THE RELATIONSHIP OF MICROBIAL ABUNDANCE TO POREWATER GEOCHEMISTRY IN CORAL REEF SEDIMENTS, HAWAI'I

A THESIS SUBMITTED TO
THE GLOBAL ENVIRONMENTAL SCIENCE
UNDERGRADUATE DIVISION OF THE
UNIVERSITY OF HAWAI'I AT MANOA

IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF

BACHELOR OF SCIENCE

IN

GLOBAL ENVIRONMENTAL SCIENCE

MAY 2007

by

Sean K. Ogata

Thesis Advisors

Antje Rusch

Eric Gaidos

We certify that we have read this thesis and that, in our opinion, it is satisfactory in scope and quality as a senior thesis for the degree of Bachelor of Science in Global Environmental Science.

THESIS ADVISORS

_____

Antje Rusch, Ph.D.
Department of Geology and Geophysics

_____

Eric Gaidos, Ph.D.
Department of Geology and Geophysics

ACKNOWLEDGMENTS

I would like to acknowledge my thesis advisors Antje Rusch and Eric Gaidos. Antje was absolutely invaluable as she worked with me extensively everyday I entered the laboratory, teaching and guiding me throughout my entire research. Eric mentored me in the research and as well as offered valuable advice during the course of my experiment.

Other individuals who assisted me throughout my research includes Angelos Hannides who providing and assisting me with the use of his microcosm set-up, Friends of He'eia for providing the use of their kayaks, Jane Schoonmaker for the academic stimulation and guidance, and the GES faculty and students.

I would also like to thank my family and friends for their unconditional support and interest.

ABSTRACT

Coral reef sediment microbes are considered important parts of nitrogen cycling in coral reef ecosystems. Research in the past, however, has focused mainly on microbes found on the coral reef proper and overlooked microbes existing within the sediments. This research attempted to investigate the relationship coral reef sediment microbes have with the geochemistry of sediment porewater by determining if microbial abundances are correlated to oxygen, nitrogen, or organic matter content of the sediments. Porewater and sediment samples were taken from various depths at Checker Reef, Hawaii, and sediment from Kilo Nalu, Hawaii was used in an artificial microcosm set-up. Porewater profiles of O_2, NH_4^+, NO_3^-, and NO_2^- were measured from each sediment array along with organic matter content measurements and DAPI cell enumerations. Cell abundances were then graphically analyzed versus each geochemical parameter and tested for correlations using a two-tailed t-test with a 95% confidence interval. Cell abundance and NH_4^+ were found to significantly correlate in the microcosm sediments but not in the Checker Reef sediment arrays. Significant correlations were not found between cell abundances and organic matter content, O_2, NO_3^-, or NO_2^-. Microbial abundances were also compared against cell abundances in silicate sands and muddy sediments, representing sediments of different material and grain size, respectively. Cell abundances of the carbonate sediment samples exhibited similar numbers to silicate sands and lower numbers than muddy sediments. Analyzing and comparing cell abundances in coral reef sediments with their geochemical environment is just the first step in understanding the impact coral reef sediment microbes have on the coral reef ecosystem.

TABLE OF CONTENTS

LIST OF FIGURES

Page

CHAPTER 1. INTRODUCTION

Most coral reefs in the world are found in tropical waters where nitrogen is commonly a limiting nutrient in primary production. However, despite their widespread existence in highly oligotrophic waters, coral reef ecosystems are areas of high biological productivity relative to surrounding waters (Capone et al, 1992; Entsch et al., 1983; Corredor and Capone, 1985). The cause of this discrepancy has been linked to the biological fixation of nitrogen carried out by microorganisms and the efficient recycling of organic matter (Capone et al, 1992).

Majority of the research focusing on microbial nitrogen cycling has concentrated on the microbes (i.e. bacteria and archaea) found on and around the coral reef proper. However, the coral reef sediments have been relatively ignored. Very little is known about the microbes that exist within coral reef sediments, and how much of an influence they have on nitrogen cycling in the coral reef ecosystem (Capone et al, 1992; Sorenson et al, 2007).

Coral reef sediments create a unique environment. The physical characteristics of carbonate sediments provide a situation that favors microbial processes (Wild et al., 2006). Due to their biological source, carbonate sand grains are characterized as exhibiting skeletal features, such as small channels and crevices (Figure 1.1). This offers more external and internal surface area for the growth of bacteria and archaea (Sorenson et al., 2007). This also contributes to the high permeability and porosity of coral reef sediments, which in turn improves the mixing of overlying seawater and porewater (Wild et al., 2006).

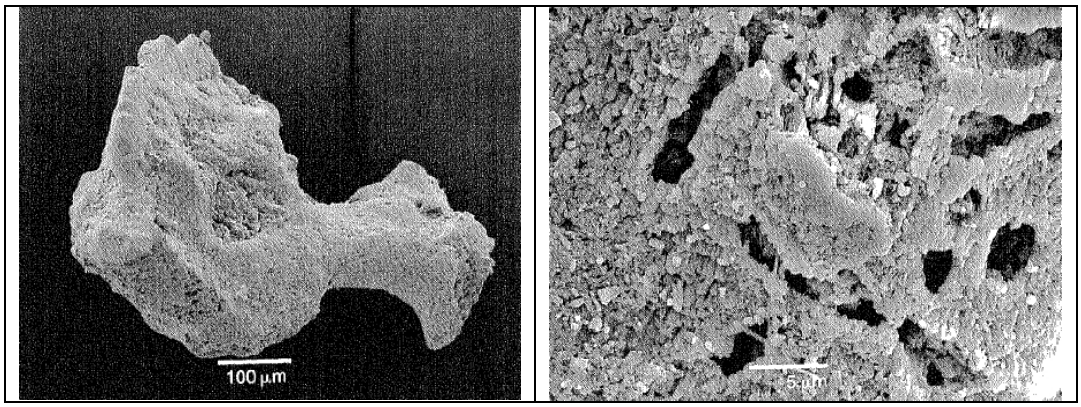


Figure 1.1. Carbonate sand grain exhibiting skeletal features (left). Close up view of the channels and crevices that characterize carbonate sands and thus provide a high external and internal surface area (right) (Wild et al, 2006).

Hydraulically induced advective porewater flow also characterizes coral reef sediments. The highly porous carbonate sands permit advective porewater flow, which is driven by pressure gradients, over the permeable sediment bed. This flow has been found to enhance particulate and solute transport by several orders of magnitude compared to molecular diffusion (Precht and Huettel, 2004; Falter and Sansone, 2000a). With increased transport of porewater, the sediments can act as a large filter, capturing organic matter and dissolved compounds, thus promoting microbial activity (Precht and Huettel, 2004).

Advective porewater flow also allows oxygen to penetrate deeper into the sediment column. Increased flow rates of overlying, oxygenated water into the sediments will lower the oxic/anoxic interface and coupled with increased organic matter transport, allow more aerobic microbial processes (Falter and Sansone, 2000a). When compared to other types of sediments as in Figure 1.2, oxygen penetration (signaling the penetration of overlying water) is found to be much deeper in carbonate sands due to advective porewater flow.

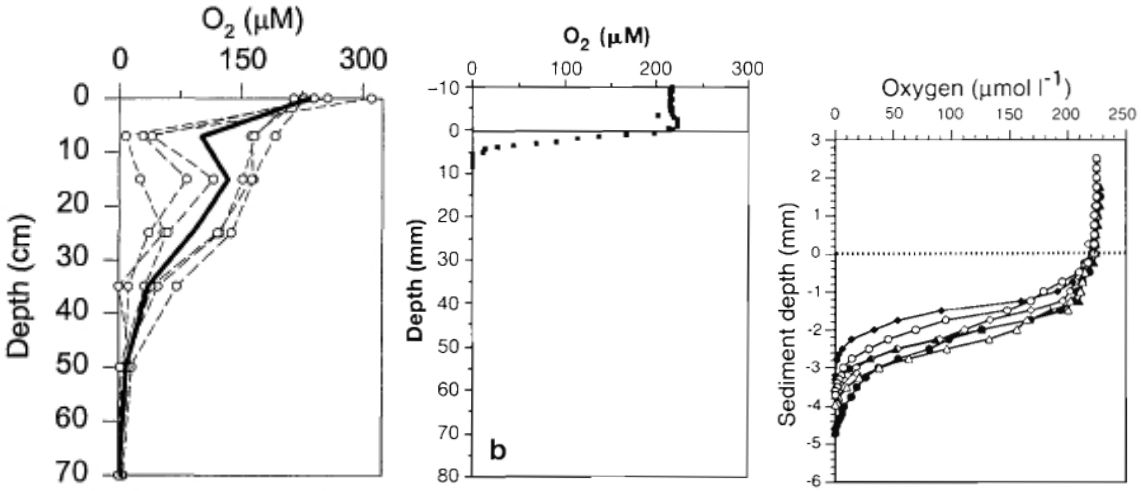


Figure 1.2. Sediment profiles of oxygen taken from (a) carbonate sand (Falter and Sansone, 2000a), (b) silicate sand Tahey et al., 1994), and (c) muddy sediments (Ziebis et al., 1996). Note oxygen penetration in carbonate sediments measured in cm; silicate sand and muddy sediments measured in mm. Advective porewater transport in carbonate sediments is significantly more efficient than molecular diffusion occurring in the silicate sands and the muddy sediments, which exhibit both lower porosity and permeability.

Microbes exert much control over the nitrogen concentrations found in sediment porewater (Capone et al, 1992; Corredor and Capone, 1985). Microorganisms are capable of transforming nitrogen between various compounds through processes such as nitrogen fixation, nitrification, and denitrification. Past studies done by Capone et al (1992) have shown upper sediment layers as likely sites of nitrogen fixation and nitrification where diatomic nitrogen (N_2) and oxygen (O_2) are available via the overlying water. The accumulation of nitrate and nitrite in the top, oxygenated sediment layers is a common occurrence due to the oxidation of ammonium (Corredor and Capone, 1985). Ammonification is a process more likely to occur deeper in the sediment column as shown by sharp increases in ammonium coupled with the depletion of oxygen. Nitrogen

cycling is unquestionably known to occur in coral reef sediments, however the magnitude of these processes has not yet been quantified (Capone, 1992; Sorenson et al., 2007).

Increasing our knowledge about these sediment dwelling microorganisms is vital to our overall understanding of their importance. Coral reef sediment microbes are vital to coral reef nitrogen cycling, which is what essentially allows coral reefs to be such an active area of biological production in a seemingless oligotrophic desert. Determining the relationship coral reef microbes have with their environment is just the first step toward understanding their impact on coral reef porewater geochemistry and nitrogen cycling.

The objectives of this study were to: (1) test bacterial and archeal abundances for any spatial correlation with oxygen and nitrogen species concentrations, (2) test bacterial and archeal abundances for any spatial correlation to organic matter content of the sediments, and (3) compare microbial abundances of coral reef sands to silicate sands and muddy sediments.

CHAPTER 2. BACKGROUND

2.1. Nitrogen Cycling

Nitrogen Fixation: Nitrogen is the most abundant element found in earth's atmosphere. It comprises approximately 78 percent of the atmosphere, found in the form of diatomic nitrogen (N_2). It is used by all organisms to build essential chemical compounds such as proteins and nucleic acids (Hubbell and Kidder, 2003). However atmospheric nitrogen is not readily available to most organisms and must first be chemically "fixed" before any incorporation into a biological system. This creates the paradox where the most abundant element found in earth's atmosphere is often a limiting nutrient in both aquatic and terrestrial environments (Capone, 2001).

Diazotrophs, or nitrogen fixers, "fix" nitrogen by the process of reducing atmospheric nitrogen to ammonia. Using the enzyme nitrogenase, diazotrophs carry out the chemical process of biological nitrogen fixation:

$$N_2 + 8H^+ + 8e^- + 16\ ATP \rightarrow 2NH_3 + H_2 + 16ADP + 16$$

The product of this reaction, ammonia, will then ionize with water to produce ammonium (NH_4^+). Cyanobacteria are the major nitrogen fixers in the coral reef ecosystem.

Nitrification: Another process in the nitrogen cycle is nitrification, the oxidization of ammonia into nitrate (NO_3^-) via nitrite (NO_2^-). Microorganisms carry out this process and obtain energy through oxidation. Nitrification is a two-step process involving two types of microorganisms, ammonium oxidizers (e.g. *Nitrosomonas* sp.) and nitrite

oxidizers (e.g. *Nitrobacter* sp.). The former catalyze the first step, using ammonium monooxygenase to oxidize ammonia to nitrite:

1) $NH_3 + O_2 \rightarrow NO_2^- + 3H^+ + 2e^-$

The latter catalyze the second step, oxidizing nitrite to nitrate using the enzyme nitrite oxidase.

2) $NO_2^- + H_2O \rightarrow NO_3^- + 2H^+ + 2e^-$

Denitrification: Denitrification, another step in the nitrogen cycle, is the reduction of nitrate (NO_3^-) and nitrite (NO_2^-) to dinitrogen (N_2). It is the reversal of nitrogen fixation, turning fixed nitrogen back into the inert, diatomic nitrogen and nitrous oxide (N_2O). Performed by microbes, denitrification is common in anoxic environments, since oxygen is a more favorable electron acceptor elsewhere. Some known denitrifiers include *Pseudomonas sp.*, *Shewanella sp.*, and Planctomycetes. The microorganisms extract energy by reducing NO_3^- and converting it to nitrous oxide (N_2O) and dinitrogen (N_2) (Zumft, 1997).

$2NO_3^- + 10e^- + 12H^+ \rightarrow N_2 + 6H_2O$

Organic Matter Diagenesis: Organic matter diagenesis is believed to be facilitated by coral reef sediment microbes during nitrogen transformations. Evidence of this is seen in the low organic matter levels, high rates of oxygen consumption, and dissolved inorganic carbon production frequently found in carbonate sediment porewater (Rusch, 2006).

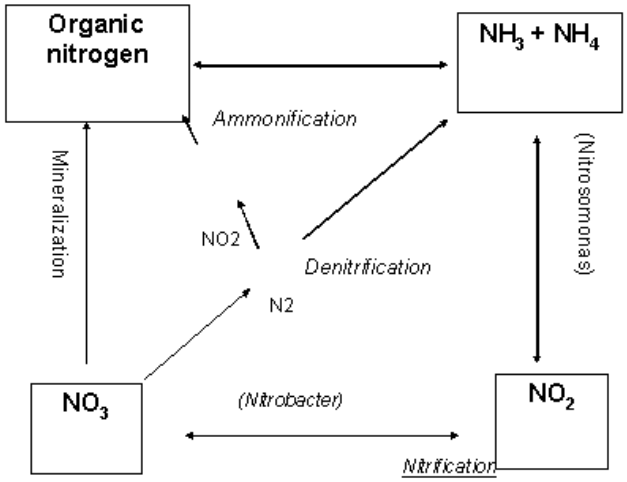


Figure 2.1. The nitrogen cycle **(**Thy, 2003**).**

These characteristics all point to the uptake and remineralization of organic matter by benthic microbes (Ducklow, 1990).

Microbes are capable of processing organic matter in both aerobic and anaerobic environments. In aerobic environments, oxygen is used as an electron acceptor to mineralize organic matter into H_2O, CO_2, and inorganic nutrients. Anaerobic mineralization of organic matter by microbes occurs through a series of steps where alternate electron acceptors are used. Due to this, anaerobic mineralization has been found to be less efficient then aerobic mineralization (Kristensen, 2000).

2.2. Advective Porewater Flow

Porewater flow is an important process in the biogeochemistry of coral reef sediments. Flow of water through the pore spaces of a permeable sediment bed is responsible for the transport of particulate matter and solutes from overlying water into the sediments (Falter and Sansone, 2000a; Tribble et al, 1992). Molecular diffusion is

another prominent force that can drive porewater flow, however, advective flow has been shown to exceed molecular diffusion by several orders of magnitude (Precht and Huettel, 2004; Falter and Sansone, 2000a).

Advective porewater flow is controlled by hydrodynamic forces such as waves propagating over the coral reef or bottom currents in contact with the sediments. Relationships between the size of the waves and advective porewater flows have been established in past studies (Falter and Sansone, 2000a). As currents pass over ripples or various sediment topographies, pressure gradients are formed and advective transport of porewater is induced (Precht and Huettel, 2004). Flow rates driven by currents may depend on ripple formations and physical properties such as sediments transitioning from rough to smooth textures. It is the difference in pressure that controls the magnitude of the advective transport of porewater (Precht and Huettel, 2004).

The effect porewater flow has on the biogeochemistry of the sediment porewater also varies on the flow rates and the permeability of the sediments. A larger degree of porewater flow will transport more particulates and dissolved constituents deeper (decimeters) into the sediments. This has been found to have effects on oxic/anoxic zonation, nutrient cycling within the sediments, and other biogeochemical processes (Falter and Sansone, 2000a).

CHAPTER 3. MATERIALS AND METHODS

3.1. Study Sites

Checker Reef: Checker Reef is a lagoonal patch reef found in Kaneohe Bay on the northeast side of the island of Oahu, Hawaii. The reef is approximately 750 m by 350 m (Tribble et al., 1992). Upper layers of the reef are poorly lithified and consist of biogenic calcareous sediments. Low energy waves and currents progress over the reef from a northeasterly direction, forereef to backreef, commonly throughout the year (Falter and Sansone, 2000a). Checker Reef is described more extensively by Tribble et al. (1992) and Falter et al. (2000a).

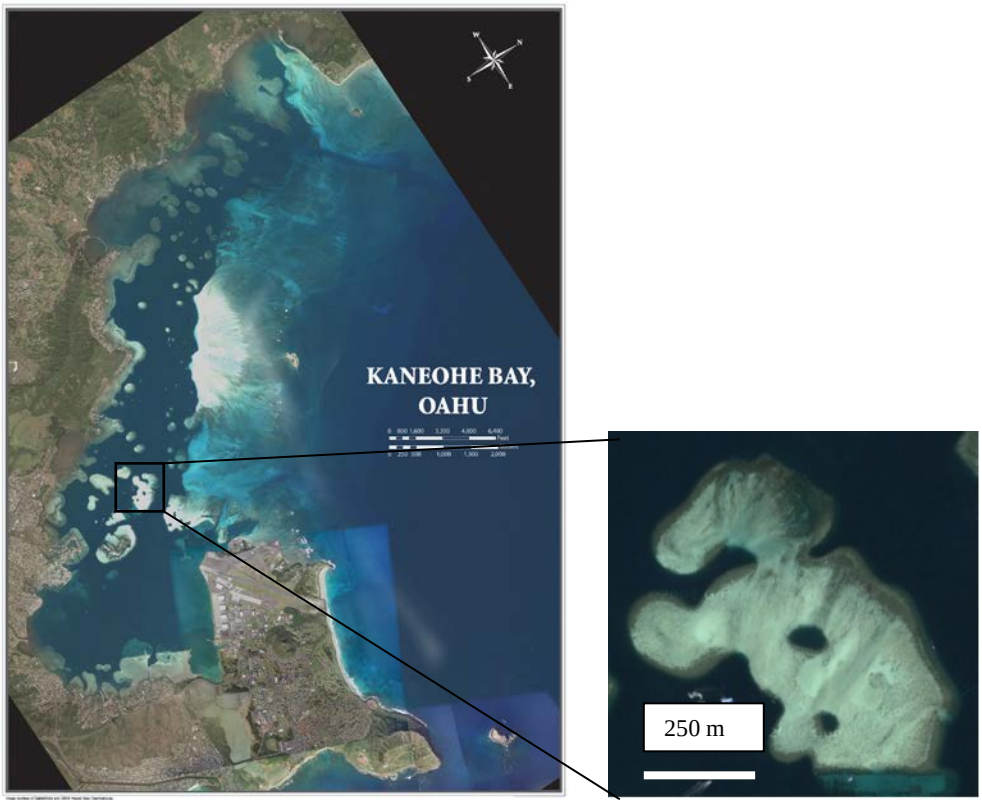


Figure 3.1. Map of Kaneohe Bay (courtesy Coastal Geology Group) and Checker Reef, Oahu (courtesy Google Map).

Kilo Nalu: Kilo Nalu Observatory is located on a reef off the south shore of the island of Oahu, Hawaii. The Kilo Nalu reef consists of unconsolidated sandy beds, limestone pavement, and active coral beds. Sediments are primarily carbonate and considered highly permeable. A detailed summary of the Kilo Nalu Nearshore Reef Observatory is located at http://www.soest.hawaii.edu/OE/KiloNalu/.

Kilo Nalu sediments were collected on-site, sieved to retain the fraction > 250 µm in the laboratory, and used in a microcosm experiment. Filtered seawater was acquired from the Waikiki Aquarium. A detailed description of the microcosm setup is given by Hannides et al. (2005).

Figure 3.2. Microcosm set up (right) and close up view of porewater sampling ports (left) (courtesy A. Hannides).

3.2. Field Sampling

Using a GPS locator, the field site at 21°26.6' N, 157°47.6' W was located on Checker Reef, Oahu during a low tide. A metal sheath with removable rod was driven into the coral reef sediment to the desired depth. The rod was then removed, and a rubber stopper connected to tubing and a three-way stopcock was placed at the end of the hollow sheath. A 60 ml syringe was connected to the stopcock, and 60 ml of porewater was withdrawn. This was checked for sulfidic odor and then discarded. Another 60 ml of porewater was drawn and immediately measured for oxygen concentration using an Orion820 oxygen probe. A third 60 ml of porewater was drawn and transferred into a 50 ml Falcon tube for porewater analysis (ammonium, nitrate, and nitrite).

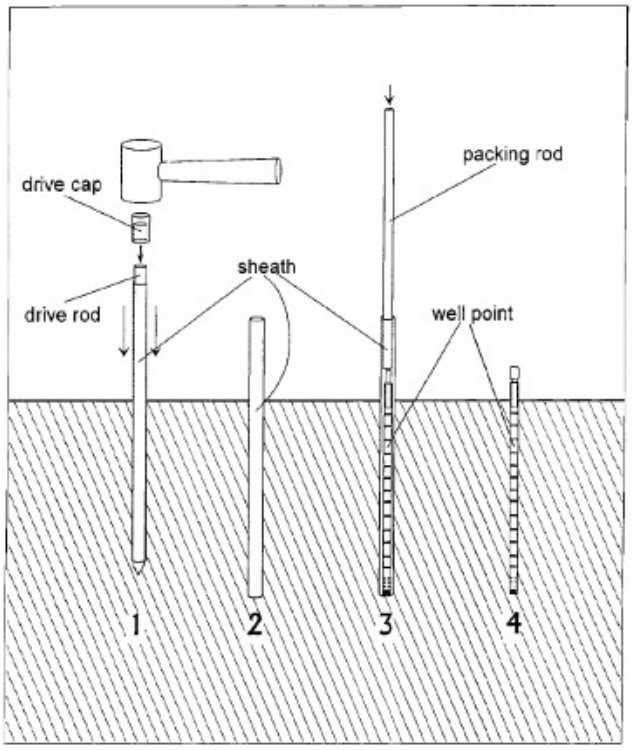


Figure 3.3. Diagram of well point sampling (Falter and Sansone, 2000b).

The rubber stopper was then removed, and the sleeve was driven 1-2” further into the sediment. The sheath was removed with a sediment core contained in the lower section of the sheath and placed in a 15 ml Falcon tube. A diagram of this process in pictured in Figure 3.3.

These steps were repeated for various depths in sampling wells in the same vicinity, including an oxygen concentration reading of the overlying seawater. Samples were kept on ice during transport to the laboratory. Upon arriving, 2 g of each sediment sample were placed into a 10 ml serum bottle with 3 ml of a preservative solution consisting of 2% formaldehyde and 2% acetic acid in filtered seawater. The serum bottles were sealed and refrigerated for later sediment analysis (Falter et al., 2000b).

3.3. Nutrient Analysis

Ammonium Analysis: Ammonium was measured using a spectrophotometer and calibration standards. Porewater samples were filtered (pore size 0.2 μm), split into 1 ml subsamples, and solutions of NH_4Cl in filtered seawater were used for calibration standards. Each subsample and calibration standard was mixed with 40 μl of phenol solution (10% phenol in 95% ethanol), 40 μl of sodium nitroprusside solution (16.8 mM $Na_2[Fe(CN)_5NO]$), and 100 μl of oxidizing reagent (4:1 ratio of an alkaline reagent (0.25 M NaOH, 0.77 M Na_3-citrate) and Clorox). Samples and standards were vortexed and left to react for 1 hour. After developing a blue color, a Genesys 5 (Manufacturer) spectrophotometer was used to measure the absorbance of each sample at 640 nm. Using the calibration standards, a linear calibration curve was generated and used to compute $[NH_4^+]$ in each subsample from its absorbance (Solórzano, 1969).

Nitrite Analysis: Porewater samples were split into subsamples of 1 ml. Calibration standards were prepared using $NaNO_2$ and filtered seawater. Each subsample and calibration standard was combined with 20 µl of sulfanilamide (58 mM in 10% HCl) and 20 µl NEDA (3.9 mM N-(1-naphthyl)ethylenediamine). The reactions were vortexed and left to react for 10-20 minutes. The absorbances at 543 nm were measured, and concentrations were calculated just as described for ammonium (Bendschneider and Robinson, 1952).

*Nitrate Analysis***:** Nitrate was measured using a cadmium column, which reduced the nitrate into nitrite, which could then be analyzed using the nitrite analysis procedure. The cadmium column was constructed using a 5 ml syringe barrel without plunger. 4-5 grams of cadmium granules were washed in 10% HCl and rinsed with DI water until a pH greater than 5.5 was reached. The cadmium granules were then immersed and mixed in $CuSO_4$ (80 mM) solution for 2 minutes. Once removed from the $CuSO_4$ solution, the granules were submersed in a NH_4Cl (117 mM) solution. Glass wool was placed into the syringe, filled with NH_4Cl (117 mM), cadmium granules added, and capped with glass wool. Tubing was attached to the outlet of the syringe. Porewater samples were passed through this column, converting all nitrate into nitrite. Nitrite concentrations were then subtracted to yield the final nitrate data (Morris and Riley, 1963).

3.4. Cell Extraction and Enumeration

*Cell Extraction***:** A Fisher Sonic Dismembrator 100 (22.5 kHz) ultrasonic probe was used to extract microbial cells from the sediment samples. The serum bottle with preserved sediment sample was placed in an ice bath, and the ultrasonic probe was immersed into the sample avoiding contact with the sediment. Sediment samples were pulse sonicated at a power setting of 3 (output power of 7-8 W). Five cycles of 5 seconds on and 10 seconds off were performed. The sand grains were allowed to settle, and the supernatant was collected with a Pasteur pipette.

3 ml of 2% acetic acid in filtered seawater were added to the settled sediment and shaken. Once again the sand grains were allowed to settle and the supernatant collected. This process was repeated 6 more times for a total combined volume of 21 ml of supernatant. This entire process was then repeated four times for a total of five sonicating cycles yielding 105 ml of extract. The extract was stored in a refrigerator for cell counts within 24 h from extraction (Wild, 2006; modified after Rusch, unpubl.; Epstein and Rossel, 1995).

Cell Staining and Enumeration: Cell extracts of volumes ranging from 125-300 µl were placed in a glass tower fitted with a black polycarbonate filter (pore size 0.2 µm). An equal amount of 4 µg ml^{-1} DAPI (4',6'-diamidino-2-phenylindole) was added to the sample and allowed to stain for at least nine minutes. Small volumes of deionized water were added to enhance the uniform distribution of cells over the filter area. The sample was then drawn through the filter using a vacuum pump. The filter was removed from

the tower and placed on a slide with immersion oil added both below and on the filter. A cover slip was placed on top. Each sample was processed in duplicate.

Epifluorescence microscopy was used to observe bacterial cells. Cell enumerations were performed using an Olympus BX51 epifluorescent microscope. Fitted with a UV lamp and under 1300X magnification, direct counts were taken within a grid at twelve randomly selected fields. Under the UV light, the DAPI stained cells fluoresced blue against the black filter (Wild, 2006; modified after Rusch, unpubl.; Epstein and Rossel, 1995). The abundance of microbes per sediment was calculated using equation 3.1.

EQUATION 3.1. $$\frac{\text{cells}}{\text{g sed.}} = \frac{\text{cells}}{\text{grid}} \times \frac{\text{grids}}{\text{filter}} \times \frac{\text{filter}}{\text{V filtered}} \times \frac{\text{V filtered}}{\text{V extracted}} \times \frac{\text{V extracted}}{\text{g sed.}}$$

3.5. Organic Matter Measurement

Organic content was measured using the "Loss on ignition method." Ceramic crucibles were cleaned and dried, and then combusted in an oven at 550°C for 4 hours. The crucibles were then weighed and numbered. About 10 g of sediment samples were placed in each crucible and weighed again. All crucibles were then left under a fume hood for 72-96 hours to allow any moisture retained within the sediment samples to evaporate. After drying, the crucibles with the dried sediment samples were weighed and combusted once again at 550°C for 4 hours. The combusted sediment samples were

allowed to cool down before they were reweighed. The organic content of the sediment samples was calculated using Equation 3.2. Both the percentage of organic matter per dry sediment and per wet sediment was computed.

EQUATION 3.2. $$\% \text{ Organic Matter} = \frac{\text{Dry weight} - \text{Combusted weight}}{\text{Dry weight}} \times 100$$

3.6. Correlation Calculations

The correlations between cell counts and each of five geochemical parameters (O_2, NH_4^+, NO_3^-, NO_2^-, organic matter content) were calculated by plotting each parameter against cell counts using Microsoft Excel. A linear regression line was fitted for each data set, along with an r^2 value. Using this r^2 value, critical t-values were calculated using Equation 3.3 and correlations were determined using a two-tailed t-test and an $\alpha = 0.05$.

Equation 3.3. $$t = r \sqrt{\frac{(n-2)}{(1-r^2)}}$$

CHAPTER 4. RESULTS

4.1. Porewater Profiles

The results of porewater nitrogen analyses and oxygen measurements were compiled in depth profiles for each of the three coral reef sediment arrays; one set of profiles is shown in Figure 4.1. All profiles exhibited somewhat similar profile shapes, however the depth scales varied along with minor differences in concentration. For example, oxygen penetrated down to 35 cm and 25 cm in the Checker Reef sediments taken in July 2006 and February 2007, respectively, and only down to 10 cm in the microcosm.

In Figure 4.1 displaying porewater profiles from Checker Reef of July 2006, dissolved oxygen (O_2) was abundant in the top sediment layers and declined sharply with depth until it was depleted at approximately 30 cm. Due to a calibration offset in the oxygen probe, 24 µM and 19 µM were subtracted from raw data taken at Checker Reef and in the microcosm measurements, respectively, to obtain final oxygen concentrations

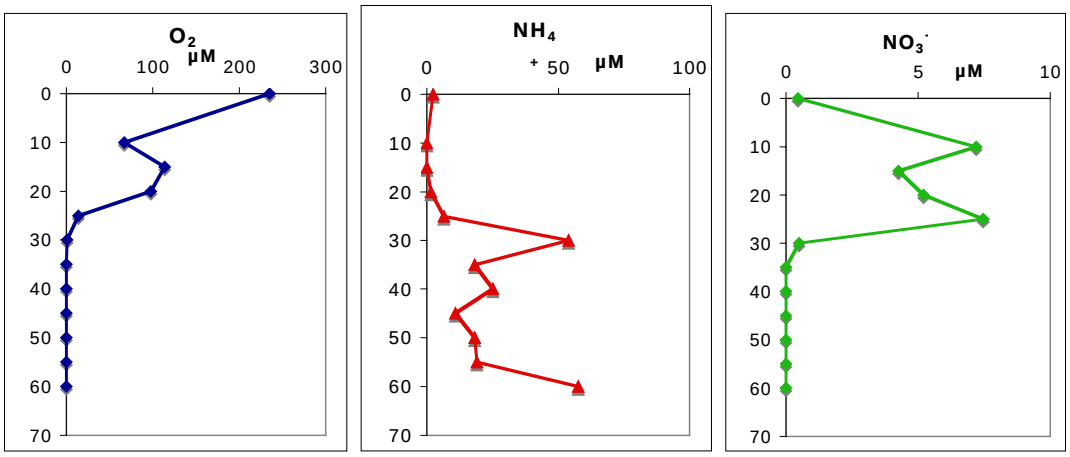
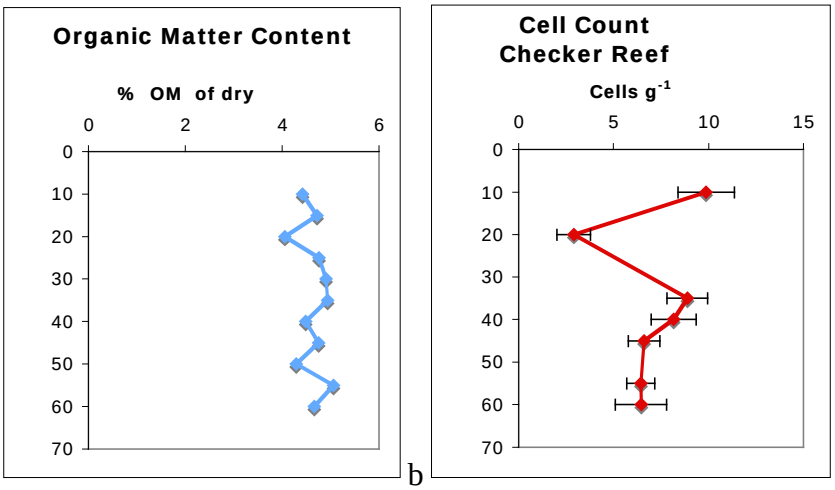


Figure 4.1. [O_2], [NO_3^-], and [NH_4^+] of porewater and overlying water of samples taken from Checker Reef, Oahu in July 2006.

(A. Hannides, personal communication). Ammonium (NH_4^+) concentrations were minimal in the top layers. A sharp increase in ammonium occurred at approximately the same depth oxygen was depleted (30 cm), and ammonium continued to increase with depth. Nitrate (NO_3^-) concentrations were relatively small compared to oxygen and ammonium. Maximum nitrate concentrations were found to occur in the oxygenated sub-surface layers. Nitrate then fell back to minimal concentrations, mirroring the depletion of dissolved oxygen. Nitrite concentrations were found in extremely small concentrations and mirrored the nitrate profile. However due to the minute quantities, nitrite exhibited some variations and was almost nonexistent in the microcosm sediments. Porewater profiles for each sediment array can be found in Appendix A.

4.2. Cell Abundance

Three depth profiles of bacterial cell counts were taken, two from Checker Reef, one from the microcosm. The profile from Checker Reef of July 2006 (Figure 4.2 a)



a b

Figure 4.2. (a) Organic matter content and (b) cell count profiles of sediment samples taken from Checker Reef, Oahu in July of 2006.

revealed a maximum cell count of 9.8 x 10^8 cells g^{-1} in the upper sediment layer with a sudden mid-depth minimal cell count of 2.9 x 10^8 cells g^{-1} occurring at the 20 cm depth. Cell abundance then increased back to 8.8 x 10^8 cells g^{-1} and from there, slowly declined with depth.

The sediment profile of Checker Reef of February 2007 consisted only of 5 samples. Similar to the July 2006 Checker Reef profile, at the 20 cm depth, cell abundance were also found to be minimal: approximately 3.3 x 10^8 cells g^{-1}. Cell abundance then increased to 6.6 x 10^8 cells g^{-1} and then gradually declined with depth. The microcosm sediment array had maximum cell abundance in the top layers (9.2 x 10^8 cells g^{-1}) and steadily decreased until 4.8 x 10^8 cells g^{-1} at a depth of 50 cm. At the 60 cm depth, cell count rose to 7.4 x 10^8 cells g^{-1}. All cell count data are tabulated in Appendix A and microbial abundance profiles for the three sediment arrays is found in Appendix B.

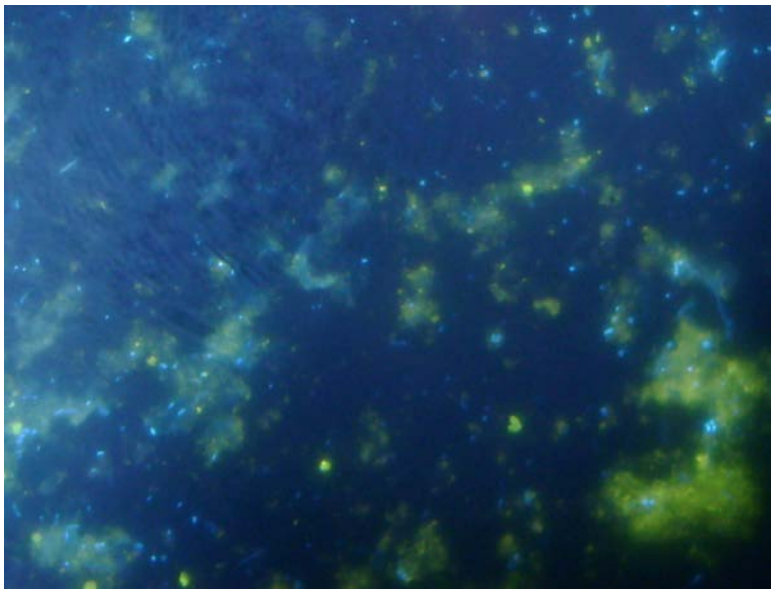


Figure 4.3. Epifluorescence microscopy. Light blue dots represent DNA-bound DAPI cell stains. Organic matter is pictured as neon green.

4.3. Organic Matter

Organic matter content ranged from approximately 3.9% to 5.1% of dry sediment. There did not appear to be any trend with depth, and this was consistent in all three sediment arrays. Figure 4.2(b) is an organic matter profile taken from Checker Reef of July 2006 and exhibits typical measurements for all three sediment arrays. All organic matter data can be found in Appendix A.

4.4. Correlations

In sediment samples taken from Checker Reef in July 2006 and February 2007, no significant correlation was found between cell abundance and any of the geochemical parameters. Correlation coefficients and degrees of freedom are shown in Table 4.1 and Table 4.2 for July 2006 and February 2007, respectively. Microcosm sediments, shown in Table 4.3, exhibited one significant correlation. Cell abundance and ammonium concentrations were found to be negatively correlated. A t-value of 3.98 with 6 degrees of freedom was also found to be higher than a critical value of 3.707 corresponding to a 99% confidence interval. Figure 4.4 shows the scatter plot and regression line of the negative correlation between cell abundance and ammonium concentration.

Table 4.4 is the cumulative data set taken from all three sample groups. No significant correlations were found in the combined graphical analysis. Figure 4.5 shows the scatter plot exhibiting the non-correlation between cell abundance and organic matter content. Complete sets of scatter plots can be found in Appendix B.

TABLE 4.1. Checker Reef of July 2006.

CH July	**R^2**	**n**	**t-value**	**95% t-value**
CC vs. O_2	0.1616	6	0.878	2.776
CC vs. NH_4^+	0.0002	7	0.032	2.571
CC vs. OC	0.1622	7	0.984	2.571
CC vs. NO_3^-	0.0007	7	0.059	2.571
CC vs. NO_2^-	0.0017	7	0.092	2.571

TABLE 4.2. Checker Reef of February 2007.

CH Feb	**R^2**	**n**	**t-value**	**95% t-value**
CC vs. O_2	0.2996	5	1.133	3.182
CC vs. NH_4^+	0.0238	5	0.270	3.182
CC vs. OC	0.1916	4	0.688	4.303
CC vs. NO_3^-	0.0715	5	0.481	3.182
CC vs. NO_2^-	0.0013	5	0.062	3.182

TABLE 4.3. Microcosm

Microcosm	**R^2**	**n**	**t-value**	**95% t-value**
CC vs. O_2	0.0439	8	0.525	2.447
CC vs. NH_4^+	**0.7256**	**8**	**3.983**	**2.447**
CC vs. OC	0.0064	8	0.197	2.447

TABLE 4.4. Cumulative data from all three sediment arrays.

Cumulative	**R^2**	**n**	**t-value**	**95% t-value**
CC vs. O_2	0.0127	19	0.468	2.11
CC vs. NH_4^+	0.0654	20	1.122	2.101
CC vs. OC	0.0316	19	0.745	2.11
CC vs. NO_3^-	0.0596	12	0.796	2.228
CC vs. NO_2^-	0.0285	12	0.542	2.228

* CC = cell counts & OM = organic matter content.

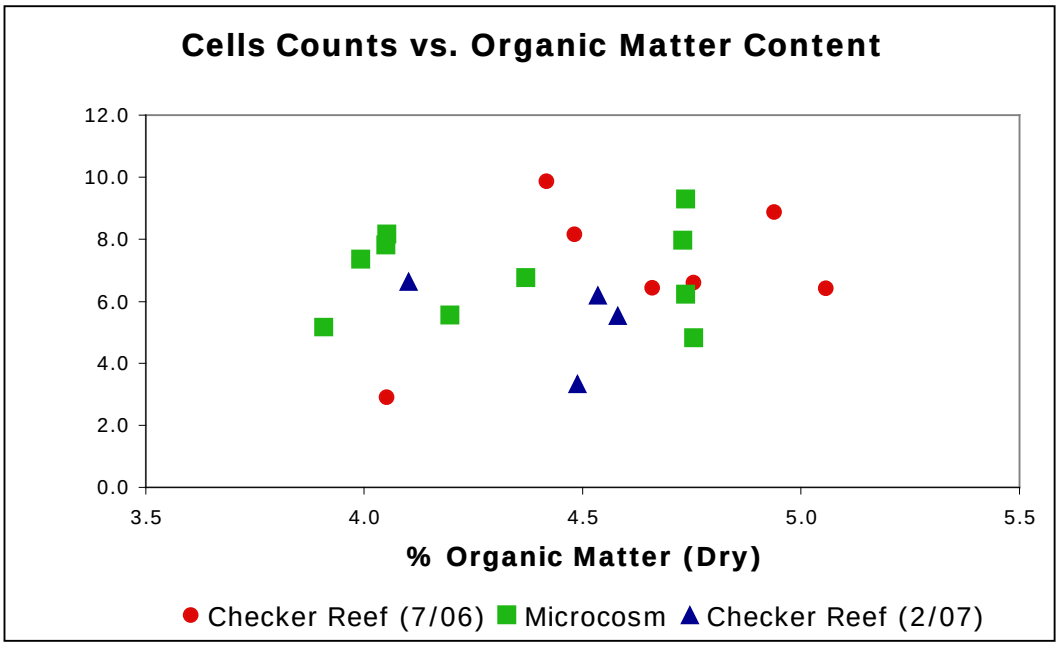


Figure 4.4. Scatter plot of cell counts vs. organic matter content of cumulative data taken from all three sediment arrays. No significant correlation was found in this plot.

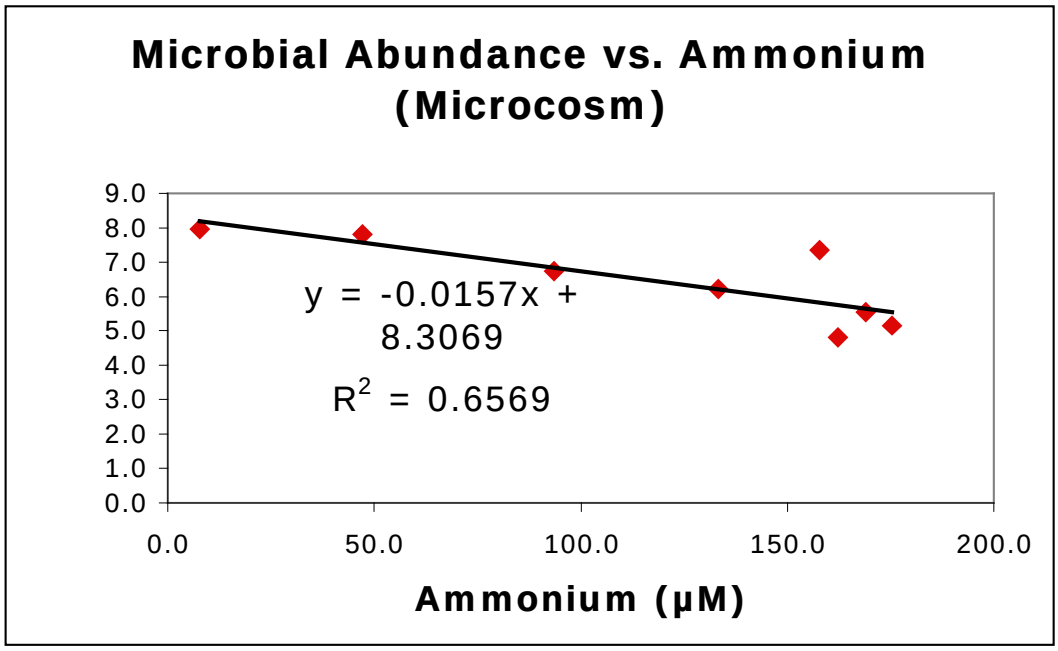


Figure 4.5. Scatter plot of cell counts vs. ammonium in the microcosm array. This was the only significant correlation out of all data sets. The regression line and R^2 value is displayed.

CHAPTER 5. DISCUSSION/CONCLUSION

It has been proposed that coral reef sediment microbes exert much control over the nitrogen distributions and concentrations found in coral reef sediments (Capone et al., 1992). The relative lack of information on these microorganisms compared to other microbes found on and around coral reefs is the result of the limited research focused on coral reef sediment microbes. The main objectives of this research were to determine if a correlation could be found between microbial abundances and the geochemical characteristics of coral reef sediments and to compare microbial abundances of carbonate sands with silicate sands and muddy sediments.

Microbial Abundance & Nitrogen Species: Using a 95% confidence interval, only one statistical correlation in the three sediment arrays was found. Microbial abundance and ammonium (NH_4^+) were found to be significantly correlated in the microcosm array. However, microbial abundance and ammonium were not significantly correlated in the two Checker Reef sediment arrays or cumulative sediment data. Microbial abundances and the remaining measured geochemical parameters (O_2, NO_3^-, and NO_2^-) did not exhibit any significant correlations.

The lack of correlations between oxygen, nitrate, and nitrite with cell abundance may be attributed to the nature of where these three nutrients are formed and found in the sediment column. Nitrate and nitrite are formed through the process of nitrification, which is the oxidation of ammonium and thus consequently only occurs in aerobic environments. Meanwhile dissolved oxygen is primarily found within the sediments due

to the penetration of overlying, oxygenated seawater. Therefore, oxygen, nitrate, and nitrite concentrations are all found in the sediment layers which are continually being flushed with overlying seawater. So in addition to microbial processes, porewater flushing is also affecting nutrient concentrations and may have contributed to the findings of this experiment.

The single correlation found between cell abundance and ammonium occurred in the microcosm array. The major difference in the microcosm sediments from the Checker Reef sediments was the penetration of oxygen, which signals the penetration of overlying seawater. In the microcosm array, oxygen penetrated 10 cm into the sediment column, compared to 35 cm and 45 cm in the Checker Reef arrays. Therefore, in the 60 cm sediment column of the microcosm, oxygen along with nitrate and nitrite were depleted within 10 cm from the surface and ammonium concentrations increased considerably in the remaining 50 cm. It is possible that due to the absence of substantial porewater flushing in a considerable section of the sediment column (which characterized the two Checker Reef arrays), an isolated analysis of strictly ammonium and microbial abundance was available and accounted for the significant correlation found only in the microcosm.

Upon further inspection and analysis of the two Checker Reef sediment arrays, it appears that two cell counts taken at a depth of 20 cm in each array contained much lower cell abundances. It is important to note, that these two sediment samples were processed on the same day and with a slightly altered procedure from the rest of the samples. Nevertheless, these alterations in procedure should not have affected the end

results nor is there independent evidence that these two cell counts are outliers. More sediment sampling would need to be sampled to confirm these results. However, it can be seen that these two samples by themselves would not have destroyed or decreased any correlations between cell counts and geochemical parameters, if they did not exist in the first place. This is displayed in the scatter plot of cell counts vs. ammonium found in Appendix B.

Microbial abundance and organic matter content were also not found to significantly correlate. The sediments from both Checker Reef and the Kilo Nalu microcosm sediments contained approximately 4 -5% organic matter. The homogeneity of organic matter throughout the sediment column in all three sediment arrays was the main reason why there existed no correlation to microbial abundance.

It is interesting to note that these organic rich sediments at Checker Reef were not depleted of oxygen. Often one result of organic matter diagenesis is the consumption of oxygen. It is likely that advective pore water flow enhances the exchange of oxygenated overlying water, as well as transporting organic matter deep into the sediments, keeping the sediment column oxygenated despite the presence of abundant organic matter.

The lack of correlations between cell abundances and nitrogen concentrations and organic matter content do not seem to agree with the concept that microbes are major contributors of nitrogen cycling in coral reef sediments. Nevertheless, the lack of significant correlations may be attributed to the method used to enumerate bacterial cells. In the enumeration process, DAPI staining does not allow differentiation of cell type. Therefore total cell numbers were counted, which includes bacteria and archaea as well

as eukaryotes. Also with the extensive variations of microbes existing within coral reef sediments, which carry out different processes such as nitrogen fixation, nitrification, and denitrification, it may be likely that bacteria species are differentiated with respect to their environment. For example, various bacteria prefer and perform certain processes in specific environments such as aerobic or anaerobic sediments. The same is true for environments containing various levels of nutrient concentrations. If this is the case, different bacteria populations may occur at different sediment depths. Yet another factor not accounted for in DAPI staining, is the activity of microbial cells. DAPI binds to the DNA of any intact cell, however not all cells are actively metabolizing and affecting porewater geochemistry.

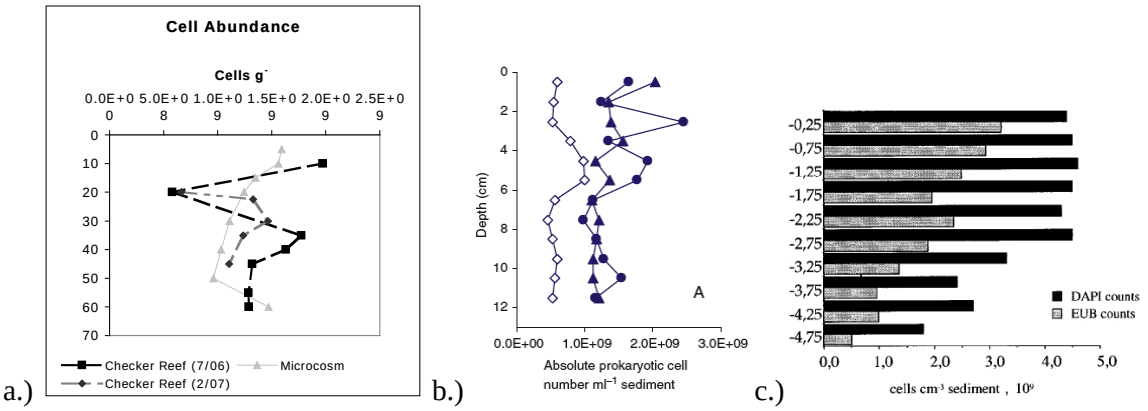


Figure 5.1. Sediment profiles of cell abundances taken from (a) carbonate sediments from Checker Reef and microcosm, (b) silicate sand from Sylt Island, Wadden Sea (Musat et al., 2006), and (c) muddy sediments from Jadebusen Bay, Wadden Sea (Llobet-Brossa et al., 1998). Note that sediment profiles from the (a) carbonate sands have been converted to cells per cm^3 (1 g carbonate sediment = 0.5 cm^3).

Comparison of microbial abundances in carbonate, silicate, and muddy sediments: Carbonate sediment profiles of cell counts shown in Figure 5.1 (a) were taken from the two Checker Reef arrays and the one microcosm array. Maximum abundances occurred in the top sediment layers at ~10^9 cells g^{-1}. Cell numbers decreased with depth minimizing at approximately 4 x 10^8 cells $(cm^3 \text{ sed})^{-1}$. Checker Reef sediments showed somewhat higher abundances of cells compared to the microcosm sediments. Note that carbonate sediment profiles were enumerated in cells per gram of dried sediments and converted to cells per cm^3.

Comparing our cell abundance profiles from carbonate sands with sandy silicate sediments and muddy (fine-grained) sediments at other sites is seen in Figure 5.1. The sandy silicate sediment profile of cell abundance, (Figure 4.6 b.) taken from Musat et al. (2006); profiles from October 1999 and March and July 2000, were taken from an intertidal sand flat on the Sylt island located in the northern Wadden Sea. Cell abundances ranged from 0.4 x 10^9 to 3.3 x 10^9 cells $(\text{ml sed.})^{-1}$. There did not seem to be any noticeable trend with depth.

A muddy sediment profile of cell abundance (Figure 4.6 c.) was taken from Llobet-Brossa et al. (1998). The profiles were taken from Jadebusen Bay of the Wadden Sea in November 1997. Cell counts ranged from 4.5 x 10^9 to 1.8 x 10^9 cells $(cm^3 \text{ sed.})^{-1}$ over a depth of 0-5 cm. Cell abundances appeared to decrease with increasing depth. EUB counts were also taken however for our purposes we will ignore them.

Carbonate sands sampled from Checker Reef and Kilo Nalu represent sediments dominated by advective pore water flow due to their high permeability. Silicate sands

and muddy sediments are less permeable, limiting the flow of pore water through the sediments. Despite the differences in the depths of the profiles, a comparison of microbial abundances can be analyzed with the assumption that the depths of these profiles are relative to the depths of oxygen penetration.

Analyzing the sediment profiles, carbonate sediments have abundances similar to those of silicate sands, ranging approximately from 0.5×10^9 to 3.0×10^9 cells cm^{-3} sed. However, cell abundances decreased with depth in carbonate sediments compared to the relatively constant cell abundances in the silicate sand. The similarity of microbial abundance in carbonate sands and silicate sands can be expected due to the similarity in the grain sizes of each. However, the irregular features of carbonate sands make them more susceptible to advective pore water flow due to increased permeability and porosity. The increased oxygen penetration and organic matter transport is likely to support higher rates of microbial activity. Therefore carbonate and silica sands may have similar microbial abundances, but it is likely carbonate sands experience higher rates of microbial activity.

Cell abundances in muddy sediments appear to be approximately twice as high as in carbonate sediments. Though both sediments do share similar sediment profile shapes, maximizing in the top sediment layers and decline with depth. The comparatively high abundance of microbes found in muddy sediments can be attributed to the high grain surface area of muddy sediments. The fine grains of muddy sediments provide more substrate for microbes to latch onto and grow. However, the lack of benefits advective

porewater flow provides will mean less microbial activity when compared to carbonate sediments possibly off-setting the difference in microbial abundance.

Future Study: Future studies in determining the relationship of coral reef sediment microbes with porewater geochemistry would certainly be enhanced with the increase in sample number and frequency. The coral reef sediment environment is an ever-changing one. Concentrations of nutrients are constantly in flux with the variability of weather, currents, and benthic fauna. Porewater and sediment sampling methods take all but a mere snap shot of the continually changing environment. By increasing sampling, temporal variations are more likely to be eliminated, revealing a more normalized picture of sediment and porewater geochemistry.

The next step in determining relationships between microbes and porewater geochemistry should be the identification of specific microbes and examining their individual effect in the sediments. This can be accomplished using the enumeration procedure known as fluorescent in situ hybridization (FISH) (Ravenschlag et al., 2000). FISH counts are capable of identifying specific monophyletic group of microbes. Being able to distinguish specific groups of bacteria from other bacterial and non-bacterial cells will allow the examination of the specific relationships microbial species have with sediment geochemistry.

Cell activity is another feature that FISH analysis is able to determine. The activity or non-activity of cells in the sediments can be established using FISH and RNA analysis. By examining RNA, the expression of genes can reveal whether a cell is active

or inactive. Consideration of cell activity while determining correlations between microbial abundances and their environment will definitely provide a clearer insight in the relationship between microbes and coral reef sediments.

Conclusion: This research has provided a small step towards better understanding the intimate relationships of coral reef sediment microbes with their environment. The findings in this experiment conclude that microbial abundances are not correlated (with the exception of the microcosm sediment array) with nitrogen species found in sediment pore water or organic matter content, an observation that we interpret to be the partial result of insufficiencies in the methods used to enumerate microbial abundance. Comparison of microbial counts of carbonate reef sediments with silicate sands and muddy sediments shows that the carbonate sands have similar microbial abundances to silicate sands and less microbes than muddy sediments.

APPENDIX A

TABLE 1. Compiled raw data taken from porewater and sediment measurements.

sample	depth	$[O_2]$	$[NO_3^-]$	$[NH_4^+]$	$[NO_2^-]$	$[NO_3^-]+[NO_2^-]$	TOM	TOM	cells (g sediment)$^{-1}$	
Checker Reef	cm	μM	μM	μM	μM	μM	% wet	% dry	mean	stdev
water - July	-1	259.00	0.45	2.43	0.02	0.47				
reef-41	10	91.00	7.21	0.00	0.07	7.27	3.24508	4.41755	9.865E+08	1.486E+08
reef-39	15	138.00	4.26	0.00	0.06	4.32	3.77407	4.71759		
reef-37	20	122.00	5.20	1.62	0.09	5.29	3.28975	4.05183	2.904E+08	8.877E+07
reef-35	25	38.00	7.46	6.48	0.34	7.80	3.87054	4.75566		
reef-33	30	25.00	0.50	53.86	0.00	0.50	3.67748	4.90804		
reef-31	35	13.00	0.00	18.22	0.11	0.00	3.47376	4.93851	8.877E+08	1.069E+08
reef-29	40	13.00	0.00	25.11	0.00	0.00	3.20934	4.48208	8.154E+08	1.191E+08
reef-27	45	13.00	0.00	10.93	0.00	0.00	3.70033	4.75346	6.604E+08	8.264E+07
reef-25	50	6.00	0.00	18.22	0.00	0.00	3.33435	4.29312		
reef-23	55	9.00	0.00	19.03	0.00	0.00	3.82830	5.05796	6.427E+08	7.338E+07
reef-21	60		0.00	57.51	0.00	0.00	3.48662	4.65977	6.440E+08	1.347E+08
Microcosm										
B-Before	-1	191		1.39		0.50	2.89115	4.05270	8.164E+08	3.390E+08
B-11	5	19		7.70		2.29	3.78443	4.73010	7.962E+08	4.044E+08
B-12	10	16		47		0	3.42993	4.05109	7.810E+08	3.907E+08
B-13	15	19		94			3.24689	4.37080	6.747E+08	2.984E+08
B-14	20	19		133			3.75044	4.73629	6.216E+08	1.909E+08
B-15	30	19		169			3.41364	4.19733	5.552E+08	2.973E+08
B-16	40	16		175			3.32639	3.90838	5.154E+08	2.025E+08
B-17	50	22		162			3.88393	4.75459	4.801E+08	1.951E+08
B-18	60	41		158			3.40414	3.99312	7.357E+08	2.035E+08
Checker Reef										
water - Feb	-1	241	1.85	2.98	0	1.85				
reef-39	15	159	7.28	2.98	0.06	7.34				
reef-37	20	116	9.36	4.95	0.25	9.61	3.40530	4.48761	3.325E+08	1.129E+08
reef-36	22.5	97	10.66	5.45	0.22	10.88	3.22625	4.10145	6.640E+08	7.920E+07
reef-35	25	31	3.41	18.4	0	3.41				
reef-33	30	34	3.97	17.4	0.26	4.23			7.328E+08	1.155E+08
reef-31	35	38	2.70	35.7	0	2.70	3.26192	4.53522	6.188E+08	6.717E+07
reef-27	45	19	0.33	50.6	0	0.33	3.33848	4.58056	5.530E+08	1.036E+08

APPENDIX B

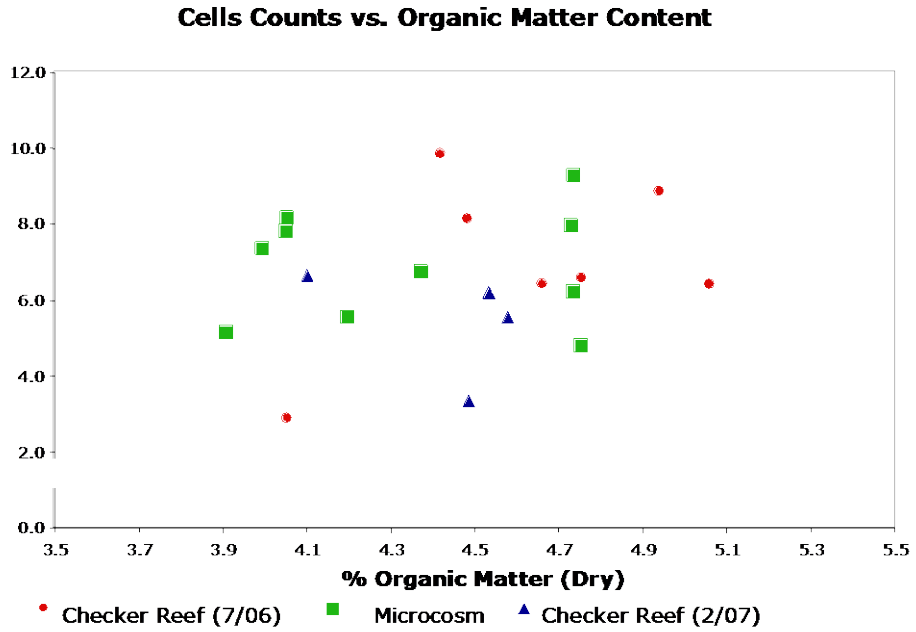


Figure 1. Scatter plot of cell counts vs. organic matter content. No significant correlation was determined.

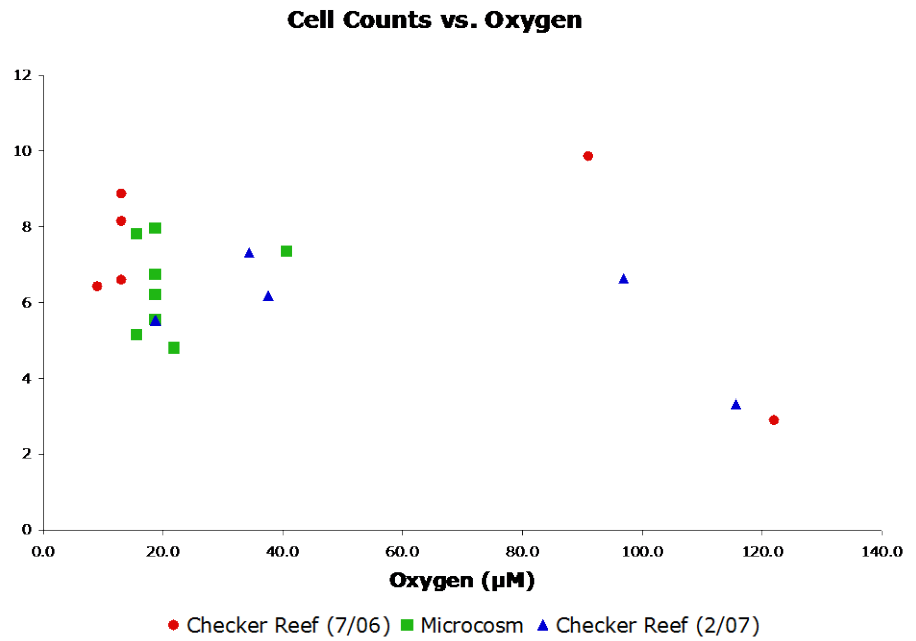


Figure 2. Scatter plot of cell counts vs. oxygen. No significant correlation was determined.

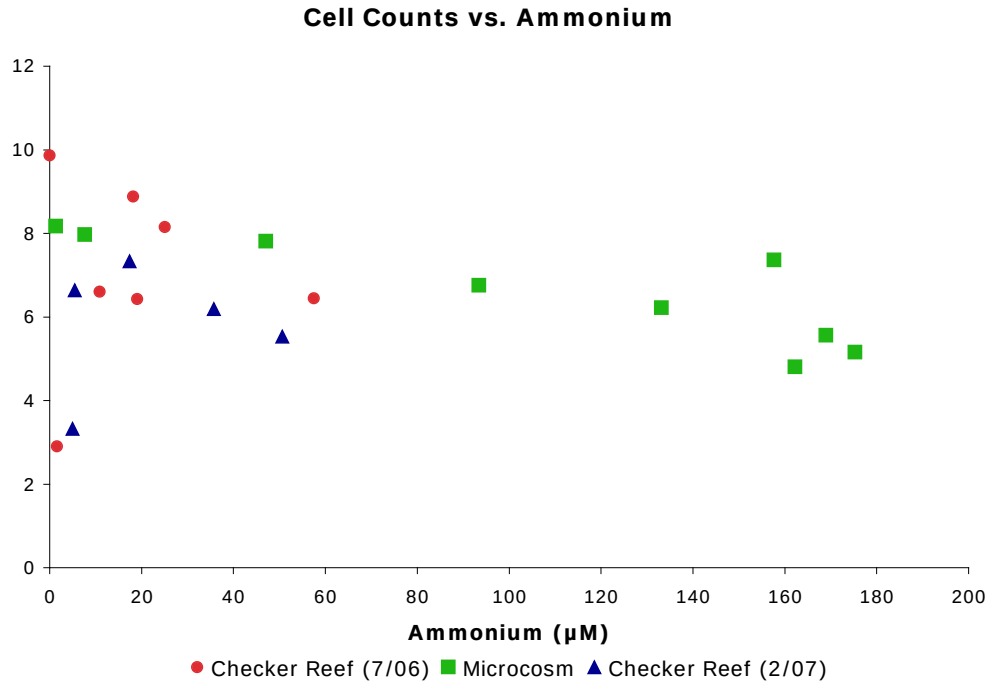


Figure 3. Scatter plot of cell counts vs. ammonium. No significant correlation was determined.

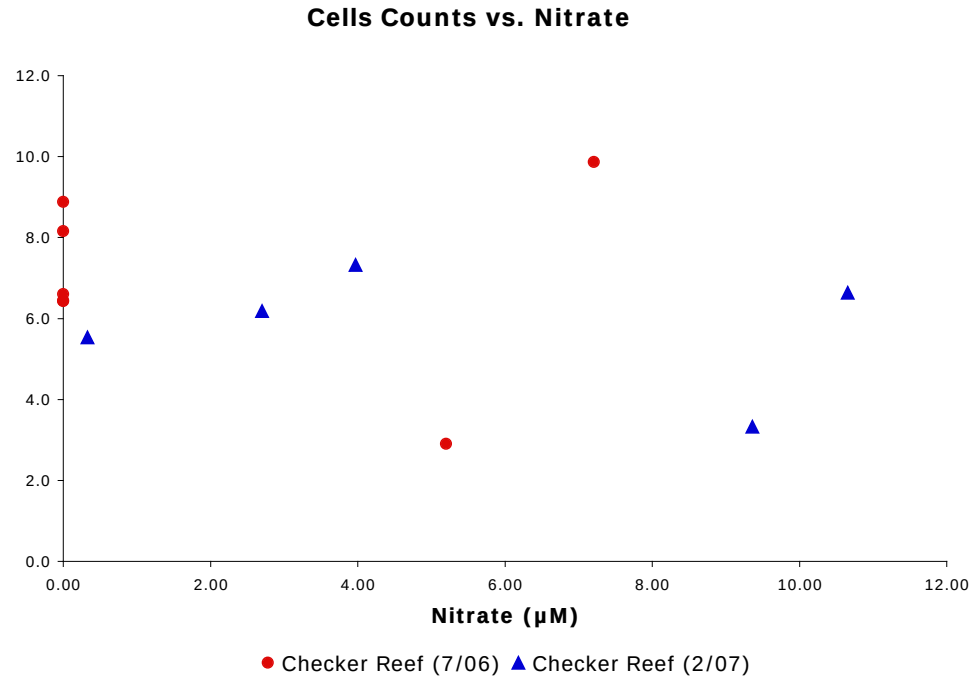


Figure 4. Scatter plot of cell counts vs. nitrate. No significant correlation was determined.

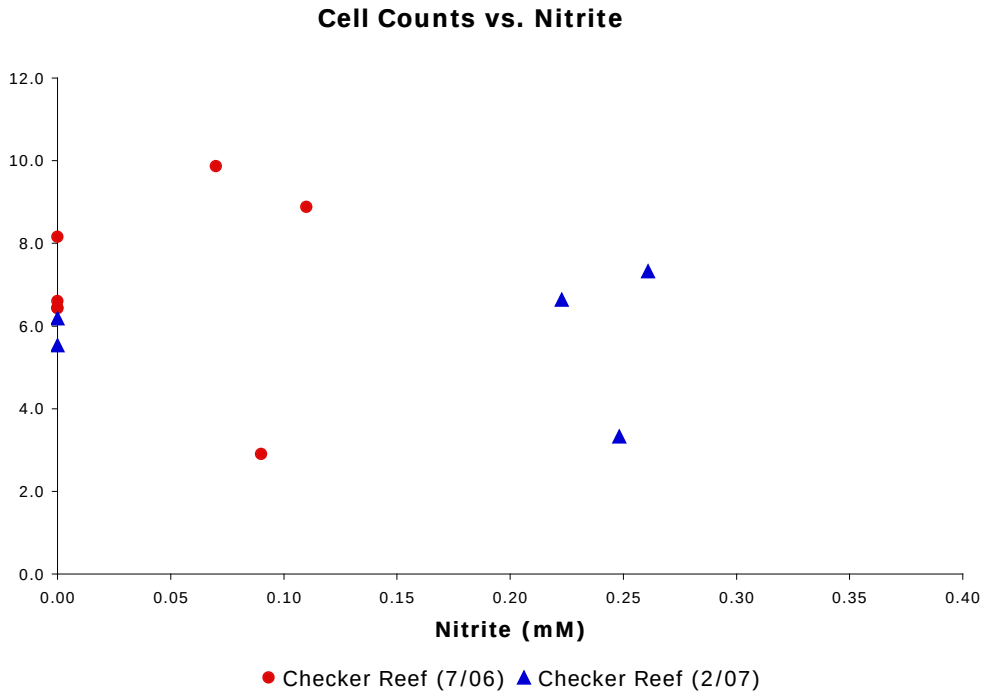


Figure 5. Scatter plot of cell counts vs. nitrite. No significant correlation was determined.

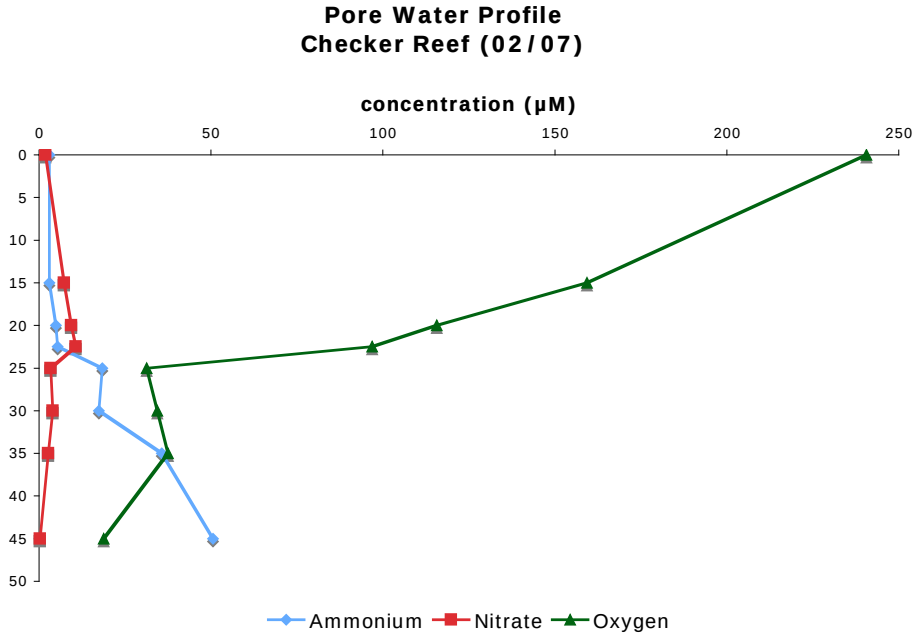


Figure 6. Porewater profiles for the Checker Reef February 2006 sediment array. Nitrite is omitted due to minute concentrations.

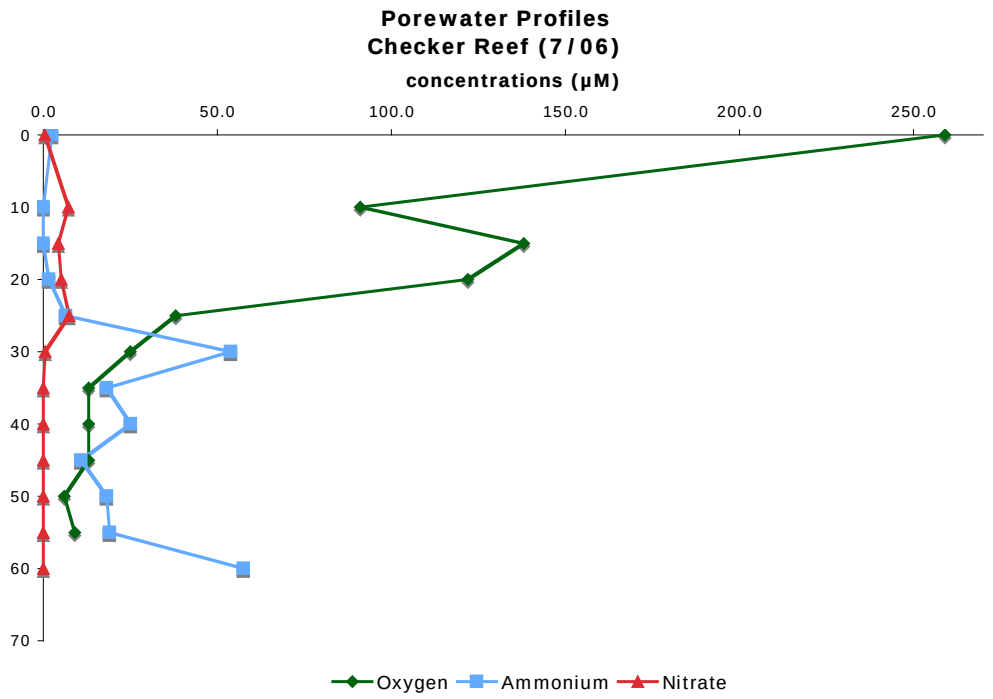


Figure 7. Porewater profiles for the Checker Reef July 2007 sediment array. Nitrite is omitted due to minute concentrations.

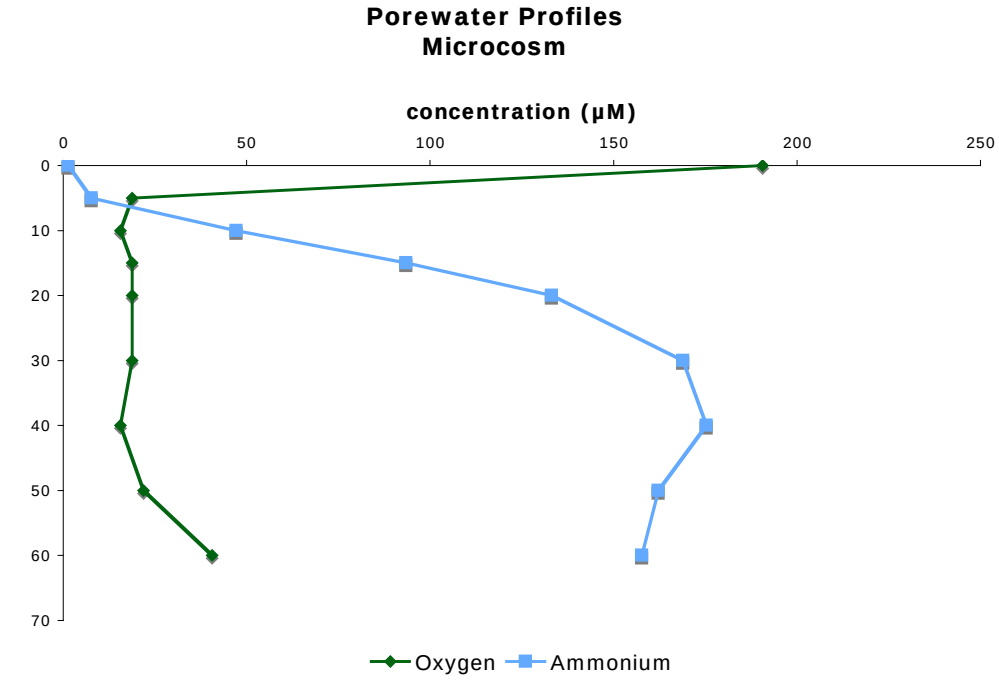


Figure 8. Porewater profiles of the microcosm sediment array. Nitrate and nitrite concentrations were not measured due to depletion within a depth of 5 cm.

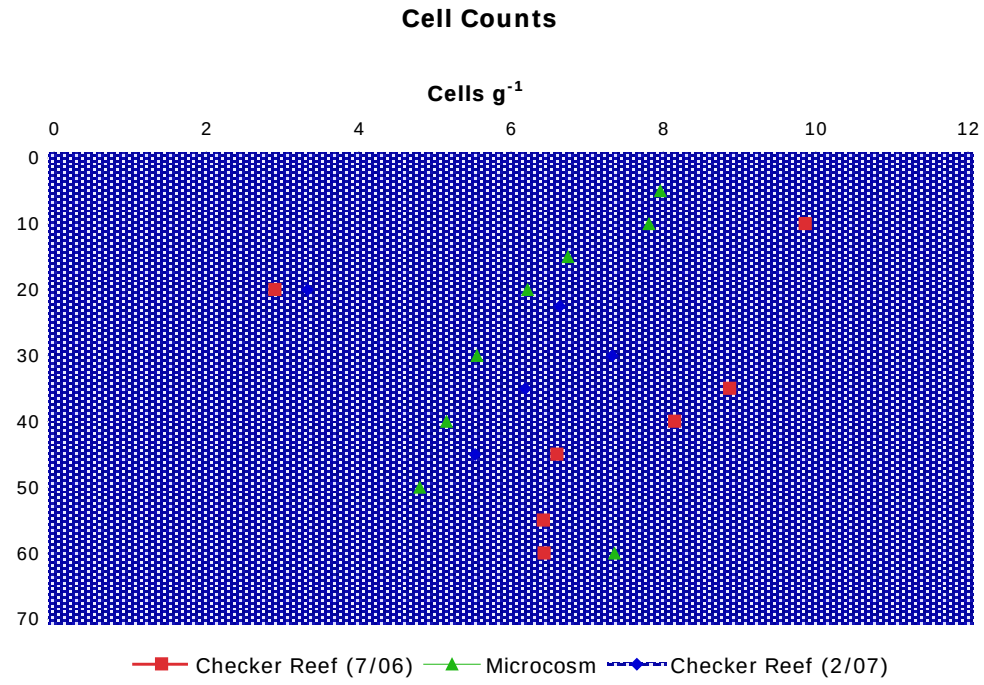


Figure 9. Cell count profiles for the three sediment arrays.

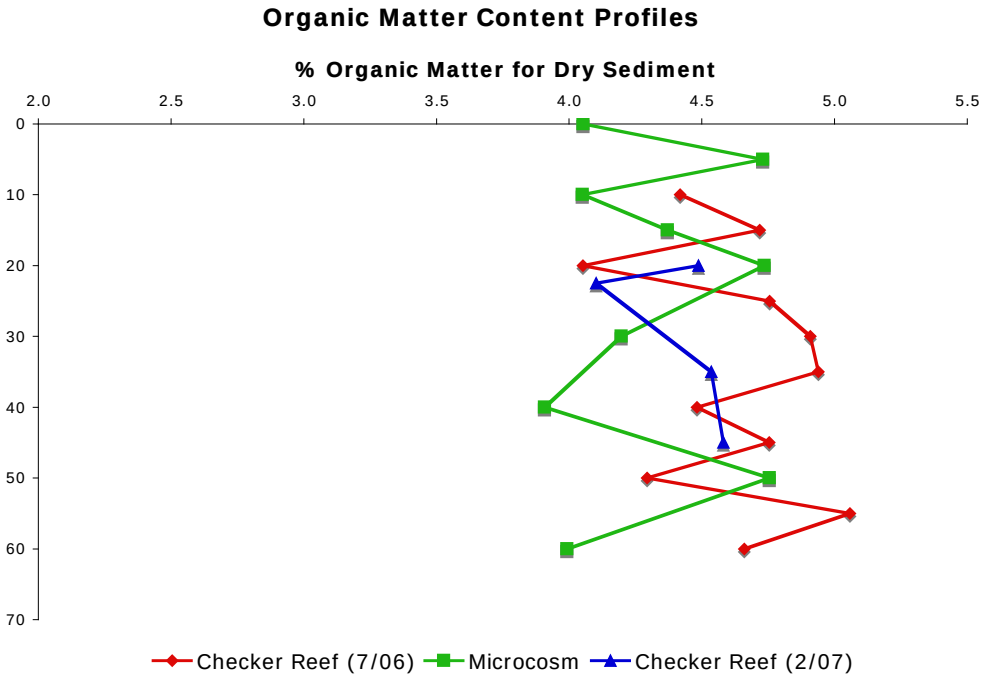


Figure 10. Organic matter content profiles for the three sediment arrays.

REFERENCES

Bendschneider, K. and Robinson, R.J. A new spectrophotometric method for the determination of nitrite in sea water. *Journal of Marine Research*. 11: 87-89 (1952).

Capone, D.G. Marine nitrogen fixation: what's the fuss? *Current Opinion in Microbiology*. 4:341-348 (2001)

Capone, D.G., Dunham, S.E., Horrigan. S.G., and Duguay, L.E. Microbial nitrogen transformations in unconsolidated coral reef sediments. *Marine Ecology Progress Series. 80*: 75-88 (1992).

Corredor, J.E., and Capone, D.G. Studies on nitrogen diagenesis in coral reef sands. *Proceedings of the Fifth International coral Reef Congress; Tahiti, 26 May-1 June 1985*. *3*: 395-399 (1985).

Ducklow, H.W. The biomass, production and fate of bacteria in coral reefs. *Coral reefs*, Z. Dubinsky, Editor. 1990, Elsevier: New York. p. 265-290.

Entsch, B., Boto, K.G., Sim, R.G., and Wellington, J.T. Phosphorus and nitrogen in coral reef sediments. *Limnology and Oceanography. 28(3*): 465-476 (1983).

Epstein, S.S., and Rossel, J. Enumeration of sandy sediment bacteria: search for optimal protocol. *Marine Ecology Progress Series.* 117: 289-298 (1995)

Falter, J.L., and Sansone, F.J. Hydraulic control of pore water geochemistry within the oxic-suboxic zone of a permeable sediment. *Limnology and Oceanography. 45*(3): 550-557 (2000).

Falter, J.L., and Sansone, F.J. Shallow pore water sampling in reef sediments. *Coral Reefs.* 19: 93-97 (2000)

Hubbell, D.H. and Kidder, G. Biological Nitrogen Fixation. The Institute of Food and Agricultural Sciences. (2003).

Kristensen, E. Organic matter diagenesis at the oxic/anoxic interface in coastal marine sediments, with emphasis on the role of burrowing animals. Hydrobiologia. 426: 1-24 (2000).

Llobet-Brossa, E., Rossello-Mora, R., and Amann, R. Microbial community of Wadden Sea sediments as revealed by fluorescence in situ hybridization. *Applied and Environmental Microbiology*. 64(7): 2691-2696 (1998).

Morris, A.W. and Riley, J.P. The determination of nitrate in sea water. *Analytica Chimica Acta*. 29: 272-279 (1963).

Musat, N., Werner, U., Knittel, K., Kolb, S., Dodenhof, T., van Beusekom, J.E.E., de Beer, D., Dubilier, N., and Amann, R. Microbial community structure of sandy intertidal sediments in the North Sea, Sylt-Romo Basin, Wadden Sea. *Systematic and Applied Microbiology*. 29: 333-348 (2006).

Precht, E., and Huettel, M. Rapid wave-driven advective pore water exchange in permeable coastal sediment. *Journal of Sea Research*. 51: 93-107 (2004).

Ravenschlag, K., K. Sahm, and R. Amann. Quantitative molecular analysis of the microbial community in marine arctic sediments (Svalbard). *Applied Environmental Microbiology*. 67: 387-395 (2001).

Rusch, A., Huettel, M., Wild, C., and Reimers, C.E. Benthic oxygen consumption and organic matter turnover in organic-poor, permeable shelf sands. *Aquatic Geochemistry*. 12:1-19 (2006).

Solorzano, L. Determination of ammonia in natural waters by the phenolhypochlorite method. *Limnology and Oceanography*. 14: 799-801 (1969).

Sorensen, K.B., Glazer, B., Hannides, A., and Gaidos, E. Spatial structure of the microbial community in sandy carbonate sediment. To appear in *Marine Ecology Progress Series*. (2007).

Tahey, T.M., Duineveld, G.C.A., Berghuis, E.M., and Helder, W. Relation between sediment-water fluxes of oxygen and silicate and faunal abundance at continental shelf, slop and deep-water stations in the northwest Mediterranean. *Marine Ecology Progress Series*. 104: 119-130 (1994).

Thy, S. Management and utilization of biodigesters in integrated farming systems. *MSc Thesis, MEKARN-SLU.* (2003)

Tribble, G.W., Sansone, F.J., Buddemeier, R.W., and Li, Y. Hydraulic exchange between a coral reef and surface sea water. *Geological Society of America Bulletin*. 104: 1280-1291 (1992).

Wild, C., Laforsch, C., and Huettel, M. Detection and enumeration of microbial cells within highly porous calcareous reef sands. *Marine and Freshwater Research*. 57: 415-420 (2006).

Ziebis, W., Huettel, M., and Forster, S. Impact of biogenic sediment topography on oxygen fluxes in permeable seabeds. *Marine Ecology Progress Series*. 140: 227-237 (1996).

Zumft, W.G., Cell biology and molecular basis of denitrification. *Microbiology and Molecular Biology Reviews*. 61(4): 533-616 (1997).