Chemical Composition of Anthropogenically Influenced Groundwater

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ABSTRACT

I examined the oxygen and nitrogen components of groundwater. I looked at groundwater from a pristine site, a human impacted site, and Title V treated wastewater. All of the water that was collected was fractionated, using ultrafiltration, into DOM<1000 da water, 1000<DOM<10,000 da water, DOM>10,000 da, and total water with DOM of all sizes. All of the water fractions went through a one-week incubation with bacteria. All chemical analyses took place on water pre and post incubation. Nitrate and ammonium were measured colormetrically. The total dissolved nitrogen (TDN) was measured using a persulfate digestion technique. Oxygen consumption was measured using the Winkler technique. The total dissolved inorganic nitrogen (DIN) and total dissolved organic nitrogen (DON) were calculated from the measured data. Significant chemical differences were found between the three sites and avenues for future research are discussed.

KEY TERMS: dissolved inorganic nitrogen, dissolved organic nitrogen, molecular weight fractionation, oxygen consumption, total dissolved nitrogen, persulfate digestion, ultrafiltration.

INTRODUCTION

Fertilizer production and use, fossil fuel burning, and cultivation of N-fixing crops has doubled the rate of N-fixation on the globe (Galloway, 2002). Much of the reactive nitrogen fixed entered coastal and marine waters, where nitrogen is often the primary limiting nutrient (Vitousek and Howarth, 1991). The delivery of excess nitrogen to coastal waters has resulted in toxic algal blooms (Anderson, et. al. 2002), eutrophication, and declining water quality in estuaries worldwide (Bricker et al 1999; Howarth et. al. 2002). Nutrients are delivered to esturaries in river flow, runoff, and through groundwater (McClelland and Valiela, 1998; Valiela, 1997).

There has been considerable work evaluating the effects of enrichment with inorganic nutrients on coastal and marine producers (Ryther and Dunstan. 1971; Fisher et. al. 1992) but less attention has been paid to the role of dissolved organic nitrogen. Up to 70% of DON may be liable (Sietzinger and Sanders, 1997). DON comprises approximately 60% of the nitrogen concentration in the groundwater at the seepage face of Green Pond (Kroeger, 1999). Therefore, DON is a significant portion of the labile nitrogen polluting our coastal waters. DON may stimulate primary producers, as nitrogen is often the limiting element capping the growth of the primary producers in freshwater ecosystems.

Understanding the chemical composition of DON can help to illustrate the rationale behind the growth of microbial communities and primary producers. Access to bioavailable N is a common limiting factor to primary production (Stephanauskas et. al. 1999). Knowing the chemical composition of groundwater DON could help indicate the chemical composition of rivers.

I hypothesize that the chemical composition and availability of organic matter will be different in pristine groundwater, impacted groundwater, and wastewater. The chemical composition of dissolved organic matter (DOM) in marine waters has been extensively explored via tangential flow ultrafiltration (Benner et. al. 1997). Rainer and Benner (1996) have studied how bacteria utilize different size classes of DOM. High molecular weight DON has recently been implicated to be utilized more by bacteria and be more bioreactive than low molecular weight DON in freshwater and marine ecosystems (Amon and Benner, 1996).

Valiela (2000) has implicated residential areas with changes in the DON and DIN pools. He has found that an increase in residents corresponded with a decrease in DON and an increase in DIN (nitrate plus ammonium) (Figure 1). I examined the groundwater at two very different sites that represent two extremes: a pristine standard with no residents, and a pond surrounded by two heavily populated peninsulas. As an endpoint, I also examined the water from a leach field of a standard septic tank.

In this experiment, I attempt to characterize DON and its labiltiy via fractionating the DOM in groundwater into three molecular weight ranges: DOM<1000da, 1000<DOM<10,000da, DOM>10,000da, and total water. All chemical analyses were done on the fractionated and total water before and after a one-week dark incubation with native bacteria from the two sites. To assess the organic matter lability I measured the oxygen consumption in water confined to BOD bottles. To determine the changes in the DON pools the total dissolved nitrogen (TDN) and dissolved inorganic nitrogen (DIN) components of the water were measured. The DON equaled the TDN minus the DIN component.

METHODS

I examined the groundwater from two sites: Washburn Island, a pristine standard, and Green Pond, a pond surrounded by two heavily populated peninsulas. I also looked at treated wastewater from a leach field of a standard septic tank. I chose Washburn Island as my pristine standard because it is the most pristine local area. Washburn Island is a forested island nature preserve in Waquoit Bay (Figure 2). Green Pond is a small coastal embankment on the Ackapesket Peninsula on Cape Cod (Figure 3). Green Pond is a long narrow Pond with two heavily populated peninsulas on either side of it. Green Pond receives nitrogen from wastewater disposal via septic tanks, fertilization use, and atmospheric deposition. The Massachusetts Military Reservation's Ashamet Valley wastewater plume may be an additional source of nitrogen to Green Pond (Kroeger et. al. 1999).

I removed one liter from each of fourteen different groundwater sites on the perimeters of both Washburn Island and Green Pond in order to qualitatively collect a composite sample of the groundwater in these two locations. The groundwater was sampled by inserting a drive-point piezometer into the water table at the shoreline. The salinity of the groundwater was checked with a refractometer and all groundwater that was collected had a salinity equal to or below 2 parts per thousand. This groundwater was hand pumped into a collection vessel.

The Title V treated wastewater was collected from the Massachusetts Alternative Septic System Testing Facility. The treated wastewater was collected from lysimeters at 1m, 2m, and 5m below the leach field of three standard septic tanks. This collection method allowed for a composite sample of Title V treated wastewater.

All of the water went through multiple filtration stages. Firstly, the water was filtered through a 0.7 GFF filter using high pressure nitrogen filtration in order to separate out and remove the particulate matter. Next, the water was filtered through a 0.22 micron membrane filter pack (Millipore Steripak) in order to remove all bacteria. The groundwater was then separated into four size fractions using a Millipore Stirred Cell 8400 Ultrafiltration unit: water with dissolved organic matter (DOM) less than 1,000 daltons, water with DOM between 1,000 and 10,000 daltons, water with DOM greater than 10,000 daltons, and total water with DOM of all sizes.

The fractionated water was inoculated with mixed bacteria from Waquoit Bay and Green Pond in the ratio 50mL of inoculum per liter of water. Twelve BOD bottles were prepared for each site and were overflowed twice to prevent the inclusion of air bubbles in the incubation. One bottle from each size fraction was fixed and analyzed for oxygen consumption immediately. The other eight bottles went through a one-week incubation. After the incubation one bottle from each size fraction was fixed and analyzed for oxygen consumption. The remaining four bottles were saved for further chemical analyses. All chemical analyses were done on the four different size fractions before and after the one-week dark incubation (Figure 4).

Oxygen was measured using the Winkler technique (Limnological Analyses, 1991). Total dissolved nitrogen (TDN) was measured using a persulfate digestion technique (D'Elia, et. al. 1997) which oxidizes all of the nitrogen present into nitrate. DON was estimated by subtracting the inorganic nitrogen, in the forms of nitrate and ammonium, from the measured TDN. Nitrate was measured colorimetrically using Cadmium reduction in a lachat Quickchem 8000 Flow Injector Analyzer. I measured the ammonium colorimetrically using the phenol-hypochlorite method on a Shimadzu 160 UV/VIS spectrophotometer with the absorbency set at 640nm (Strickland and Parsons, 1972).

RESULTS

First, I looked at the DON and DIN components of the initial total water at all sites. Washburn Island had the greatest percentage of DON compared to DIN. Washburn Island had 65.6% of its TDN in the form of DON, with the remaining 34.4% of its TDN in the form of DIN. Green Pond had approximately equal quantities of DON and DIN, with slightly greater DIN present than DON. Green Pond's DON component of its TDN was 46.3%, and its DIN component was 53.7%. The Septic tank TDN was dominated with 96.4% DIN and only 3.6% DON. The DON to DIN ratios more starkly illustrates this pattern of an increased DIN component of TDN with increased anthropogenic inputs. The Washburn Island DON:DIN ratio equaled 1.91. The Green Pond DON:DIN ratio equaled 0.86. The Septic tank DON:DIN ratio equaled 0.04 (Figure 5).

For all sites, the oxygen consumption was greatest in the fraction with DOM<1000 da. This indicates that the bacterial growth was greatest in the DOM<1000 da water fraction. In all size fractions, except the 1,000<DOM<10,000 da fraction, the oxygen consumption was greatest in the Washburn Island groundwater. The septic tank water oxygen consumption was largest in the 1000<DOM<10,000 da size fraction. The septic tank water had the second greatest oxygen consumption, after Washburn Island groundwater, in the DOM<1000 da and the total water size fractions. Washburn Island groundwater had the second greatest oxygen consumption in the 1,000<DOM<10,000 da water fraction, after the Septic tank water. Green Pond had the second greatest oxygen consumption in the DOM > 10,000 da size fraction.

The oxygen consumption for the total water, for all sites, was nowhere near what I would have expected. I expected that the oxygen consumption of total water would be approximately equal to the sum of the oxygen consumption in the fractionated water. Perhaps there is some sort of inhibition to bacterial growth that is present in the larger size fractionated and total water that is not present in the smallest size fractions (Figure 6).

I compared the oxygen consumption to the DON. I found a correlation between increased oxygen consumption and increased DON. This correlation does not include three of the septic tank fractions, as their DON values were not accurately measured. This correlation had an r-squared value of 0.69 (Figure 7).

I anticipated that the TDN values would remain constant over the course of the incubation. My results indicate that the TDN increased in all of the Washburn Island size fractions. This result is probably an artifact. The TDN seemed to remain relatively constant in the Green Pond groundwater. In the Septic tank water, the TDN seemed to decrease slightly in all of its size fractions except the DOM<1000da size fraction. In this fraction almost all of the TDN vanished over the course of the incubation. This is probably due to the fact that this sample went anoxic and underwent denitrification.

The larger picture of the TDN results is what I expected. The TDN in the Washburn Island groundwater ranges from 1.0 μM to 27.0 μM. The TDN in the Green Pond groundwater ranges from 6.2 μM to 54.5 μM. The Septic tank TDN values range from 4.6 μM to 1138.3 μM. These data illustrate that Green Pond had more nitrogen in its groundwater than the Washburn Island groundwater, and that this increase in nitrogen in the Green Pond groundwater is primarily due to nitrogen inputs from septic tanks (Figure 8).

There was a decrease in the DIN in all of the size fractions in all of the sites. This decrease indicates that the nitrogen was immobilized over the course of the incubation. There are stark trends in the DIN components in the various sites. The Washburn Island DIN ranged from 1.2 μM to 9.6 μM. The Green Pond DIN ranged from 2.1 μM to 34.6 μM. The Septic tank DIN ranged form 2.9 μM to 972.7 μM. The Green Pond DIN range is approximately five times higher than the Washburn Island DIN range. The septic tank DIN range is roughly thirty times greater than the Green Pond DIN range. This trend supports the hypothesis that DIN is being added to Green Pond via septic tanks (Figure 9).

The DON value for each site was calculated by subtracting the DIN component from the TDN component. The DON calculated for the Septic tank samples were discarded due to inaccuracy in the methodology with multiple dilutions. The Washburn Island DON values ranges from 0.8 μM to 23.8 μM. The Green Pond DON values ranged from 6.3 μM to 38.4 μM. The general trend from the DON data is that DON was produced over the course of the incubation. This is illustrated by the fact that the final DON for all of the Washburn Island and Green Pond water samples were greater than the initial DON values (Figure 10).

Lastly, I compared the DON difference (final DON minus initial DON) to the DIN difference (final DIN minus initial DIN). I found a positive correlation between decreasing DIN and increasing DON. This correlation had an r-squared value of 0.99. This comparison included all water samples from all sites. These data indicate that DIN was converted into DON over the course of the incubation (Figure 11).

DISCUSSION

Washburn Island groundwater had the least relative DIN and greatest relative DON component of the three sites. This follows from the assumption that Washburn Island is a pristine standard. However, the presence of any DIN in the Washburn Island groundwater is surprising, as I am unclear as to where the DIN would be originating from. I would have expected to see a greater proportion of DIN in the Green Pond groundwater, due to its heavy load of septic tanks surrounding the pond. The Septic tank nitrogen source being primarily comprised of DIN is exactly what I expected to see.

The oxygen consumption results imply that the DOM<1000da fraction is the most labile fraction, as hypothesized. It is unclear as to why the total water oxygen consumption values are nowhere near the sum of the fractionated water samples. It seems possible that there is some sort of inhibitory process that inhibits microbial activity in the larger size fractions and in the total water that is not present in the smallest size fraction. This surprising result warrants further investigation if it is confirmed by further experimentation.

Both the TDN and DIN measurements indicate that the majority of the nitrogen in the water at all sites is in the DOM<1000da size fraction. This is what I expected to see, given that most nitrogen compounds are small. Secondly, the oxygen consumption data indicates that Washburn Island groundwater was the most labile in three of the four size fractions. The greater lability of the DOM in the pristine site suggests that anthropogenic effects are decreasing the lability of DOM. These results require further investigation if they are confirmed by repeat experimentation.

The results of this study suggest that over the course of the incubation the DIN was converted into DON. Since DIN is not labile and DON is labile, the microbial communities in my experiment converted DIN into DON over the course of the incubation. This conversion of nitrogen from DIN into DON must be further explored.

On Cape Cod, groundwater carries the majority of nitrogen into aquatic ecosystems. As such, groundwater is a key component in contributing to the harmful changes occurring in coastal waters. Wastewater and fertilizer inputs from houses can decrease the lability of the DOM. More research in needed to test the theories suggested in this paper. The chemical composition of groundwater is a relatively new field that must grow in order to preserve our coastal ecosystems.

ACKNOWLEDGEMENTS

I would like to thank Dr. Kenneth Foreman, Dr. Joseph Vallino, and Jen Bowen for their help in the design of this research. I would like to thank Leslie Graham, and Ian Washbourne for their assistance in the field and with nutrient analyses. Lastly, I would like to thank Nicole Chiota for her collaborative research.

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FIGURES

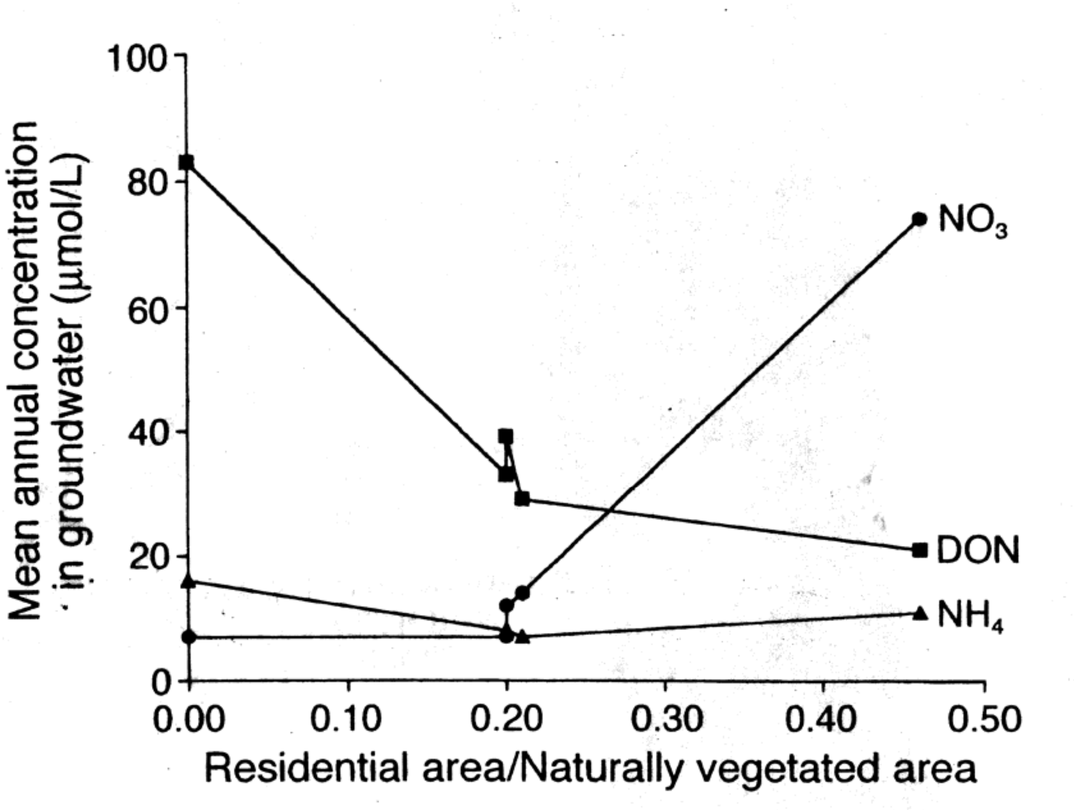

Figure 1: Changing land use may alter the form of N-loading as well as the amount (from Valiela, et. al. 2000)

Figure 2: Aerial photograph of Washburn Island

Figure 3: Aerial photograph of Green Pond

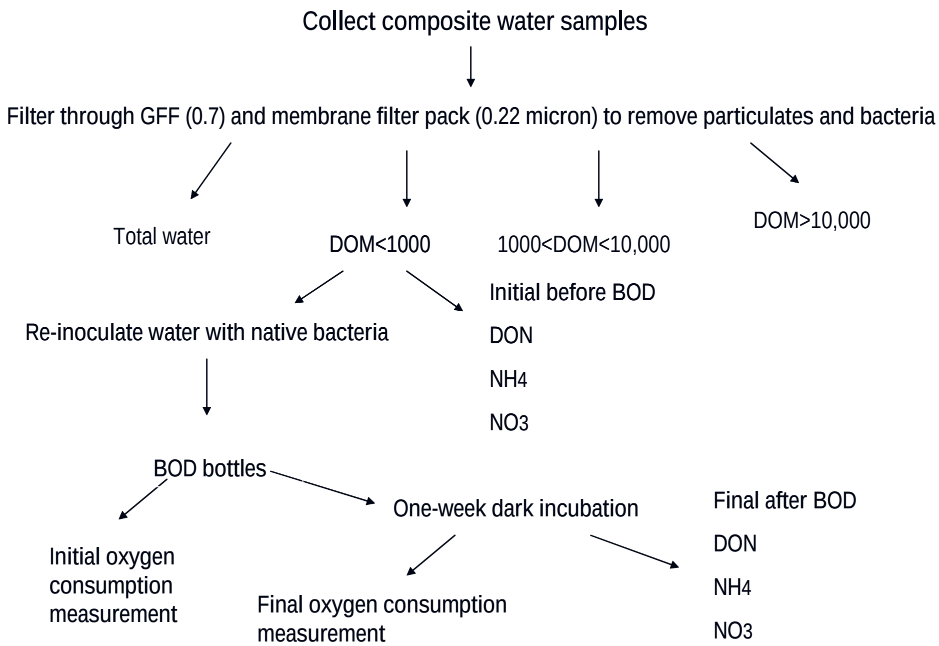

Figure 4: Protocol for methods of one representative size fraction

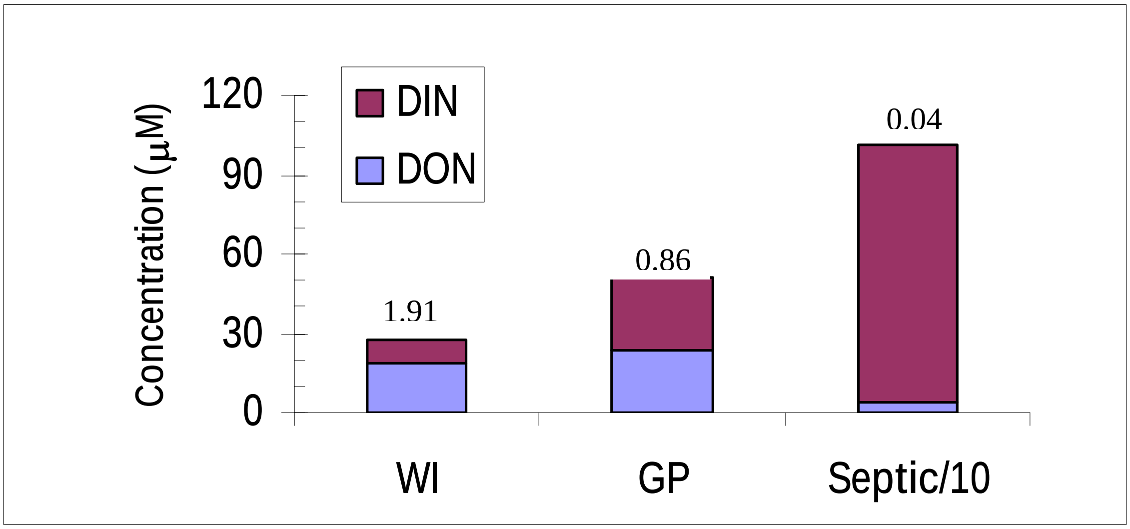

Figure 5: Nitrogen sources for the total initial water at all sites including DON:DIN ratio
Note: Septic tank DON and DIN values on graph are 1/10 of their actual values

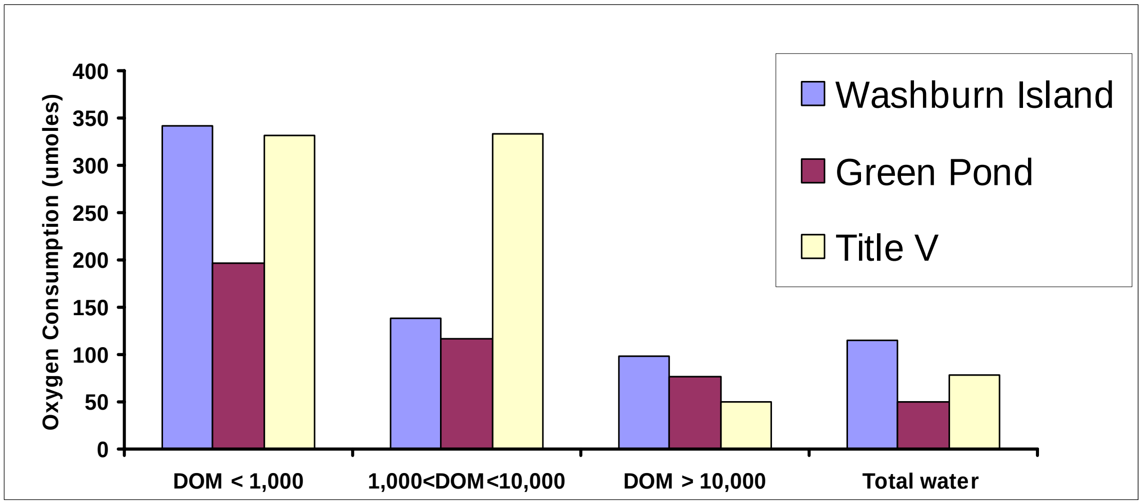

Figure 6: Oxygen consumption comparison among sites and size fractions

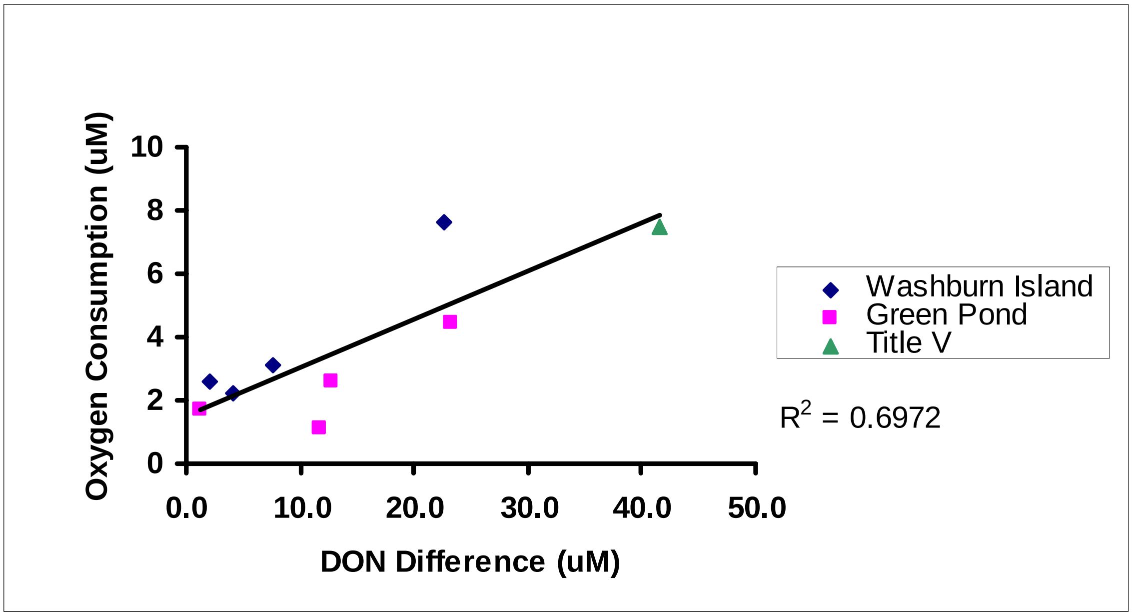

Figure 7: Oxygen consumption vs. DON Difference for all sites and size fractions
Note: Some Septic Tank data is not plotted because it was inconclusive

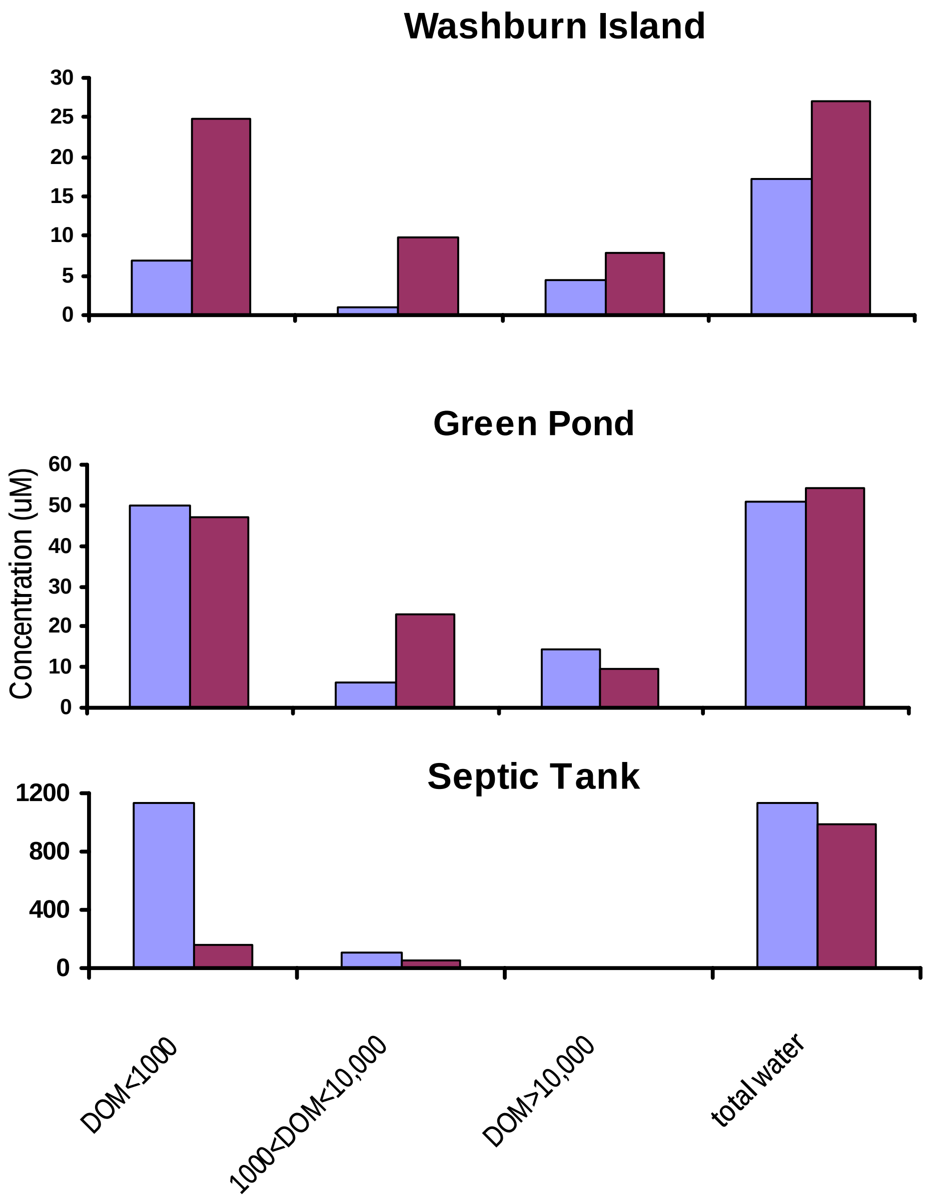

Figure 8: TDN for all sites and size fractions

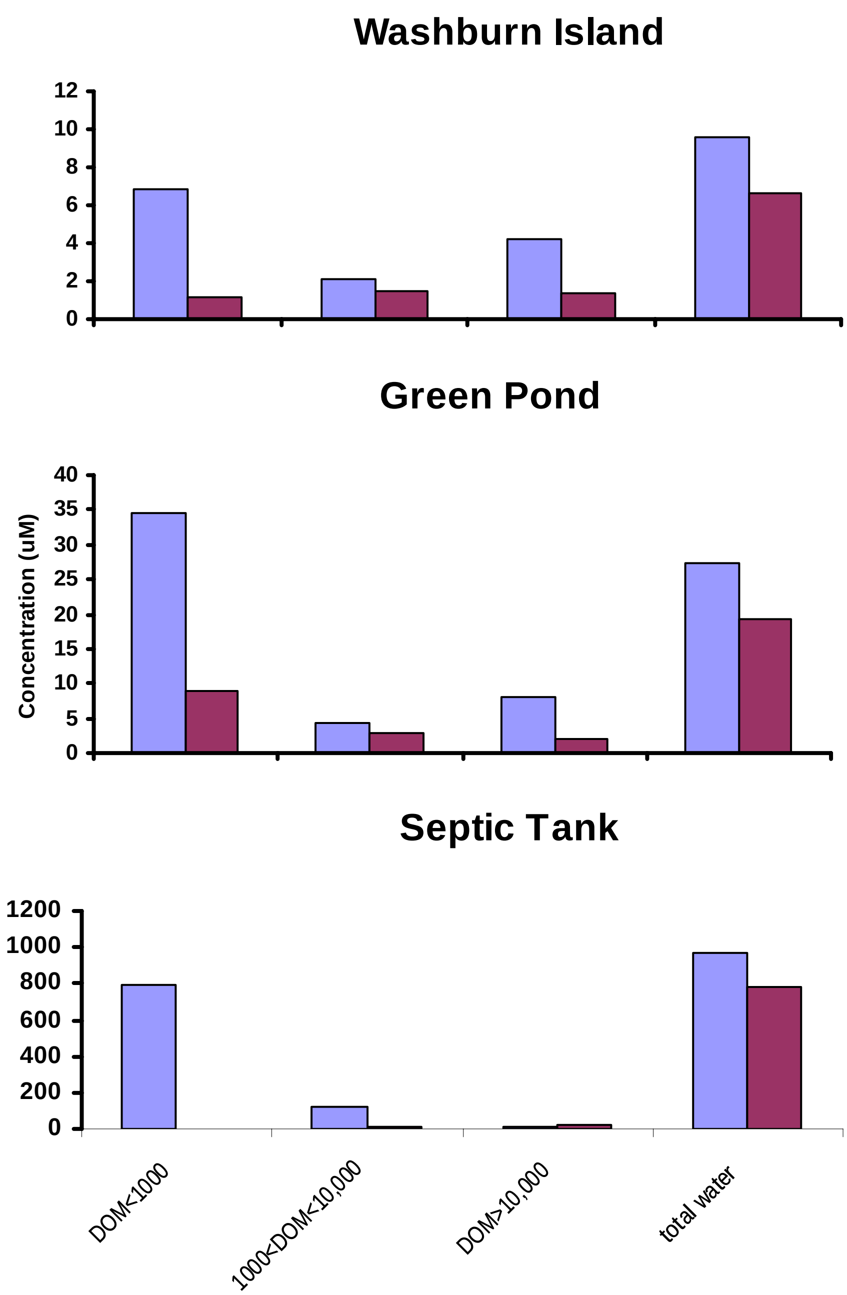

Figure 9: DIN for all sites and size fractions

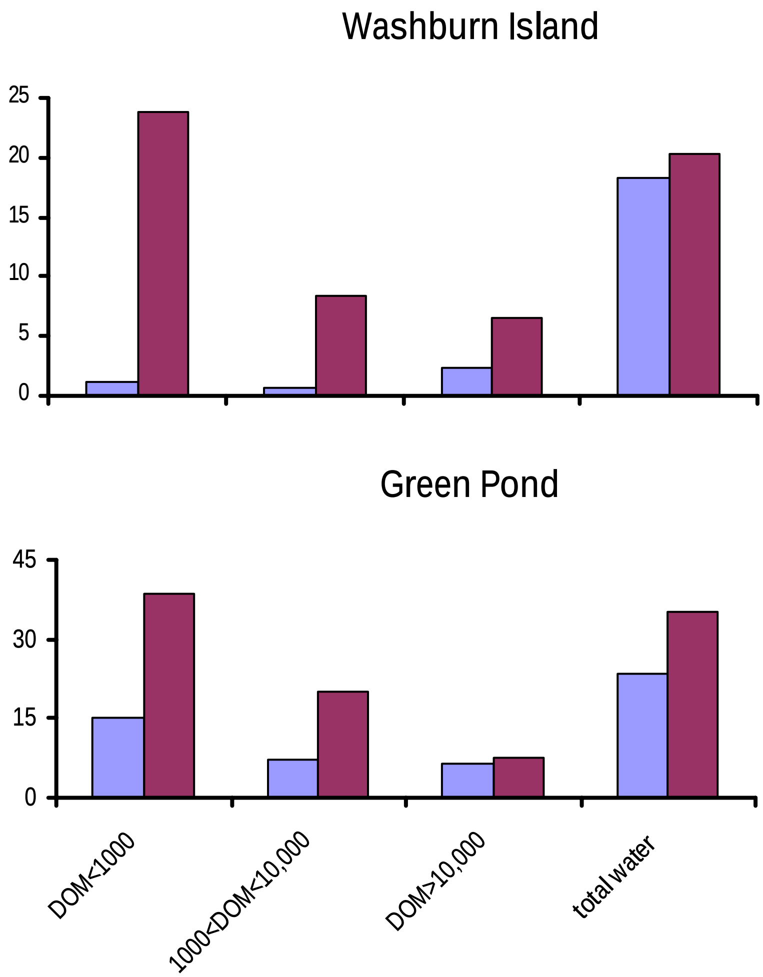

Figure 10: DON for Washburn Island and Green Pond
Note: Septic Tank DON data was inconclusive

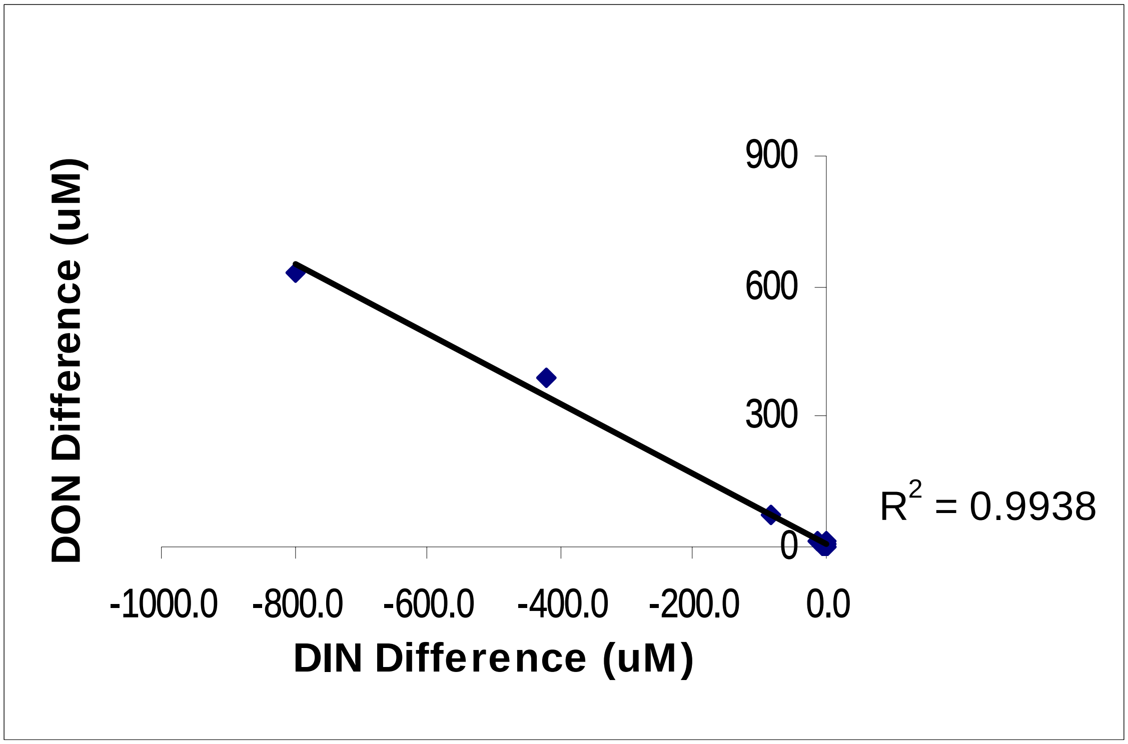

Figure 11: DON Difference vs. DIN Difference for all sites and size fractions