

**The Effects of Seawater Intrusion on Microbial Nitrate and Sulfate Reduction  
within a NITREX<sup>TM</sup> Permeable Reactive Barrier Designed to Mitigate  
Groundwater N-Pollution**

**Angela Vincent  
Grinnell College, Grinnell IA  
December 11, 2006**

**Advisor: Joe Vallino  
Collaborators: Whitney Eng and Kaitlyn Lucey**

## Abstract

Over the last 50 years, nitrogen loading to Waquoit Bay has been steadily increasing from anthropogenic inputs including wastewater, fertilizer, and atmospheric deposition. In an attempt to reduce estuarine nitrogen loading, two test NITREX™ Permeable Reactive Barriers (PRBs) have been installed along the shores of Childs River and Waquoit Bay. In this study, I focused on sampling the Waquoit Bay PRB. Composed of a highly permeable reactive matrix, the NITREX™ PRB is designed to intercept and denitrify incoming nitrate-rich groundwater prior to its seeping into the estuary. Since the NITREX™ PRB is the first of its kind to be implemented in an estuarine setting, the extent and influence of saltwater intrusion on the PRB has not been well documented. In this study, calculation of organic matter mineralization from sulfate reduction indicates that sulfate reduction has a dominant role in carbon oxidation within the PRB. Consequently, sulfate reduction may reduce the longevity of the PRB carbon media by accelerating decomposition. As evident from movement of the nitrate bloom underneath the PRB, nitrate was not efficiently intercepted, particularly under spring tide conditions.

## Introduction

Estuarine and coastal eutrophication caused by nitrogen loading is a growing national concern (Vallino 2006). The increasing density of human populations in coastal zones is a primary contributor to increased nitrogen loads (Valiela et al 1992). Primary sources of anthropogenic nitrogen loading include wastewater inputs, agricultural and residential fertilizers, and atmospheric deposition (Bowen and Valiela 2001).

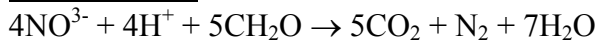
Between 1938 and 1990, nitrogen loading into Waquoit Bay more than doubled (Bowen and Valiela 2001). In 2004 (Serveiss et al.), the workgroup for the Waquoit Bay Watershed ecological risk assessment identified nutrient enrichment as the major anthropogenic stressor in the Waquoit Bay watershed. Nitrogen-induced eutrophication in Waquoit Bay has led to replacement of eelgrass beds with macroalgae and caused a significant decline in scallop harvest (Serveiss et al. 2004).

In a new approach to mitigating estuarine nitrogen loading, Principle Investigator Joseph Vallino with the Ecosystems Center is leading a study on the *Effectiveness of Reactive Barriers for Reducing N-Loading to the Coastal Zone*. Beginning in September 2004, funding from the Cooperative Institute for Estuarine and Coastal Environmental Technology (CICEET) was received to design and build NITREX™ Permeable Reactive Barriers (PRBs) along the shores of Waquoit Bay and Childs River (Vallino 2006) (Figure 1). As the first attempt to implement this technology in an estuarine context, the NITREX™ PRB may have broad application in restoring water quality, if it is shown to be a viable means of mitigating estuarine nitrogen loading (2006).

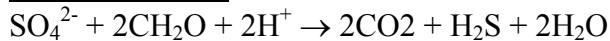
Composed of a highly permeable wood chip matrix, the NITREX™ PRB is designed to intercept nitrate-rich groundwater and supply a slowly decomposable carbon source that promotes nitrate removal via denitrification (Robertson et al. 2005). One concern is that seawater intrusion of the PRB may alter the flow path of groundwater nitrate. Depending on the extent of seawater intrusion in the PRB, the flow path of groundwater may be redirected underneath rather than up through the PRB. Another concern of seawater intrusion is that it stimulates sulfate reduction (Vallino 2006). Sulfate reduction is a concern for two reasons: 1) in the presence of sulfide, nitrate may

undergo dissimilatory nitrate reduction to ammonia (DNRA) rather than denitrification (Brunet et Garcia-Gil 1996, Vallino 2006) and 2) significant carbon oxidation in the PRB via sulfate reduction may reduce the longevity of the woodchip media (Robertson et al. 2005). Furthermore, since DNRA is also a carbon oxidizing process, it may also reduce the longevity of the woodchip media, if it is a dominant process in the PRB. Below are reactions for the abovementioned carbon oxidation processes:

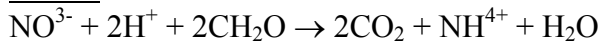
#### Denitrification



#### Sulfate reduction



#### DNRA



In this study, I sought to assess tidal intrusion in the Waquoit Bay NITREX™ PRB and the influence of this on biogeochemical processing. Processes of sulfate reduction, dissimilatory nitrate reduction to ammonia (DNRA), and nitrate removal were assessed in and around the PRB. Since it is thought that sulfate-rich seawater inundation of the PRB may be stimulating the growth of sulfate reducing bacteria (SRB), I assessed the distribution of SRB in relation to their functioning. Finally, the flow path of groundwater nitrate was examined to determine the extent to which the PRB is intercepting groundwater nitrate.

### **Methods**

#### *Site description*

A NITREX™ Permeable Reactive Barrier (PRB) is located at the head of Waquoit Bay, Falmouth MA (Figure 1). Installed below the water table, the PRB is approximately 3 m wide, 2 m deep, and 30 m long filled with a limestone buffered woodchip media (Vallino 2006).

#### *Sample collection*

Groundwater was pumped from multi-depth sampling wells up gradient, within, down gradient, and below the PRB using a peristaltic pump (Figure 2). At each sampling depth, a 1 L Nalgene™ polycarbonate bottle was used to collect water for nucleic acid extraction. While filling the 1 L bottle, Quanta measurements of temperature, conductivity, dissolved oxygen, and pH were obtained. Samples for sulfate/chloride, ammonium, and nitrate were collected in a 60 ml syringe and filtered using Swinex filter heads packed with Whatman™ 0.25 mm GFF filters. Sulfate/chloride samples were filtered into non acid-washed glass scintillation vials, while nitrate and ammonium samples were filtered into acid-washed plastic scintillation vials. At each sampling well, 250 µl of water for sulfide analysis was pipetted from a 60 ml BOD bottle into a non-acid washed vial pre-filled with 6 ml of 2% zinc acetate. The 60 ml BOD bottle was flushed with water from the sampling well before pipetting out 250 µl and filling an additional 60 ml syringe for DIC analysis. An open water sample was obtained about 50 m down shore

from the PRB, in an area uninfluenced by down gradient conditions. All samples were stored on ice.

The majority of wells were sampled 11/16 with additional down gradient samples collected 11/21. Wells underlying the PRB (not sampled 11/16 or 11/21) were collected 12/5 specifically for nitrate analysis. All well sampling was done at mid to low tide.

#### *Nutrient analysis*

Upon returning from the field, nitrate samples were frozen and sulfide samples refrigerated. Sulfate/chloride samples were promptly bubbled with N<sub>2</sub> gas for sulfide removal and then refrigerated as well. Samples were bubbled for approximately 20 minutes until no sulfides were smelt. Ammonium samples were acidified with 15 to 20 µl of 5 N hydrochloric acid at approximately 1 µl per ml of sample. Acidified ammonium samples were refrigerated with sulfate and sulfide samples until later analysis.

Sulfate/chloride samples were loaded on the AS40 Automated Sampler in 0.5 ml Dionex™ vials and analyzed on the Dionex™ DX-120 Ion Chromatograph (Staff, 2006). Sample dilutions were scaled within the range of a 400x dilution for 32 PSU seawater and no dilution for groundwater. Salinity values were based on the chloride concentration in each sample.

Prior to analysis, sulfide samples were diluted to 2/3 and 1/3 the concentration of the stock solution. Following a method for sulfide analysis adapted from N. Gilboa-Garber (1971), 5 ml of 3.728 g n,n-dimethyl-p-phenylene diamine mono hydrochloride (6 g FeCl<sub>3</sub> x 6 H<sub>2</sub>O) solution was added to each sample. After addition of the dye solution, samples were shaken and left to develop in the dark for about an hour. Samples were read on the UV-Visible 1601 Shimadzu spectrophotometer at 670 nm.

Ammonium samples were processed on the Shimadzu 1601 spectrophotometer based on a method modified from Solarzano (1969). A set of artificial seawater and deionized water standards were made using a 10,000 µM ammonium chloride stock solution at concentrations of 0, 0.5, 1, 5, 10, 50 and 100 µM. Standards and samples were pipetted into test tubes pre-reacted with reagents. These reagents included a phenol solution, sodium nitroprusside solution, and oxidizing solution. Prepared fresh daily, the oxidizing solution is a mix of 100 ml alkaline reagent with 25 ml of sodium hypochlorite solution. Diluted standards and samples were prepared with 0.12 ml of phenol, 0.12 ml of sodium nitroprusside solution, and 0.3 ml of oxidizing reagent. After reagent addition, standards and samples were read on the Shimadzu 1601 at 640 nm.

A couple hours before analysis, nitrate samples were removed from the freezer and allowed to thaw. Samples were diluted according to a 20 µM target concentration with estimates based on former sampling of the PRB. Nitrate samples were processed on the Lachat Flow Injection Analyzer (FIA) using a method adapted from Wood et al (1967).

#### *Dissolved Inorganic Carbon (DIC)*

Samples were analyzed for DIC content with the Shimadzu Gas Chromatograph GC-14A (refer to Lucey, 2006 unpublished manuscript).

#### *Determining sulfate consumption and DIC from respiration*

Expected sulfate and expected DIC were calculated using a standard mixing equation employing chloride as a conservative tracer.

$$\text{(Eq 1) Expected sulfate} = (\text{Cl} - \text{Cl}_{\text{GW}}) * (\text{Cl}_{\text{SW}} - \text{Cl}_{\text{GW}})^{-1} * (\text{SO}_4^{2-}{}_{\text{SW}} - \text{SO}_4^{2-}{}_{\text{GW}}) + \text{SO}_4^{2-}{}_{\text{GW}}$$

Cl = measured chloride  
Cl<sub>GW</sub> = chloride in groundwater  
Cl<sub>SW</sub> = chloride in Waquoit Bay seawater  
SO<sub>4</sub><sup>2-</sup><sub>GW</sub> = sulfate in groundwater  
SO<sub>4</sub><sup>2-</sup><sub>SW</sub> = sulfate in Waquoit Bay seawater

Expected DIC was the same equation, except groundwater and seawater sulfate were replaced with groundwater and seawater DIC. Sulfate consumed and DIC from respiration were obtained from the difference between the expected and measured values.

#### *Determining respired DIC attributed to sulfate reduction*

The percent respired DIC attributed to sulfate reduction was computed as follows:

$$\text{(Eq 2) DIC from SO}_4^{2-} \text{ Reduction (\%)} = (2 * [\text{H}_2\text{S}]) / (\text{DIC from respiration}) * 100$$

#### *Determining abundance of bacteria containing dsrAB and universal 16S rRNA genes*

The following Sterivex Filtration System (SFS) and Puregene kit nucleic acid extraction methods were recommended by Secretariat of International Census of Marine Microbes (ICoMM), Linda Amaral-Zettler.

##### *Water sample filtration through 0.22 micron Sterivex Filters*

Attach tube to input connector of a 10L carboy and attach output to a vacuum pump. Attach male end of the Sterivex filter to the top of the female adapter and the sterile syringe to the top of the Sterivex (see Figure 3). Secure connections, invert water sample over the syringe, and turn on pump. Open t-valve, run the manifold until Sterivex filter is dry, and then close t-valve removing Sterivex filter. Cap the male end of the Sterivex filter and add 2 ml of Puregene Lysis Buffer. Cap the female end of the Sterivex filter and seal both ends of the filter with parafilm. Place into sterile Whirlpack bag and store at -20 °C.

##### *Nucleic Acid extraction with Puregene Kit*

Thaw samples for 20 min and add 10 µl Lytic Enzyme. Incubate at 37 °C for 30 min on rotating carousel. Add 10 µl refrigerated Proteinase k and vortex for 10 sec. Attach a 3 cc syringe to female end of Sterivex filter and plunge liquid into sterile 2 ml labeled microfuge tube. Subdivide this volume into 3 tubes at 700 µl each. Cool for 20 min. Add 233 µl of Protein Precipitation Solution to each tube. Vortex 20 sec to mix ppt with cell lysate. Incubate on ice for 15 min. Centrifuge for 5 min. Pipette supernatant into tube with 1 volume 100 percent IPA. Invert 50 times. Centrifuge for 5 min. Remove supernatant and drain on paper towel. Add 700 µl of 70 percent ethanol inverting several times. Centrifuge for 5 min and remove supernatant. Invert and drain

tube on paper towel allowing it to air dry. Resuspend each pellet in 10  $\mu\text{l}$  of hydration buffer for about 30  $\mu\text{l}$  concentrated DNA per filter sample.

#### *Quantification of 16S rRNA and dsrAB genes by real-time PCR*

Total bacteria were quantified using a SyBrGreen assay targeting 16S rRNA genes (338F and 907R) (Table 1) with a primer set described by Risgaard-Petersen et al. (2004). Sulfate-reducing bacteria were quantified by a SyBrGreen assay targeting the dsrAB genes using a primer set (DSR1-F+ and DSR-R) (Table 1) described by Leloup et al. (2006).

The reaction mix ( $V_f = 25 \mu\text{l}$ ) was composed of: 12.5  $\mu\text{l}$  of MasterMix (SyBrGreen 2X, Eurogentec, Seraing, Belgium), 10.3  $\mu\text{l}$  of water, 0.1  $\mu\text{l}$  of each primer, and 2  $\mu\text{l}$  of template DNA. Quantitative PCR was carried out by the Opticon Monitor 2 (Bio-Rad Laboratories, Hercules CA) as follows: 10 min at 95  $^{\circ}\text{C}$ , 30 sec at 95  $^{\circ}\text{C}$ , 1 min at 55  $^{\circ}\text{C}$ , 30 sec at 72  $^{\circ}\text{C}$ , 39 more cycles of 30 sec at 95  $^{\circ}\text{C}$ , 1 min at 95  $^{\circ}\text{C}$ , 1 sec at 55  $^{\circ}\text{C}$ , melting curve from 55  $^{\circ}\text{C}$  to 95  $^{\circ}\text{C}$  read every 0.5  $^{\circ}\text{C}$ , hold 30 sec, 10 min at 21  $^{\circ}\text{C}$ . The starting template concentration of dsrAB and 16S rRNA genes in each sample was quantified using Real-time PCR Miner 2.0 software (Sheng, Z, Fernald, R.D. 2005). The process of quantification implements an algorithm that includes exponential phase determination, efficiency estimation, cycle threshold (CT) calculation, and comparison among samples.

## **Results**

### ***Salinity***

Salinity in the PRB ranged from approximately 2.0 to 9.5 PSU with the highest concentration at well 28 in the central portion of the PRB (Figure 4). Groundwater salinity up gradient of the PRB (averaged from sampling depths in well 26) was about 0 PSU, while down gradient measurements (wells 22, 23) ranged from about 2.5 to 7.5 PSU. Salinity of the open water in Waquoit Bay was calculated as 28.2 PSU.

### ***Sulfate***

Distribution of sulfate showed the same pattern as salinity. Sulfate concentrations in the PRB ranged from about 1.5 to 6.5 mM with the highest concentration at well 28 in the central portion of the PRB (Figure 5). Groundwater sulfate was about 0.1 mM, while down gradient measurements ranged from about 2.5 to 5.5 mM.

### ***Hydrogen sulfide***

Hydrogen sulfide concentrations peaked at 1500  $\mu\text{M}$  in the upper region of well 27 (Figure 6). The majority of the PRB had sulfide concentrations ranging from about 500 to 1300  $\mu\text{M}$ . Trace amounts of sulfide were detected in groundwater samples from well 26. Up gradient wells had sulfide concentrations ranging from about 100 to 500  $\mu\text{M}$ .

### ***Dissolved oxygen***

Conditions within the PRB were fairly anoxic (less than 1 mg/L DO), while groundwater in well 26 was fairly oxic ( $\sim 5$  mg/L) (Figure 7).

### *Ammonium*

Ammonium concentrations peaked at about 30  $\mu\text{M}$  in well 23 immediately down gradient from the base of the PRB (Figure 8). Overall, the ammonium concentration in the PRB was fairly low ( $\sim 0\text{-}12\ \mu\text{M}$ ).

### *Sulfate consumed*

The relationship between sulfate consumed (mM) and hydrogen sulfide produced is nearly a 1:1 relationship (Figure 9). This is consistent with the stoichiometry of sulfate reduction to sulfide.

### *Dissolved inorganic carbon (DIC)*

Peak total DIC was 2800  $\mu\text{M}$  at well 28 in the central portion of the PRB (Figure 10). The range within the PRB was from 1000 to 2800  $\mu\text{M}$ , while up gradient it was about 700  $\mu\text{M}$ , and down gradient it ranged from 700 to 1600  $\mu\text{M}$ .

### *DIC from respiration*

Peak DIC from respiration was 2000  $\mu\text{M}$  at well 28 in the central portion of the PRB (Figure 11). DIC from respiration was about 100  $\mu\text{M}$  up gradient, 400 to 2000  $\mu\text{M}$  within the PRB, and 100 to 600  $\mu\text{M}$  down gradient the PRB.

### *Respired DIC from sulfate reduction*

Since sulfate consumed and hydrogen sulfide produced showed approximately a 1:1 relationship, the concentration of sulfide was used to determine the percent respired DIC from sulfate reduction.

The percent respired DIC from sulfate reduction showed a similar pattern to DIC from respiration (Figure 12). The highest percentages were within the PRB peaking at the uppermost depth of well 27. The contour shows that in a large portion of the PRB, sulfate reduction accounts for all DIC from respiration.

### *Percent bacteria carrying the dsrAB gene*

The relative abundance of dsrAB divided by the relative abundance of 16S rRNA in the same sample was used to estimate the proportion of bacteria carrying the dsrAB gene. The dsrAB gene ranged from 0.002 to 3.7 percent amongst collected samples (Figure 13). The areas of the PRB highest in dsrAB gene abundance were located at well 29 in the mid to lower region of the PRB and well 23 at the shallowest sampling depth.

### *Nitrate*

Since tidal conditions influencing the PRB were significantly different on 11/16 and 12/5 sampling dates (Figure 14), two separate contours are shown. While samples collected 11/16 were during a neap tide, samples collected 12/5 were during a spring tide (K. Lucey 2006, unpublished). While the 11/16 contour shows a portion of the nitrate plume flowing underneath the PRB, there is insufficient sampling to accurately trace the plume. However, on 12/5, the plume clearly flows underneath the PRB.

## Discussion

Since incoming groundwater was significantly less saline (~0 PSU) than the salinity range observed in the Permeable Reactive Barrier (PRB) (~2.5-9.6 PSU), it appears tides periodically infiltrate the entire PRB. Based on lunar cycling and documentation of Woods Hole tides (Figure 15), a spring tide occurred on 11/5, eleven days prior to our sampling the PRB. Considering peak salinity in the PRB was about a third (~9.6 PSU) that in the Bay, it appears we may have been observing residual effects from this spring tide event in our 11/16 sampling under neap tide. Although additional down gradient sampling on 11/21 occurred during spring tide (i.e. new moon), the low and high tidal range of this spring tide (11/21) was narrower compared to that of the 11/5 spring tide (Figure 15).

Since seawater is sulfate-rich (in Waquoit Bay ~26 mM) and groundwater sulfate poor (~0 mM), I predicted similar profiles for salinity and sulfate in the PRB. Contours of salinity and sulfate supported this relationship (Figures 4 and 5) and indicated a strong tidal influence on the PRB.

Hospitable areas to sulfate reducing bacteria (SRB) are anoxic and contain a variety of organic compounds in addition to sulfate (Leloup et al. 2005, Barton 1995). Although studies show that SRB can also survive under both oxic and sulfate depleted conditions (Ravenschlag et al. 2000, Leloup et al. 2005, Barton 1995), these are generally less favorable. The sulfate reduction zone in estuarine sediments is usually between 10 and 50 cm (Day et al. 1989). This is consistent with results in our study indicating highest hydrogen sulfide production in the uppermost sampling wells about a meter underground (Figure 6). According to dissolved oxygen measurements, these areas were also fairly anoxic (Figure 7).

Although Brunet and Garcia-Gil (1996) found that high levels of hydrogen sulfide stimulated dissimilatory nitrate reduction to ammonia (DNRA), no such relationship was apparent in this study (Figure 8). Higher concentrations of ammonium at lower depths in well 23 and upper depths in well 26 showed no correlation with the profile of measured hydrogen sulfide.

Although measurements of hydrogen sulfide may provide a crude estimate of sulfate reduction, a more accurate measure also considers sulfate consumed. Considering hydrogen sulfide in sediments often reacts with iron to precipitate FeS, a 1:1 molar ratio between sulfate consumed and sulfide produced is not always observed (Schlesinger 1997). However, in plotting sulfate consumed and measured hydrogen sulfide, we saw from the slope of the fitted line that the 1:1 molar relationship appeared to apply (Figure 9).

As stated by Leloup et al. (2004), sulfate reduction in marine sediment often accounts for over 50% of the mineralization of organic matter. In this study, mineralization of organic matter was calculated as dissolved inorganic carbon (DIC) from respiration. Since seawater is generally high in DIC, it was necessary to correct for outside inputs of DIC in estimating DIC from respiration. This was accomplished using a standard mixing equation and measured DIC values as described above (Eq 1). A rough comparison of DIC and DIC from respiration concentrations within the PRB indicate that over 60 percent of DIC may be attributed to respiration in most regions (Figures 10, 11). This shows that carbon oxidizing processes constitute a large fraction of total DIC in the PRB.

For the purpose of this study, I sought to determine the proportion of carbon oxidation attributed to sulfate reduction (Eq 2). Results suggest that sulfate reduction accounts for all DIC from respiration in over half the PRB (Figure 12). These results support the findings of Leloup et al. (2004) that sulfate reduction may account for over 50% of mineralized organic matter in marine sediments. Organic matter mineralization from sulfate reduction was particularly high in this case, probably as a result of carbon supplied from the woodchip media.

The partial decoupling of respired DIC from sulfate reduction (Figure 12) and abundance of *dsrAB* carrying bacteria (Figure 13) was not initially hypothesized. A probable explanation for this decoupling relates to the dynamic nature of the PRB under neap versus spring tide conditions (K. Lucey, 2006 unpublished). Lucey (2006) found that significant portions of the PRB, previously anoxic under neap tide conditions became oxic under spring tide conditions (12/5). Even daily fluctuations in tidal infiltration of the PRB could influence SRB distribution; for example, wells highest in percent *dsrAB* also receive a more constant supply of tidal seawater sulfate over time. Since some species of SRB only partially oxidize carbon in reducing sulfate (e.g. end product is acetate rather than CO<sub>2</sub>) (Barton 1995), this creates another source of variability in measurements. Ultimately, since the system is not stable, too many variables exist to attempt an explanation for why distributions of *dsrAB* carrying bacteria and DIC from sulfate reduction do not directly coincide. The PRB must be observed from a more temporal perspective to begin assessing relations between SRB abundance and function.

Contours depicting flow paths of groundwater nitrate indicate the PRB is not intercepting large portions of the plume (Figure 14). Samples underneath the PRB that were missed on the 11/16 sampling under neap tide were obtained 12/5 under spring tide. Particularly under spring tide conditions, it appears that dense seawater infiltration is forcing the groundwater nitrate plume underneath the PRB. Less seawater intrusion of the PRB under neap tide conditions may enable the PRB to intercept more groundwater nitrate. Full assessment of groundwater flow paths under each set of tidal conditions (neap and spring) is necessary to assess whether a difference exists.

## **Conclusions**

Salinity measures indicate that the PRB receives significant tidal inundation. Furthermore, it is this sulfate-rich tidal inundation that stimulates growth of sulfate reducing bacteria (SRB). Since sulfate reduction appears to account for all respired DIC in over half the PRB, results indicate that sulfate reduction is a dominant carbon oxidizing process in the PRB. Unlike sulfate reduction, dissimilatory nitrate reduction to ammonia (DNRA) does not appear to be occurring in the PRB. Finally, extensive saltwater intrusion of the PRB (i.e. particularly under spring tide events) appears to be forcing the groundwater nitrate plume underneath the PRB.

In order for the NITREX™ PRB to be considered a cost-effective approach to mitigating estuarine nitrogen loading, the woodchip media must provide long-term nitrate removal without the need for frequent carbon replenishment. In a study by Robertson et al (2000), PRBs processing significantly higher daily concentrations of nitrate than the Waquoit Bay PRB were expected to last at least a decade before needing carbon replenishment. Since the Waquoit PRB processes less nitrate, it appears its carbon

reserves should last longer. However, carbon oxidation by sulfate reducers may affect this outcome in accelerating decomposition.

From this study, tidal inundation appears to stimulate sulfate reduction and inhibit nitrate removal in the Waquoit PRB. These effects support the need for structural modifications to the PRB that limit seawater infiltration. One such modification might include a water-resistant membrane lining the exterior of the PRB. Alternatively, repositioning of the PRB deeper and more up gradient may also be a possible solution. In either case, more frequent monitoring of the PRB is a necessary step to better understand biogeochemical processing in the system.

### **Acknowledgements**

I would like to extend a special thanks to Joe Vallino for advising me throughout the project. I would also like to thank Ken Foreman for inciting my initial interest in this topic and providing additional guidance along the way. To all of those at the Bay Paul Center (Linda Amaral Zettler, Susie Theroux, and Erika Del Castillo) and Marine Research Center (Steven Roberts and Christina Romano) who helped me with either DNA extraction or Real Time Quantitative PCR, I am very grateful for your assistance and enthusiasm. Also, I would like to thank Kaitlyn Lucey and Whitney Eng for their collaboration and field assistance during the project. For all the TAs that assisted me in this project (especially Rich McHorney, Clara Funk, Beth Bernhardt, and Jen Culbertson), I am very appreciated of all your help with lab techniques.

### **Works Cited**

- Barten, L. (Ed). 1995. Sulfate Reducing Bacteria. Plenum Press, New York, USA.
- Bowen, J., I. Valiela. 2001. The ecological effects of urbanization on coastal watersheds: historical increases in nitrogen loads and eutrophication of Waquoit Bay estuaries. *Canadian Journal of Fisheries and Aquatic Science* **58**:1489-1500.
- Brunet, R., and L. Garcia-Gil. 1996. Sulfide-induced dissimilatory nitrate reduction to ammonia in anaerobic freshwater sediments. *FEMS Microbiology Ecology* **21**:131-138.
- Day, J., C. Hall, W. Kemp, A. Yanez-Arancibia. 1989. Estuarine ecology. A Wiley-Interscience publication, New York, USA.
- Gilboa-Garber, N. 1971. Direct spectrophotometric determination of inorganic sulfide in biological materials and in other complex mixtures. *Analytical Biochemistry* **43**:129-133.
- Leloup, J., L. Quillet, C. Oger, D. Boust, F. Petit. 2004. Molecular quantification of sulfate-reducing microorganisms (carrying *dsrAB* genes) by competitive PCR in estuarine sediments. *FEMS Microbiology Ecology* **47**: 207-214.
- Leloup, J., F. Petit, D. Boust, J. Deloffre, G. Bally, O. Clarisse, and L. Quillet. 2005. Dynamics of sulfate-reducing microorganisms (*dsrAB* genes) in two contrasting mudflats of the Seine Estuary (France). *Microbial Ecology* **50**: 307-314.
- Leloup, J. 2006. Diversity and abundance of sulfate-reducing microorganisms in the sulfate and methane zones of marine sediment, Black Sea. *Environmental Microbiology*: 1-12.
- Lucey, K. 2006. Permeable reactive barriers as long-term solutions for groundwater remediation: concentrations of methane and dissolved inorganic carbon as indicators

- of the rates of carbon oxidation processes across the NITREX™ permeable reactive barrier in Waquoit Bay, Cape Cod (unpublished, PowerPoint SES Symposium and manuscript).
- Ravenschlag, K., K. Sahm, C. Knoblauch, B. Jorgensen, R. Amann. 2000. Community structure, cellular rRNA content, and activity of sulfate-reducing bacteria in marine arctic sediments. *Applied and Environmental Microbiology* **66**:3592-3602.
- Risgaard-Petersen, N., M.H. Nicolaisen, N.P. Revsbech, and B.A. Lomstein. 2004. Competition between ammonia-oxidizing bacteria and benthic microalgae. *Applied and Environmental Microbiology* **70**:5528-5537.
- Robertson, W., D. Blowes, C. Ptacek, J. Cherry. Long-term performance of in-situ reactive barriers for nitrate remediation. *Groundwater* **38**:689-695.
- Robertson, W., G. Ford, P. Lombardo. 2004. Wood-based filter for nitrate removal in septic systems. *Soil & Water of ASAE* **48**:121-128.
- Schlesinger, W. 1997. *Biogeochemistry: an analysis of global change*, 2<sup>nd</sup> ed. Academic Press, Amsterdam.
- Serveiss, V., J. Bowen, D. Dow, I. Valiela. 2004. Using ecological risk assessment to identify the major anthropogenic stressor in the Waquoit Bay watershed, Cape Cod, Massachusetts. *Environmental Management* **33**:730-740.
- Sheng Zhao, Russell D. Fernald. 2005. Comprehensive algorithm for quantitative real-time polymerase chain reaction. *Journal of Computer Biology* **12**(8):1045-62.
- Solarzano, L. 1969. Determination of ammonia in natural waters by the phenohypochlorite method. *Limnology and Oceanography* **14**:799-800.
- Staff, SES. 2006. Dionex-120 Manual. Laboratory Manual for Semester in Environmental Science, Marine Biological Laboratory.
- Valiela, I., K. Foreman, M. LaMontagne, D. Hersh, J. Costa. 1992. Couplings of watersheds and coastal waters: sources and consequences of nutrient enrichment in Waquoit Bay, Massachusetts. *Estuaries* **15**:443-457.
- Vallino, J. 2006. MIT Sea Grant Preliminary Proposal (unpublished).
- Wood, E.D., F.A.G Armstrong, and F.A. Richards. 1967. Determination of nitrate in seawater by cadmium-copper reduction to nitrite. *Journal Marine Biological Association. U.K.* **47**:23.

## **Figures and Tables**

Figure 1. Map of Falmouth, MA showing sites of the NITREX™ PRBs.

Figure 2. Cross-section of PRB.

Figure 3. Sterivex filtration design.

Figure 4. Contour of salinity.

Figure 5. Contour of sulfate.

Figure 6. Contour of hydrogen sulfide.

Figure 7. Contour of dissolved oxygen.

Figure 8. Contour of ammonium.

Figure 9. Sulfate consumed versus measured hydrogen sulfide

Figure 10. Contour of dissolved inorganic carbon (DIC).

Figure 11. DIC from respiration

Figure 12. Contour of the percent respired DIC from sulfate reduction.

Figure 13. Contour of the percent bacteria carrying the dsrAB gene.

Figure 14. Contour of nitrate.

Figure 15. Woods Hole Tides (2006)

Table 1. Primer sequences



Figure 1. Map of Falmouth, MA indicating sites of the test NITREX™ Permeable Reactive Barriers along Childs River and Waquoit Bay.

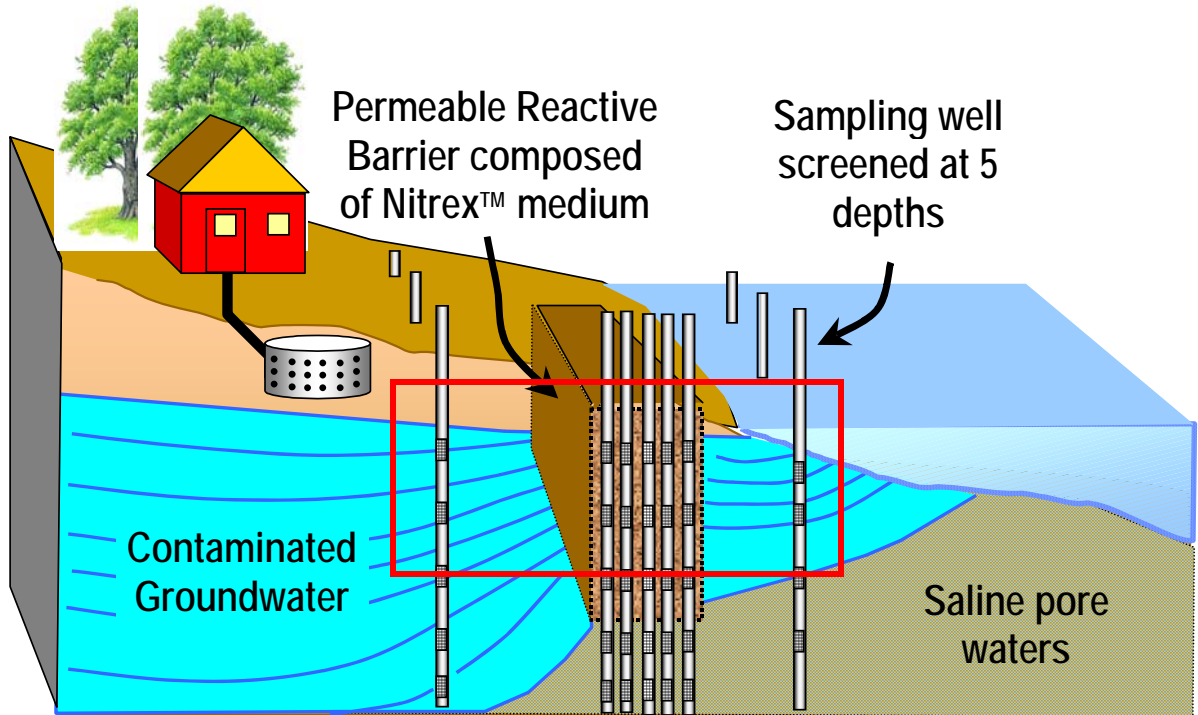


Figure 2. NITREX™ Permeable Reactive Barrier (PRB). Samplings wells are located within, up gradient (left), and down gradient (right) the PRB. Red box indicates cross-section of the PRB.

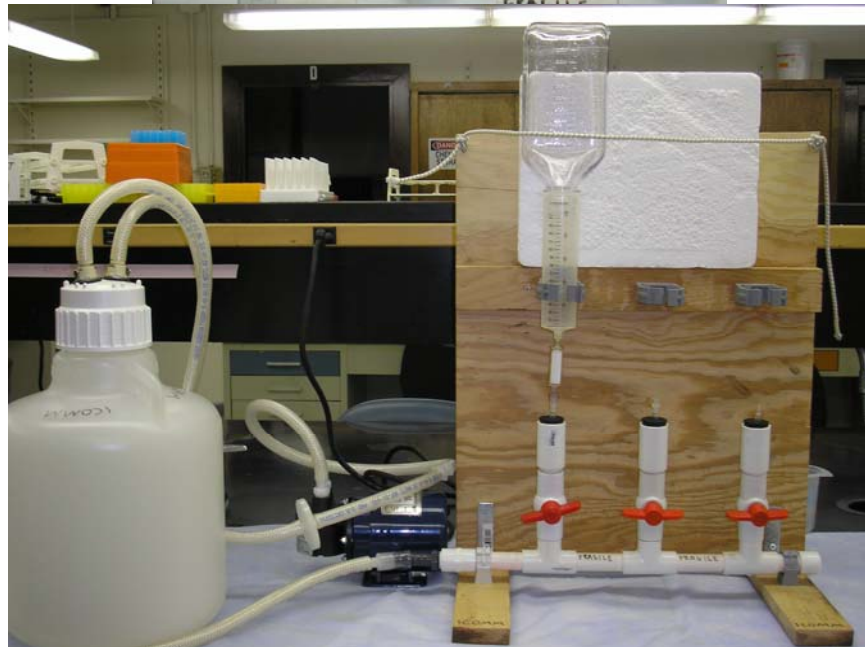
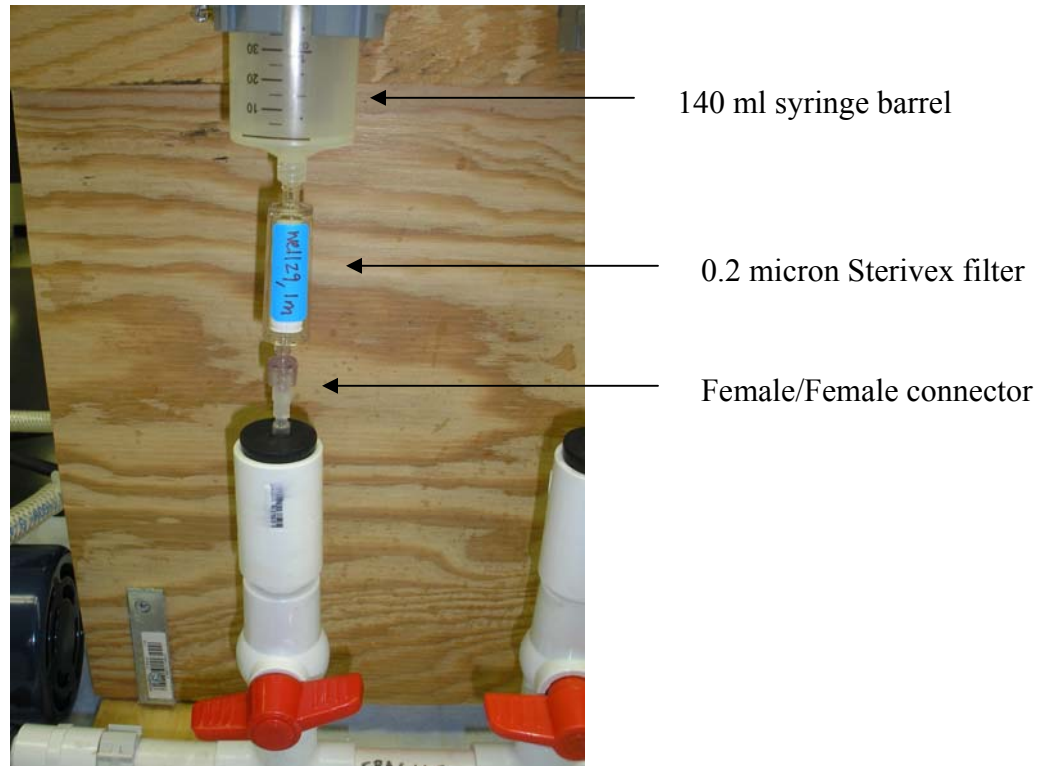


Figure 3. Sterivex filtration system with details for Sterivex filter connections.

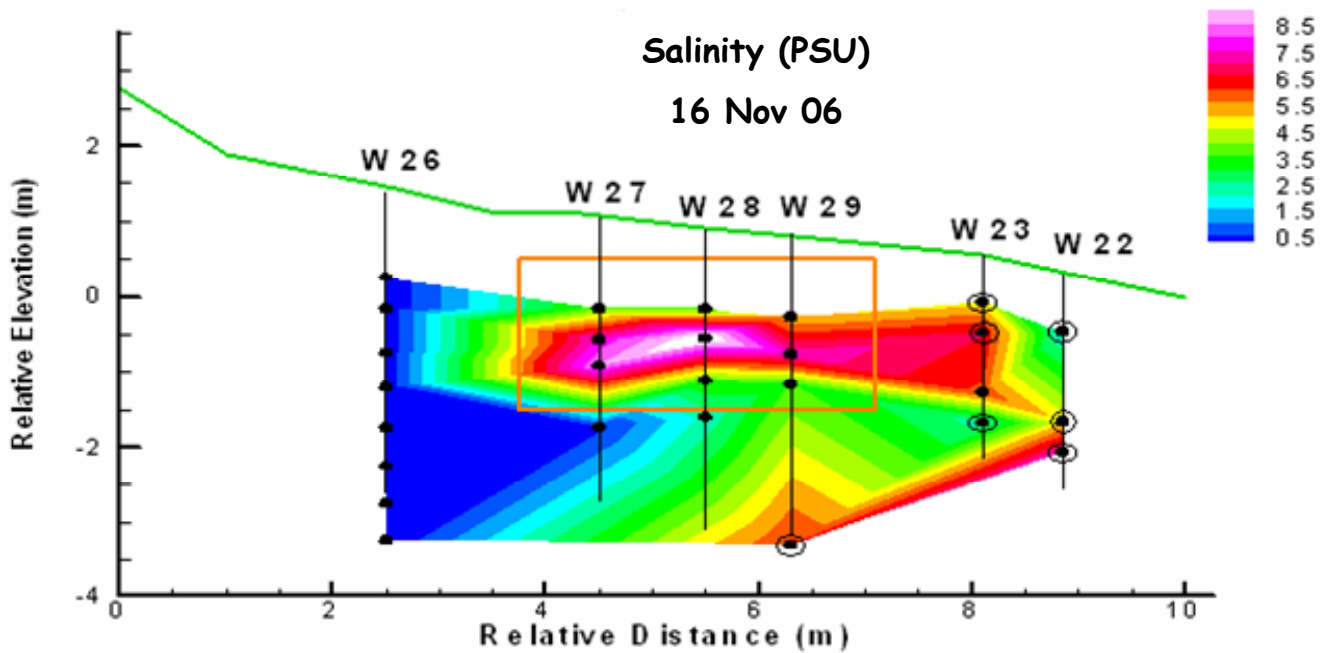


Figure 4. Salinity (PSU) in wells sampled in and around the NITREX™ PRB (shown as orange box) on 11/16/06. Well transect is perpendicular to PRB and coastline. Green line is the relative elevation of grade, upland is on the left side, and Waquoit Bay is on the right side of the image. Circled wells were sampled 11/21/06.

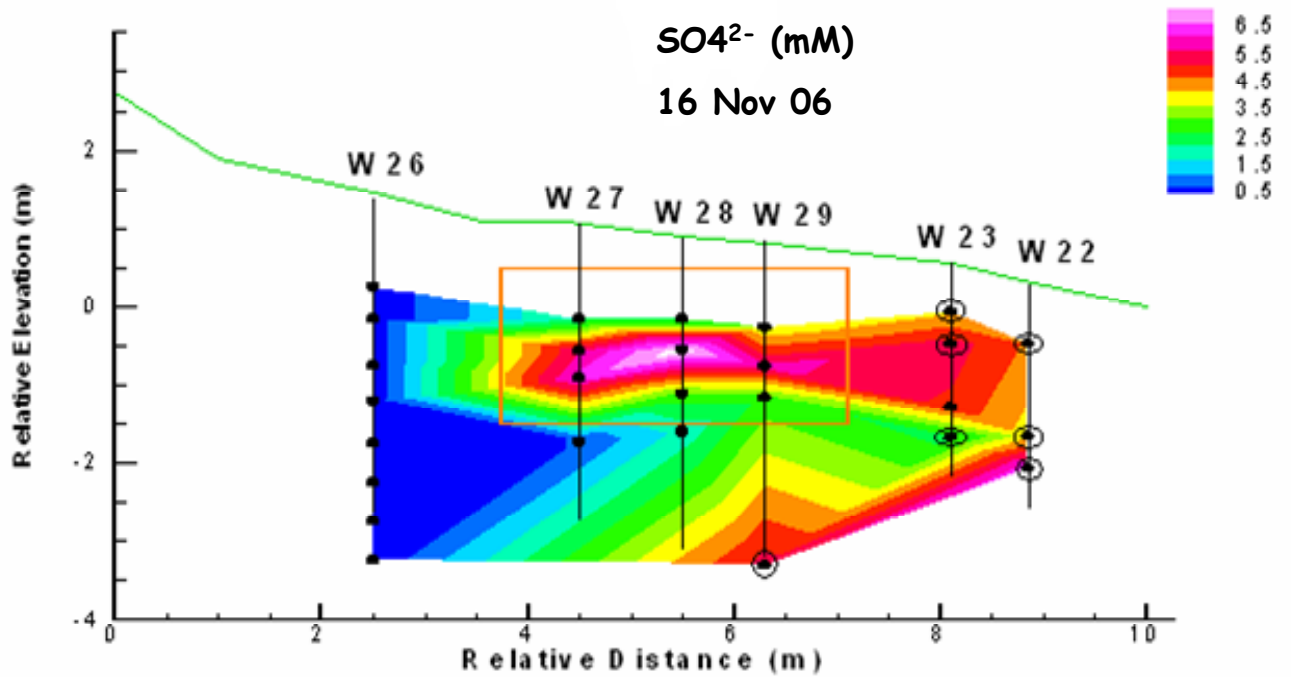


Figure 5. Sulfate concentration (mM) in wells sampled in and around the NITREX™ PRB (shown as orange box) on 11/16/06. Well transect is perpendicular to PRB and coastline. Green line is the relative elevation of grade, upland is on the left side, and Waquoit Bay is on the right side of the image. Circled wells were sampled 11/21/06.

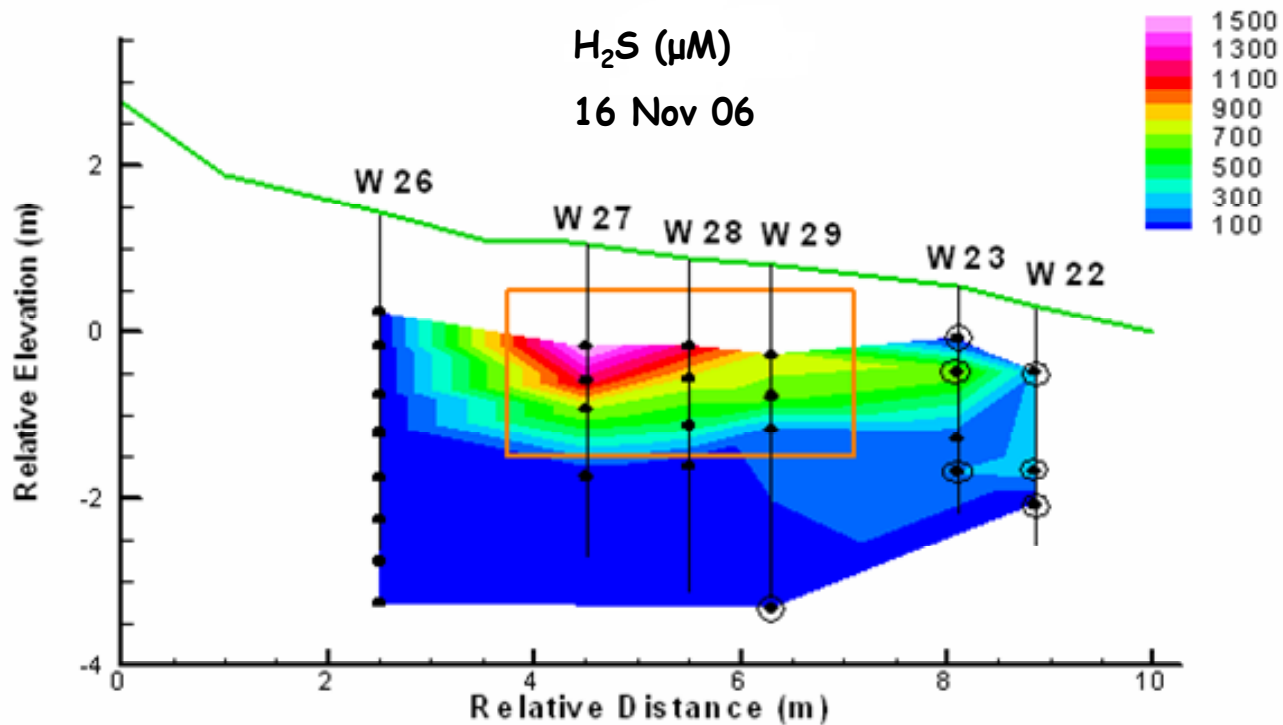


Figure 6. Hydrogen sulfide concentration ( $\mu\text{M}$ ) in wells sampled in and around the NITREX<sup>TM</sup> PRB (shown as orange box) on 11/16/06. Circled wells were sampled 11/21/06.

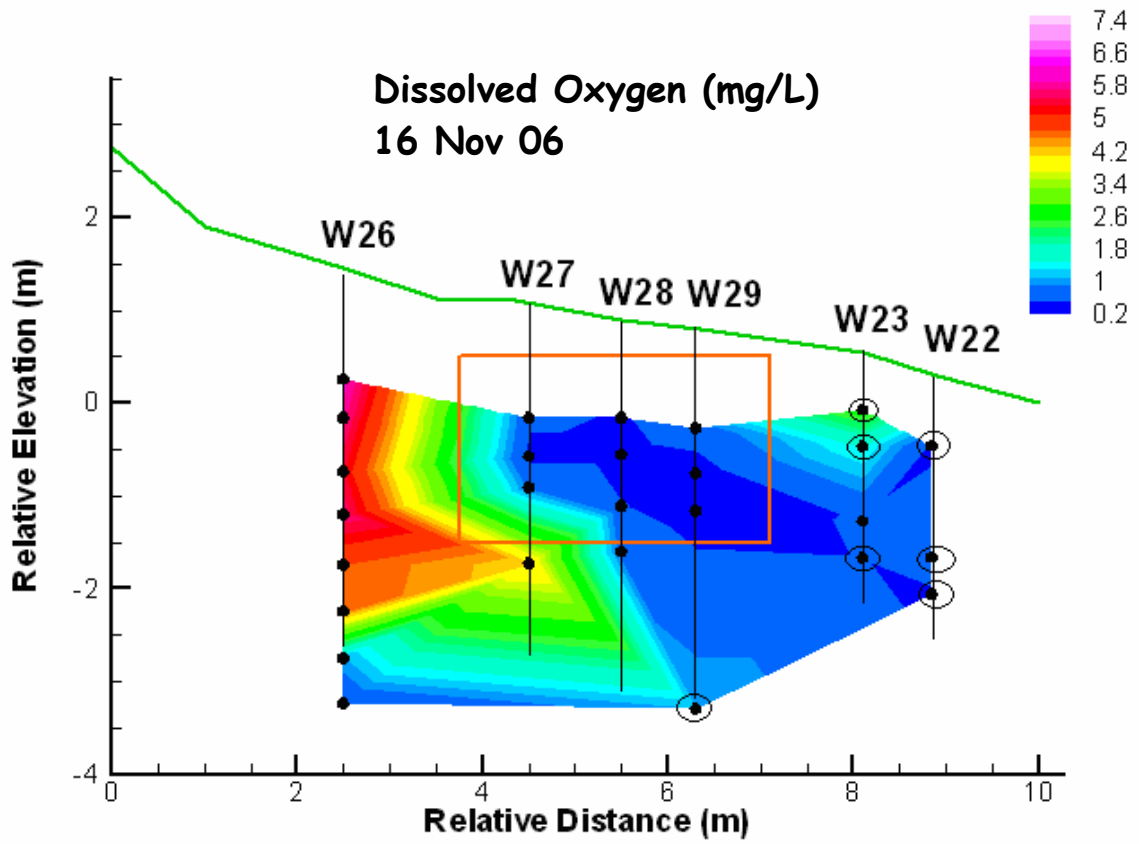


Figure 7. Dissolved oxygen (mg/L) in wells sampled in and around the NITREX™ PRB (shown as orange box) on 11/16/06. Circled wells were sampled 11/21/06.

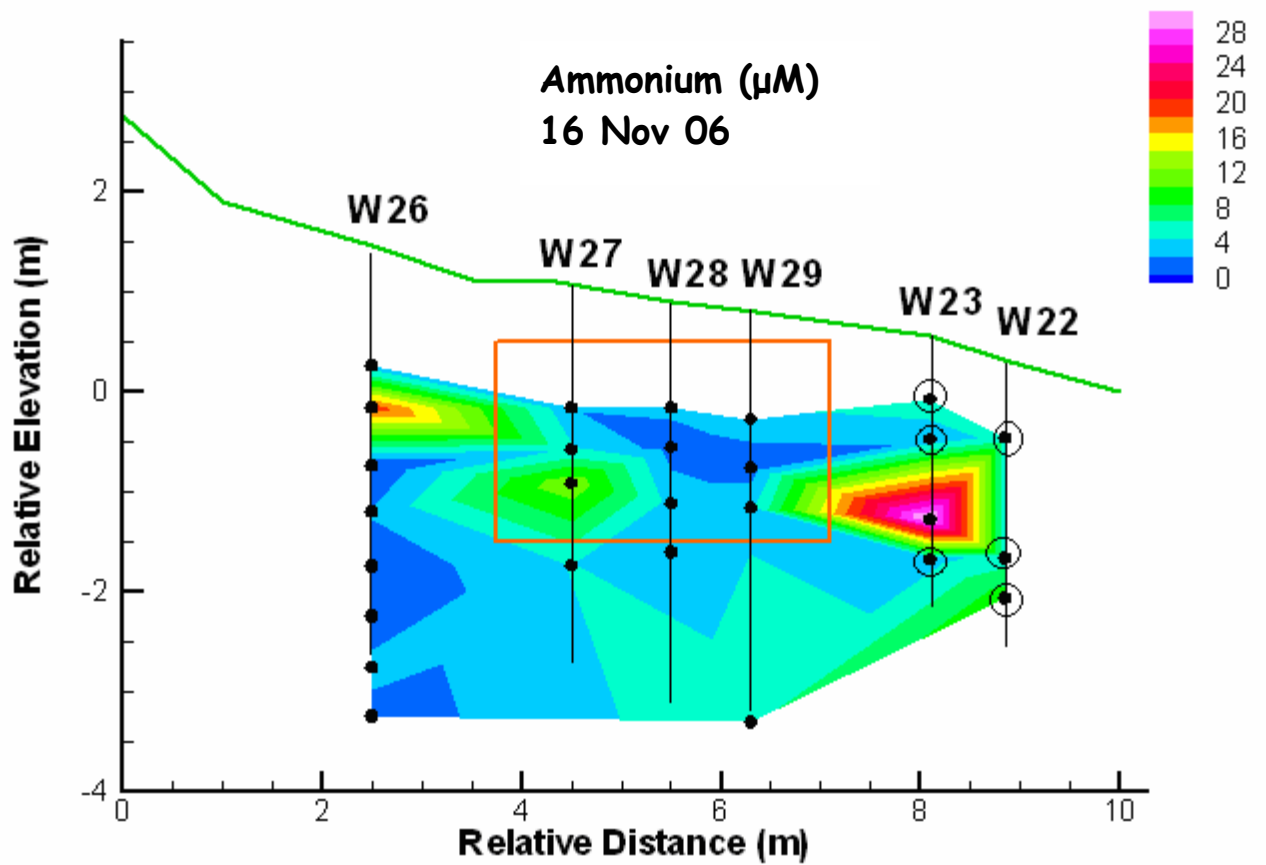


Figure 8. Ammonium concentration ( $\mu\text{M}$ ) in wells sampled in and around the NITREX™ PRB (shown as orange box) on 11/16/06. Circled wells were sampled 11/21/06.

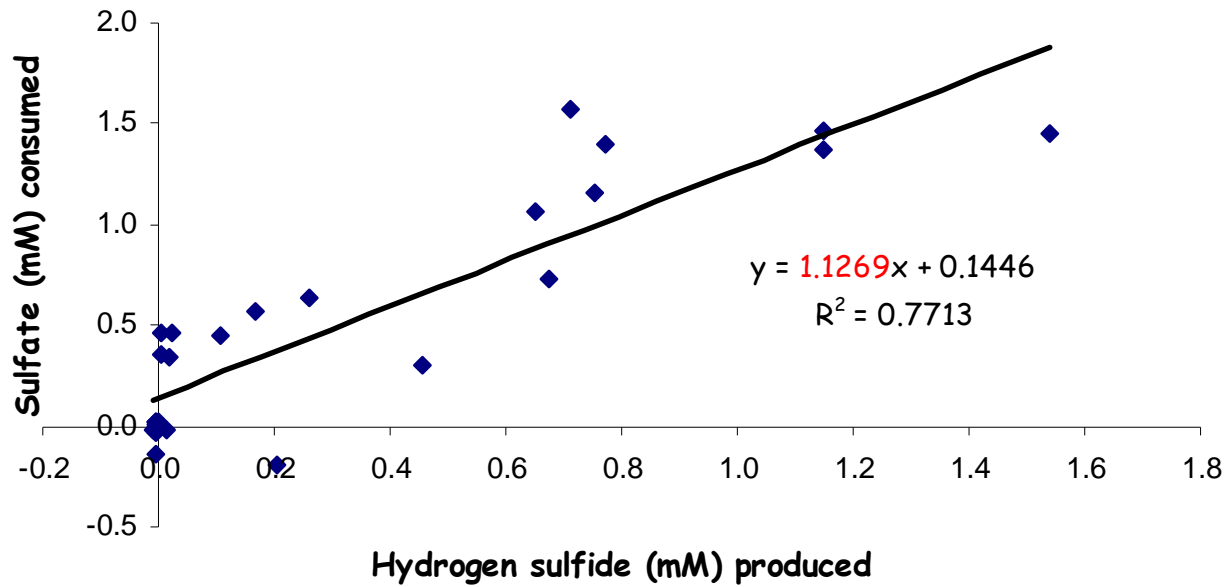


Figure 9. Sulfate consumed (mM) and hydrogen sulfide produced (mM) in and around the PRB. Slope of linearly fitted line shows approximate 1:1 stoichiometric relationship.

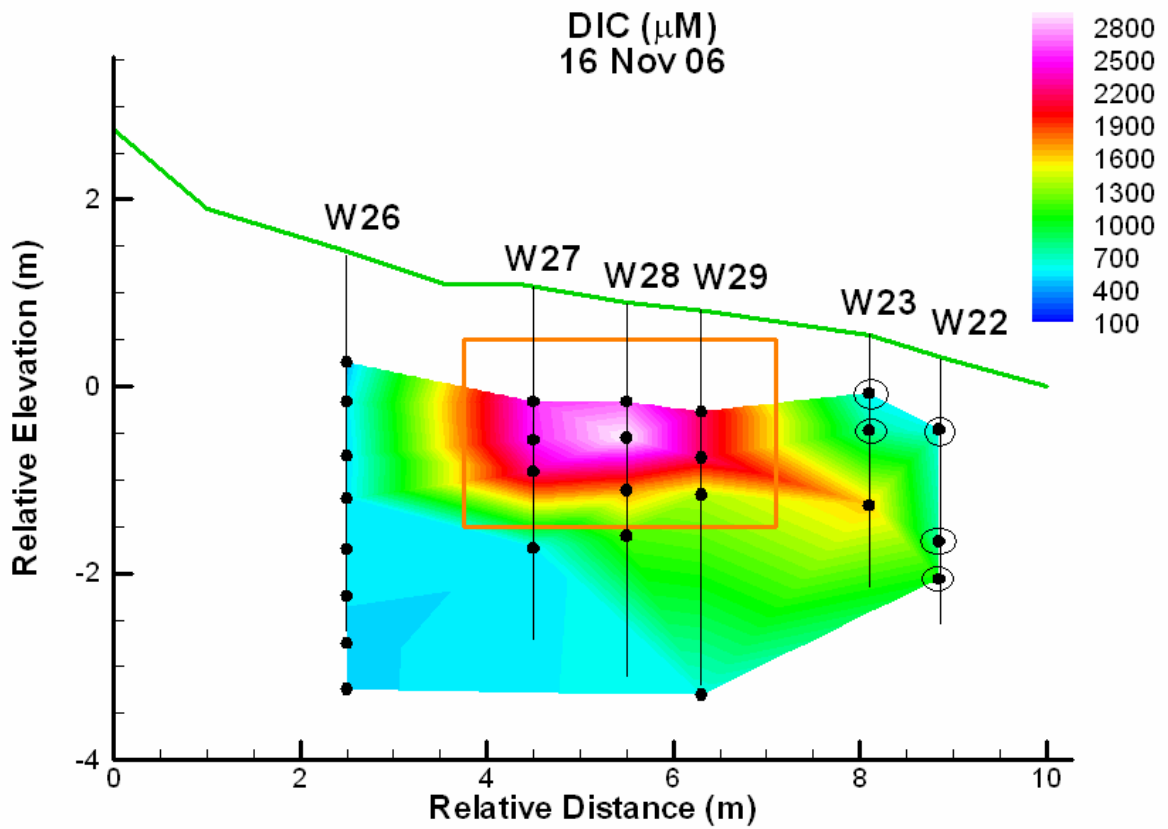


Figure 10. Dissolved inorganic carbon ( $\mu\text{M}$ ) in wells sampled in and around the NITREX<sup>™</sup> PRB (shown as orange box) on 11/16/06. Circled wells were sampled 11/21/06.

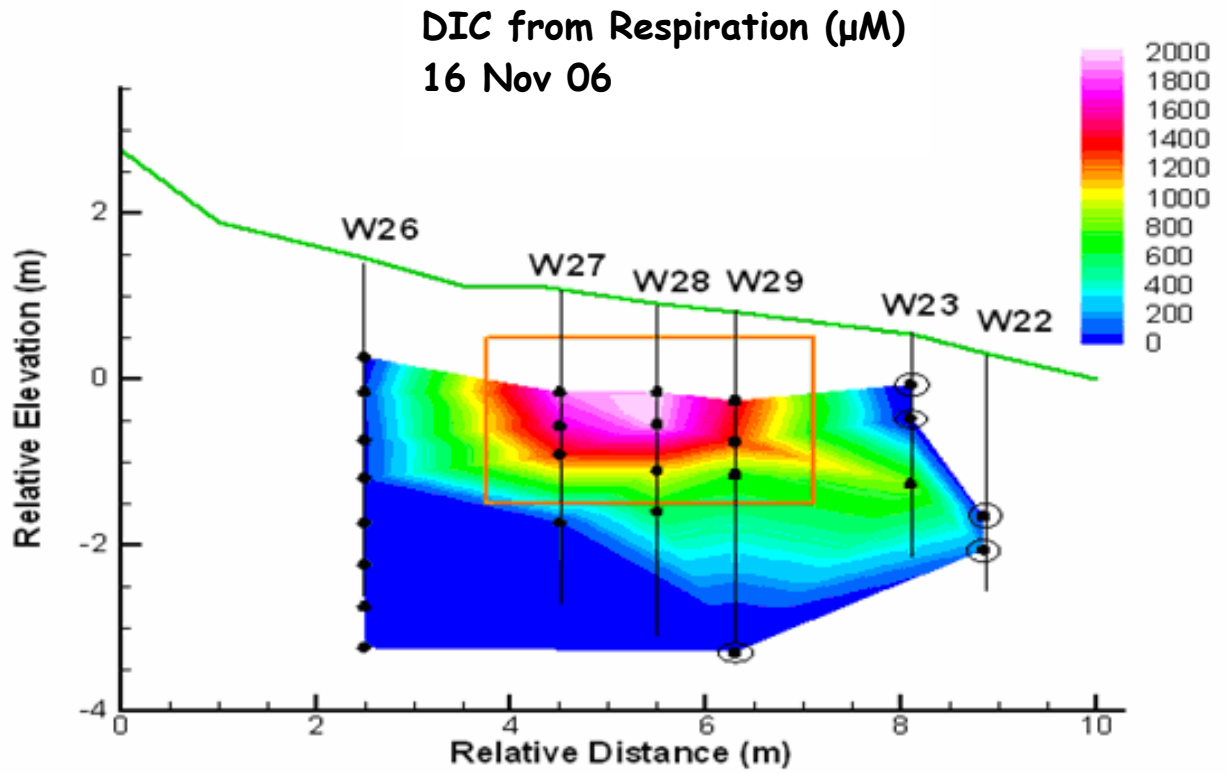


Figure 11. DIC from respiration ( $\mu\text{M}$ ) in wells sampled in and around the NITREX<sup>TM</sup> PRB (shown as orange box) on 11/16/06. Circled wells were sampled 11/21/06.

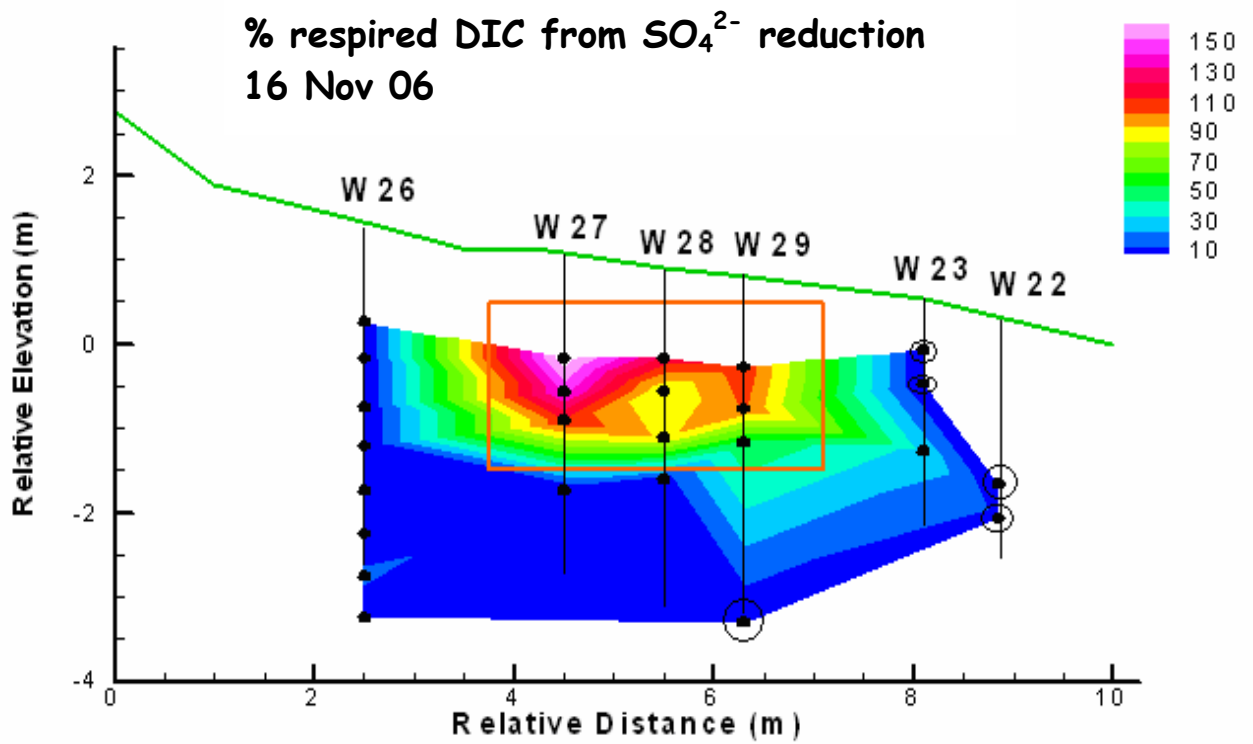


Figure 12. Percent respired DIC from sulfate reduction in wells sampled in and around the NITREX™ PRB (shown as orange box) on 11/16/06. Circled wells were sampled 11/21/06.

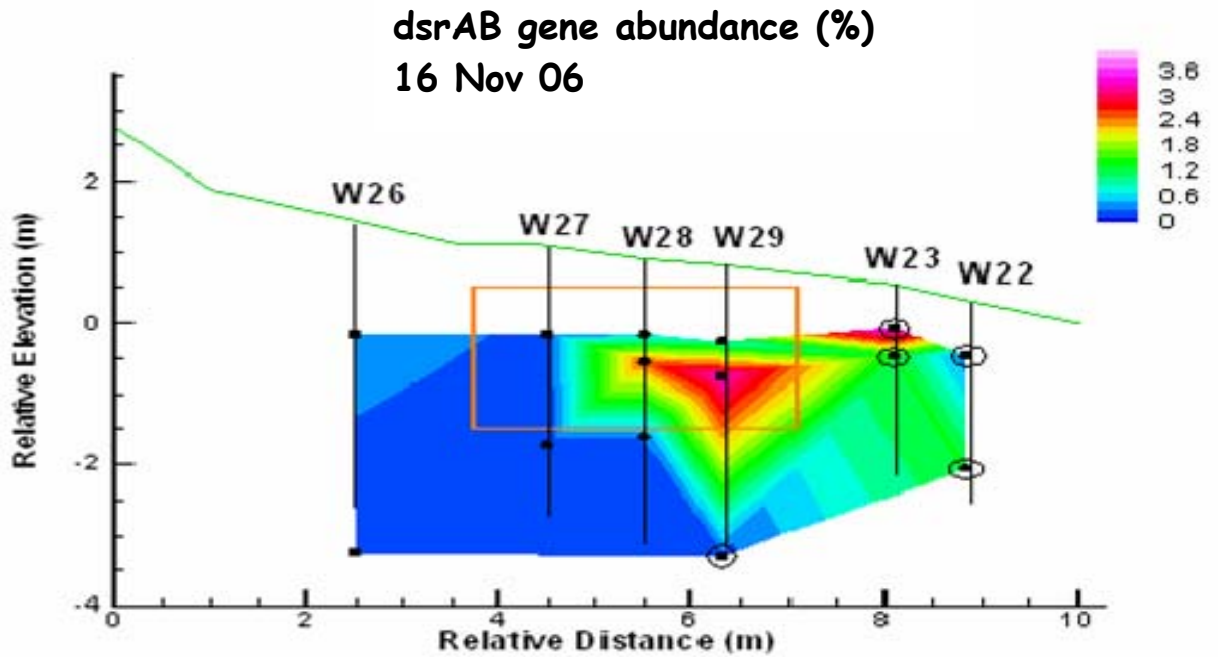


Figure 13. Percent bacteria containing dsrAB gene from wells sampled in and around the NITREX™ PRB (shown as orange box) on 11/16/06. Circled wells were sampled 11/21/06.

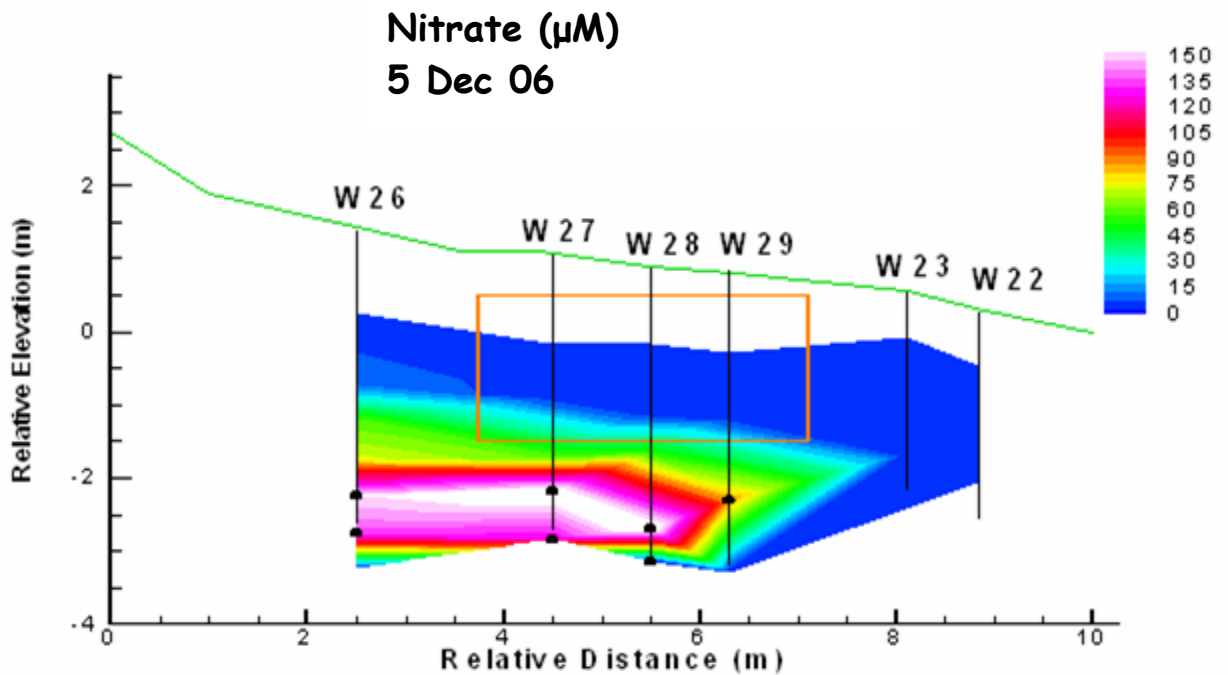
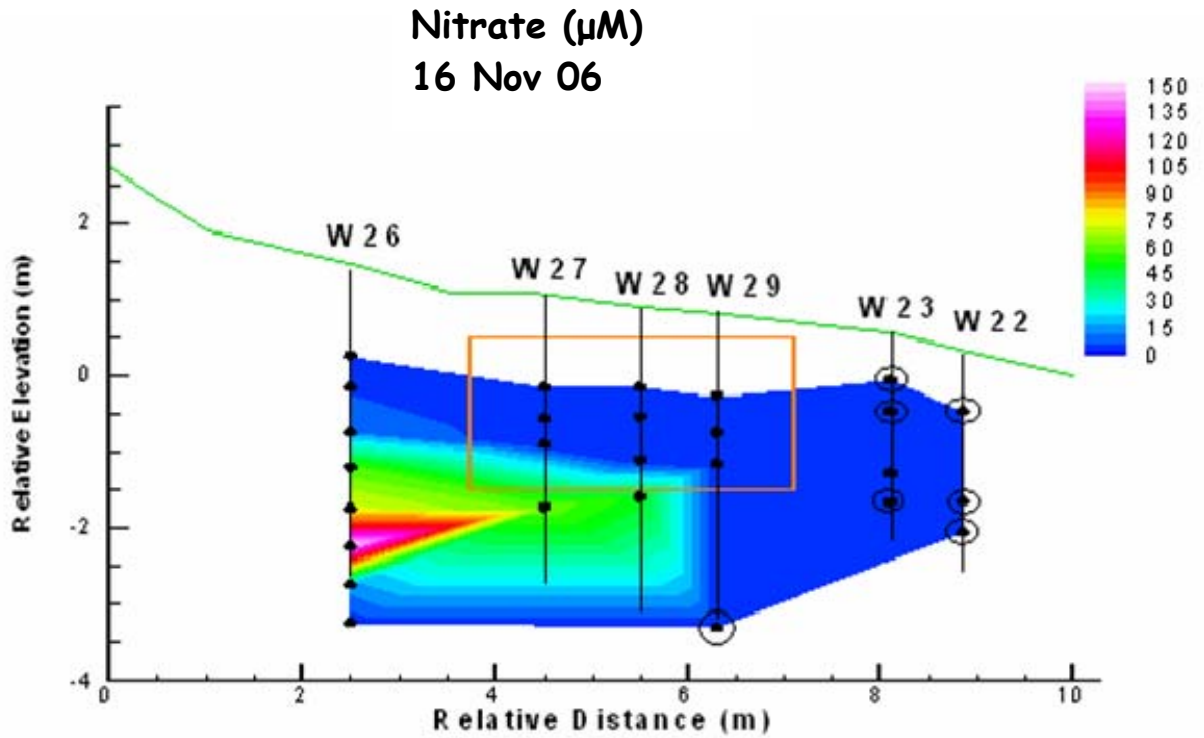


Figure 14. Nitrate concentration ( $\mu\text{M}$ ) from wells sampled in and around the NITREX<sup>TM</sup> PRB (shown as orange box) on 11/16/06 and 12/5/06. Circled wells were sampled 11/21/06.

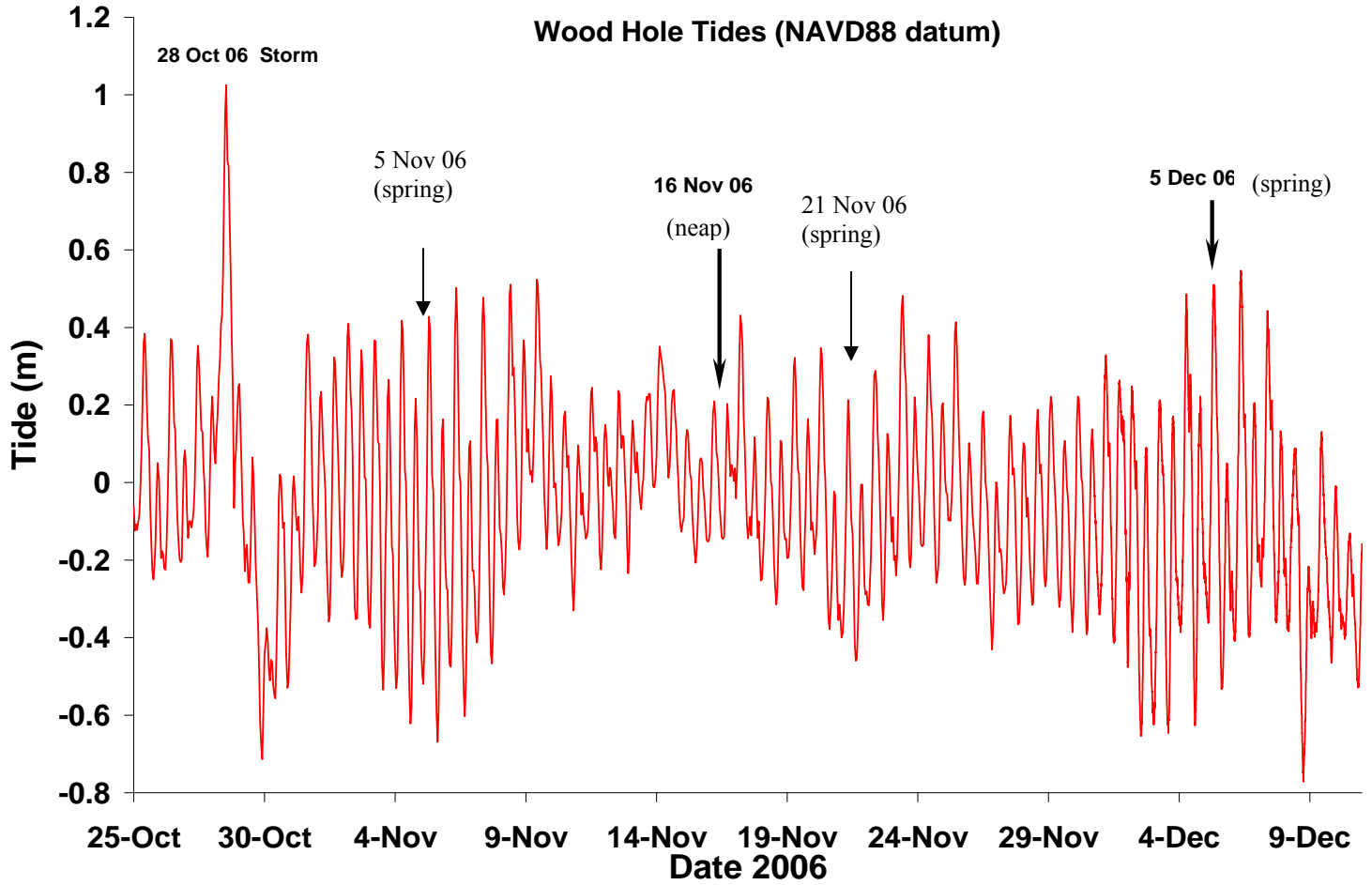


Figure 15. Arrows indicate sampling date and neap or spring tidal cycle.

**Table 1. Primer sequences**

<b>Primer</b>	<b>Nucleic sequence (5'-3')</b>	<b>Target</b>
Eub 338 F	ACT CCT ACG GGA GGC AGC	Bacteria
Univ 907 R	CCG TCA ATT CCT TTR AGT TT	Bacteria
DSR1-F+	ACS CAC TGG AAG CAC GGC GG	SRB (dsrAB gene)
DSR-R	GTG GMR CCG TGC AKR TTG G	SRB (dsrAB gene)

Table 1. The following are the nucleic sequences for degenerative primer sets targeting most known bacteria (Risgaard-Petersen et al. 2004) and sulfate reducing bacteria (SRB) (Leloup et al. 2006).