Effects of a Nitrex Permeable Reactive Barrier on Pigment Levels, Sediment Characteristics, and Organism Abundance

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**Abstract:**

Permeable reactive Nitrex Barriers, like the one that was installed in Waquoit Bay, are good for the town of Falmouth because they have the effect of removing nitrate from groundwater. This study will examine the other effects the barrier may have on the biomass of chlorophyll and the abundance of animals by adding sulfides and changing organic matter content. Examining differences in chlorophyll biomasses, sediment quality and organism distribution and abundances will tell us how the barrier is affecting each of these different factors, and how these compare to a similar control site.

CHN analysis showed that the control site is richer in organic matter, having higher amounts of nitrogen and higher total amounts of nitrogen and carbon. Sulfide data shows that sulfide levels at the barrier site are higher than those at the control site, but not high enough to be toxic to polychaetes, oligochaetes, nematodes, bivalves, and gastropods. Isotopic analysis shows that carbon signals in the control and barrier site are similar throughout the depths of the cores from each site, but nitrogen signals are lighter at the barrier.

Pigment analysis showed that there was slightly more chlorophyll a and phaeophytin in the control site than in the barrier site. The barrier and control site differed more in organism distribution and animal abundance than in chlorophyll biomass. The barrier site also has greater species diversity. I found that the barrier has an effect on sediment organic matter quantity and quality as well as organism abundance and diversity, while chlorophyll biomasses are less affected than expected from a drastic reduction in nitrate levels. By studying these effects that the barrier is having on the ecosystem, we can better understand the effects that Nitrex barriers will have if the town of Falmouth chooses to use them more extensively in their efforts to reduce eutrophication.

**Key Words:** Nitrex barriers, sulfides, benthic infauna, nitrate removal, pigment levels, stable isotopes, organic matter, Waquoit Bay.

**Introduction:**

There is a large amount of eutrophication occurring in the Waquoit Bay watershed. This is mainly due to a larger number of houses surrounding the location of the barrier in the head of Waquoit bay, and their septic systems, in addition to fertilizer and atmospheric deposition, load a lot of nitrogen into the bay. This addition of nutrients can cause increases in algae and phytoplankton biomasses. An experiment has already been put in place that has started to mitigate the effects of this nitrogen addition. A barrier of wooden chips and lime has been buried in a 2m deep, 20m wide trench in a segment of beach along Waquoit Bay. This barrier “provides a carbon source that drives the PRB anaerobic” (Vallino 2008). Groundwater that flows into the bay passes through
this barrier, where microbes use the carbon in the wood for energy and are able to convert a lot of the nitrate and ammonium that are flowing through the groundwater. For example, denitrifying bacteria use nitrate to produce nitrogen gas. The barrier reduces concentrations of nitrate in the groundwater from 200-500 uM to about 1 uM (Vallino 2008). Also, there are higher concentrations of sulfides in the barrier site, which could decrease the suitability of the sediments for sustaining life, particularly in more mobile organisms. These changes in water chemistry likely have some effect on the abundance benthic organisms that live downgradient from the Nitrex barrier, as well as the concentrations of chlorophyll pigments in benthic algae and phytoplankton. In addition, the quality of the organic matter is important because detrital feeders such as some species of nematodes would likely be more abundant in places with a higher quality of organic matter.

I analyzed the concentrations of pigments at both sites. Since the area surrounding Waquoit is eutrophied, an increased amount of chlorophyll a might be occurring at the control site (Cedarwall 1990). To test for this, I extracted pigments from several sediment samples from both sites and ran them through a spectrophotometer to compare them. Since the barrier has been shown to reduce nitrate concentrations (Foreman), I expected the pigment levels to be lower at the barrier site.

I tested several different sediment characteristics at both the barrier site and a control beach site to look at any differences that may be occurring in sediment composition. CHN analysis showed the differences in carbon and nitrogen content in the sediments at the two sites, and allows for better analysis and comparison of the differences between them. I also tested the isotopic signals of the sediment organic matter collected from both sites. Differences in the 13C signals allowed me to determine whether organic matter is coming from wood chips in the barrier site versus dead phytoplankton and other decomposing organisms. Nitrogen isotopes allowed me to see if there were differences in fractionation occurring at the barrier and control sites. I also tested the presence of sulfides in the sediments, because high concentrations of sulfides can be toxic to organisms, particularly more mobile crustaceans. Sediment-dwellers such as polychaetes are less affected, and nematodes and bivalves are least affected by high
sulfide concentrations (Gray 2002). If the barrier does increase sediment sulfide concentrations, it may have a toxic effect on the organisms in this region.

I also looked at the differences in abundances of organisms between the barrier and control sites. The reduced amounts of nitrogen and increased amounts of sulfides may change the abundance of organisms relative to the control site. Different taxa tolerate these differences in sulfides and organic matter differently (Sagasti 2001) and so I looked at abundances at several different distances away from the barrier region at both sites for all of the samples I collected. Also, in taking abundance counts, I identified all the organisms from one replicate core from each distance at each site down to the species level. This allowed me to compare differences in species diversity at each site and determine differences in the lower trophic levels at each site.

The analyses described above will give a clearer look at what the barrier is doing to chlorophyll concentrations, sediment organic matter, and animal abundance at three different distances downgradient from the barrier. The town of Falmouth is considering spending hundreds of millions of dollars to install new sewer systems along the Waquoit Bay watershed to reduce eutrophication, but the Nitrex barrier is a possible solution to this problem. We know it reduces nitrate levels, but more study is needed to determine its effects on the chlorophyll concentrations and animal populations downgradient from the barrier. In this study, I hope to shed some light on this subject, as well as compare differences in species composition and several ecological effects that differences in species diversity and abundance between the two sites might have.

**Methods:**

**Sample Collection**

I went to Waquoit Bay during low tide so that enough of the beach would be exposed for sample collection. I made three transects at both the control and barrier sites at distances of 8, 11, and 14 meters away from the bluff (Figure 1). I collected three cores for abundance counts along each of these transects at both the barrier and control sites. I extruded the top 10cm of each core and put each separate core into its own jar. In
this way, I had 18 abundance jars in total. I collected three cores in each transect for pigment analysis as well. I extruded the top 2cm (where most chlorophyll is generally found) from each core into separate jars. On a separate occasion, I returned to collect three cores from the 11m transects at both sites for isotopic and CHN analyses at each site to a depth of 14cm.

*Pigment Analysis*

In lab, I placed the 18 pigment samples in the freezer for several days. When I was ready for analysis, I removed them from the freezer and homogenized them. I then took a syringe with a 2.8cm diameter and took subcores 2cm in depth. I extruded these cores into 25ml Falcon tubes with 25ml of 100% ethanol (the pore water in the sediment samples will dilute it to 90%). I then sonicated each sample at 2/3 power for one minute. After sonication, I wrapped each tube in aluminum foil and placed them in a test tube rack inside a cooler with ice. I let them sit overnight, and in the morning I analyzed them on a spectrophotometer.

I took off the aluminum foil, shook each tube, and centrifuged them to remove the sediment from the fluid. I then pipetted liquid from the tubes into a 1cm cuvette until it was ¾ full. I ran it in the spectrophotometer at wavelengths of 665nm and 750nm for chlorophyll and turbidity measurements. I acidified the sample with about 8 drops of HCl, let it sit for one minute, then re-ran it at the same wavelengths.

*Sediment Chemistry (CHN and isotopic analyses)*

I took the six intact cores I had collected for organic matter analysis and sectioned them into five segments (0-2cm, 2-4cm, 4-6cm, 6-10 cm, and 10-14cm). I put each segment into a separate jar and put them in the drying oven for several days. I then weighed each jar with the sediment inside. I took out about 2.5 grams of sample from each jar and ground it with a mortar and pestle, then weighed it and acidified it in a dessicator with 25ml of concentrated HCl. I allowed the samples to fume in the dessicator for 60 hours, then removed the samples from the dessicator. I allowed them to sit in a fume hood for two hours to remove the last of the HCl fumes, then reground and reweighed them and placed them back in the drying oven for a day. I then weighed out
20-30mg of sample from each and packed it in a tin sample holder. I then ran them on
the CHN analyzer. I used sediment from the middle organic matter core from each site
for isotopic analysis as well. I sectioned the cores, dried, ground, acidified, and weighed
them as described in the CHN procedure. I chose 10 samples (one from each depth at
each site) to send in for del13C and del15N analysis.

**Abundance Analysis**

Upon returning to the lab with the 18 abundance jars, I filled them with 10%
formalin to preserve the organisms. I then added Rose Bengal dye that stains the
organisms and makes them more visible. I let the jars sit for several days. Then, for each
jar, I sieved out the contents into three size fractions (2mm, 1mm, and 250 um). I washed
the two larger sizes into finger bowls. Once the sediment was separated into the three
size fractions, I examined the two largest sizes under a dissecting microscope. I removed
the organisms I found and put them into separate vials for polychaetes, oligochaetes, and
“others” which were mainly clams and oyster drills. I put 70% ethanol in the vials to
preserve the organisms.

For the smallest size fraction, there was the greatest amount of sediment to be
sorted through. I used Ludox with a density of 1.12 to help separate the small organisms
from the sediment. I diluted the ludox 1:1 with 300ml of ludox and 300ml of tap water
and placed this into a 1L beaker. I washed half of the sediment into this beaker with a
squirt bottle of ludox with the same dilution. I poured the contents of the beaker into a
1L graduated cylinder and used the same squirt bottle to wash all the sediment in as well.
I repeated this procedure for the other half of the sediment into another 1L graduated
cylinder. I let the cylinders sit for an hour to allow the organisms to float to the top and
the sediment to settle to the bottom. After settling, I poured the Ludox through a fine
sieve (63 um) and caught the worms on the sieve. I then poured the sediment into the
Ludox one more time to remove any organisms that may have been trapped in the
sediment. I poured it through the fine sieve again, and took all the material caught on the
sieve to pour it into an Elmigrin subsampler and let it settle for another hour. This device
separates the worms into eight subsamples by dividing the bottom of the sampler into
eight segments, each with its own small rubber stopper. When the subsampler was done
settling, I removed the stoppers from three of the segments and let the contents fall into collection tubes below. I used tap water to wash the remaining contents of the segment into the collection tubes.

I poured the contents of the collection tubes one at a time into separate, gridded petri dishes. I examined them under the dissection microscope and used a Pasteur pipette to remove organisms (nematodes and copepods) from the petri dish. I put them into small finger bowls, then counted them with a clicker counter. I then transferred them into small vials with 70% ethanol for preservation.

Identification

I chose one replicate from each transect at each site to identify down to the species level. For each transect at each site, I separated out the worms by species and used published literature to help in identification. I then counted them after I was finished.

Results:

Pigment Analysis

By transect, the barrier starts out with low chla and phaeophytin concentrations at the transect closest to the bluff (8m) with 129 mgchla/m². It then spikes in the middle transect of 11m to over 400 mg/m², then decreases again in the 14m transect (Figure 2). The control site follows a similar pattern of starting off low in the 8m transect with 373 mg chla/m², spiking in the middle, and then decreasing again in the 14m transect to 1,099 mg chla/m² (Figure 2). The control site starts out at higher chla and phaeophytin levels, and maintains these higher levels throughout all transects. Phaeophytin levels in the control site are consistently higher than chl a levels by 300-400 mg/m² in the control site, and 200-300 mg/m² higher in the barrier site.

Sediment Chemistry
The percent nitrogen in the sediment at the barrier site starts out at 0.065 (molar) at the shallowest 1cm depth, then drops to 0.026 at the 5cm and 8cm depths before rising to 0.030 at the lowest 12cm depth (Figure 4). The control site starts out at a higher value of 0.083 before dropping to 0.047 at the 12cm depth. It follows a similar trend as the barrier site, but the control site had more percent nitrogen than the barrier. The percent carbon at the barrier site starts at 0.446 at the 1cm depth, then drops to 0.188 at the 5cm depth. It then rises to 0.414 at the deepest depth (Figure 4). The control site follows a similar trend. It starts at 0.482 then drops in the middle depth before rising to 0.586 at the 12cm depth. Overall, the control site has more carbon than the barrier. The C:N ratios at both sites were similar throughout each core depth (Figure 5). The barrier started out at 6.91 and then increased as the core got deeper to 13.65. The control site had a similar trend, starting out at 5.82 and increasing to 12.46.

The carbon isotopes at both sites were similar throughout each core depth (Figure 6). The barrier started out at -17.5 and then decreased in signal to -24.8 at the 12cm depth. The control site followed a similar trend, starting out at -16.5 then decreasing in signal in a similar trend to the barrier site until it reached -25.9 at the 12cm depth. The nitrogen isotopes on the sediment organic matter at the barrier and control showed different trends (Figure 6). The signals at the 1cm and 12cm depths are similar, but through the middle depths at 3cm, 5cm, and 8cm, the barrier site’s signals drop. The lowest recorded was 2.5 at the 5cm depth. The control site shows no such drop in signal, staying at around 5.5 at the 5cm depth.

Abundance Analysis

In both the barrier and control sites, nematode abundance is similar at the 8 and 11m transects, and then nearly doubles in the control site (Figure 7). In the barrier site, the nematode abundance exhibited a much larger increase from 21,780 to 56,134 worms/m². Total nematode abundance was higher at all transects in the barrier site. At the barrier site, copepod numbers were consistently much lower than those in the control site, but did increase as the distance from the barrier increased. At the control site, the copepod abundance started out high at the 8m transect at 27,009 copepods/m², dropped
slightly at the 11m transect, and then disappeared almost entirely as the 14m transect was reached (Figure 7).

The nematode:copepod ratio (Ne:Co) shows that while sulfide concentrations are increasing, the Ne:Co also increases. Both the sulfide concentrations and the Ne:Co ratios increase by an order of magnitude from the control site to the barrier site (Figure 8). At the barrier site, Ne:Co values are up to 24, and sulfide concentrations are up to 0.985 (mM). The control site’s highest Ne:Co value is 0.889 and the highest sulfide concentration is 0.074 (mM).

Oligochaetes at the control site were very abundant closest to the beach at the 8m transect at 2,835 worms/m$^2$, then decreased as we moved further away from the beach (Figure 9). The barrier showed a similar pattern, except that it started out with a lower abundance of 1,993 worms/m$^2$ at the 8m transect and had higher abundances than the control at the other two transects. Overall polychaete numbers were lower in the control site than at the barrier (Figure 9). The 8m control transect had very few polychaetes at 112 worms/m$^2$, then increased to the highest abundance of 898 worms/m$^2$ in the middle transect before decreasing slightly at the final transect. The barrier had higher overall polychaete abundances, with 926 worms/m$^2$ at the 8m transect. The barrier site also showed a spike in abundance in the middle transect. Both the control and barrier sites had very similar numbers for bivalve abundance, and these increased as the transects progressed out from the beach. Gastropods also showed similar abundances and trends in both sites, except they were more abundant closest to the beach (Figure 9). There was more species richness occurring in the barrier site (Figures 10). The barrier had a total of 8 species, with highest species richness at the middle transect. The control had 3 species, and the highest richness was also at the middle transect (Figure 11). The highest species richness coincided with the highest pigment levels.

**Discussion:**

*Pigment Analysis*
Due to the nutrient-loading occurring in Waquoit, more algae and phytoplankton blooms are able to occur because nitrate is often the limiting nutrient in coastal systems. The groundwater transports nutrients such as nitrate from fertilizer and sewers to the bay, where nutrient concentrations are significantly lower than those in the groundwater (Valiela 1999). This could be why pigment levels are slightly higher at the control site than at the barrier (Figure 2), but I would have expected to see larger differences if eutrophication was causing major algal and phytoplankton blooms. The time of year should be taken into consideration, however, because in November and December the algal blooms have mostly died, and phytoplankton biomasses have decreased. Figure 3 shows the beach at Waquoit during July. The algae is covering the beach, except where the barrier is. This is not as pronounced in late fall/early winter. If the study was undertaken in summer, much larger differences in pigment levels between the two sites would likely have been seen. As for pigment levels over transects, the lowest values are seen at the 8m transect, possibly because these transects are above the low tide line and are exposed for parts of the day. Also, the pigment levels are highest at the middle 11m transect because this is where most of the nitrate-laden seepage water is flowing in.

Sediment Chemistry

Environmental factors such as organic matter content can have an impact on the distribution of benthic fauna (Miron 1993). Polychaetes, for example, need medium to high levels of organic matter to survive. The control site has more nitrogen and carbon than the barrier site. Nitrate is leaching into this section of the beach from the groundwater, and this increases primary producer biomass. When they die, they contribute to organic matter content. I would have expected, however, that the barrier site would have higher carbon percentages, since the barrier is made of oak wood chips and would likely leach some carbon downgradient of the barrier site. This may be the case, but it is still not more than the carbon contributed by phaeophytin and chlorophyll at the control site, not to mention the algae that is present at the control site but not at the barrier. This algae could contribute a significant amount of carbon and nitrogen to detritus at the control beach. Also, the molar C:N ratios look fairly similar to one another (Figure 5), so overall organic matter quality is similar at both sites because the amount of
nitrogen relative to carbon is similar at both sites. Also, more organisms were found at the barrier site, and several species were detritivores. The reduced total amounts of nitrogen and carbon could be due to grazing by these organisms.

The isotopic signals in the barrier and the control site indicate that in the top three depths of the sediment, the carbon likely originated from benthic algae, which has a delta 13C signal of -17. Both sites show similar trends in the dropping carbon signals (Figure 6). Whether this is because both sites have similar original carbon sources, or it is due to normal fractionation during sedimentation and decomposition remains unclear. The nitrogen signals in organic matter at the barrier site are lighter than the control site, indicating a difference in fractionation occurring somewhere. It may be due to processes occurring in the barrier, such as denitrification. However, we would expect denitrifying bacteria to favor lighter isotopes and therefore make the nitrogen atoms exiting the barrier have an overall heavier signal. This needs more study to be fully understood.

Abundance Analysis

Nematodes occur in higher abundances in the barrier because they have a high tolerance to sulfide concentrations. In the 10cm depth range in which I measured abundances, sulfide concentrations were not high enough to be toxic, even to polychaetes. This, therefore, is not what directly influences nematode distribution. In fact, nematode distribution is not affected by pollution, such as that occurring from sewer and fertilizer runoff at Waquoit (Sutherland 2007). Tietjen asserts that organic matter content did not affect the distribution of nematodes either (Tietjen 1969), and this is consistent with the data collected at Waquoit because the barrier site actually has less percent nitrogen and carbon than the control site. Other factors such as sediment particle size could influence the distribution of nematodes if it is in fact the case that nematodes are not influenced by organic matter content.

Copepods require different environmental conditions in order to survive. At low copepod abundances, such as those found in the barrier site (Figure 7), the sediments tend to have higher organic matter concentrations as well as higher sulfide concentrations (Sutherland 2007). Although the organic matter content is not as high as the control site, there are more sulfides in the porewater at the barrier. This could be preventing high
populations of copepods from developing. Copepods are much less tolerant of sulfides than benthic worms, so although polychaetes, oligochaetes, and nematodes may be able to live at the barrier site, the copepods experience reduced abundances there.

The nematode:copepod ratio can be a measure of pollution in an area because of the differences in environment tolerated by each species, and gives a closer look at nematode and copepod abundances relative to sulfide concentrations. There is more sulfate reduction occurring at the barrier, so this is why sulfide concentrations are higher at this site than the control. Sulfides can be toxic to certain taxa (Gray 2002). Copepods, for example, are susceptible to high sulfide concentrations while nematodes are one of the more tolerant species. Therefore, the nematode:copepod ratio can be used as an indicator of differing sulfide concentrations. As the ratio increases, this indicates higher abundances of nematodes relative to copepods, and higher sulfide concentrations should be seen as the ratio increases. This was the case, as shown in Figure 8. The Ne:Co ratios and sulfide concentrations are an order of magnitude lower at the control site, which is why they are all clustered into a single point.

Polychaetes and oligochaetes are found in higher abundances at the barrier site (Figure 9). Since sulfide concentrations are not high enough in the top 10cm of sediment to limit polychaete or oligochaete abundance, another factor must be affecting their numbers at the barrier site. Some of the species identified, including glycera, marphysa, and paraonis worms are carnivores (Fauchald 1979). Their prey items may be more abundant at the barrier site. Glycera, for example, feeds on small polychaetes, which are more abundant at the barrier site. Glycera populations could be facing bottom-up food web controls on their populations. Small polychaetes certainly have higher abundances at the barrier. Paraonis is also known to feed on diatoms, and since the chlorophyll a biomasses at the two sites are similar, it may not be a limiting factor in its abundance at the barrier site. Several of the species found can also survive on detritus, particularly ninoe nigripes and syllis gracilis. It seems counterintuitive to find higher abundances of these species at the barrier, where the organic matter less rich in carbon and nitrogen. However, other factors may increase their abundances at the barrier site. For example, it could be that some of their predators, such as crustaceans, have a difficult time surviving under barrier conditions (Gray 2002). This would allow their populations to increase due
to top-down food web controls. Also, the C:N ratios were similar at both sites, so the detritus is equally nutritious at the barrier as it is at the control. The control and barrier sites both showed low abundances and diversities (Figure 10) at the 8m transect because it is not always inundated by the tides. This could reduce the number of species and individuals living there.

**Conclusions:**

The chlorophyll a and phaeophytin levels were similar at the two sites, most likely due to the time of year at which the samples were taken. If the samples had been taken in summer when algal blooms are at their highest, I expect that very different results would have been seen. The barrier site had higher overall worm abundances than the control site. This seems counterintuitive because the barrier site has more toxins (higher sulfide concentrations), less total organic matter, and lower pigment levels. Some other factors must be at work that are causing the worms to be more abundant at the barrier. It could be prey availability for some species, or reduction in predator population sizes. More research is needed to fully address these issues. It is also important to note that this year had more rain than usual, so salinity was lower in the barrier, resulting in lower sulfide concentrations than usual. This reduction in sulfate levels may have allowed more polychaetes to inhabit the sediment downgradient from the barrier. This, in conjunction with other possible factors such as prey availability, could be contributing to higher worm abundances in the barrier site. Finally, the organic matter had similar C:N ratios at both sites, despite the barrier having less total carbon and nitrogen.

In order to say whether the Nitrex barriers can be implemented on a large scale, further research is needed. Samples should be taken year-round about once a month to ensure that temporal changes are being accounted for as well. Also, if worm populations are increasing downgradient from barriers all around Waquoit, the effects of this increase in population should be explored in an ecological context.
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