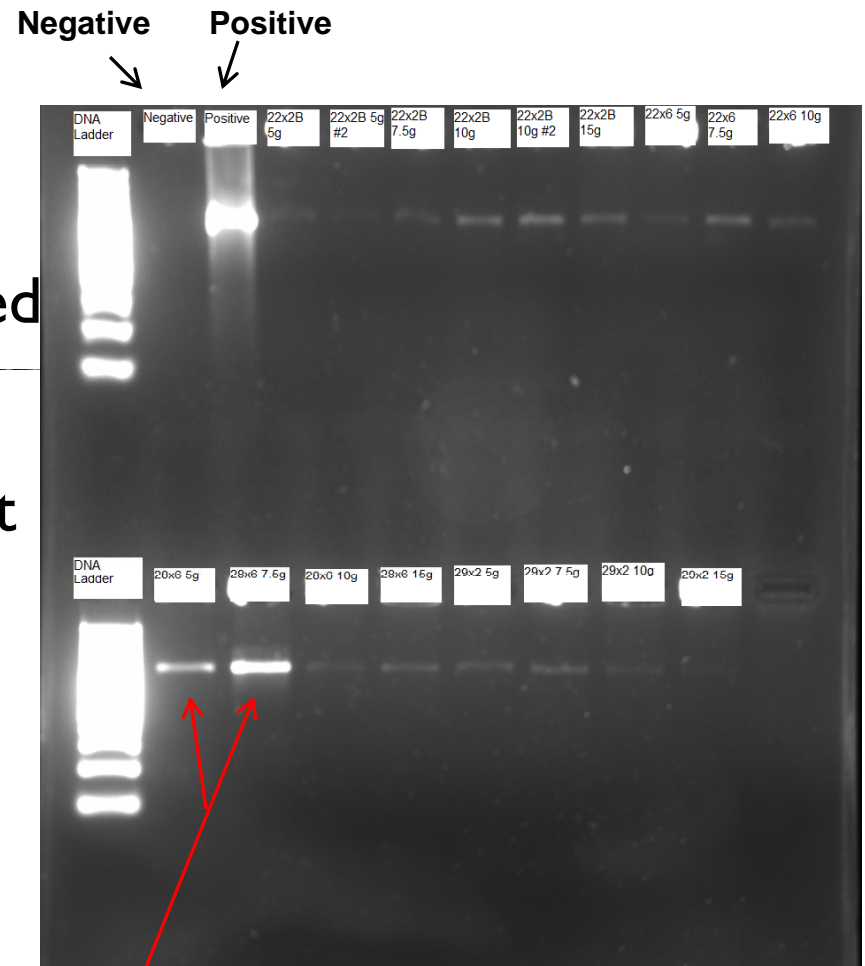
# DNA Extraction

- Both MoBio PowerSoil and PowerMax kits were used for extractions.
- 5g, 7.5g, 10g, and 15g of sediment from each core sample were used to extract DNA.
- Samples were then dried out in a speed vac followed by re-suspension in 100 $\mu$ l of water.



# PCR Amplification

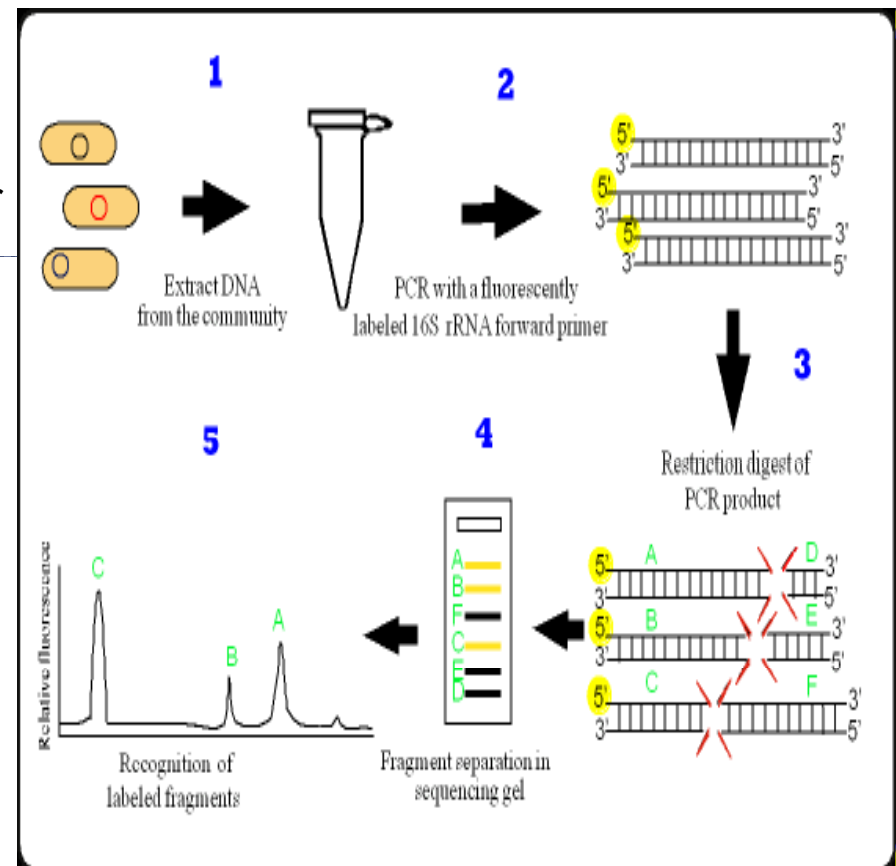
- 5  $\mu$ l of each sample was amplified using PCR.
- Results showed reamplification was required for some samples.
- Concentrating DNA with spin columns provided best results.
- After spin column concentration, hardly any PCR inhibitors could be detected.



Ex: 28x6 5g & 7.5g

# Terminal Restriction Fragment Length Polymorphism Analysis

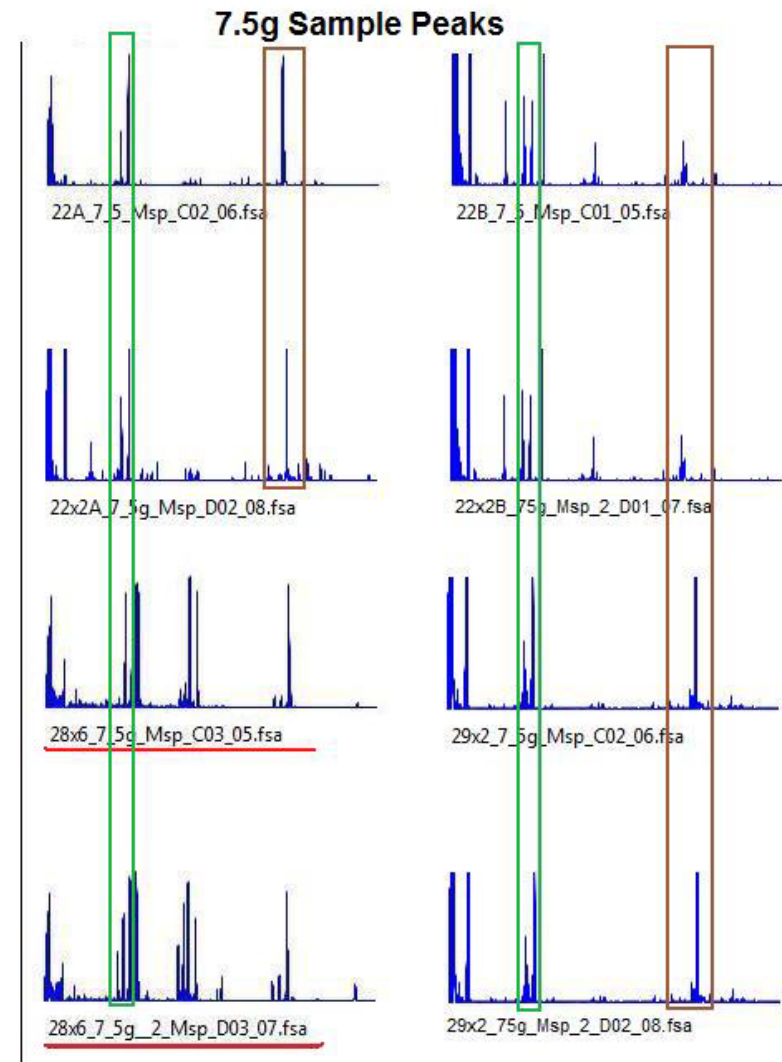
- DNA is extracted from sample (1)
- The gene of interest is amplified using PCR with a fluorescently labeled primer (2).
- PCR produces a mixture of amplicons that is digested with a restriction enzyme.
- This generates fragments of different sizes (3).
- Fragments are then separated through gel or capillary electrophoresis (4).
- A laser reader reads the labeled fragments and generates a profile based on fragment lengths (5).



Gruntzig et al. 2002

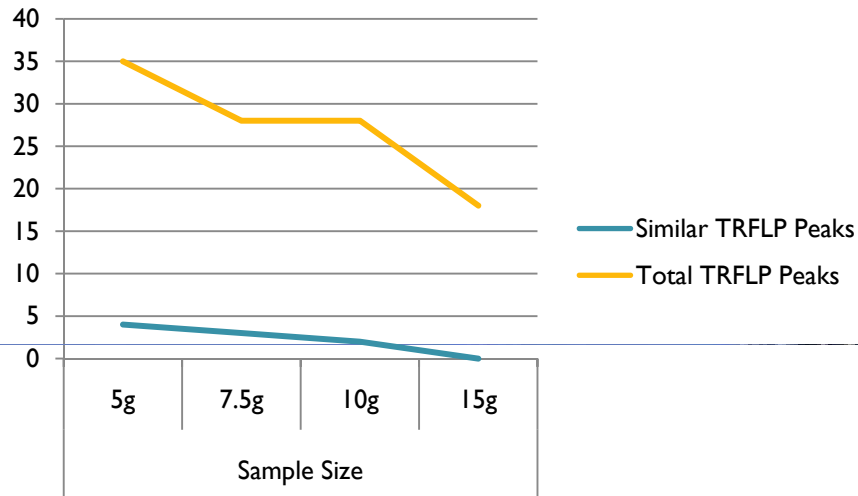
# Final Analysis

- TRFLP Peaks were analyzed in three ways:
  1. Individual samples and their duplicates
  2. Depth comparison between the five cores
  3. Sample sizes within cores
- Overall, most similarity was seen between sample sizes of 7.5g, including the gas hydrate core.
- Without the gas hydrate core, best similarity was between the 5g samples and their duplicates.
- Larger samples had very little similarities.



# TRFLP Peaks – Sample Size and Depth of Cores

### Sample Size vs. TRFLP Peaks



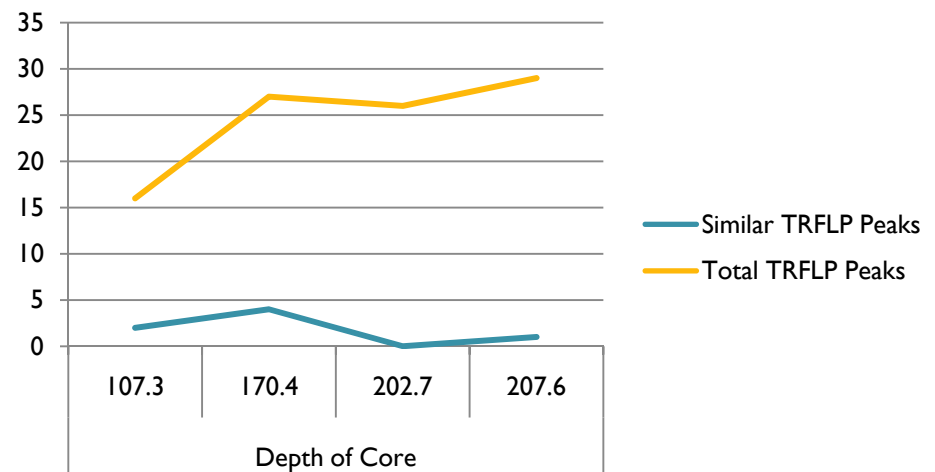
### Sample Size Comparison:

- Similarity of TRFLP peaks between replicates of each sample size decreases as the samples increase in size.
- Total TRFLP peaks decrease as sample size increases

### Depth of Cores Comparison:

- In general, similarity of TRFLP peaks decreased as core depth increased.
- Similar peaks were best seen at 170.4m.
- No common TRFLP peaks were seen in the gas hydrate core

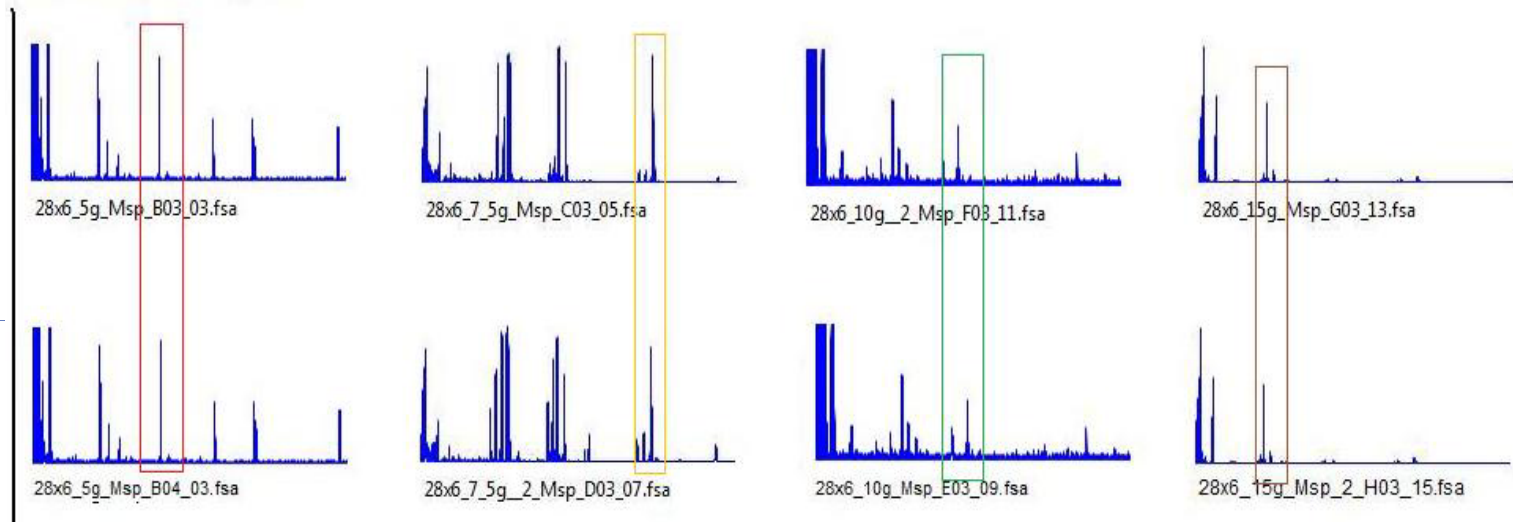
### Depth of Cores vs. TRFLP Peaks





# Final Analysis – Gas Hydrate Core

28x6 Core Samples

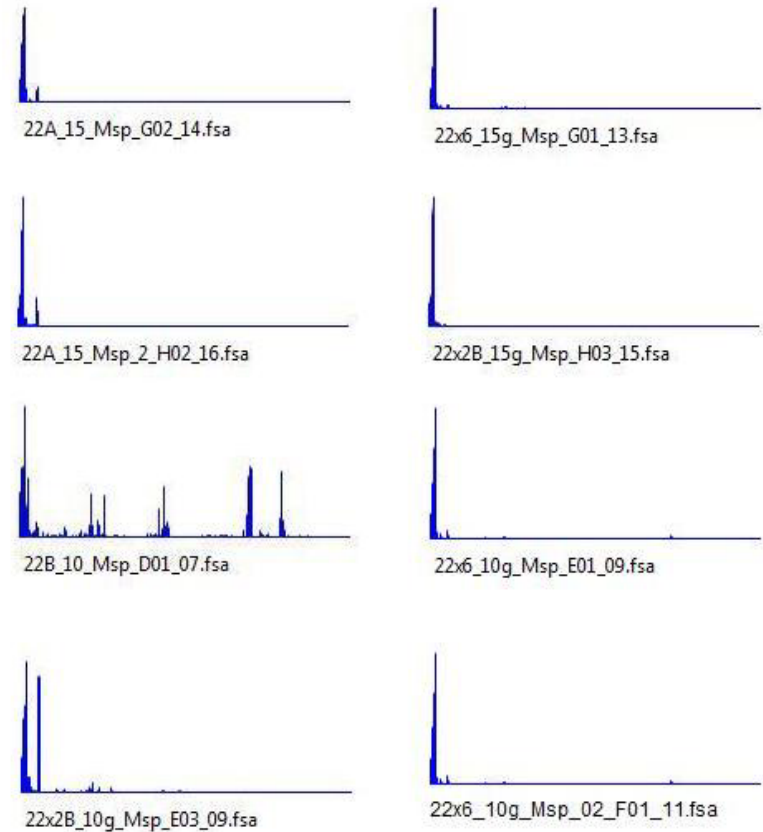


- Gas hydrate samples showed the most dissimilarity between the other cores and samples.
- No consistency from one sample size to the next within the gas hydrate core.
  - Between replicates → very compatible

# Interesting TRFLP Results

- Suggested smaller sample sizes produced more TRFLP peaks.
- The 10g and 15g samples showed nearly no TRFLP peaks.
- qPCR was run to check for the amplification ability of the samples.

Various 10g and 15g Samples





## After qPCR Check...

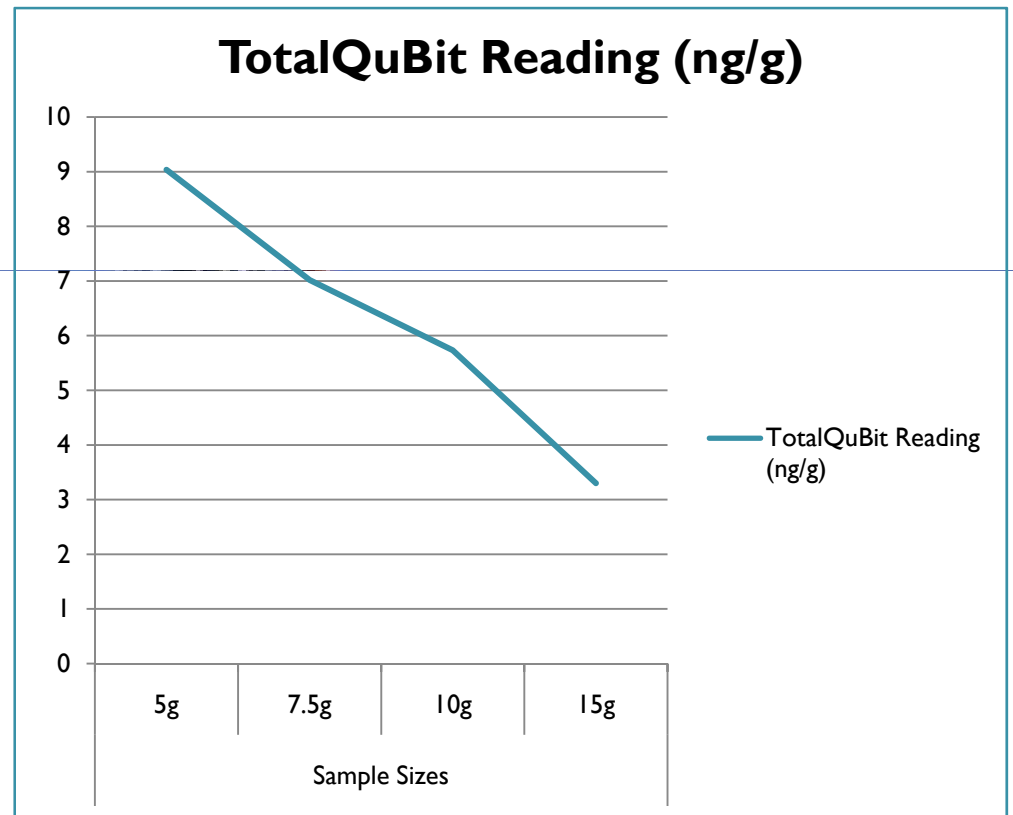
- Low copy numbers were found in many samples and some samples came back with no results.

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- After reviewing experimental process, it was realized that the SpeedVac temperature was incorrect for concentrating the DNA.
  - Could have lead to the troubles with amplification and the low copy numbers.

# DNA Concentration Analysis

- Compare general DNA concentration from all samples to find a general trend.
- Qubit readings taken right after drying of DNA in SpeedVac.
- Supported idea that SpeedVac might have impacted the final DNA concentration and ultimately, the TRFLP data.
- Inverse relationship was seen:
  - As sample size increased, the average DNA concentration reading decreased.





# Conclusions...

- Smaller sample sizes appear to have more consistent results between all cores.
  - Indicates higher probability of similar results with smaller samples.

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- Presence of gas hydrates could be a factor in consistency.
- Depth appears to be an aspect of microbial diversity.
  - At shallower depths, similarity of TRFLP peaks were greater.



## Further Questions

- How do the 5g and 7.5g TRFLP peak results change in different geological settings, such as sand or silt? Would there be more consistency in larger sample sizes?
- How do other environmental factors, such as pressure and temperature, play a part in microbial diversity?
- How does the SpeedVac temperature affect concentration of the DNA extracted? When does sample size become a factor?



# Acknowledgements

- Dr. Rick Colwell
  - Brandon Briggs
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  - COAS
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