MISSISQUOI BAY SEDIMENT PHOSPHORUS CYCLING:

THE ROLE OF ORGANIC PHOSPHORUS AND SEASONAL REDOX

FLUCTUATIONS

A Thesis Presented

by

Lydia Gene Smith

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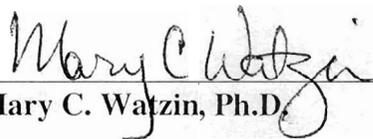**Specializing in Geology**

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Accepted by the Faculty of the Graduate College, The University of Vermont, in partial fulfillment of the requirements for the degree of Master of Science, specializing in Geology.
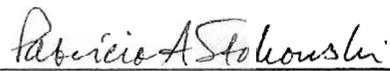
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ABSTRACT

Missisquoi Bay, Lake Champlain is a eutrophic, northern shallow freshwater bay that experiences toxic cyanobacteria blooms during the summer months, largely as result of high nutrient (P and N) loading from the agricultural watershed. The sediments, which contain minerals that readily sorb P, can act as a sink or source of water column nutrients. Phosphorus, both inorganic and some organic forms, sorbs to metal oxides at neutral pH in the sediment, thus P release into overlying and pore water can be significantly affected by the reduction and subsequent solubilization of these oxides. This study addresses novel aspects of nutrient cycling in lake sediments as part of a larger study to better understand the link between phosphorus forms, mobility, and cyanobacteria blooms. These aspects include: 1) diel and seasonal sediment redox fluctuations and 2) the role of organic P (P_{org}) in overall P mobility within sediments as a function of depth and time.

Missisquoi Bay sediment porewater redox chemistry was monitored across diel and seasonal cycles over the course of two summers (May-October, 2007 and 2008) by using *in-situ* voltammetry. Redox chemistry was monitored at the sediment-water interface (SWI) continuously over diel cycles, and the vertical concentration profiles of several key redox species (O_2, Mn^{2+}, Fe^{2+}, and $FeS_{(aq)}$) were obtained from cores collected at different times. The sediments were then analyzed for Total P (TP), Reactive P (RP), P_{org}, Mn, Fe, Ca, Al, Total Organic C and N.

A bloom did not occur in Missisquoi Bay during the summer of 2007, but did in summer of 2008, providing an opportunity to compare the sediment chemistry between non-bloom and bloom conditions. Increasingly anoxic SWI conditions across summer 2008 were observed but the SWI remained oxic for the duration of summer 2007. Significant changes in diel cycle redox chemistry at the SWI were also detected in both summers. Reactive P in the surface sediments decreased across the 2008 season but not in 2007. A strong correlation found between RP and RFe (operationally defined as Fe(III)OOH) suggests that a significant portion of sediment P (30-40%) is closely associated with Fe(III)OOHs, which are susceptible to reduction in anoxic conditions. Phosphorus mobility from the sediment into the water column can be limited by the amount of Fe(III)OOH at the surface, thus P flux from the sediments would be greatest when reducing conditions promote solubilization of these minerals. Completely anoxic surface sediments were only observed during the presence of a bloom, explaining the loss of RP in the surface sediments in 2008 in the late summer. Organic P species represent 18-26% of the P in sediments and the lack of a definite, consistent trend of P_{org} fractionation across the season suggests that there is variable mobility and degradation of these complex organic compounds on small timescales.

The loss of RP from the sediment in 2008 could have contributed to an estimated water column P increase on the order of thousands of µg/L, which in addition to measured increases in NH_4^+ gradients and subsequent N flux estimates in the upper sediment, could have sustained the bloom for an extended period of time. The relationship between the bloom and reducing sediment conditions suggest that bloom dynamics enhance nutrient release from the sediments, allowing for proliferation and sustainability of the bloom.

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CHAPTER 1: INTRODUCTION

Anthropogenic eutrophication (nutrient enrichment) of lakes and water bodies is a global issue regarding the quality of water resources and the health of aquatic ecosystems. Excessive nutrients (specifically P and N) in lakes often result in toxic blue-green algae blooms, the growth of weedy plants, and taste, odor, and water supply filtration problems (Smith, Tillman, & Nekola, 1999). Approximately 47% of assessed lakes in America are listed as impaired and eutrophication is the primary source of impairment (US EPA, 2000). Agricultural runoff was reported as the main source of nutrient enrichment for 41% of the impaired lakes (US EPA, 2000).

Lake Champlain is a large freshwater lake spanning 120 miles north to south with portions in New York, Vermont, and Quebec, Canada (LCBP, 2004). The majority of the watershed and basin population is in Vermont (56% and 68%, respectively). The lake supplies drinking water to approximately 200,000 people. Although only 16% of the land use in the basin is agricultural, 38% of P load is attributed to agricultural runoff. Missisquoi Bay is the most eutrophic section of the lake and 26% of the Total P (TP) load into Lake Champlain originates from its watershed (LCBP, 2008). The average total DP concentration in Missisquoi Bay (1994-2007) is 49 µg/l, with a range between 6 and 111 µg/l (VT ANR, 2009); lakes are classified as eutrophic if the average total DP concentration in the water is 35 µg/l. As a result, toxic cyanobacteria blooms typically form in the bay in the summer months.

Although efforts are in place to reduce point and non-point source nutrient loading into Missisquoi Bay and Lake Champlain, (e.g. VT Clean and Clear Action Plan) a massive amount of nutrients are tied up in the sediments (e.g. Druschel et al. 2005) which can be released under specific conditions (internal loading), perpetuating the eutrophic state and the presence of cyanobacteria blooms. Internal loading has been found to reduce the recovery rate of other eutrophic lakes despite drastic reductions in external loading via river drainage (e.g. Penn, 2000; Perkins & Underwood, 2000; Peticrea & Arocena, 2001; Søndergaard, et al. 2003).

Phosphorus exists in sediments as inorganic (PO_4^{3-}, P_i) or organic ($R\text{-}C\text{-}O\text{-}PO_3^{2-}$ of $R\text{-}PO_3^{2-}$, P_{org}) forms, whose mobility and bioavailability within the sediments are controlled by association with minerals (especially by sorption) and the recalcitrance of certain organic P (P_{org}) moieties. Fundamental processes that affect internal nutrient loading in lakes are largely processes that affect P-mineral association and include changing redox conditions, pH, and nutrient speciation. Phosphorus readily sorbs to metal oxides at neutral pH in the sediment, thus P release into overlying and pore water can be significantly affected by the reduction and subsequent solubilization of these oxides. This study addresses novel aspects of nutrient cycling in lake sediments as part of a larger study to better understand the link between phosphorus mobility and cyanobacteria blooms. These aspects include: 1) diel and seasonal sediment redox fluctuations and 2) the role of organic P in overall P mobility within sediments as a function of depth and time. To our knowledge no attempt to describe the diel and

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seasonal variation in redox chemistry in sediments and at the sediment water interface

(SWI) during a cyanobacteria bloom exists.

CHAPTER 2: LITERATURE REVIEW

Missisquoi Bay Study Site

Missisquoi Bay is located in the northeastern quadrant of Lake Champlain with 58% of its watershed in Vermont and 42% in the Province of Quebec, Canada (Troy, et al, 2007). The entire watershed (Figure 1) is 3,105 km^2 (767,246 ac), the surface area of the bay is approximately 77.5 km^2 (19,150 acres), and the maximum depth is 4 m (Hegman, et al. 1999). The

Figure 1. Missisquoi Bay Watershed Map (LCBP, 2007)**.** 58% the watershed in is in Vermont, USA and 42% is in Quebec, Canada.

Missisquoi, Pike, and Rock Rivers are the major tributaries and Dead Creek is a minor tributary (VT ANR DEC, 2004) of Missisquoi Bay; Missisquoi River is the largest tributary draining an area of ~850 mi^2. The average discharge of the Missisquoi River at Swanton, VT (USGS station 04294000) from 1990-2006 was 1,734 cfs and the average peak discharge was 22,150 cfs (USGS, 2007); the lowest peak discharge was in September of 1999 (11,900 cfs) and the highest peak discharge was in January of 1998 (32,300 cfs). The Pike River is the second largest tributary with an average discharge of 282 cfs from May 1990 to October 2005 (MDDEP station No. 030420, obtained from Eric Smeltzer, 2008). The data collected for this study was obtained at GPS Coordinates N44° 59.5'03" W73° 06.7'98", the site is referred to as Highgate Springs.

Phosphorus loading from the Missisquoi Bay watershed is by far the highest of Lake Champlain's nineteen watersheds (Figure 2, LCBP, 2008). Although only 25% of the watershed is agricultural land, 65% of the total P load (97,680 kg/year out of 149,628 kg/year total) originates from agricultural runoff (Table 1, Troy et al., 2007). The average total phosphorus (TP) concentration, 49 µg/L, (VT DEC, 2008) within the bay for the past 14 years (1993-2007) has been well above the goal of 25 µg/L set by Vermont and New York to meet the guidelines of the EPA Clean Water Act (LCBP, 2008). The average nitrate (NO_3-N) concentration for the same time period was 750 µg/L, and the State of Vermont Water Quality Standards mandate that nitrates do not exceed 5.0 mg/L in lakes, ponds, and reservoirs (VT NRD, 2008).

Table 1: 2001 Phosphorus loading into Lake Champlain by lake segment and land use type as calculated by Troy et al. (2007) using the loading method. The Vermont portion of Missisquoi Bay's watershed is the leading contributor of P to the lake.

LCB 2001 – Loading Method		Pollution Load (year)				Source of Load			Land Use			Area Load
Lake Segment	Area (ha)	URB (kg)	AG (kg)	FOR (kg)	Total (kg)	URB (%)	AG (%)	FOR (%)	URB (%)	AG (%)	FOR (%)	kg/ha
Burlington Bay	1,419	1,683	8	10	1,702	98.9	0.5	0.6	83.6	1.6	13.5	1.2
Cumberland Bay	174,186	15,884	4,106	8,031	28,020	56.7	14.7	28.7	6.1	6	78.5	0.16
Isle La Motte-NY	97,332	8,908	9,043	3,255	21,206	42	42.6	15.4	6.9	26.3	62.5	0.22
Isle La Motte-QUE	6,022	708	1,348	87	2,142	33	62.9	4	8	57.6	26.3	0.36
Isle La Motte-VT	6,889	1,091	1,361	103	2,554	42.7	53.3	4	11.8	54.8	29.3	0.37
Main Lake-NY	257,944	19,450	5,915	11,443	36,808	52.8	16.1	31.1	5.6	6.5	85.3	0.14
Main Lake-VT	281,542	55,098	13,302	14,249	82,649	66.7	16.1	17.2	11.8	10.8	75.4	0.29
Malletts Bay	201,022	34,501	14,416	11,344	60,260	57.3	23.9	18.8	9.2	14.3	74.1	0.3
Missisquoi Bay-QUE	131,645	14,148	26,259	6,248	46,654	30.3	56.3	13.4	6	24.3	67.9	0.35
Missisquoi Bay-VT	180,900	23,162	71,421	8,391	102,974	22.5	69.4	8.1	7	25.1	64.8	0.57
Northeast Arm	23,350	3,717	3,689	498	7,904	47	46.7	6.3	11	40.6	38.3	0.34
Otter Creek-NY	1,071	42	8	55	105	40.2	7.8	51.9	2.8	2	93.8	0.1
Otter Creek-VT	286,559	35,214	30,798	10,199	76,211	46.2	40.4	13.4	8.2	27.7	60.5	0.27
Port Henry-NY	23,857	2,417	955	954	4,325	55.9	22.1	22.1	7.6	11.2	79.4	0.18
Port Henry-VT	3,166	793	738	28	1,560	50.9	47.3	1.8	18.1	63.1	17.1	0.49
Shelburne Bay	17,940	6,249	2,909	380	9,538	65.5	30.5	4	23.5	39.2	35.2	0.53
South Lake A-NY	96,487	8,015	2,214	3,845	14,075	56.9	15.7	27.3	5.8	6.1	73.6	0.15
South Lake A-VT	17,394	1,688	4,052	248	5,987	28.2	67.7	4.1	7	63.1	26.6	0.34
South Lake B-NY	98,565	12,636	10,968	3,185	26,788	47.2	40.9	11.9	8.7	28.9	58.5	0.27
South Lake B-VT	100,314	11,192	8,043	3,980	23,215	48.2	34.6	17.1	7.4	20	68.3	0.23
St. Albans Bay	13,056	3,529	3,011	231	6,771	52.1	44.5	3.4	16.4	53.3	28.4	0.52
Total	2,020,660	260,124	214,563	86,762	561,449	46.3	38.2	15.5	8.1	18.3	69.5	0.28

Figure 2: Average Total P concentrations in Lake Champlain by lake segment (LCBP, 2008). Missisquoi Bay has not met target P concentrations in the last 17 years and P concentrations appear to be increasing.

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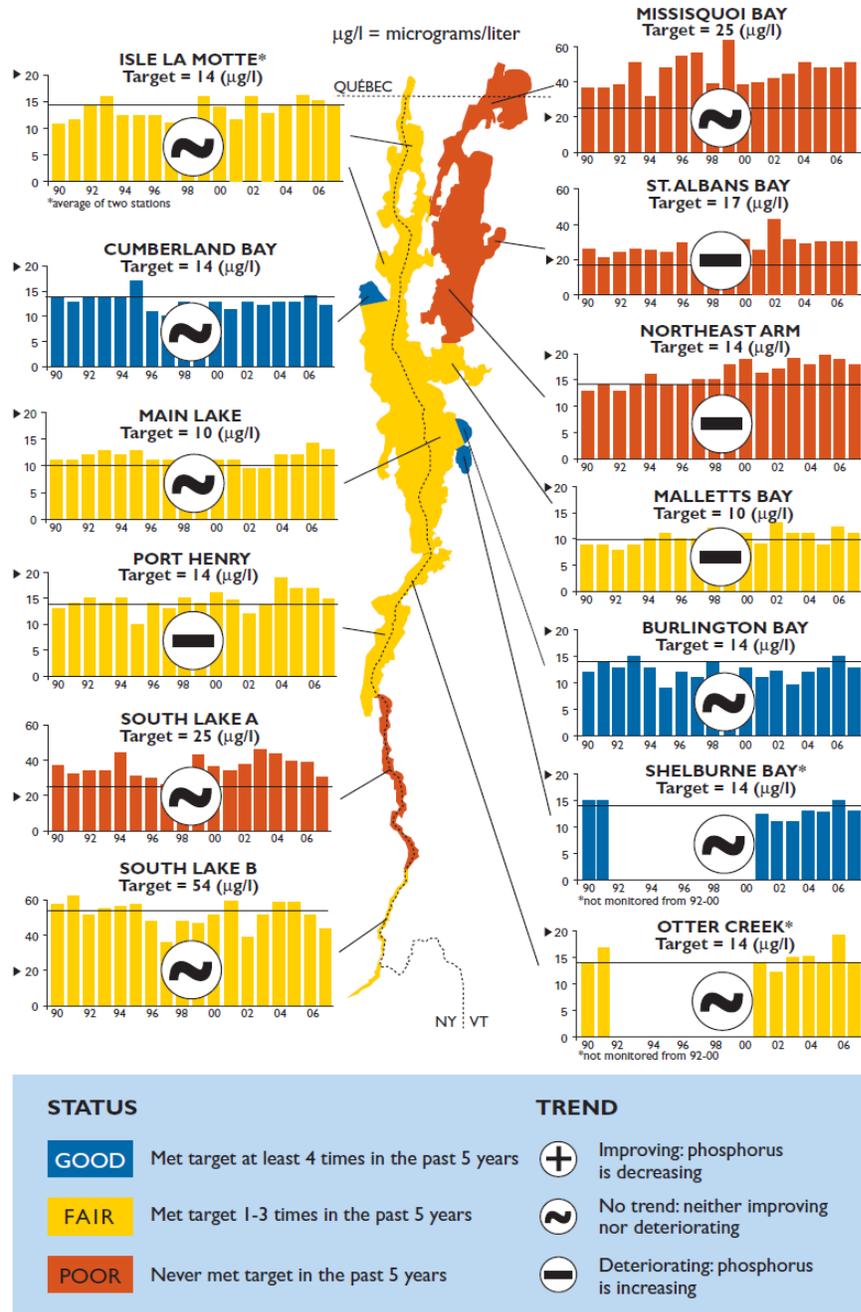
Brief Overview of the Geology of Lake Champlain Basin

The underlying bedrock of Missisquoi Bay is predominately shale, of the Iberville Shale unit (VGS, 1961). Contacting bedrock units making up the eastern shoreline consist of a dolomite and sandstone unit (Dunham Dolostone) overlying an undifferentiated slate, shale, limestone, and phyllite unit (Iberville Shale); the Champlain Thrust Fault separates these units. The western shore geology consists mostly of a dolomite, sandstone, limestone, and a shale unit, making up the Champlain Isles (Glens Falls Unit). The Missisquoi River basin and its tributaries drain through a wide array of surficial geologic materials consisting of mostly glacial till toward the eastern edge of the watershed (not including the river channel) which changes to lacustrine and marine silts, clays, sands, and gravels to the west (in closer proximity to the bay) (VGS, 1970). The surficial geology of the river channel itself is comprised of ice contact gravel, lacustrine and marine clays, silts, sands and gravels, recent stream alluvium, and peat and muck. The bedrock units underlying the watershed consist of (generally from east to west): schistose greywacke; light and dark colored plutonic igneous rocks; quartzite, shale, limestone and dolomite; interbedded slate, dolomite, limestone, sandstone, and shale; dolomite; limestone; and undifferentiated slate, limestone, and phyllite.

The oldest sediments in the Lake Champlain basin, including Missisquoi Bay are the remnants of the relatively recent glacial history. Overlying the bedrock is glacial till (mix of gravel, sand, silt, and clay) resulting from the occurrence of the Laurentide Ice Sheet (20,000-12,500 ya) (Wright, 2004). The glacial till is overlain by laminated silt and clay layers that were deposited during the existence of Glacial Lake Vermont which

covered the entire Champlain Basin after the retreat of the ice sheet about 12,500 ya. By

12,000 ya, the glacial dam forming Lake Vermont failed and most of the lake drained.

As ice continued to retreat sea water from the Atlantic mixed with the fresh water via the

St. Lawrence estuary and the Champlain Sea was formed, resulting in marine sediment

deposition ("massive grey silt and clay" (Wright, 2004) with abundant marine fossils

such as clams and the famous 'Charlotte Whale', a beluga whale). As ice continued to

retreat, the land rebounded, cutting off the estuary and saltwater influx and the lake

gradually returned to freshwater. It is estimated that Lake Champlain has been

freshwater for about 9,000 years.

Eutrophication

Phosphorus and nitrogen are essential nutrients for plant growth (including

phytoplankton) in both aquatic and terrestrial systems; the lack of either of these nutrients

within a system will limit plant growth (e.g. von Liebig, 1855; Schlesinger, 1991;

Vitosek and Howarth, 1991). Lakes and water bodies will naturally progress from a

nutrient lacking state (oligotrophic) to a nutrient rich state (eutrophic) as they age

(eutrophication). Although eutrophication is a natural process, anthropogenic inputs of P

and N into aquatic systems expedites the process and can result in undesirable conditions

relatively quickly.   Anthropogenic eutrophication of lakes can result in: the reduction of

phytoplankton biodiversity (taxa composition may shift to becoming toxic or inedible);

increased biomass of phytoplankton and periphyton (e.g. blue green algae blooms);

reduced water clarity; public health issues; growth of weedy plants; taste, ordor, and

water supply filtration problems; elevated pH; dissolved oxygen depletion in the water column; increased fish production and harvest; increased probability of fish kills; and overall decline in the health of a lake's ecosystem and water quality (Smith, 1998; Smith, Tilman, & Nekola 1999).

In 2000, water quality assessments of the lakes in America (43% of total lake acres were assessed) concluded that 47% were impaired, with eutrophication being the predominant contributor of the impairment (US EPA, 2000). Agriculture was reported as the main source of nutrient enrichment for 41% of the impaired lakes. Anthropogenic sources of nutrients have become detrimental to water quality and aquatic ecosystem health; thus, much research has gone in to identifying the sources and mechanisms of nutrient supply to lakes. Anthropogenic nutrient inputs are classified as point sources or non-point sources. Point sources are considered to be localized and more easily controlled whereas non-point sources are more difficult to regulate or monitor because they are diffuse (Smith, Tilman, & Nekola 1999); a comprehensive list of possible sources, both point and non-point, are included in Table 2.

Missisquoi Bay is currently impaired from the high amount of nutrient loading from its watershed (see previous section on Missisquoi Bay Study Site) and as such a remarkable decrease in the phytoplankton diversity and increase in total plant biomass (weeds and dominant phytoplankton species) has been observed (Brown et al. 1991, 1992; Watzin et al. 2005). Specifically, cyanobacteria species *Microcystis aeruginosa* and *Microcystis wesenergii* now dominate the phytoplankton community and often form surface scums in the summer months (Watzin et al., 2003). These blue-green algae

blooms often result in the production of cyanotoxins (such as microcystin) which poses a

threat to human health and enjoyment of the bay.

Table 2: Potential point and nonpoint sources leading to nutrient enrichment in water supplies.
Modified from Smith, Tilman, & Nekola, 1999.

Point sources	Nonpoint sources
- wastewater effluent (municipal and industrial) runoff and leachate from waste disposal sites - runoff and leachate from animal feedlots - runoff from mines, oil fields, and unsewered industrial sites - storm sewer outfalls from cities with populations - overflows of combined storm and sanitary sewers - runoff from construction sites with an area	- runoff from agriculture (including return flows from irrigated agriculture - runoff from pastures and rangelands - urban runoff from unsewered areas and sewered areas with populations <100,000 - septic tank leachage and runoff from failed septic systems - runoff from construction sites with an area <2 ha - runoff from abandoned mines - atmospheric deposition over a water surface - activtities on land that generate contaminates such as logging, wetand conversion, construction and development of land or waterways

Cyanobacteria respond to environmental characteristics such as phosphorus,

nitrogen, and iron concentrations, light, wind, and temperature (e.g., Visser et al., 2005).

A debate as to whether P or N is the limiting nutrient for cyanobacteria and other

phytoplankton exists, although the most common perception is that P is the limiting

nutrient (Hecky & Kilham, 1988). Schindler (1977, 2007, 2008) found a significant

relationship between mean annual P concentration and mean annual chlorophyll (*chl*)

concentrations in The Experimental Lakes Area, Ontario. He determined that P

ultimately limits phytoplankton growth in lakes because they can fix N and C from the

atmosphere. Several other studies were extensively reviewed by Hecky & Kilham (1988)

and they concluded that excessive P was the most likely cause for algal blooms. Despite

these findings, the limiting nutrient for Missisquoi Bay is still in question, although

Smeltzer et al. (2008, unpublished) found a significant relationship between TP and *chl* a

and TN and *chl* a values in Missisquoi Bay from 1995 to 2007, indicating that P and N

are both important controls of phytoplankton abundance (Figure 3).

Blue-green algae blooms have been a persistent threat to many impaired water

bodies for years yet the specific reasons for bloom duration, intensity and toxicity remain

unclear. Most likely, the onset of algal blooms is due to a number of complex

interactions among biota, nutrients, and climate.
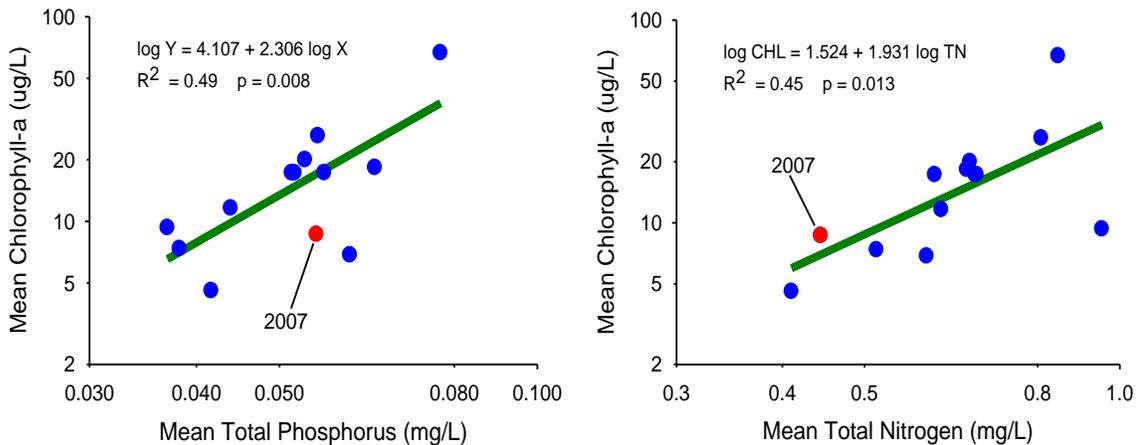
Figure 3: From Smeltzer et al. 2008. Linear regression of yearly mean July-September chl-a vs. TP (left) and TN (right) in Missisquoi Bay, 1995-2007.

Internal Loading

Internal loading occurs when conditions within sediments of a lake allow for P

and N release into the water column, thereby increasing the TP (specifically available or

reactive P (RP)) and TN of the water column, regardless of external loading. Penn et al.

(2000) estimated internal loading can contribute up to 80% of the TP input of a lake

under specific circumstances. Several examples exist where recovery speed is reduced

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and eutrophic conditions persist despite the reduction of external loading of nutrients (Druschel, 2005; Peticrea & Arocena, 2001; Penn, 2000; Perkins & Underwood, 2000, Jeppesen et al. 1991). Shallow lakes are particularly susceptible to internal loading due to the high sediment surface to water column ratio. Often, the P pool in the sediments is 100 times higher than the water column in eutrophic lakes and P can be released from as deep as 20 cm below the SWI (Sondergaard, Jensen, & Jeppesen, 2003), although Hosomi & Sudo (1992) suggested that the top 3 cm of the sediment surface are the most active with respect to P cycling. In general, the extent and significance of internal loading is dependent on the type of lake and its associated morphology and biological structure.

Lake sediments, comprised of complex mineral aggregates, water, and inorganic and organic chemical components, can act as both a sink and a source of P (Christophoridis and Fytianos, 2006). P readily sorbs to Fe, Mn, and Al oxyhydroxide surfaces in sediments which can permanently or temporarily remove it from the water column. Complex physical, chemical, and biological processes contribute to phosphorus release from the sediment including: desorption, ligand exchange, dissolution of particles, mineralization processes, release from living cells, and autolysis of cells (Christophoridis and Fytianos, 2006). Physical and chemical parameters such as temperature, pH, redox potential, nitrates, sulfates, bioturbation, and the presence of Ca, Mn, Fe, Al and Mg have been found to control or play a part in P release (Kleeberg and Kozerski, 1997). These physical, chemical, and biological controls associated with internal loading are discussed further in the following sections.

Physical Controls

Physical controls of P release from the sediment include resuspension, bioturbation, and advective transport. Temperature and light may be included as a physical control, however the influence of temperature and light in P release is mostly due to regulation of biological activity (Spears et al. 2008). As a result of underwater currents and mixing, P bound particulate matter can be resuspended indefinitely before permanently settling (Ekholm et al. 1997). Resuspension increases the amount of time and surface area of contact between sediment and water (Kristensen et al., 1992). Several studies (e.g. Jones & Welch,1990; Søndergaard, Kristensen, & Jeppesen, 1992) found that the internal P loading variation in a shallow lake was largely due to wind mixing resulting in variable amounts of resuspended particulate P. However, P release from resuspended sediments depends on water-phosphorus equilibrium concentrations and the biological demand for phosphorus; therefore, the extent to which resuspended particles contribute to the overall TP in the water may be dependent on seasonal P demand and P loading (Sondergaard et al. 1992; Horppila & Nurminen, 2001). Bioturbation effects the potential P release in sediments by increasing the water:sediment ratio and burrowing may provide a transport pathway for P released into the sediment porewater to escape into the overlying water (Fukuhara & Sakamoto, 1987; Søndergaard, 2003)

Jahnke et al (2003) found that advective transport due to pressure gradients played a role in nutrient input to the Satilla River Esuary, GA. Although the pressure gradient in this study was determined to be mostly tidally influenced, the water table level in a lake's surrounding watershed may influence potential nutrient loading via springs. The

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relationship between springs and nutrient loading in lakes has not been well studied but factors such as sediment type and depth of accumulation, bedrock type (susceptibility to controlled fractures), precipitation, and aquifer storage would need to be considered. The underlying bedrock of Missisquoi Bay is shale, which presumably would allow for preferential water flow based on hydrologic gradient. However, deeper overlying sediments are very clay rich, which may reduce the influence of springs at our site. Areas near the shore where the sediments are sandier would be more susceptible input of underground water due to hydrologic gradients and ease of flow through sand.

Chemical - Redox Conditions and P Release from Sediments

P Related Chemical Components of Sediments

Phosphorus exists in sediments as a soluble anion (orthophosphate, PO_4^{3-}), a precipitated phosphate salt, or as part of a mineral or organic compound. Possible phosphate minerals in lake sediments include: stregnite [$FePO_4 \cdot 2H_2O$], vivianite [$Fe_3(PO_4)_2 \cdot 8H_2O$], hydroxyapatite [$Ca_5(PO_4)_3(OH)$], monelite [$CaHPO_4$], and variscite [$AlPO_4 \cdot 2H_2O$] (Böstrom, 1988; Søndergaard, 2001; Christophoridis & Fyiantos, 2006). The average total and reactive amounts of P, Fe, Mn, and Al for the sediments from Missisquoi Bay are listed in Appendix G. Phosphorus, both inorganic (orthophosphate and polyphosphates) and organic forms (phosphate compounds with carbon, e.g. monoester phosphates), can also sorb to Fe(III), Mn, and Al (hydr)oxide surfaces, calcite, and clays (Søndergaard, 2001). Phosphorus sorption to these surfaces occurs by ion exchange (electrostatic attraction) or ligand exchange (hydroxyl substitution) although ligand exchange is more typical in sediments (Rhue & Harris, 1999). The extent of P

sorption and release from mineral surfaces is sensitive to redox conditions of the sediment (e.g., Roden and Edmonds, 1997; Van der Zee et al., 2003; Shenker et al., 2005; Druschel et al, 2006) and pH (Lijklema, 1976; Koski-Vahala & Hartikainene, 2001; Wang et al 2007). The redox reactive components in sediments in addition to pH fluctuations and equilibrium gradients are the primary chemical controls of phosphorus cycling from and within sediments.

Chemical and Biological Mn/Fe Reduction Pathways

Inorganic and organic P sorption and release is largely a function of the solubility of Fe and Mn oxides and hydroxides, which is controlled by the redox state of the sediment. Under oxic conditions (>200 mv at pH 7), Fe(III) oxides and hydroxides are insoluble and P sorption is favorable (Einsele, 1936; Mortimer, 1941; Lijklema, 1980; Søndergaard, et al. 2003) Anoxic conditions promote the reduction of insoluble FeOOH minerals to soluble Fe(II) resulting in P release from the surfaces into the surrounding water (Lijklema, 1980; Böstrom, 1982; Christophoridis and Fytianos, 2006). Mn oxides become reduced and dissolve more rapidly and under higher redox potentials than Fe(III) oxides, but otherwise behave similarly (Lovely, 1991). Redox controlled release is a function of the redox value, Fe:TP ratio, and the presence of other redox active compounds such as sulfates and nitrates (Böstrom et al., 1982; Perkins and Underwood, 2001;Søndergaard et al., 2003). The Fe:TP ratio corresponds to the number of available sites on the Fe mineral to which P can sorb; P sorption and release in sediments with an Fe:TP ratio greater than 15 by weight are considered to be controlled by sorption and release to iron as a function of oxygen supply (e.g. Jensen et al. 1992). In Missisquoi

Bay sediments, the average Fe:TP ratio is 34, with a range from 23-42, suggesting that Fe

plays a significant role in the sorption and release of P. Christophoridis and Fytianos

(2006) found that P release was most favorable under extremely reductive conditions (-

200mV) and at high pH values (~9).

Mechanisms for Fe and Mn reduction and subsequent P release include both

abiotic and biotic processes; examples of reduction pathways are included in equations 1-

6. Direct reduction is assumed to occur by abiotic Fe/Mn reduction or by anaerobic

Fe/Mn reducers. Abiotically, Fe and Mn can be reduced by organic compounds (eq. 1),

reduced sulfur compounds (eq. 2), and nitrite (eq. 3) in natural environments (Lovely,

1991). Fe(II) has also been shown to rapidly reduce Mn(IV) to Mn(III) or Mn(II) (eq. 4)

at pH 7 in sediments (Lovely & Phillips, 1988). It is generally believed that the presence

of nitrate inhibits Fe(III) reduction because it is the preferred terminal electron acceptor

for nitrate-and Fe(III) reducing organisms (Lovely, 1991). On the other hand, Ahl (1979)

found that the concentration of nitrate before complete denitrification directly

corresponded to the magnitude of P release from Fe. Jansson (1987) proposed that nitrate

stimulates microbial growth, but once depleted, nitrate reducers can utilize Fe(III) as an

electron acceptor instead.

Microorganisms can either reduce Fe and Mn through assimilatory or

dissimilatory pathways in which the Fe or Mn is reduced when assimilated into enzymes

or when serving as an electron acceptor. Sediment microorganisms can obtain energy for

growth through the oxidation/reduction coupling of organic compounds and Fe(III) or

Mn(IV) or through indirect metabolic processes, such as sulfur-oxidizing (eq. 5),

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hydrogen-oxidizing (eq. 6), and organic acid oxidizing Fe(III) and Mn (IV) reduction

(Lovely, 1991).

Roden and Edmonds (1997) found a correlation between phosphate release,

sulfate reduction, and iron-sulfate formation, suggesting another important Fe reduction

pathway (2). They attribute P release from Fe(III) oxides to the formation of insoluble

FeS by sulfate reducing bacteria, resulting in a terminal sink for Fe and release of PO_4^{3-}

to sediment pore water. Examples of Fe and Mn reduction equations include:

$$(1)\ 2\ \text{cysteine} + 2\ Fe(III) \rightarrow \text{cystine} + 2\ Fe(II) + 2\ H^+$$

$$\text{Pyruvate}^- + MnO_2 \rightarrow \text{acetate}^- + MnCO_3$$

$$(2)\ 2\ FeOOH + H_2S + 4H^+ \rightarrow 2\ Fe(II) + S^o + 4\ H_2O$$

$$3\ H^+ + MnO_2 + HS^- \rightarrow Mn^{2+} + S^o + 2H_2O$$

$$(3)\ NO_2^- + MnO_2 + 2H^+ \rightarrow Mn^{2+} + NO_3^- + H_2O$$

$$NO_2^- + 2MnO_2 \rightarrow Mn_2O_3 + NO_3^-$$

$$(4)\ 2Mn(IV) + 2\ Fe(II) \rightarrow 2\ Mn(II) + 2\ Fe(III)$$

$$(5)\ S^o + 6\ Fe(III) + 4\ H_2O \rightarrow HSO_4^- + 6\ Fe(II) + 7\ H^+$$

$$(6)\ \text{pyruvate}^- + 2Fe(III) + 2H_2O \rightarrow \text{acetate}^- + HCO_3^- + 2Fe(II) + 3H^+$$

$$\text{pyruvate}^- + Mn(IV) + 2H_2O \rightarrow \text{acetate}^- + HCO_3^- + Mn(II) + 3H^+$$
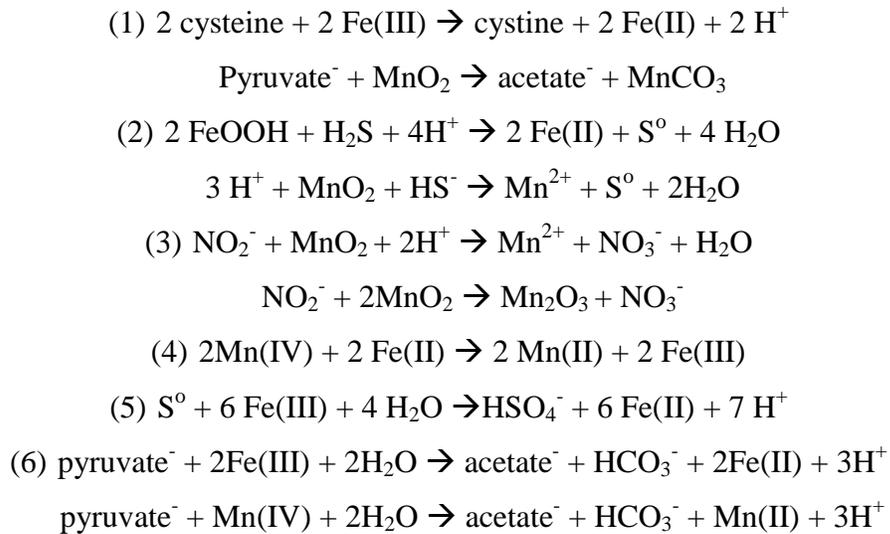
The pH can influence P sorption and release to sediments. At higher pH, OH^- are

likely to displace PO_4^- sorbed to hydroxides. The relationship between pH and P sorption

to FeOOH is represented by the following equation (Lijklema, 1980):
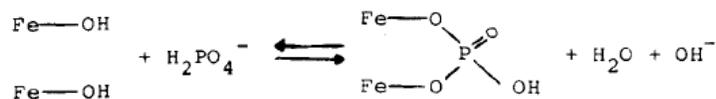
Additionally, the pH affects the surface charge of metals, where the zero point

charge (ZPC) is dependent on the pH ($pH_{ZPC.}$, Bebie et al., 1997). At pH above the pH_{ZPC}

of a given metal, the surfaces will be negatively charged. The pH_{ZPC} for FeS, FeOOH,

MnO_2, and MnO are 2, 9.70, 4.60, and 8.61, respectively. The pH in the water column of

Missisquoi Bay ranges from 6 to >9, which may affect the surface charge on FeOOH

minerals in the sediment surfaces. FeS has a low pH_{ZPC} of 2, therefore, when FeS is

formed as proposed by Roden and Edmonds (1997), PO_4^- is released because it is

repelled by the negative surface charge. Subsequent papers cite this as a valid P release

mechanism for suboxic to anoxic sulfidic sediments (e.g. Rozan et al., 2002, Wang et al.

2007). On the other hand, sediments may be relatively buffered; Cai et al. (2002) found

that pH actually drops from 7.2 to 6.8 in the top 10 cm of Lake Champlain sediments.

The pH drop corresponded to O_2 penetration depths where H^+ could be produced by:

$NH_4^+ + 2O_2 \rightarrow NO_3^- + H_2O + 2H^+$.

Sediment Organic Phosphorus Cycling

Aquatic organisms are comprised of phosphorus containing macromolecules such as nucleic acids (DNA and RNA), phospholipids membranes, and phosphate monoesters which enter the organic phosphorus pool upon death of the organism (Wetzel, 1999). Studies have shown that total organic P (P_{org}) constitutes about 1/3 of the TP in lake sediments (Wetzel, 1999; Williams and Mayer, 1972; Bostrom et al., 1982); however, P_{org} content varies among sediments according to the lake's morphology, hydraulic loadings, hydrodynamics, trophic state, and other factors (Wetzel, 1999). When assessing nutrient availability within natural systems, organic forms of phosphorus, such as inositol phosphates, are often considered immobile, refractory, or bio-unavailable. This is mainly because they do not react with molybdate, used in a colorimetric test that determines the concentration of bioavailable P, or soluble reactive P (McKelvie, 2007). However, sediment bacteria and microalgae enzymatically hydrolyze terminal phosphate groups of exogenous P_{org} compounds such as glycerophosphate, adenosine 5'-monophosphate (AMP), guanosine 5'-monophosphate (GMP), adenylic acids, phosphonate compounds, and others (Wetzel, 1999; Cambella et al., 1983, 1984). This mineralization process involving different P_{org} compounds, and the resulting release of inorganic phosphate plays an important role in the P cycle by contributing to the bioavailable P pool; an aspect that is not well characterized in lake sediments. Additionally, bacteria and algae are able to directly utilize dissolved P_{org} (DOP) (Bentzen & Taylor, 1992; Cotner & Wetzel, 1992). The bioavailability of organophosphates or the potential for organophosphates to be transformed into more bioavailable forms is dependent on biogeochemical reactions,

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mobility, and speciation within the sediment; all of which may be different in comparison to orthophosphate (P_i) mobility and bioavailability.

Rodel et al. (1977) found that P_{org} sorption and microbial catalyzed hydrolysis rates in lake sediments are related to the form of P_{org}. They compared additions of glycerophosphate, nucleotide P, and inositol hexakisphosphate (IHP) to lake sediment and found that although each quickly sorbed to the sediment, they did so at different rates and bonding strengths. Glycerophosphate and nucleotide P sorbed more slowly and hydrolyzed in the sediment much faster than IHP. Inositol hexakisphosphate did not hydrolyze at all in the length of their study. This was determined to be due to the strong interaction between the sediment and the six phosphate groups on the IHP molecule. They concluded that hydrolysis activity of microbes is impeded by P_{org} sorption affinities to the sediment, which is dependent on the compound. Preferential sorption of some P_{org} compounds over P_i is generally attributed to the higher number of phosphate ester groups in P_{org} compounds resulting in stronger attraction to the positive surface charge of oxidized minerals.

The rates of cycling between organic and inorganic forms are dependent on P_i concentrations, the molecular weight of the P_{org} moiety, and redox conditions (Wetzel, 1999). As P_i concentrations decrease, the microbial cycling rate between organic and inorganic forms tends to increase as phosphatase (enzyme) activity is activated. Molecular weight (MW) of the P_{org} moiety ultimately governs the rate in which it is mineralized; low MW compounds cycle on the time scale of hours, much more rapidly than high MW moieties. With respect to burial and sediment depth, half lives of mono-

and diester ortho-P have been estimated to be between 20-23 years whereas the half-life of pyrophosphate is about 10 years in some sediments (Alghren et al. 2005).

Recycling rates of P_{org} species could also be related to whether the environment is anoxic or oxic (Carman, Edlund, and Damber, 2000). For example, pyrophosphate and polyphosphate have been found in oxic lake sediments but not in anoxic sea water (Alghren et al. 2005; Sannigrahi & Ingall, 2005; Carman, Edlund, and Damber, 2000). When present, pyrophosphate and polyphosphate exist in the top few cm of sediment. These molecules are associated with intercellular microbial P storage which microbes tend to release under anoxic conditions. Most likely, they are not present in anoxic environments because they are rapidly hydrolyzed once released. Redox controlled microbial storage and release of P in the sediments may play a role in the well-observed trend of increased P release under anoxic conditions (Sannigrahi & Ingall, 2005; Søndergaard, Jensen, & Jeppesen, 2003; Hupfer, Gächter, &Ruegger, 1995). Carman et al. (2000) noted that orthophosphate di-esters were present in higher concentrations in reduced sediments of the Baltic Sea than in anoxic/suboxic sediments, suggesting that aerobic environments promote degradation of di-esters.

In the sediments of mesotrophic Lake Erken, Sweden, Reitzel et al. (2007) observed several forms of P_{org} using ^{31}P-NMR such as orthophosphate monoesters, teichoic acids, P lipids, DNA-P, and pyrophosphate. They found a trend in the ratios of inorganic to organic forms with depth as well as a trend in the relative abundance of different P_{org} forms. Orthophosphate comprised ~50% of surface sediment P and decreased to 20-30% with depth. Orthophosphate monoesters (form of P_{org}), on the other

hand, dominated the sediment P fraction (up to 40%) below about 2 cm. DNA-P, pyrophosphate, and polyphosphate concentrations decreased with depth, indicating that they were decomposing over time, most likely by microbial activity. Although this study provided detailed characterization of P_{org} forms with depth, samples were collected at one point in time and redox conditions were not reported. Additionally, the lake was mesotrophic and deeper than Missisquoi Bay, which may result in different P_{org} dynamics.

In general, the redox state of the sediments, among the other factors discussed, will control whether P_{org} molecules will sorb to Fe or Mn (hydr)oxides which will ultimately control their bioavailability. It is plausible that if dissolved P_i (DIP) in the water column becomes exhausted, P_{org} in the sediments will become an important P source to sediment and aquatic organisms. The fact that increased productivity and subsequent water column OM decomposition instigates anoxic conditions suggests that P_{org} degradation and utilization would increase throughout the summer.

Seasonal/Diel Controls

The location of the redox front, defined by the transition from oxic to suboxic conditions, is established by the limits of oxygen supply to the benthic environment and the presence of alternative electron acceptors such as NO_3^-, Mn^{4+}, Fe^{3+}, or SO_4^{2-} . The redox front migrates vertically depending on conditions such as temperature and productivity and is therefore subject to move over seasonal and diel timescales (e.g. Rozan et al., 2002; Sundby, 1986; Anschutz et al. 1998; Roden and Edmonds, 1997). The redox condition of sediments and subsequent P release is closely tied to seasonal and

diel parameters. Rozan et al. 2002 observed that Rehoboth Bay sediments were most anoxic during the late summer months due to respiration rates exceeding O_2 production rates. The redox front in St. Alban's Bay, Lake Champlain, indicated by the lack of O_2 and the presence of Mn^{2+} and Fe^{2+} reduction, moved upward in the sediment column over the course of a summer (Druschel et al., 2005). The onset of bottom water anoxia and subsequent upward migration of the redox front in sediments of eutrophic lakes and estuaries is generally attributed to temperature, sunlight, and respiration rate increases (Tyler, 2009; Beck and Bundland, 2001; D'Avanzo and Kremer, 1994).

The diel trend of the redox front movement in freshwater systems is lesser known yet very important to P and nutrient cycling in shallow aquatic systems. It is conceivable that the redox front within the sediment would be subject diel fluctuations as well due to the lack of oxygen production at night while night time respiration continues to consume O_2. Beck and Brundland (2001) found that DO concentrations were lowest between 3:20 AM and 7:00 AM in a tidal salt marsh and highest at 2:00 PM. The diel DO fluctuations measured by Beck and Bundland (2001) coincided with fluctuations in nutrient (N and P) and trace metal (I, Fe, and Mn) concentrations in the water column but emphasis was not placed on the role of sediments in these changes. Additionally, the tidal flushing within the salt marsh system influenced the nutrient loading and transport making the link between water column O_2 and internal loading more complicated.

Because P release is tied to the location of the redox front, the maximum P diffusion from the sediment into the water column can be inferred by determining when the front is above the sediment-water interface (Druschel et al. 2005, Rozan et al. 2002).

Cyanobacteria, specifically *Microcystis*, may use sediment nutrient diffusion to their advantage because they can regulate their buoyancy (Hyenstrand et al., 1998). Unlike other phytoplankton, they can sink to the bottom to uptake nutrients at night and return to the surface to photosynthesize during the day, an ability that may allow them to out-compete other organisms. Rozan et al. 2002 found that P released from sediments in reducing conditions fueled a benthic algae bloom, but the diel relationship between P flux, redox chemistry, and phytoplankton vertical mobility was not examined.

Methodology Review
Au-Hg Amalgam Microelectrodes and *in-situ* Voltammetry

In-situ voltammetry is a quantitative electrochemical method that provides valuable information of chemical speciation within an aqueous environment (Brendel & Luther, 1995; Luther et al., 1999; Lorenson, 2006; Druschel et al., 2003, 2005). The ability to record data *in-situ* is invaluable to understanding the complex geochemistry of an aqueous environment, as many species tend to degrade or oxidize once removed from that environment. The use of Au-Hg amalgam microelectrodes and *in-situ* voltammetry can precisely determine the location of the redox front within the sediment column. The glass microelectrodes used in this study to electrochemically profile sediment cores have a tip of <0.8 mm in diameter and can be lowered throughout out a sediment core in 1 mm increments with the use of a micromanipulator. Microelectrodes are more sensitive than oxygen probes in anoxic to suboxic environments and they characterize the presence and relative concentration of pore water chemical species such as O_2, H_2O_2, Fe^{3+}, Fe^{2+}, H_2S,

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HS$^-$, S$_2$O$_3$$^{2-}$, Mn^{2+}, Cu^{2+}, and others *in situ* and in real time (Druschel et al., 2003, 2005; Luther et al., 2003; Luther et al., 1999; Brendel and Luther, 1995). Druschel et al. (2005) successfully used microelectrodes to monitor the seasonal movement of the redox front in sediment cores from St. Alban's Bay, Lake Champlain.

The general voltammetric system used for this study consisted of three microelectrodes: a Au-Hg amalgam solid state working electrode (glass for core profiles, PEEK for SWI monitoring), a platinum wire counter electrode, and a Ag/AgCl reference electrode (Figure 4). Detailed descriptions of this set-up and theory behind the method can be found elsewhere (e.g. Druschel, 2005; Lorenson, 2006; Brendel & Luther, 195). Briefly, the working electrode measures the resulting current relative to the reference electrode at certain potentials applied between the working and counter electrodes. The potential is varied by computer software (Analytical Instrument Systems DLK 60) and a DLK 60 electrochemical analyzer. Current peaks at specific potentials indicate the presence of specific analytes; specific potentials between the counter electrode and the working electrode induce the reduction reaction of an analyte species, A, to form a product B, at the mercury surface of the microelectrode. The half-reaction in a solution containing an electrolyte, a known concentration of A (C_A), and a known product (B) can be generalized as:

$$A + ne^- \leftrightarrow B$$

This reaction is assumed to have a standard potential (E^o) which is related to Gibb's free energy of the reaction, ΔG°_R, through the equation:

$$E^\circ = \Delta G^\circ_R/-nF$$

where F is Faraday's constant. Assuming the reaction is reversible and rapid, the electrode potential, E_p, is related to the concentrations of species A and B (C_A and C_B, respectfully) and the standard potential of the reaction E_A° through the Nerst Equation:

$$E_p = E_A^\circ - (0.0592/n)\log (C_B^0/C_A) - E_{ref}$$

Not all reactions occur at the thermodynamically defined electropotential for a reaction, however, the overpotential is the difference between the thermodynamically determined reduction potential and the experimentally observed potential of a reaction (Skoog and Leary, 1998). Mercury is an ideal metal for aquatic in-situ voltammetry because it has a high overpotential for water, allowing electrode potentials to become more negative (0 to -1.8V) than the thermodynamic potential for reduction of water into $H_{2(g)}$ (0V). Therefore, chemical species with more negative half reaction potentials can be reduced on the mercury surface in aqueous solutions. E_p values for Au-Hg microelectrodes have been empirically determined (Figure 5) and can be used to identify specific electrochemical species based on current peak locations on a voltammogram.
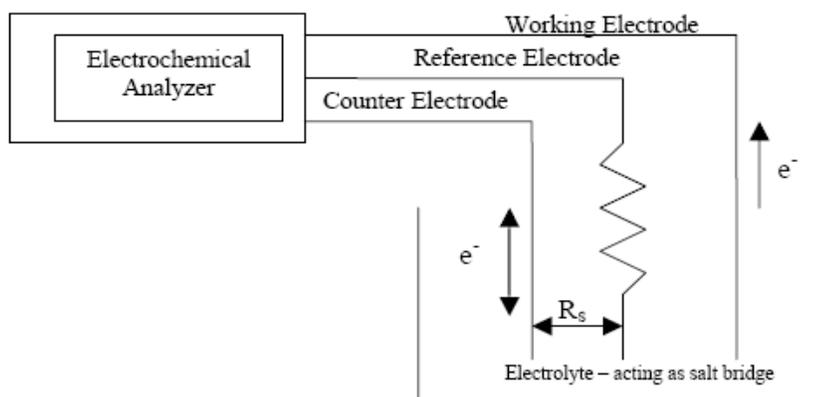
Figure 4: Schematic representation of 3 electrode voltammetry system. From Lorenson 2006.

		E_p ($E_{1/2}$) (V)	MDL (μM)
1a)	$O_2 + 2\,H^+ + 2\,e^- \rightarrow H_2O_2$	-0.30	5
1b)	$H_2O_2 + 2\,H^+ + 2\,e^- \rightarrow H_2O$	-1.2	5
2a)	$HS^- + Hg \rightarrow HgS + H^+ + 2\,e^-$	adsorption onto Hg <-0.60	
2b)	$HgS + H^+ + 2\,e^- \longleftrightarrow HS^- + Hg$	~ -0.60	<0.1
3a)	$S(0) + Hg \rightarrow HgS$	adsorption onto Hg <-0.60	
3b)	$HgS + H^+ + 2\,e^- \longleftrightarrow HS^- + Hg$	~ -0.60	<0.1
4a)	$Hg + S_x^{2-} \rightarrow HgS_x + 2\,e^-$	adsorption onto Hg <-0.60	
4b)	$HgS_x + 2\,e^- \longleftrightarrow Hg + S_x^{2-}$	~ -0.60	<0.1
4c)	$S_x^{2-} + x\,H^+ + (2x-2)\,e^- \rightarrow x\,HS^-$	~ -0.60	<0.1
5)	$2\,RSH \longleftrightarrow Hg(SR)_2 + 2\,H^+ + 2\,e^-$	typically more positive than H_2S/HS^-	
6)	$2\,S_2O_3^{2-} + Hg \longleftrightarrow Hg(S_2O_3)_2^{2-} + 2\,e^-$	-0.15	10
7)	$2\,I^- + Hg \longleftrightarrow HgI_2 + 2\,e^-$	-0.30	<0.1
8)	$FeS + 2\,e^- + H^+ \longrightarrow Fe(Hg) + HS^-$	-1.1	molecular species
9)	$Fe^{2+} + Hg + 2\,e^- \longleftrightarrow Fe(Hg)$	-1.43	10
10)	$Fe^{3+} + e^- \longleftrightarrow Fe^{2+}$	-0.2 to -0.9 V	molecular species
11)	$Mn^{2+} + Hg + 2\,e^- \longleftrightarrow Mn(Hg)$	-1.55	5
12)	$Cu^{2+} + Hg + 2\,e^- \longleftrightarrow Cu(Hg)$	-0.18	<0.1
13)	$Pb^{2+} + Hg + 2\,e^- \longleftrightarrow Pb(Hg)$	-0.41	<0.1
14)	$Cd^{2+} + Hg + 2\,e^- \longleftrightarrow Cd(Hg)$	-0.58	<0.1
15)	$Zn^{2+} + Hg + 2\,e^- \longleftrightarrow Zn(Hg)$	-1.02	<0.1

Figure 5: Electrode reactions at the Au-Hg amalgam electrode surface with empirical E_p (reaction potentials) that are used to identify current peaks in a voltammogram. MDL is the minimum detection limit. From Luther et al. (2005).

Nuclear Magnetic Resonance Spectroscopy

Considering the forms of P in a system is important because of the inherent reactivity of different P_i and P_{org} moieties. The use of ^{31}P-NMR spectroscopy is extremely useful for the determination of P_i and P_{org} species and relative concentrations within environmental samples. Organic P concentrations can be determined through sequential extraction techniques, but ^{31}P-NMR is the only method that characterizes and quantifies P_i and P_{org} (Turner, 2002; Cade-Menun 2005). This method is quantitative because ^{31}P is the only naturally existing P isotope (e.g., Cade-Menun, 2004; Gorenstein, 1984). The premise of ^{31}P-NMR is that the structure of the organic molecule correlates to the adsorption of radio-frequency radiation by the ^{31}P nucleus in a magnetic field (Skoog et al., 2007). When a magnetic field is applied to the sample, the ^{31}P nuclei align with the field. The energy associated with the adsorption of the radiofrequency radiation causes the nuclei to align against the field resulting in an unstable high energy state (Ault, 1998; Cade-Menun, personal comm. 2007; Skoog et al. 2007). As the nuclei relax, a Free Induction Decay (FID) signal is released and recorded by a radio receiver coil and subsequently digitized and stored in the computer. The data are then converted to frequency-domain by Fourier transformation. The resulting data are plotted as chemical shift, a relative measure from a reference resonance frequency (usually 85% H_2PO_4 for ^{31}P-NMR). For ^{31}P-NMR, the integration of the peak area is proportional to the concentration of that particular species and can be defined quantitatively by comparison to TP analysis. To do this, the total integrated areas for all the peaks are assumed to represent 100% of the total P concentration from the extraction. The area under each

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peak therefore represents the relative concentration of a specific P species in which its concentration can be calculated based on the TP concentration from analysis from ICP_OES or a similar technique.

Samples can be analyzed either through solid-state or liquid state ^{31}P-NMR. Solid state ^{31}P-NMR is advantageous because the sample is unaltered, a small sample size is required, and minimal preparation is necessary (Cade-Menun, 2005). On the other hand, solid state ^{31}P-NMR has a higher minimum detection limit and produces spectra with poor resolution compared to liquid state ^{31}P-NMR. For our study, we used liquid state ^{31}P-NMR to obtain better spectra resolution and because P_{org} concentrations of the sediments were not known.

Several extraction schemes have been used to prepare environmental samples for liquid state ^{31}PNMR. Samples are typically pre-extracted, lyophilized, and then post-extracted immediately prior to being analyzed. Extractants usually include NaOH to solubilize P from soils or sediments in addition to a chelating agent such as Chelex or ethylenediaminetetraacetic acid (EDTA) to minimize the interference of Fe or other paramagentics. Alghren et al. (2007) reviewed different pre-extractant solutions and determined that NaOH-EDTA yielded almost 50% more P from sediments than the second most effective extractant (originally suggested by Cade-Menun & Preston, 1996). We chose to use this method for P_{org} ^{31}P-NMR pre-extraction and followed the procedure by Cade-Menun & Preston (1996) in which 25 ml of 0.50 M NaOH 0.25 M Na_2EDTA solution was added to 3 g (dry weight equivalent) of wet sediment. Although ^{31}P-NMR is a powerful tool for analyzing environmental samples, studies of P_{org} characterization and

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importance have been limited to terrestrial and marine settings and very little work (e.g.

Reitzel et al. 2007; Alghren et al. 2005; Carman, et al. 2000) has addressed their role in

lake sediments.

CHAPTER 3: Paper for Submission to Journal of Limnology and Oceanography

Relating Sediment Nutrient Mobility to Seasonal and Diel Redox Fluctuations at the Sediment-Water Interface in a Eutrophic Freshwater Lake

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ABSTRACT:

The relationship between nutrient cycling and redox conditions in the sediments of eutrophic Missisquoi Bay, Lake Champlain were investigated over diel and seasonal timescales. Data was obtained from two consecutive summers (2007 and 2008), one of which (2007) did not experience a cyanobacteria bloom for the first time in a decade. Sediment extraction data showed that reactive P (RP) is strongly correlated to reactive Fe (RFe) indicating that the mobility of a large portion (30-40%) of the P pool in the sediment is influenced by redox conditions. RP concentrations in the top sediments tended to increase throughout the season in 2007 but decreased in 2008, indicating that a portion of the P was released into the water column in 2008. Additionally, the percent RP of Total P (TP) was greater in 2008 than 2007 in the surface sediments (34% vs. 42% respectively). Ammonium (NH_4^+) also demonstrated seasonal fluctuations associated with more reducing conditions and concentrations tended to be the highest during peak bloom in the pore water of the 0-1cm sediment segment. Redox conditions were measured both within the sediment column and at the sediment water interface (SWI) at different stages of the season using in-situ voltammetry. The SWI redox conditions were measured continuously over selected 24 hr periods and the SWI remained relatively oxic in 2007 compared to 2008. Redox conditions at the SWI become progressively reduced across the season, and were significantly more reducing in the presence of a bloom. Diel trends were also observed with increasingly more reducing conditions at the SWI overnight in both years. Observed redox conditions suggest that any flux of P from the sediments to the water column may be variable over diel cycles and be strongly influenced by the presence of a bloom.

INTRODUCTION

Anthropogenic eutrophication of surface waters often results in undesirable

conditions such as algae blooms (e.g. Schindler, 1977; Smith, Tilman, & Nekola 1999;

HARNESS, 2005) that create detrimental ecosystem imbalances, human health concerns

(some algae blooms release toxins (e.g. Rosan et al. 2001)), and the loss of income to

recreation-based economies. Although eutrophication and resulting cyanobacterial

blooms have been studied for some time (e.g. Hecky & Kilham, 1988; Smith et al, 1999;

Xie & Xie, 2002), many questions remain about the dynamics of coupled sediment-water

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column systems and the response of algal blooms to these changes over seasonal and diel scales. High lake P concentrations are often linked to increased biomass of cyanobacteria (Xie & Xie, 2002; Schindler et al., 2008). Lake P concentrations reflect not just agricultural and urban runoff (external loading) but also P released from the sediments into the water column through internal loading processes controlled by redox conditions, pH, temperature, light, and physical disturbances (e.g. Ryding, 1985; Jensen and Andersen, 1992; Søndergaard, Jensen, & Jepsen, 2003).

Sediment P release mechanisms are related to physical, biological, and chemical processes within the sediment and overlying water. P release can occur when the surface sediments are disturbed by bioturbation or underwater currents (Philips et al. 1982; Kristensen et al. 1992). Biological P release processes also contribute, including cell lysis and bacterial mineralization (Gächter, Meyer, and Mares, 1988). Sediment P mobility is largely controlled by the sorption of P forms to Fe and Mn oxides and hydroxides (e.g., Mortimer, 1941; Lijklema, 1980; Jensen et al. 1992). The sorption capacity of these minerals is governed by their surface area, the local pH, and the redox conditions of the environment (e.g. Jensen et al. 1992; Jensen and Andersen, 1992). Under oxic conditions (redox potentials >200 mV at pH 7) Fe(III) oxides and hydroxides are insoluble and P sorption is favorable (Mortimer, 1941; Lijklema, 1980; Søndergaard, et al. 2003). Anoxic conditions promote the reduction of insoluble FeOOH minerals to soluble Fe(II) resulting in P release from the surfaces into the surrounding water (Lijklema, 1980; Böstrom, 1982; Christophoridis and Fytianos, 2006). For example, Sundby et al. (1986) found that as oxygen concentrations approached zero in a benthic

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chamber in the sediments of Gullmarsfjorden, Sweden, dissolved inorganic P (DIP,

PO_4^{3-}) and Fe^{2+} were released simultaneously in equivalent concentrations. Kostka and

Luther (1995) and Anshutz et al. (1998) determined that the portion of reactive P

(ascorbate extractable) in sediments are highly correlated to amorphous Fe(III) oxides

(ascorbate extractable). Mn oxides are thought to contribute to sediment P sorption and

release in a similar fashion although Mn oxides become reduced and dissolve more

rapidly and under higher redox potentials than Fe(III) oxides, thus making them

potentially important in P cycling especially closer to the SWI (Lovey, 1991). The role

of Mn and Fe in biogeochemical cycling is also significant because they are important

trace metals for phytoplankton (Roitz et al., 2000; Frausto da Silva and Williams, 1991).

Mechanisms for sediment iron reduction and subsequent P release include a

number of abiotic and biotic processes. Direct reduction of FeOOH is assumed to occur

by microbial anaerobic Fe reduction, with the mineral serving as the electron acceptor

(eg. Cristophoridis and Konstantinos, 2006; Sunby et al. 1986; Böstrom et al. 1982).

Roden and Edmonds (1997) suggested an indirect reduction pathway, attributing P

release from Fe(III) oxides to sulfate reducing bacteria which enhance the formation of

soluble FeS. FeS has a low pH_{ZPC} of 2, meaning that above pH 2, this mineral has a net

negative surface charge and will therefore repel PO_4^{3-} upon formation (Bebie et al. 1997).

Rozan et al. 2002 found that this was a dynamic process controlling P cycling in sulfidic

Rehoboth Bay estuary sediments, but sulfide may not be as important in freshwater P

release because significantly less sulfate is present (Gunnars and Blomqvist,1997). The

pH of the environment also influences P release from Fe and Mn oxides and hydroxides;

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higher pH environments promote ion exchange of PO_4^{3-} with OH^- on the mineral surfaces (Andersen, 1975). However, pH controlled ion exchange in sediments may be minimal because mineral interactions may buffer large pH shifts (Drake and Heaney, 1987). Cai et al. (2002) observed a pH drop (7.2 to 6.8) in the top cm of sediments as a result of Mn^{2+}, Fe^{2+}, and NH_4^+ oxidation (e.g. $2NH_4^+ + 3O_2 \rightarrow 2NO_3^- + 8H^+$). The Fe:P ratio has been suggested to be a measure of free sorption sites on iron hydroxide surfaces and the ratio may be a fairly reliable indicator of the P release potential of sediments (Jensen, et al. 1992). Jensen et al. (1992) suggested that an Fe:P ratio greater than 15 by weight suggests P will be retained by Fe in oxic sediments.

The location of the redox front, defined by the transition from oxic to suboxic conditions, is established by the limits of oxygen supply to the benthic environment and the use of alternative electron acceptors such as NO_3^-, Mn^{4+}, Fe^{3+}, or SO_4^{2-}. The redox front migrates vertically depending on the balance of O_2 supply into the water column (from the atmosphere and primary production), O_2 consumption by aquatic organisms, and availability of alternate electron acceptors in the sediment. This balance is influenced by physical, chemical, and biological factors controlling O_2 supply such as temperature, wind, waves, sediment resuspension, light intensity (driving photosynthesis), and light penetration versus O_2 consumption via respiration and reaction with reduced species such as Fe^{2+} and H_2S. The redox front is therefore subject to vertical migration over seasonal and diel timescales (e.g. Rozan et al., 2002; Sundby, 1986; Anschutz et al. 1998; Roden and Edmonds, 1997). Druschel et al. (2005) found that the redox front, indicated by the lack of O_2 and the presence of Mn^{2+} and Fe^{2+}

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reduction, moved upward in the sediment column over the course of a summer in Saint Albans Bay, Lake Champlain.

The presence of an iron oxyhydroxide microlayer at the SWI when the redox front is above the SWI has been suggested to be significant sink of P (Mortimer, 1941; Sundby et al. 1992; Jensen et al. 1995; Penn et al. 2000). However, under anoxic conditions, these oxyhydroxides can be dissolved by use of sediment microorganisms as an alternate electron acceptor which may result in P release from the sediments at specific times dependent on O_2 availability in the water column (Anschutz et al. 1998; Canfield et al. 2005). In general, the redox condition of sediments and subsequent P release is closely tied to seasonal biogeochemical changes in freshwater systems, but the trend of redox front movement on a diel scale is less well known. The potential for diel P fluxes from sediments may be advantageous to cyanobacteria, specifically some *Microcystis* spp., because they can regulate their buoyancy in order to utilize nutrients diffusing from the sediments during water column anoxia (Hyenstrand et al., 1998). Unlike other phytoplankton, they can sink to the bottom to uptake nutrients at night and return to the surface to photosynthesize during the day, an ability that may allow them to out-compete other organisms.

This study evaluates how redox front fluctuation responds to seasonal and diel variation to affect P mobility in shallow freshwater lake sediment. Redox fluctuations were measured within the sediment column and at the sediment water interface (SWI) over diel cycles at different points across the season when cyanobacteria (blue-green algae) bloom.

Au-Hg amalgam microelectrodes were used to measure the redox conditions of the sediments. Microelectrodes are more reliable than oxygen probes in anoxic environments (H_2S interferes with many oxygen probes) and they characterize the presence and relative concentration of chemical species such as O_2, H_2O_2, Fe^{3+}, Fe^{2+}, $FeS_{(aq)}$, H_2S, HS^-, $S_2O_3^{2-}$, Mn^{2+}, Cu^{2+}, and others *in situ* and in real time (Brendel and Luther, 1995; Luther et al., 2003; Druschel et al., 2005; 2008). The use of these microelectrodes provided detailed, *in-situ*, real-time redox chemistry data at highly resolved time periods and depths within the sediment column, and were coupled to porewater and sediment analyses (P, N forms, Fe, Mn, Al, and Ca).

METHODS

Study Site Background

Missisquoi Bay is located in the northeastern quadrant of Lake Champlain (N44 59.503 W73 06.798) with 58% of its watershed in Vermont and 42% in the Province of Quebec, Canada (Troy, et al, 2007). The entire watershed is 3,105 km^2 (767,246 ac), the surface area of the bay is approximately 77.5 km^2 (19,150 acres), and the maximum depth is 4 m (Hegman, et al. 1999). Phosphorus loading from the Missisquoi Bay watershed is by far the highest of Lake Champlain's nineteen watsheds (LCBP, 2007) resulting in an average total phosphorus (TP) concentration of 49 µg/L (ranges from 6-111 µg/L from 1994-2007, VT DEC, 2008), which is well above the eutrophic status level of 35 µg/L.

The sediments are described in detail by Burgess (2007), briefly the top 30 cm are mottled brown-gray sediments, slightly coarser than clay, with C_{org} between 2-3%. The

top sediments are organic rich and become more clay rich (dark gray and fine-grained) below about 60 cm. The recent sedimentation rate is approximately 1 mm/per (Burgess, 2007). We chose a study site on the eastern side of the Missisquoi River delta in the drainage area of Rock River. The sediments at our study site contained few, sparsely dispersed plants and few, sparsely dispersed native mussels.

Field Methods

General Field Methods

A "diel sampling event" consisted of continuously monitoring the redox fluctuations at the sediment water interface for ~24 hours using *in-situ* voltammetry in addition to collecting dive cores (gravity cores in 2007 using a Glew corer) in the afternoon, dusk, and dawn (2 at each time). Two and a half inch diameter core tubes were used for coring (dive cores were capped underwater and gravity cores sealed with caps immediately upon collection) and stored without headspace until analysis. They were electrochemically profiled within one hour of collection and covered by Al foil during profiling to prevent photosynthetic stimulation.

Electrochemical profiling was carried out to determine the redox reactive chemical species (through the top 10 cm of sediment). Two glass Au-Hg amalgam microelectrodes (about 2 cm apart) were lowered at 1-5 mm increments vertically through a sediment core using a micromanipulator. The electrodes were constructed in the lab according to methods published in Brendel and Luther (1995). A sequence of ten cyclic voltammograms (-0.1 to -1.8 V vs. Ag/AgCl at 1V/sec with 2 second deposition at -0.1 V) was obtained from each electrode at each depth using DLK-60 (AIS Instruments)

software. The current response signals of the last 5 scans of each sequence were measured and averaged (AIS Instruments DLK-60 Analysis program). The variability between measurements of one sequence is extremely small (typically less than 1%). For the SWI voltammetry, 10 cyclic voltammograms at the same conditions were obtained every 8 minutes and the last 5 scans of a sequence were analyzed. Oxygen peaks exist at -1.3V and -0.3V (O_2 and H_2O_2), Mn^{2+} at -1.6V, Fe(II) at -1.4V, Fe(III) at -0.6 V, and HS- at -0.8 V. The electrodes were calibrated using 2-point O_2 calibrations (air-saturated and N_2 purged) and standard additions of MnCl to N_2 purged water, with calibration for other ions, relative to Mn^{2+} accomplished using the pilot ion method (Brendel and Luther, 1995).

After profiling, the cores were sectioned into segments of 0-1, 1-2, 2-3, 3-4, 4-6, & 6-10 cm, each of which was divided into separate falcon tubes for pore water analysis, sediment extractions, and ^{31}P-NMR analysis (data not presented). These samples were immediately frozen and stored until analysis in the laboratory. In addition to pH measurements, Hach DR/890 portable spectrophotometer was used to measure NH_4^+, NO_2^-, and Fe^{2+} of filtered water collected with the cores (using published protocols from Hach). Water column chemistry, cell density, chlorophyll, and DNA measurements were acquired at the same times at five depths by a collaborating group (Watzin et al., 2009, in progress).

2007 Sampling Dates and Field Work

Sediment cores were collected 6/13, 7/11, 8/14, 9/17-9/18, and 10/11 during 2007. In September, the cores were collected and electrochemically profiled at 4 PM and 8 PM

on 9/17 and 6 AM and 12 PM on 9/18 as part of a diel sampling event. The University of

Vermont's Research Vessel, *Melosira*, served as a platform for the electrochemical

equipment (computer, potentiostat, etc.) that controlled the diel measurements of the

redox chemistry at the SWI. HOBO temperature and light intensity data loggers were

also placed at -11cm, 0cm, 1m, and 2 m with respect to the sediment-water interface, and

recorded from 6/13/07 to 10/1/07.

2008 Sampling Dates and Field Work

A floating research platform was anchored on the site in May 2008 to serve as the

base for the diel electrochemical equipment instead of the *R/V Melosira*, as the vessel

was subject to position shifts due to wind changes. HOBO temperature and light

intensity data loggers were also placed at -11cm, 0cm, 1m, and 2 m with respect to the

sediment-water interface and recorded from 5/20/08 to 10/2/08. In June 2008, we

installed a sediment grid using posts and strings to establish a reference point for dive

core collection and redox monitoring. Sediment cores were collected by SCUBA divers,

profiled, and sectioned during these initial events. The 2008 diel sampling events took

place June 25th & 26th (pre-bloom), July 29th & 30th (bloom onset), August 27th & 28th

(peak bloom), and October 1st & 2nd (bloom decline). During the 2008 diel sampling

events, the cores were collected as close to the following times as possible: 1 PM, 7 PM,

and 6 AM to capture peak productivity (i.e. oxygen production via photosynthesis),

downswing of productivity, and lowest productivity (i.e. oxygen consumption greater

than production), respectively.

Diffusive Gradient in Thin Film (DGT) probes were used to measure the spatial distribution of P concentrations across the SWI in the sediments in 2007 but were replaced with "peepers" in 2008 due to difficulties with preserving the physical integrity of the DGT device diffusion membrane. Peepers are polypropylene blocks with 20 10-ml pockets spaced 1 cm apart vertically, constructed after Hesslein (1976). The pockets were filled with N_2-purged DI water and covered with a 0.2μm filter membrane which would chemically equilibrate with its environment over time. The peepers were installed vertically in the sediments by divers with about 5 cm of the peeper above the sediment water interface (15 cm in the sediment) and allowed to equilibrate for several weeks at a time. They were put in the sediment for a total of 3 equilibration periods (May-June, June-July, and September-October). Upon retrieval, the water was immediately extracted from each pocket and sectioned into 3 aliquots for anion and cation analysis using Ion Chromatography (Dionex IC 2500) and total phosphorus using colorimetric techniques.

Lab Analysis - Sediment Extractions and Fe, Mn, P, Ca, and Al analysis

The sediments collected during 2007 and 2008 were analyzed using the same methods, except the 2007 sediments were oven dried prior to extractions and the 2008 sediments were freeze-dried. Triplicate extractions comparing oven drying versus freeze drying produced statistically agreeable results for both extraction techniques (Student's t test showed no significant difference, $p < 0.05$). An aqua regia digest (EPA method 3050B) which consists of a 3:1 ratio of $HCl:HNO_3$ was used to extract total P (TP), Fe (TFe), Mn (TMn), Ca (TCa), and Al (TAl) from 0.25-0.50 g of dry sediment. The acid and sediment were then refluxed for at least 1 hour at 80-90°C, the supernatant was

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diluted 10-fold and then measured using Inductively Coupled Plasma Optical Emission

Spectroscopy (ICP-OES; Horiba Ultima 2C). To determine the reactive portions of these

components (RP, RFe, RMn, RCa, and RAl) an ascorbic acid extraction (Kostka &

Luther, 1995; Anschutz, et al 2007) was performed. The ascorbic acid solution is made

by dissolving 4 g sodium bicarbonate and 4 g sodium citrate in 200 ml N_2 purged water

with 2 g ascorbic acid slowly added, the resulting pH should be 8 (if not it was adjusted

using NaOH or HCl, appropriately). The prepared solution was then added to ~0.50 g of

dry sediment, rotated for 24 hours, centrifuged, decanted, diluted 10-fold, and analyzed

using the ICP-OES. Total organic carbon (TOC) and total organic nitrogen (TON) were

determined by analyzing ~0.50 mg of homogenized freeze dried sediments in an

elemental analyzer (NC 2500 CE Instruments) with B2150 and B2152 standard soil as

reference standards.

RESULTS

Spatial/ Sediment Core Redox Variability

The SWI and core profile voltammetry data are largely in agreement, for example,

when Mn(II) was detected with the SWI system, Mn(II) was also detected in the

overlying water of the sediment core. Variability with depth was noticeable when two

separate cores were profiled, most likely due to spatial heterogeneity (Figure 6). When

two electrodes were used simultaneously to profile vertically 1-2 cm apart, redox species

were generally detected at the same depth in each profile but sometimes in different

concentrations, which is most likely due to localization of specific analytes. Generally,

the redox profile was homogenous within one core. Efforts were made to reduce

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disturbance and time of exposure to air when profiled; though removing samples will inevitably affect the redox chemistry to some degree, these measurements would only underestimate the degree to which the sediment is reduced, and the agreement with *in situ* measurements suggests they represent the redox profile very well.
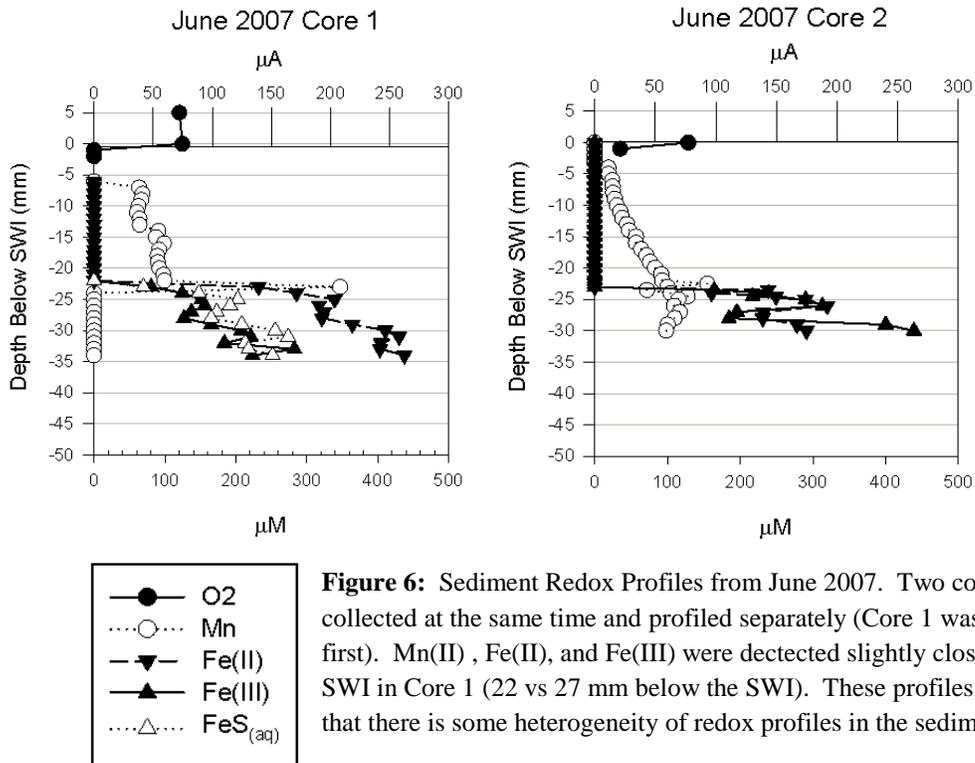
Figure 6: Sediment Redox Profiles from June 2007. Two cores were collected at the same time and profiled separately (Core 1 was profiled first). Mn(II) , Fe(II), and Fe(III) were dectected slightly closer to the SWI in Core 1 (22 vs 27 mm below the SWI). These profiles suggest that there is some heterogeneity of redox profiles in the sediments.

2007 Electrochemical Sediment Profile Data – Seasonal Trends

The depths of oxygen penetration and redox appearance in cores collected from 2007 are listed in Table 3. Fe(II) and Fe(III) were detected concurrently but in different relative concentrations in all core profiles. In June and July, O_2 penetration was restricted to the surface of the sediment, with low concentrations detected 1 mm below the surface in July. In August, Mn(II) was detected in the overlying water of the core in addition to O_2, although O_2 did not penetrate the SWI. The cores collected at 4 PM and 8 PM during

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the diel cycle sampling showed O_2 penetration to 3 and 2 mm below the SWI, respectively. The overlying water of the core (estimated at +5 mm above the SWI) collected at 06:00 contained low O_2 concentrations and Mn(II). Mn(II), Fe(II), and Fe(III) were present in detectable concentrations in the overlying water of the core collected at 12:00. The accompanying continuous SWI electrochemical monitoring also detected Mn (II), Fe(II), and Fe(III) at the SWI (starting at 11:45 on 9/18) although the intense noise in preceding redox scans (11:45 to 11:50) led us to believe that the electrode system was physically disturbed (therefore those scans were not analyzed), possibly by accidental interaction of divers with the system or shifting of the research vessel due to wind. In October, O_2 was detectable at the SWI but Mn(II), Fe(II), and Fe(III) were not detected until 29 and 46 mm below the SWI (Table 4, all profiles included in Appendix E).

The sediment surface was relatively oxic in June and July of 2007 and suboxic in August and September as indicated by the presence of Mn(II) in the overlying core water. The general trends indicate that the redox front shifted slightly upward from June to August and over the course of the diel cycle based on the O_2 penetration and Mn reduction appearance. The diel system indicated that the sediment surface remained oxic in September 2007, although O_2 concentrations were higher in the afternoon to late evening (05:00 to 01:50), dropped in the late night to early morning (01:50 to 11:00), and increased again in the late morning (11:00 onward).

2007 Depth of O_2 Penetration and Redox Appearance									
	6/13	**6/13**	**7/10**	**8/14**	**9/17**		**9/18**		**10/11**
	13:00	13:00	13:00	13:00	16:00	20:00	06:00	12:00	12:00
O_2	0	-1	-1	0	-3	-2	5	5	0
Mn(II)	-7	-4	-7	5	-16	-4	5	5	-29
Fe(II), Fe(III)	-23	-23.5	-28	-5	nd	nd	0	5	-46
FeS$_{(aq)}$	-23	nd	nd	nd	nd	nd	nd	nd	nd

Table 3: Depth (mm) of O_2 penetration and appearance of redox species relative to the SWI in sediment cores collected in 2007. Positive numbers for Mn(II) and Fe(II) & Fe(III) indicate that the analyte was detected in the overlying core water. A positive number for O_2 indicates it was only detected in the overlying water. nd = not detected in the depth that the sediment column was profiled.

2008 Electrochemical Sediment Profile Data – Seasonal Trends

The timing of sampling events in 2008 corresponded well with different population growth stages of the cyanobacteria bloom (population data not shown; Watzin et al., 2009 in progress). Sediment redox conditions showed distinct differences in comparison to 2007. Oxygen was detected at the surface of all sediment cores in 2008, although concentrations varied greatly (Figure 7). The depth of O_2 penetration and appearance of Mn(II), Fe(II), Fe(III), and FeS$_{(aq)}$ for all cores profiled in 2008 are listed in Table 4.

Generally, the redox profiles of the cores collected during summer 2008 indicated an upward movement of the redox front, with some variability in concentrations with time. Although O_2 was present in measurable amounts in the water above the SWI collected with each core, the presence of Mn(II) indicates that the environment was slightly reducing. The diel SWI electrochemical system which recorded the redox fluctuations of one point in the sediment at the SWI for about 24 hours at highly resolved time intervals generally agreed with the information obtained from the surfaces of the sediment cores (Figure 8a-e). The sediment surface was the most oxic in June and July,

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although Mn(II) reduction was detected at the SWI in July. No O_2 was detected at the SWI in August, only Mn(II) and small, scatted amounts of Fe(II) were measured. In October, only Mn(II) was consistently detected during the diel cycle, although O_2 was measured sporadically in the early morning.

Depth of O_2 Penetration and Redox Appearances in Sediment Cores - 2008														
	5/20	6/19	June 25 & 26			July 29 &30			August 27 & 28			October 1 &2		
	12:00	12:00	13:00	17:30	6:30	13:30	19:00	6:15	13:00	19:00	6:15	11:46	18:30	6:30
O_2	-1	1	0	0	-5	0	-1	0	0	0	0	-2	-3	1
Mn	-5	0	-7.5	-8	-5	-13	0	5	-1	10	10	-20	-3	0
Fe(II) & Fe(III)	-17	-32	-7.5	-36	-8	-19	-12	-12		-4	-60	-45	-22	-10
$FeS_{(aq)}$	-72	-110	-34.5	-42	-40	nd	nd	-27	nd	-28	nd	nd	nd	nd

Table 4: Depth of O_2 penetration and appearance of redox species relative to the SWI in cores collected in 2008. A positive number, 0, or negative number indicates that the analyte was detected above, at, or below the SWI, respectively.
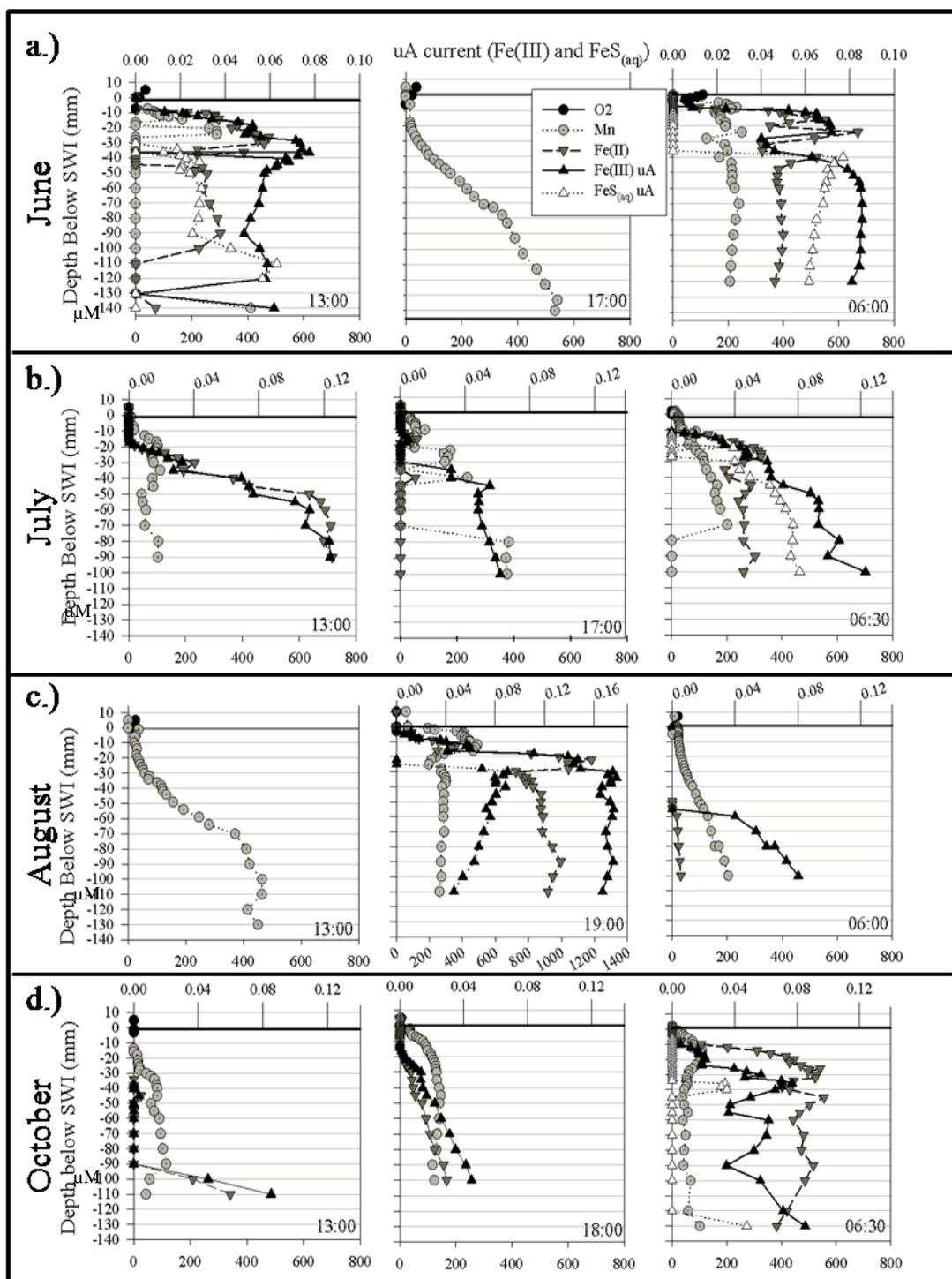
Figure 7 (a-d): 2008 sediment core redox profiles from diel sampling in June (a), July (b), August (c), and October (d). The top horizontal axes correspond to current values (µA) for Fe(III) and FeS$_{(aq)}$. The bottom axes correspond to concentrations (µM) of O$_2$, Mn(II), and Fe(II). Each set of three profiles corresponds to cores collected at noon, dusk, and dawn of the respective diel sampling event.

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2008 Diel Cycle Data

June Diel Cycle (Figure 8a)

In June, the sediments remained oxic and O_2 concentrations ranged from 170-200 μM assuming saturation = 262 μM (Millero et al., 2002, .1 Molal $MgCl_2$ at 15°C). Unfortunately, difficulties with the electrochemical software resulted in only capturing redox conditions between 05:40 and 11:11. However, when the system was checked on manually at various points throughout the 24-hour cycle, all scans appeared to contain oxygen peaks. Also, based on the data collected from the rest of the summer, this pre-dawn-late morning time period seems to be the most dynamic, variable, and most reduced.

July Diel Cycle (Figure 7b)

The July diel cycle demonstrated highly variable O_2 and Mn(II) concentrations from 13:30 to 19:30, followed by more stable concentrations from 19:30 – 23:00, decreasing O_2 concentrations with increasing Mn(II) concentrations from 13:00 to 06:15, and then more variable concentrations of both until the system was removed. In general, this diel cycle measurement captured the relationship between O_2 and Mn(II) concentrations at one point in space over 22.5 hours at the SWI. Specifically, when the O_2 levels decreased, the Mn(II) concentrations increased simultaneously. The overall observed trend demonstrates that the sediments were more reducing in the late night – early morning time period. The variability and fluctuations of concentrations are most likely due to physical disturbances such as underwater currents or bioturbation, however,

surface wind speed recorded from nearby weather station (Freighlisberg, Quebec) tended to correlate with some of the variability (APPENDIX D).

August Diel Cycle (Figure 8c)

Oxygen was only detected for a short period of time (14:33 -15:46 PM) and at relatively low concentrations (25-85 μM) at the SWI during August diel sampling. Mn(II) current concentrations ranged from 37-127 μM, with the highest variability between 13:00 and 19:52. Between 20:00 and 11:20 the Mn(II) values ranged between 63 and 103 μM. Fe(II) was also detected from 19:16 to 02:20 and again at 06:47 until the sampling ended. Sampling ended at 11:20, but both Mn(II) and Fe(III) values appeared to be trending upward at this time. Compared to July, the sediments were much more reducing, indicated by the lack of oxygen the presence of higher concentrations of Mn(II) and Fe(II).

October Diel Cycle (Figure 8d)

In October, Mn(II) was the dominant redox species detected at the SWI, but low concentrations of O_2 were also present at specific time periods. The diel system started sampling at 11:20, in which Mn(II) was detected at concentrations near 113 μM. From this time, the Mn(II) values steadily dropped to 61 μM by 14:20 and fluctuated between 61 and 70 μM until 20:30. After 20:30, the Mn(II) values steadily increased to 113 μM until 03:02 and then fluctuated between 55 and 96 μM from 03:02-07:20. The diel cycle analysis indicates that the sediments were still fairly reduced in October and a diel trend was observed with increasing Mn(II) concentrations from late evening to early morning.

2008 Diel SWI Redox Cycles
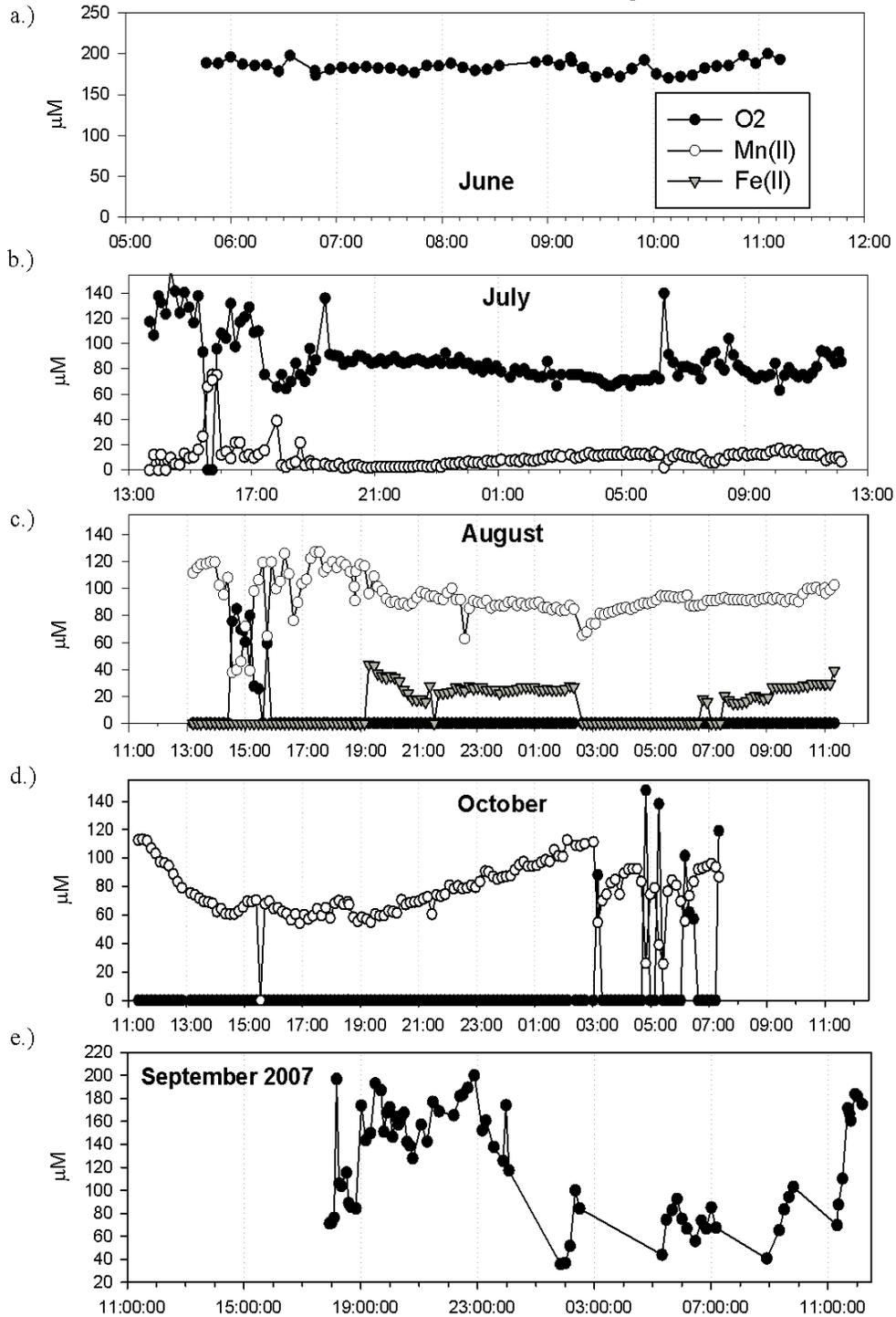
Figure 8 (a-e): Diel redox measurements at the SWI from June(a), July (b), August (c), and October (d) of 2008 and September (e) of 2007. Note that in June, measurements were only collected from 05:00 to 11:00.

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Water Column pH 2007 vs 2008

The water column pH (taken at a depth of 1 m below the surface) tended to be higher (~9) for a longer duration in the summer of 2007 (Figure 9). More variability was observed in 2008 but ranged between 5.62 (mid-July) and 8.8 (beginning of August). Overlying core water pH values were obtained for cores collected at each sampling time in June 2008 and are as follows: 7.45 at 13:00, 8.29 at 17:30, 7.61 at 06:30. These data show that the pH changes on a diel scale near the sediments.
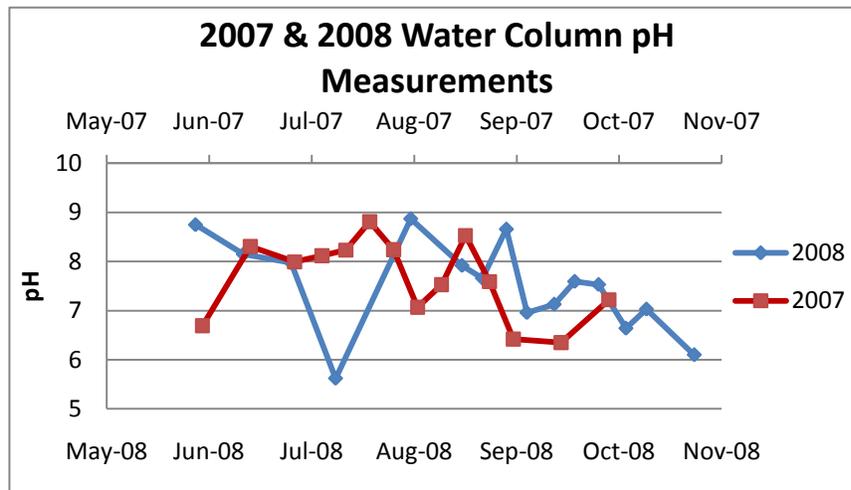
Figure 9: pH measurements in the water column from 2007 & 2008 that were obtained separately. Water column pH influences the potential for P release from mineral surfaces in the sediment. At higher pHs (above 9) FeOOH minerals are less likely to sorb P. On the other hand, sediment mineral reactions may buffer pH changes in the sediment.

Sediment Chemistry – Extractions and Redox Reactive Components

Regression R-square and *p*-values comparing the relationships between P and Fe, Mn, Al, and Ca are listed in Table 6. The sample number (n) differs between tests because the extractions were originally only analyzed for Fe, Mn(II), and P using the ICP-OES based on the assumption that these components would have the most control with P dynamics. However, other studies (e.g. Lijklema, 1980; Istvánovis, 1988; Olila

and Reddy, 1997) have determined that Al and Ca in sediments also play a role in P

cycling so a portion of the extractions were re-analyzed for Al and Ca (>50% of the 2007

and >90% of the 2008 sample extractions).

A One-Way nonparametric ANOVA (Kruskal-Wallis) showed that the RP in the

0-1 and 1-2 cm sediments in May and June 2008 were significantly different compared to

August and October 2008 ($p < 0.001$) and from Figure 10b, it is apparent that RP content

decreased across the season. The trend appears to be opposite in 2007 with RP increasing

in the surface sediments toward the end of the summer (Figure 10a). An ANOVA was

not performed to determine the statistical significance because only 1 core was collected

and analyzed for each time period in 2007. Additionally, the percent RFe and RP of TFe

and TP (respectively) was greater in the top sediments compared to deeper sediments for

both 2007 and 2008 (Table 5).

%RFe/TFe			%RP/TP	
	2007	**2008**	**2007**	**2008**
0-1 cm	14%	22%	34%	42%
1-2 cm	6%	20%	25%	41%
2-3 cm	7%	17%	24%	37%
3-4 cm	6%	14%	24%	35%
4-6 cm	7%	13%	25%	34%
6-10 cm	6%	13%	26%	32%

Table 5: 2008 and 2007 percent RFe and RP of TFe and TP, respectively. Total Fe and TP show no trend with depth but RFe and RP tend to decrease with depth.
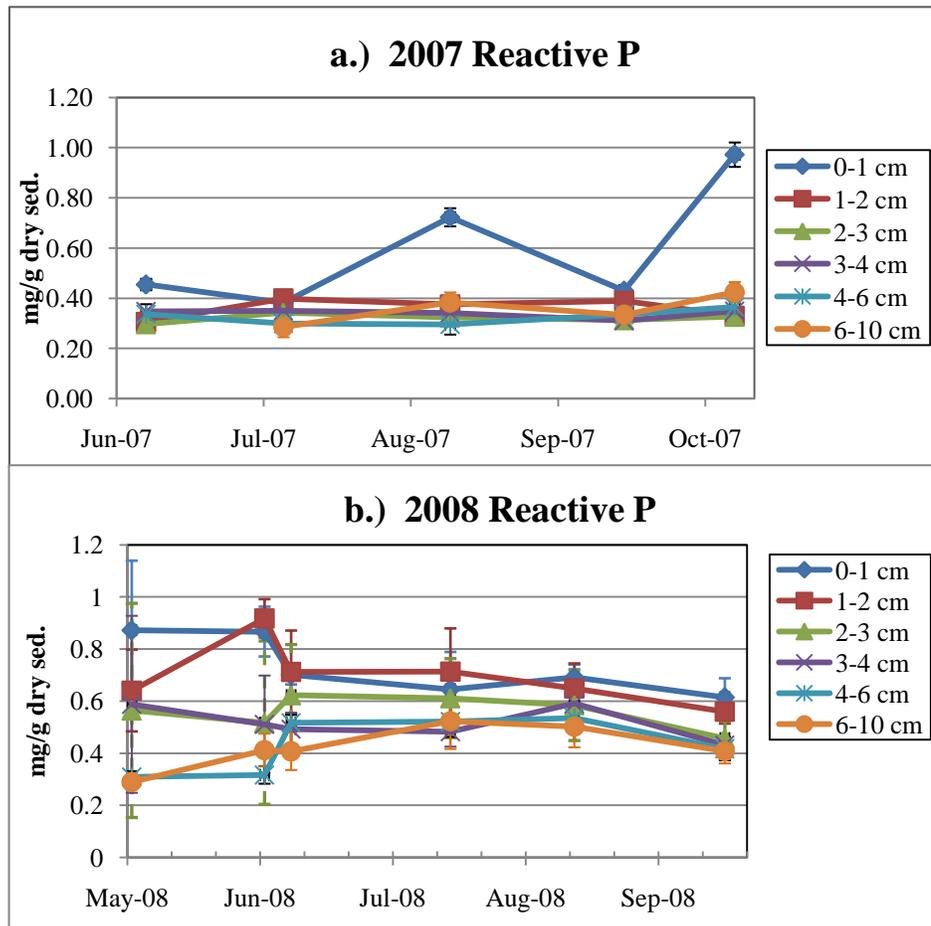
Figure 10 (a and b): Seasonal reactive P concentrations with respect to sediment depth segment across 2007 (a) and 2008 (b). Reactive P increases in the surface sediments in 2007 but decreases in 2008. Concentrations represent averages when multiple samples were extracted. Error bars for 2007 represent the average standard deviation for replicate extractions. Error bars for 2008 represent the standard deviation for the six cores that were collected at each diel sampling, except in May and June 19 when only 2 cores were collected for each time.

P, correlations with Mn, Fe, Ca, and Al

For both 2007 and 2008 samples, RP concentrations have a strong tendency to increase with increasing RFe concentrations (R^2 = 0.86 and 0.90, respectively, $p < 0.001$) (Table 6). Reactive P concentrations tend to increase with RMn and RCa concentrations but the correlation coefficients were not as strong (R^2 = 0.57 and 0.59, respectively). In

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2007, TP concentrations have a strong tendency to increase with increasing TFe

concentrations ($R^2 = 0.91$, $p < 0.001$) but the relationship is not as good for 2008 samples

($R^2 = 0.62$, $p < 0.001$).

Sediment Extraction Correlation Statistics						
	Ascorbic Acid (Reactive)			Aqua Regia (Total)		
2007	R^2	p	n	R^2	p	n
P vs. Fe	0.86	< 0.001	51	0.91	< 0.001	53
P vs. Mn	0.57	< 0.001	51	0.65	< 0.001	53
P vs. Ca	0.59	< 0.001	36	0.57	< 0.001	30
P vs. Al	0.24	< 0.01	36	0.32	< 0.001	30
2008						
P vs. Fe	0.90	< 0.001	202	0.62	< 0.001	202
P vs. Mn	0.66	< 0.001	202	0.63	< 0.001	202
P vs. Ca	0.69	< 0.001	184	0.17	< 0.001	185
P vs. Al	0.47	< 0.001	184	0.69	< 0.001	185

Table 6: Sediment extraction correlation statistics representing the linear relationship between total and reactive P, Fe, Mn, Ca, and Al.

Nitrogen

Porewater Ammonium (NH_4^+) values

demonstrated variability in the sediment column,

specifically in the 0-1 cm segment where

concentrations were greatest in August 2008 (Figure

11). Total organic N (TON) averaged $0.28 \pm .04$ %

for all samples, TOC averaged $2.8 \pm 0.3\%$, and C:N

ratios averaged around 10 ± 0.6. No significant

relationship between TON and Fe, Mn, or P (total

and reactive) was found.
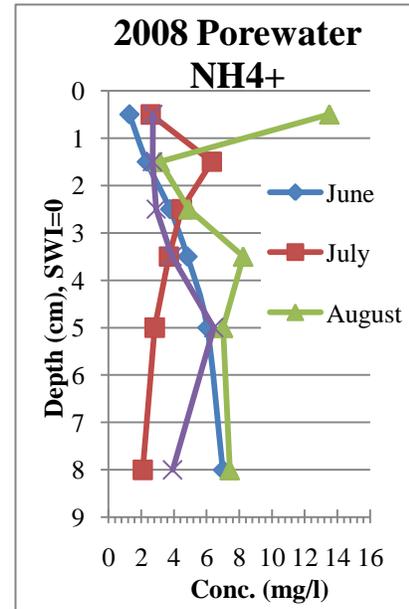
Figure 11: Pore water ammonium concentrations from cores collected in 2008 (at the noon sampling time). The y-axis represents the depth segment of the sample relative to the SWI.

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DISCUSSION

Extraction Data

Retention of P in oxic sediments is often explained by a capping effect caused by a microzone of oxidized Fe minerals on the sediment surface which sorb settling P (DIP or DOP) and prevent P from lower sediments from escaping (e.g. Mortimer, 1941; Böstrom, 1988; Penn et al. 2000). The Fe(III) oxide microlayer is formed as Fe from deeper sediments is reduced, solubilized, and transported vertically to equilibrate with water column Fe (Sundby, 1982; Penn et al. 2000). Upon contact with oxygen, either in the surface sediments or slightly above, Fe(III)oxide will precipitate. We operationally defined the reactive Fe pool as that extractable by an ascorbic acid solution. According to Kostka and Luther (1995), the Fe extracted with this method is almost completely 'amorphous' Fe(III) oxides (likely a combination of nanocrystalline 2-line and 6-line ferrihydrite (Banfield et al. 2000; Cornell and Schwertmann, 2003; Ferris, 2005; Fortin and Langley, 2005;), which are the most susceptible to reduction under reducing conditions and would have a significant available surface area for P sorption. In Missisquoi Bay, average RFe was the highest percent of average TFe in the surface sediments for both 2007 and 2008, most likely due to this process. However, in 2008 the RFe in the 0-1 cm segment consisted of a much larger percentage of TFe (seasonal average of 22%) compared to 2007 (seasonal average of 14%) suggesting a difference in the degree of Fe mobility between the two summers.

The P measured in the same supernatant of the ascorbic extraction is considered the reactive P portion, or the portion that will potentially be released with solubilization

of the minerals to which it is adsorbed. Extraction results show that the RP comprises a significant portion of the TP in the surface sediments (averaged 34 ± 1.5 % in 2007 and $42 \pm 1.0\%$% in 2008) and RP is most strongly correlated to the RFe component of the sediment for both summers ($R^2 = 0.85$ and 0.91, 2007 & 2008 respectively). The considerable portion of RP in the surface sediments of Missisquoi Bay indicates that a significant amount of P in the surface sediments is associated with reducible Fe and mobility of this P will be controlled by redox conditions. The strong correlation between the RFe and RP therefore indicate that the RP in the sediment is closely tied to and dependent on the nanocrystalline iron oxyhyrdoxide minerals. The correlation between these components is slightly stronger for 2008 compared to 2007 ($R^2 = 0.85$ vs. 0.90). Correlations between RP and RMn or RCa are fairly good ($R^2 > 0.55$) but RP and RAl are poorly correlated ($R^2 < 0.50$).

A noticeably different seasonal trend in surface sediment RP and RFe concentrations was observed in 2008 compared to 2007. The 2007 RP and RFe pool generally increased in concentration in the top layers of the sediment over the course of the summer (Figure 10a) while the trend was opposite in 2008 (concentrations in the beginning of the summer (June & July) in the 0-1cm and 1-2 cm segment were significantly different than during peak bloom (August & October) in 2008 (as determined by Student's t test, $p < 0.001$)). The RFe in the 0-1 cm segment decreased over the season of 2008, suggesting that Fe(III)oxides were reduced and solubilized from the surface sediments. The concomitant trend of decreasing RFe and RP concentrations with increasing reducing conditions demonstrates the role of Fe (III) oxide reduction in P

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release to the water column in Missisquoi Bay during a cyanobacteria bloom.

Calculations suggest that the amount of RP lost from the surface sediments to the

overlying water from May to August 2008 could have increased DP in the water column

on the order of thousands of micrograms/l (Table 7).

Units	mgP/g dw	mg RP/m^2	mg RP/m^2	µg P/ m^2	µgP/L added to overlying water (compared to 5/20/08)
	(ave, ~1/8 core D)	sed (core D= 8 cm)	Compared to May (loss to water column)	conversion to µg/m^2	3 m^2 overlying water/ 1 m^2 sed
5/20/2008	0.48	38182			
6/19/2008	0.48	37945	237	237,287	79
6/25/2008	0.39	30686	7,496	74,96,254	2,499
7/31/2008	0.36	28266	9,916	9,915,506	3,305
8/28/2008	0.38	30269	7,913	7,912,947	2,638
10/1/2008	0.34	26920	11,262	11,261,978	3,754

Table 7: Calculated loss of RP to water column based on average RP concentrations in the surface sediments (0-1 cm) in May 2008. The sediment used for the reactive extraction was estimated to comprise 1/8 of the 0-1 cm core volume and the core diameter (D) was 8 cm. The calculations were based on a 1 m^2 section of sediment with 3 m^3 of overlying water, assuming the entire 1 cm of sediment depth contributes to RP loss into the overlying water column.

Nitrogen

Nitrogen speciation and concentrations in the sediment pore water also

demonstrated fluctuations associated with seasonal changes in the bay. Ammonium

values were the highest in the pore waters in August. In the absence of O_2, some

organisms can utilize NO_3^- as an electron acceptor during ammonification (Cainfield,

Thamdrump, & Kristensen, 2005). Alternatively, NH_4^+ is liberated from organic matter

via hydrolysis of N containing components such as proteins and lipids (Cainfield,

Thamdrump, & Kristensen, 2005). It is therefore difficult to discern if the increased

amount of NH_4^+ was a result of more reducing conditions or increased organic matter

degradation in the sediments. Organic C content was not significantly different at this time, so the former pathway is more likely.

Redox Conditions

The electrochemical data from both summers show that the redox front moved upward relative to the SWI from the beginning of the summer (May) to the end of the summer (September and October) indicating progressively more reducing conditions at the SWI and within the sediment. Upward movement of the redox front was more pronounced in the core profiles and diel cycles during 2008 compared to 2007. The diel cycle from September of 2007 indicated that the sediments were oxic at this time, however August and October 2008 sediments were completely anoxic with Mn(II) and sometimes Fe reduction at the SWI. Seasonal diel monitoring in 2008 captured diel fluctuations of the redox front at the SWI where sediments tended to become more anoxic from late night to early morning in July, August, and October. These results support the hypothesis that sediments become increasingly anoxic toward the end of the summer as well as the late night to early morning time period for each diel sampling event.

Cyanobacteria photosynthetically produce O_2 during the day but also respire heterotrophically to consume O_2 at night and in low light conditions. Additionally, the establishment of thick blooms prevents light penetration and decreases the amount of photosynthesis, and thus oxygen production, in the deeper waters. Eventually, oxygen consumption can exceed production within the water column, especially in the bottom waters (less mixing), influencing the upward migration of the redox front in the sediment column (Tyler et al. 2009; Beck and Bunland 2001). In other shallow eutrophic lakes,

bottom water anoxia is a typical summer occurrence and sometimes results in fish kills due to the lack of oxygen (e.g. Breitburg, 2002). The occurrence of seasonal sediment and bottom water anoxia is well known (e.g., Carignan and Lean, 199; Jensen and Andersen, 1992; Olila and Reddy, 1997), however, diel fluctuations in lake sediments as presented in this study have not been documented at this scale before in fresh water lakes. Observed diel changes may be affected by the same process: photosynthetic activity increases oxygen concentration in the water during the day and respiration consumes oxygen at night.

The response of redox conditions at the SWI due to the proposed diel oscillations has been shown to be significant by this study. Increasing biomass across the season and the balance between photosynthetic production and heterotrophic respiration influenced the vertical migration of the redox front in the sediment on seasonal and diel timescales. The implications of this relationship are central to understanding sediment P cycling in a shallow bay because the redox state of the environment is closely coupled to P-release and sorption to Fe and Mn mineral surfaces (Roden and Edmonds, 1997; Gunnars and Blomquist, 1997; Petticrew and Arocena, 2001). As redox conditions at the SWI fluctuate over diel cycles, subsequently influencing the presence and effectiveness of the sediment surface Fe(III) hydroxide microzone, P flux from the sediments might change significantly on hourly timescales.

Our results also agree with the trends observed in the study by Beck and Brunland (2001) which showed that redox sensitive trace elements (I, Mn, and Fe), dissolved oxygen, pH, temperature, and nutrients in water collected 0.5 meters from the SWI are

subject to extreme diel variations in a salt marsh system. They determined that the balance between primary productivity and respiration was the predominate factor in diel shifts of DO concentrations in a shallow aquatic system; maximum DO concentrations were measured in late afternoon during peak photosynthesis and suboxic conditions prevailed in the early morning. A diel trend in the water column nutrients was also observed in which NH_4^+, NO_3^-, NO_2, PO_4^{3-}, Fe, and Mn concentrations changed inversely to DO. A decrease in nutrient concentration was attributed to uptake and assimilation during the hours of net primary production in addition to tidal flushing of nutrient depleted water, both processes increase water column DO. At night, respiration would consume water column DO, incurring conditions that promote the release of nutrients from the sediments via the reduction mechanisms previously discussed.

In our study, the SWI O_2 and Mn concentrations near the start and end of the diel sampling period appear to be the most variable, which could be due to a number of different physical or biological processes. Variations in average hourly wind data from a nearby station (Frelighsburg, Quebec) appear to show some influence on the fluctuations of these redox species (Appendix D). The role of bioturbation and gas ebulation from deeper sediments is another consideration. Michaud et al. (2005 & 2006) have observed the influence of benthic bioturbation and nutrient flux in the St. Lawrence tributary and found that burrows and community structure can significantly alter the nutrient flux from sediments. It is therefore plausible that biological activity and movement within the sediments could easily change the O_2 concentrations over short time scales.

2007 vs. 2008 – Implications of the Cyanobacteria Bloom

The most obvious difference in the aquatic system between the two summers was the presence of the cyanobacterial bloom. The potential for nutrients to be released from the sediments is an advantage for many cyanobacteria species because they have the ability to regulate their buoyancy (Hyenstrand, et al. 1998). It is possible for some cyanobacteria to stay near the surface and photosynthesize during the day and then sink to the bottom to utilize nutrients diffusing from the sediments. This ability may allow them to outcompete other species for nutrients. Because we collected dive cores at several points in the day during these blooms, we were able to observe that the cell density was concentrated in the top two feet of the water but cells were dispersed throughout the water column and at the SWI. This was most apparent in August and October but the cells were mostly in the top of the water column in July. Cell counts and species data are being processed and will be published in a separate paper.

Although it is highly plausible that the reducing conditions within the sediment are related to the bloom, the question remains as to whether the bloom caused the difference in the sediment redox chemistry or vica versa. Xie et al. (2003) proposed that a *Microcystis* bloom enhanced the release of DP from the sediments in a controlled study comparing mesocosms with and without sediments. They found that water column pH was the leading cause of P release; however, they did not measure pH, DO or redox potentials in the sediments.

The pH within the Missisquoi Bay water column showed a definite increase from pH 6 (May) to pH 9 (late July and August) across both 2007 and 2008 (Figure 10). Diel

changes in the pH of overlying core water were also observed. The effect of pH on P

release from Fe and Mn minerals is well known; generally, an increase in pH increases

OH^- substitution for PO_4^{3-} sorbed to mineral surfaces, changing the surface charge

density, and above pH 8 (pH_{ZPC} of FeOOH) changing the sign of the surface charge so

that PO_4^{3-} is liberated. Although our study focused on the redox fluctuations within the

sediment, changing pH conditions could also contribute to increased P release from the

surface sediments, but interstitial waters are most likely buffered by minerals (Cai et al.

2002). Cai et al. 2002 found that the pH in Lake Champlain sediments ranges between

7.2 and 6.8, with the drop in pH associated with, NH_4^+, Fe^{2+} or Mn^{2+} oxidation (i.e. NH_4^+

$+ 2O_2 \rightarrow NO_3^- + H_2O + 2H^+$).

The cyanobacteria bloom was initiated before the *in-situ* voltammetry indicated

that the SWI was completely anoxic (July). The timing of the bloom and onset of

significant sediment anoxia suggest that the bloom caused greater reducing conditions.

This concept is reinforced by the fact that there was no bloom in 2007 and topmost

sediments remained oxic throughout a late summer diel cycle but diel sediment anoxia

cycles were observed in 2008 during an intense algal bloom. The potential P released

from the sediment could have perpetuated the bloom if nutrients within the water became

limited due to the cyanobacteria population explosion. A future paper from the same

study site and times will address this in more detail. The lack of a bloom in 2007 could

be due to a number of reasons; however, it is evident that the sediment chemistry is

closely linked to the presence of a bloom.

CONCLUSION

Seasonal and diel redox cycling at the SWI is influenced by the presence of a cyanobacteria bloom which has important implications for nutrient mobility, specifically P, in the sediments. Reactive P and RFe are strongly correlated in the sediments; these components comprise about 6-22% and 30-40% of the total Fe and P pool, suggesting that a large portion of P mobility is associated with Fe oxyhydroxide minerals. This mobile P pool is therefore subject to liberation into the water column as sediments become more reduced over the season. A net loss in RP in the surface sediments was observed in 2008 when the sediments were anoxic, but not in 2007 when they were oxic, indicating that P was released into the water column under anoxic conditions. Ammonium in sediment pore water is also influenced by late summer reducing conditions as concentrations in the surface sediments were much higher in August 2008 (most reducing conditions) compared to any other month. The results of this study demonstrate that sediment chemistry is significantly influenced by seasonal and diel changes in the water column. Additionally, diel redox fluctuations at the SWI imply that P flux between the sediment and water column changes on hourly timescales, which could be important for the success and dominance of cyanobacteria species with the ability to vertically migrate.

Although the study by Beck and Bunland (2001) demonstrated a relationship between diel DO concentrations, nutrients, and redox species, water samples were collected about 0.50 m above the sediment, thus the mechanisms responsible for these patterns were not clear. This is the first study to document diel O_2 and redox fluctuation

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in freshwater lakes. The data from this study show that P mobility in the sediments is strongly correlated to RFe and that redox conditions at the SWI change significantly across diel cycles, throughout the summer, and in the presence of a bloom. Major differences in sediment chemistry were observed between summers with (2008) and without (2007) a bloom and comparison of these changes will help to build a better understanding of the relationship between sediment redox chemistry, nutrient flux, and the success of a cyanobacteria bloom.

REFERENCES

Andersen, J.M. 1975. Influence of pH on release of P from lake sediments. *Arch. Hydrobiol*. 76:411 - 419.

Anschutz, P., Sundby, B., Lefrancois, L., Luther, G. W., & Mucci, A. (2000). Interactions between metal oxides and species of nitrogen and iodine in bioturbated marine sediments. *Geochimica Et Cosmochimica Acta, 64*(16), 2751-2763.

Anschutz, P., Zhong, S. J., Sundby, B., Mucci, A., & Gobeil, C. (1998). Burial efficiency of phosphorus and the geochemistry of iron in continental margin sediments. *Limnology and Oceanography, 43*(1), 53-64.

Bainfield, J.F., Welch, S.A., Zhang, H., Thomsen Ebert,T., Penn, R.Lee. (2000). Aggregation-based crystal growth and microstructure development in natural iron oxyhydroxide biomineralization products. *Science*, 289, 751-753.

Beck, N. G., & Bruland, K. W. (2000). Diel biogeochemical cycling in a hyperventilating shallow estuarine environment. *Estuaries, 23*(2), 177-187.

Bostrom, B., Andersen, J. M., Fleischer, S., & Jansson, M. (1988). Exchange of phosphorus across the sediment - water interface. [Article]. *Hydrobiologia, 170*, 229-244.

Breitburg, D. (2002). Effects of hypoxia, and the balance between hypoxia and enrichment, on coastal fishes and fisheries. *Estuaries, 25*(4B), 767-781.

Brendel, P. J., & Luther, G. W. (1995). Development of a gold amalgam voltammetric microelectrode for the determination of dissolved fe, mn, o-2, and s(-ii) in porewaters of marine and fresh-water sediments. *Environmental Science & Technology, 29*(3), 751-761.

Burgess, H. (2007). *Geochemical indicators of productivity change in Lake Champlain, USA-CANADA*. The University of Vermont, Burlington, VT.

Cai, W. J., Zhao, P., Theberge, S.M., Witter, A., Wang, Y., Luther, G.W.(III) (2002). Porewater redox species, pH, pCO2, in aquatic sediments: Electrochemical sensor studies in Lake Champlain and Sapelo Island. In M. a. R. Taillefert, T.F. (Ed.), *Environmental Electrochemistry Analyses of Trace Element Biogeochemistry* (pp. 188-209). Washington, D.C.: American Chemical Society.

Cainfield, D. E., Kristensen, E., and Thamdrup, B. (2005). *Aquatic Geomicrobiology*. Amsterdam: Elsevier.

Carignan, R., & Lean, D. R. S. (1991). Regeneration of dissolved substances in a seasonally anoxic lake - the relative importance of processes occurring in the water column and in the sediments. *Limnology and Oceanography, 36*(4), 683-707.

Christophoridis, C., & Fytianos, K. (2006). Conditions affecting the release of phosphorus from surface lake sediments. *Journal of Environmental Quality, 35*(4), 1181-1192.

Cornell, R.M., Schwertmann, U., 2003. The Iron Oxides: Structure, Properties, Reactions, Occurrences and Uses, 2nd, Completely Revised and Extended Edition. Wiley-VCH Verlag, Weinheim, Germany, p. 703.

Davanzo, C., & Kremer, J. N. (1994). Diel oxygen dynamics and anoxic events in an eutrophic estuary of waquoit bay, massachusetts. [Article]. *Estuaries, 17*(1B), 131-139.

Drake, J. C., & Heaney, S. I. (1987). Occurrence of phosphorus and its potential remobilization in the littoral sediments of a productive english lake. [Article]. *Freshwater Biology, 17*(3), 513-523.

Druschel, G. K. H., A., Lomonaco R. and Oldrid, K. (2005). *Determination of sediment phosphorus concentrations in St. Albans Bay, Lake Champlain: Assessment of internal loading and seasonal variations of phosphorus sediment-water column cycling*.

Ferris, F.G. (2005). Biogeochemical properties of bacteriogenic iron oxides. *Geomicrobiology*, 22, 79-85.

Fortin, D. and Langley, S. (2005). Formation and occurrence of biogenic iron-rich minerals. *Earth-Science Reviews*, 72, 1-19.

Frausto da Silva, J. J. R. W., R. J. P (1991). Manganese: dioxygen evolution and glycosylation *The Biological Chemistry of the Elements/The Inorganic Chemistry of Life*. Oxford: Clarendon Press.

Gachter, R., Meyer, J. S., & Mares, A. (1988). Contribution of bacteria to release and fixation of phosphorus in lake-sediments. [Article]. *Limnology and Oceanography, 33*(6), 1542-1558.

Gunnars, A., & Blomqvist, S. (1997). Phosphate exchange across the sediment-water interface when shifting from anoxic to oxic conditions - an experimental comparison of freshwater and brackish-marine systems. *Biogeochemistry, 37*(3), 203-226.

HARRNESS (2005). *Harmful algal research and response: a national environmental science strategy 2005-2015*. Washington, DC: Ecological Society of America.

Hecky, R. E., & Kilham, P. (1988). Nutrient limitation of phytoplankton in fresh-water and marine environments - a review of recent-evidence on the effects of enrichment. [Review]. *Limnology and Oceanography, 33*(4), 796-822.

Hegmen, W., Wang, D., & Borer, C. (1999). *Estimation of Lake Champlain basinwide nonpoint source*. Grand Isle, VT: Lake Champlain Basin Program.

Hesslein, R. H. (1976). An in situ sampler for close interval pore water. *Limnology and Oceanography, 21*, 912-914.

Hyenstrand, P., Blomqvist, P., and Pettersson, A.(1998) Factors determining cyanobacterial success in aquatic systems- a lit review. *Archives Hydrobiologie Special Issues Advanced Limnology*. (51)41-62.

Istvanovics, V. (1988). Seasonal-variation of phosphorus release from the sediments of shallow lake balaton (hungary). [Article]. *Water Research, 22*(12), 1473-1481.

Jensen, H. S., & Andersen, F. O. (1992). Importance of temperature, nitrate, and ph for phosphate release from aerobic sediments of 4 shallow, eutrophic lakes. [Article]. *Limnology and Oceanography, 37*(3), 577-589.

Kostka, J. E., & Luther, G. W. (1994). Partitioning and speciation of solid-phase iron in salt-marsh sediments. [Article]. *Geochimica Et Cosmochimica Acta, 58*(7), 1701-1710.

LCBP. (2007). Lake Champlain Basin Program. www.lcbp.org

Luther, G. W., Glazer, B., Ma, S. F., Trouwborst, R., Shultz, B. R., Druschel, G., et al. (2003). Iron and sulfur chemistry in a stratified lake: Evidence for iron-rich sulfide complexes. *Aquatic Geochemistry, 9*(2), 87-110.

Mortimer, C. H. (1941). The exchange of dissolved substances between mud and water in lakes. *J. Ecol., 29*, 280-329.

Olila, O. G., & Reddy, K. R. (1997). Influence of redox potential on phosphate-uptake by sediments in two sub-tropical eutrophic lakes. *Hydrobiologia, 345*, 45-57.

Penn, M. R., Auer, M. T., Doerr, S. M., Driscoll, C. T., Brooks, C. M., & Effler, S. W. (2000). Seasonality in phosphorus release rates from the sediments of a hypereutrophic lake under a matrix of pH and redox conditions. [Article]. *Canadian Journal of Fisheries and Aquatic Sciences, 57*(5), 1033-1041.

Petticrew, E. L., & Arocena, J. M. (2001). *Evaluation of iron-phosphate as a source of internal lake phosphorus loadings*.

Roden, E. E. a. E., J.W. (1997). Phosphate mobilization in iron-rich anaerobic sediments: microbial Fe(III) oxide reduction versus iron-sulfide formation. *Arch. Hydrobiol., 139*(3), 347-378.

Roitz, J. S., Flegal, A. R., & Bruland, K. W. (2002). The biogeochemical cycling of manganese in San Francisco Bay: Temporal and spatial variations in surface water concentrations. *Estuarine Coastal and Shelf Science, 54*(2), 227-239.

Rozan, T. F., Taillefert, M., Trouwborst, R. E., Glazer, B. T., Ma, S. F., Herszage, J., et al. (2002). Iron-sulfur-phosphorus cycling in the sediments of a shallow coastal bay: Implications for sediment nutrient release and benthic macroalgal blooms. *Limnology and Oceanography, 47*(5), 1346-1354.

Ryding, S. O. (1985). Chemical and microbiological processes as regulators of the exchange of substances between sediments and water in shallow eutrophic lakes. *Internationale Revue Der Gesamten Hydrobiologie, 70*(5), 657-702.

Schindler, D. W., Hecky, R. E., Findlay, D. L., Stainton, M. P., Parker, B. R., Paterson, M. J., et al. (2008). Eutrophication of lakes cannot be controlled by reducing nitrogen input: Results of a 37-year whole-ecosystem experiment. [Article]. *Proceedings of the National Academy of Sciences of the United States of America, 105*(32), 11254-11258.

Schindler, D.W. (1974). Eutrophication and Recovery in Experimental Lakes: Implications for Lake Management. Science. (184)4139, 897-899.

Smith, V. H., Tilman, G. D., & Nekola, J. C. (1999). Eutrophication: impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems. *Environmental Pollution*. 100, 179-196.

Sondergaard, M., Jensen, J. P., & Jeppesen, E. (2003). *Role of sediment and internal loading of phosphorus in shallow lakes*.

Sondergaard, M., Kristensen, P., & Jeppesen, E. (1992). Phosphorus release from resuspended sediment in the shallow and wind-exposed lake arreso, denmark. [Article]. *Hydrobiologia, 228*(1), 91-99.

Spears, B. M., Carvalho, L., Perkins, R., & Paterson, D. M. (2008). Effects of light on sediment nutrient flux and water column nutrient stoichiometry in a shallow lake. *Water Research, 42*(4-5), 977-986.

Sundby, B., Anderson, L. G., Hall, P. O. J., Iverfeldt, A., Vanderloeff, M. M. R., & Westerlund, S. F. G. (1986). The effect of oxygen on release and uptake of cobalt, manganese, iron and phosphate at the sediment-water interface. *Geochimica Et Cosmochimica Acta, 50*(6), 1281-1288.

Sundby, B., Gobeil, C., Silverberg, N., & Mucci, A. (1992). The phosphorus cycle in coastal marine-sediments. *Limnology and Oceanography, 37*(6), 1129-1145.

Troy, A. (2007). Updating the Lake Champlain basin land use data to improve prediction of phosphorus loading. In D. Wang (Ed.), *Report to the Lake Champlain Basin Program*. Grand Isle, Vermont: LCBP Technical Report.

Tyler, R. M., Brady, D. C., & Targett, T. E. (2009). Temporal and Spatial Dynamics of Diel-Cycling Hypoxia in Estuarine Tributaries. *Estuaries and Coasts, 32*(1), 123-145.

VTDEC (2008). Lake Champlain long-term monitoring. Vermont Department of Environmental Conservation. http://www.vtwaterquality.org/cfm/champlain/lp_longterm-lakes.cfm

Xie, L. Q., Xie, P., & Tang, H. J. (2003). Enhancement of dissolved phosphorus release from sediment to lake water by Microcystis blooms - an enclosure experiment in a hyper-eutrophic, subtropical Chinese lake. *Environmental Pollution, 122*(3), 391-399.

CHAPTER 4: Paper for Submission to Environmental Science and Technology

Phosphorus Characterization in Freshwater Lake Sediments using ^{31}P NMR
Spectroscopy: Addressing Seasonal Mobility Associated with Redox Conditions

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ABSTRACT:

The phosphorus (P) content in sediments collected across two consecutive summers (2007 & 2008) from eutrophic Missisquoi Bay, Lake Champlain was characterized using ^{31}P NMR spectroscopy; a cyanobacteria bloom occurred in 2008 but not in 2007 leading to different sediment and water chemistries. Sediment cores were profiled using *in situ* voltammetry and the top ten centimeters were sectioned and analyzed for P in addition total and reactive P, Fe, Mn, Al, and Ca. More reducing conditions were observed at the sediment water interface (SWI) in 2008, particularly in August during a peak bloom. The organic P (P_{org}) content ranged from 18-26% of the total P and monoester phosphates were the dominant compound class. Phytate and *scyllo*-inositol phosphate were identified in most sediments. Monoester to diester ratios ranged from 4.6 to 13.1, the highest being in the surface sediments in August 2008. Polyphosphate was only detected in a few samples in 2007, but not in 2008. Pyrophosphate decreased across the 2008 season and was not present in the deeper sediments in October. P_{org} was strongly correlated ($R^2 = 0.89$, $p < 0.05$) to porewater Fe(II), indicating that its mobility is associated with the reduction of Fe(III) hydroxides.

INTRODUCTION

Excessive anthropogenic loading of nutrients into rivers, lakes, and ultimately the ocean is a threat to water quality and ecosystem health worldwide and eventually results in the overabundance of nutrients, or eutrophication (e.g. Smith, Tillman, & Nekola, 1999). Eutrophic surface waters often experience toxic blue-green algae blooms that are a health concern and a deterrent to recreation. External loading of nutrients from the watershed, the majority of which originates from agricultural and urban runoff, contributes to eutrophication, however, the release of nutrients from sediment (internal loading) in surface waters has been shown to perpetuate high water column nutrient concentrations regardless of decreased external loading (Marsden, 1989; van der Molen & Boers, 1994; Jeppesen et al. 1997). When assessing nutrient abundance and mobility within natural systems, organic forms of phosphorus (P_{org}) such as inositol phosphates (IPs), are often considered immobile, refractory, or bio-unavailable (McKelvie, 2007).

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This is mainly because they do not react with molybdate, a compound used in a colorimetric test that determines the concentration of operationally defined soluble reactive P (McKelvie, 2007). Characterizing and quantifying P_{org} molecules found in the environment has been difficult due to the myriad P_{org} moieties possible which are often present at relatively low concentrations. However, recent studies have shown the efficacy of [31]Phosphorus Nuclear Magnetic Spectroscopy (^{31}P-NMR) for analyzing environmental samples and clarifying the role of P_{org} in nutrient cycling (Ahlgren et al., 2005; extensive review in Cade-Menun, 2005).

Many eutrophic lakes experience algae blooms in the summer months that consume dissolved P (DP, both inorganic, P_i, and organic, P_{org}, forms) and transform it into more complex forms of P_{org} (e.g., biomass) and storage compounds such as polyphosphates (P_i). The P-containing molecules of aquatic organisms include nucleic acids (DNA and RNA), phospholipids in membranes, and phosphate monoesters, which include sugar phosphates and storage compounds. These enter the P pool upon death of the organism (Wetzel, 1999). If not consumed within the water column, these P molecules will settle to the bottom and enter the sediment P pool. P_{org} compounds originating from plants and other organic matter transported from the watershed also contribute to P_{org} in sediments (Weimer and Armstrong, 1979; Makarov, 2005). In some lake sediments, total P_{org} constitutes about 1/3 of the TP (Williams and Mayer, 1972; Böstrom et al., 1984; Wetzel, 1999); however, sediment P_{org} content varies according to the lake's morphology, hydraulic loadings, hydrodynamics, trophic state, and other factors (Wetzel, 1999).

Orthophosphate (PO_4^{3-}) is generally considered the most bioavailable and mobile form of P within sediments and the water column. However, sediment bacteria and microalgae can enzymatically hydrolyze terminal phosphate groups of exogenous P_{org} compounds such as glycerophosphate, adenosine 5'-monophosphate (AMP), guanosine 5'-monophosphate (GMP), adenylic acids, phosphonate compounds, and others (Wetzel, 1999; Cembella et al. 1983, 1984). This mineralization process involving different P_{org} compounds plays an important role in the P cycle by contributing to the more bioavailable inorganic P pool; an aspect that is not well characterized in lake sediments. Additionally, some bacteria and algae are able to directly utilize dissolved P_{org} (DP_{org}) (Bentzen et al. 1992; Cotner & Wetzel, 1992). The bioavailability of organophosphates and the potential for organophosphates to be transformed into more bioavailable forms is dependent on biogeochemical reactions, mobility, and speciation within the sediment.

Phosphorus, both P_i (PO_4^-) and some P_{org} (e.g., IHP) compounds, readily sorbs to sediment mineral surfaces under oxic conditions (e.g., Wang et al. 2007; Rodel et al. 1977; Mortimer, 1941). Common minerals in freshwater sediment which sorb P include Fe, Mn, and Al oxyhydroxides (FeOOH, MnOOH, AlOOH, respectively) in addition to calcite ($CaCO_3$) and clays (Søndergaard, 2003). Conditions influencing sediment P mobilization from sediments such as redox potential and pH have been studied although more focus has been on the sorption and release of PO_4^{3-} and not specific P_{org} compounds (e.g. Søndergaard, 2003; Roden and Edmonds, 1997; Sundby, 1992). Organic P sorption and hydrolysis rates in sediments are dependent on the form of P_{org}, which may be governed by the number of phosphate groups associated with the P_{org} molecule (Rodel et

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al. 1977), most likely because the higher number of phosphate ester groups in P_{org} compounds are more strongly attracted to the positive surface charge of oxidized minerals. Redox conditions also influence microbial storage and release of polyphosphate and pyrophosphate (P_i) in lake sediments and may play a role in the well-observed trend of increased P release under anoxic conditions (Sannigrahi & Ingall, 2005; Søndergaard, Jensen, & Jeppesen, 2003; Hupfer, Gächter, &Ruegger, 1995).

Although ^{31}P-NMR is a powerful tool for analyzing environmental samples, studies of P_{org} characterization and importance have been focused on terrestrial samples and very little work (e.g. Reitzel et al. 2007; Alghren et al. 2005; Carman, et al. 2000) has addressed their role in lake and marine sediments. This study investigated the seasonal mobility and biogeochemical cycling of P in eutrophic Missisquoi Bay, Lake Champlain sediments using ^{31}P NMR to characterize P_i and P_{org} species coupled with an investigation of sediment and porewater chemistry in lake sediments at different depths (across the top ten centimeters) and at different times to determine if P_{org} mobility and stability is different from P_i.

Study Site

Missisquoi Bay is one of the most eutrophic sections of Lake Champlain, located in the northeastern quadrant of the lake with 58% of its watershed in Vermont, USA and 42% in the Province of Quebec, Canada (Troy, et al, 2007). The entire watershed is 3,105 km^2 (767,246 ac), the surface area of the bay is approximately 77.5 km^2 (19,150 acres), and the maximum depth is 4 m (Hegman, et al. 1999). The total P (TP) in the water column from 1993-2007 ranged between 6-111 µg/L, averaging near 49 µg/L (VT

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DEC, 2008), which is well above the eutrophic status level of 35 µg/L. Our study site

was located on the eastern side of the Missisquoi River delta but more directly in line

with the drainage area of Rock River, a smaller tributary flowing into Missisquoi Bay.

The sediments are described in detail by Burgess (2007), briefly, the top 30 cm are

mottled brown-gray sediments, slightly coarser than clay, with organic-C (C_{org}) between

2-3%. The sediments become more clay rich (dark gray and fine-grained) below the

most recent deposits (60 cm). The recent sedimentation rate is approximately 1 mm/per

(Burgess, 2007). The sediments at our study site contained few, sparsely dispersed plants

and few, sparsely dispersed native mussels.

METHODS

Sample Collection

Sediment cores were collected by divers in June and October of 2007 and June,

August, July and October of 2008 in Missisquoi Bay, Lake Champlain between 11:00

and 13:00 EST. Redox measurements were obtained by electrochemically profiling the

cores using Au-Hg amalgam microelectrodes (constructed after Brendel and Luther,

1995; Luther et al. 1988; Taillefert et al. 2000; Rozan et al. 2002; Cai et al. 2002;

Druschel et al. 2005) lowered at 1-10 mm increments with a micromanipulator. The top

10 cm were then sectioned into 0-1, 1-2, 2-3, 3-4, 4-6, and 6-10 cm segments and

immediately frozen on board the research vessel (R/V *Melosira*) until analysis.

Redox Analysis

Concentrations of porewater O_2, Mn(II), and Fe(II) were calculated based on electrode calibration in the lab. Fe(III) and $FeS_{(aq)}$ were detected but are reported as current responses that are proportional to changing concentration; these species cannot currently be calibrated due to complexity of Fe(III) forms that react at the electrode surface in natural waters and the unknown molecular size of iron-sulfide molecular clusters ($FeS_{(aq)}$) (Taillefert et al. 2000; Luther and Rickard, 2005; Rickard, 2006; Roesler et al. 2007). The redox data obtained from each series of scans was averaged for each 1-cm depth segment to correspond to extraction data. For example, the redox species concentrations measured in mm increments from the top 10 mm of the core were averaged for the 0-1 cm segment.

^{31}P-NMR preparation

Wet, unaltered sediments (approximately 3 g equivalent dry weight) were extracted using a 25 ml aliquot of combined, N_2-purged NaOH (0.25 M) and Na_2EDTA (0.05 M) solution rotated end-over-end overnight (~10 hrs) (Cade-Menun and Preston, 1996). The samples were then centrifuged (1600 rpm for 20 minutes) and 1 ml of the supernatant was diluted 10-fold for analysis using ICP-OES. The remaining supernatant was frozen at -20°C and lyophilized. Subsequently, lyophilized NaOH-Na_2EDTA extracts were dissolved in 0.8 ml D_2O, 0.3 ml of 10M NaOH, and 1.2 ml of the NaOH-Na_2EDTA extraction solution, mixed, and then allowed to stand for 30 minutes with occasional vortexing. Samples were then centrifuged for 20 minutes at 1,500 g, transferred to 10-mm NMR tubes and stored at 4°C prior to analysis within 12 h.

Solution ^{31}P NMR spectra were obtained using a Bruker AVANCE 500 MHz

spectrometer equipped with a 10-mm broadband probe at Stanford University's Magnetic

Resonance Laboratory. The NMR parameters were 90° pulse, 0.68 s acquisition time,

4.32 s delay, 25°C, ~2500 scans (preparation and analysis after Cade-Menun and Preston,

1996).

Chemical shifts in parts per million were referenced to an external standard of

85% H_3PO_4. Spectra were plotted using 5 Hz line broadening and signals were assigned

to P compounds based on literature reports (Cade-Menun, 2005;Turner et al. 2003).

Relative percentages of P compounds were calculated by integration of signal areas and P

concentrations were calculated based on the total P concentration determined using ICP-

OES. A dilute phytic acid spike (.1g/100 ml NaOH-EDTA extracting solution) was used

to confirm the identification of phytate (Smernik and Dougherty, 2007).

Total and Reactive Sediment Fe, Mn, P, Al, and Ca Extractions

The sediments collected during 2007 and 2008 were analyzed for total and

reactive components, the only difference being that the 2007 sediments were oven dried

prior to extractions and the 2008 sediments were freeze-dried. Triplicate extractions

comparing oven drying versus freeze drying produced statistically agreeable results for

both extraction methods (Student's t test showed no significant difference between two

data sets, $p<0.050$.). An aqua regia digest (EPA method 3050B) consisting of a 3:1 ratio

of $HCl:HNO_3$ was used to extract total P (TP), Fe (TFe), Mn (TMn), Ca (TCa), and Al

(TAl) from 0.25-0.50 g of dry sediment. The acid and sediment were then refluxed for at

least 1 hour at 80-90°C, the supernatant was diluted 10-fold and then measured using

Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) using a JY-Horiba Ultima 2C at the University of Vermont Department of Geology. To determine the reactive portions of these components (RP, RFe, RMn, RCa, and RAl) associated with nanocrystalline metal oxyhydroxides an ascorbic acid extraction (Kostka and Luther, 1995; Anschutz, et al 1998) was performed. The ascorbic acid solution was made by dissolving 4 g sodium bicarbonate and 4 g sodium citrate in 200 ml N_2 purged water with 2 g ascorbic acid slowly added, the resulting pH should be 8 (if not it was adjusted using NaOH or HCl, appropriately). The prepared solution was then added to ~0.50 g of dry sediment, rotated for 24 hours, centrifuged, decanted, diluted 10-fold, and analyzed using the ICP-OES.

Total Organic Carbon and Nitrogen

Total organic carbon (TOC) and total organic nitrogen (TON) were determined by analyzing ~0.50 mg of homogenized freeze dried sediments in an elemental analyzer (CE Instruments NC 2500) with B2150 and B2152 standard soil as reference standards.

Statistical Analyses

Data were analyzed using Sigma Plot 11.0. Pearson product moment correlation tables were created to identify possible relationships among sediment constituents and linear regressions further examined seemingly good relationships. Multiple linear regressions were also performed to assess multivariate relationships; no statistically significant relationships were found so they are not reported. One-way ANOVA tests

compared the concentrations of P_{org} species with respect to depth and with respect to season.

RESULTS AND DISCUSSION

Redox Chemistry

Redox data obtained from voltammetric analyses indicated that O_2, if present, only penetrated 1-3 mm of the sediments at any time. Mn(II) was detected in the surface sediments (0-1 cm) in May, July, August, and October of 2008 and Fe(II) was detected in surface sediments (0-1 cm) in August only (Table 8). At other times, Fe(II) was detected in the 1-2 cm section, except in October where it was not detected until the 4-6 cm segment. Redox data from 2007 display similar trends with respect to oxygen penetration, but Mn(II) and Fe(II) were detected higher in the sediment column in June compared to October. The 2008 data indicate that the sediments were relatively reduced throughout the summer but more so in August 2008 when Mn(II) was detected above the SWI. Increasingly reduced conditions at the SWI throughout a summer have been observed in other lakes as a result of increased heterotrophic oxygen consumption in the water column during the summer months (e.g. Carignan & Lean, 1991). A cyanobacteria bloom can also contribute to summer water column and sediment anoxia through respiration at night in addition to increased sediment heterotrophic activity augmented by microbial decomposition of cyanobacteria dying and raining to the bottom.

2007 & 2008 Sediment Porewater Redox Species (µg/L)												
2008	**May**			**July**			**August**			**October**		
Depth	O2*	Mn	Fe(II)	O2*	Mn	Fe(II)	O2*	Mn	Fe(II)	O2*	Mn	Fe(II)
0-1 cm	99.4	5.3	0.0	150.0	7.0	0.0	23.0	343.3	89.7	0.0	0.0	**0.0**
1-2 cm	0.0	61.4	149.3	0.0	65.3	5.7	0.0	393.2	547.7	0.0	3.9	**0.0**
2-3 cm	0.0	121.2	284.6				0.0	238.7	994.2	0.0	23.0	**0.0**
3-4 cm	0.0	116.8	358.9				0.0	287.9	783.2	0.0	75.2	**0.0**
4-6 cm	0.0	181.4	227.8				0.0	283.7	868.5	0.0	79.6	**0.5**
6-10 cm	0.0	188.8	369.8				0.0	277.3	931.5	0.0	85.5	**9.4**
2007	**June**									**October**		
0-1 cm	125	66.3	0.0							57	0.0	**0.0**
1-2 cm	0.0	81.3	0.0							0.0	0.0	**0.0**
2-3 cm	0.0	159.1	42							0.0	40.4	**0.0**
3-4 cm	n/a	n/a	n/a							0.0	29.6	**0.0**
4-6 cm	n/a	n/a	n/a							0.0	61.6	**243.9**
6-10 cm	n/a	n/a	n/a									

Table 8: Concentration (µg/L) and appearance of redox species detected in sediment pore water by in-situ voltammetery. Values are averages of measurements made at 1-5 mm increments within each specific depth category. *O2 was only detected at the sediment-water interface, so the average value for each core at the SWI is entered in the 0-1 cm row. Detectable concentrations of O2 did not penetrate the sediments in any of the cores.

Total and Reactive Components

Sediment Total Fe, Mn, P, Al, and Ca concentrations are listed in Table 9. Total Fe, Mn, P and Ca generally decreased with depth but TAl showed no trend with depth. Reactive components were generally an order of magnitude lower but followed a similar trend with depth. Statistical analysis shows that RP is significantly linearly correlated to RFe and RMn ($R^2 = 0.95$ and 0.87, respectively, $p < 0.05$). Reactive P made up 30-40% of the TP and RFe made up 6-22% of the TFe (Table 5). Total P was also strongly correlated to TFe and TMn ($R^2 = 0.852$ and 0.925). Relationships between P and Al and Ca were significant ($p<0.05$) but not strongly correlated ($R^2 < 0.50$). This indicates that significant sediment P is associated with Fe and Mn, particularly the operationally defined reactive Fe. According to Kostka and Luther (1995), the ascorbic acid extraction reduces mostly nanocrystalline Fe(III) oxyhydroxides (amorphorous) liberating any P

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sorbed to their surfaces. The strong correlations found in this study indicate that a large portion of the sediment P that is mobile is bound to reducible Fe and Mn oxyhydroxides; their liberation into pore water and the overlying water column are thus heavily dependent on changing redox conditions.

Sediment Extraction Concentrations (mg/g dry sed.)															
		Total (AR)				Reactive (AA)					EDTA-NaOH				
	cm	Fe	Mn	P	Al	Ca	Fe	Mn	P	Al	Ca	Fe	Mn	P	Al	Ca
Jun-07	0-1	41.2	1.0	1.2	n/a	n/a	4.3	0.31	0.46	0.58	0.47	0.12	0.18	0.41	n/a	n/a
	1-2	39.9	0.8	1.0	n/a	n/a	2.7	0.14	0.31	0.55	0.41	0.13	0.17	0.41	n/a	n/a
	2-3	41.8	0.8	1.3	n/a	n/a	2.6	0.13	0.30	0.52	0.38	0.16	0.05	0.44	n/a	n/a
	3-4	58.9	1.2	1.6	n/a	n/a	3.1	0.16	0.35	0.52	0.42	0.00	0.012	0.21	0.25	0.62
	4-8	41.8	0.8	1.1	n/a	n/a	2.7	0.14	0.34	0.48	0.38	0.12	0.18	0.48	n/a	n/a
Oct-07	0-1	44.5	1.9	1.5	21.8	4.4	12.1	1.40	0.97	0.75	1.72	0.35	0.28	0.46	0.42	0.55
	1-2	67.4	1.4	1.8	19.0	2.5	2.7	0.16	0.33	0.58	0.16	0.80	0.24	0.44	0.38	0.41
	2-3	35.3	0.7	1.0	15.8	2.0	2.6	0.15	0.33	0.58	0.16	0.68	0.22	0.43	0.37	0.38
	3-4	39.5	0.8	1.1	23.0	3.5	3.1	0.16	0.35	0.60	0.13	0.34	0.12	0.26	0.48	0.44
	4-6	38.8	0.8	1.0	20.5	3.1	3.1	0.15	0.37	0.55	0.15	0.53	0.22	0.47	0.45	0.44
	6-10	50.4	1.0	1.2	22.1	2.9	3.4	0.18	0.42	0.57	0.16	0.85	0.25	0.48	0.47	0.45
May-08	0-1	44.9	1.6	1.5	20.1	4.1	14.6	1.26	1.00	0.86	1.66	0.00	0.02	0.17	0.34	2.04
	1-2	43.8	1.2	1.4	20.4	3.3	9.9	0.63	0.75	0.70	1.24	0.03	0.09	0.16	0.35	0.42
	2-3	44.1	1.3	1.4	21.0	3.2	11.0	0.77	0.86	0.74	1.18	0.05	0.05	0.16	0.34	0.44
	3-4	40.5	1.0	1.2	20.1	2.8	9.5	0.61	0.83	0.75	1.04	0.12	0.31	0.31	0.67	0.61
	4-6	39.0	0.9	1.0	20.9	2.9	2.8	0.18	0.29	0.47	0.56	0.12	0.31	0.36	0.52	0.61
	6-10	39.2	0.8	1.1	21.8	3.3	2.5	0.14	0.29	0.46	0.55	0.36	0.31	0.33	0.38	0.60
Jul-08	0-1	40.7	0.9	1.1	24.8	4.0	6.0	0.39	0.47	0.69	1.21	1.05	0.40	0.44	0.41	1.09
	1-2	39.5	0.8	1.0	21.1	2.9	5.2	0.30	0.42	0.70	1.12	1.00	0.15	0.24	0.21	0.62
Aug-08	0-1	50.7	1.9	1.7	30.2	5.2	9.7	0.97	0.74	0.73	1.42	0.56	0.80	0.13	0.22	0.31
	1-2	52.3	1.8	1.7	28.3	2.9	9.6	0.80	0.73	0.74	1.37	0.54	0.51	0.05	0.14	0.25
	2-3	46.0	1.4	1.5	29.4	2.6	10.5	0.72	0.82	0.67	1.10	0.41	0.57	0.28	0.13	0.23
	3-4	47.2	1.6	1.5	33.7	2.8	9.1	0.76	0.74	0.68	1.11	0.60	0.69	0.05	0.13	0.24
	4-6	50.7	1.5	1.5	37.0	3.4	8.4	0.65	0.65	0.65	1.46	0.36	0.63	0.19	0.12	0.23
	6-10	42.3	1.0	1.2	30.5	2.5	6.8	0.34	0.49	0.63	1.09	0.26	0.57	0.89	0.11	0.25
Oct-08	0-1	24.4	0.6	0.7	11.0	3.1	7.6	0.41	0.54	0.75	1.47	0.56	0.80	0.13	0.22	0.31
	1-2	60.5	1.5	1.7	26.3	4.1	7.9	0.45	0.57	0.75	1.31	0.54	0.51	0.05	0.14	0.25
	2-3	33.6	0.8	1.1	14.9	2.5	5.9	0.32	0.44	0.70	1.19	0.41	0.57	0.28	0.13	0.23
	3-4	34.6	0.7	0.9	15.9	2.6	5.8	0.28	0.38	0.72	1.21	0.60	0.69	0.05	0.13	0.24
	4-6	38.8	0.8	0.9	20.7	3.0	5.2	0.26	0.39	0.66	1.19	0.36	0.63	0.19	0.12	0.23
	6-10	36.7	0.7	0.9	18.8	2.8	5.2	0.24	0.38	0.67	1.30	0.26	0.57	0.89	0.11	0.25

Table 9: Sediment extraction concentrations in mg/l. AR denotes aqua regia extraction, AA is ascorbic acid extraction, and EDTA-NaOH was used for ^{31}P NMR spectroscopy preparation.

P Characterization and Quantification

The average Total P_{org} was about $22 \pm 4\%$ of the total P from all the sediments analyzed (Tables 10 and 11). Organic P compounds identified include phosphonates, monoesters and diesters. Specific monoesters were identified as inositol hexakisphosphate (IHP, phytic acid) and *scyllo*-inositol hexakisphosphate (Appendix F). In some spectra the peak in the monoester region was too broad to identify specific monoesters so the total peak area of this region was combined and categorized as "general monoesters." Monoesters were the dominate P_{org} moiety in all samples. Pyrophosphate, an inorganic polyphosphate ($P_2O_7^{2-}$) associated with microbial activity and fertilizers (Sundareshwar and Morris, 2001), was also present in most samples except for the deeper sediments of the October core. Polyphosphate, also associated with microbial activity and fertilizers, was only detected in the 0-1 and 4-8 cm segment of the June 2007 core and the 4-6 cm segment of the October 2007 core. When polyphosphate was detected, pyrophosphate and polyphosphate were of equivalent proportions. It is possible that polyP was converted to pyroP during lyophilization (Cade-Menun & Preston, 1996).

Table 10: Organic P forms and concentrations in µg/mg dry sediment for 2007 cores. "Mono gen" includes integrations from peaks that were too broad to identify specific monoesters.

| 2007 | | Inorganic P | | | | Organic P | | | | | | | | Totals | | | |
| Date | Depth (cm) | Ortho P | Pyro P | Poly P | Phosph -onates | Mono gen | IHP | scyllo P | Other Mono | Total Mono | DNA | OthDi | Total Diesters | Pi | Po | Total P | M:D |
		(µg/mg dry sediment)				Monoesters					Diesters						
June	0-1	313.4	2.8	2.8	0.0	0.0	18.7	6.1	49.9	74.7	6.1	6.1	12.2	319.1	86.9	405.9	**6.1**
	1-2	301.0	3.7	0.0	0.0	0.0	21.1	7.0	70.9	99.1	7.0	3.7	10.8	304.7	109.9	414.6	**9.2**
	2-3	324.1	3.1	3.1	0.0	0.0	22.1	11.1	58.8	92.0	13.3	6.6	19.9	330.3	111.9	442.2	**4.6**
	3-4	228.6	2.1	0.0	0.0	0.0	15.9	5.1	41.7	62.7	4.5	2.1	6.6	230.7	69.3	300.0	**9.5**
	4-8	374.9	0.0	0.0	3.4	0.0	30.8	5.3	56.9	93.0	7.2	3.4	10.6	374.9	107.0	481.9	**8.8**
Oct	0-1	357.8	6.9	0.0	0.0	0.0	24.7	8.2	49.4	82.3	6.9	3.7	10.5	364.6	92.9	457.5	**7.8**
	1-2	343.7	3.1	0.0	0.0	0.0	21.2	7.1	56.0	84.2	6.6	3.1	9.7	346.8	93.9	440.7	**8.7**
	2-3	331.0	3.0	0.0	3.0	0.0	19.1	6.4	53.6	79.1	6.4	3.0	9.4	334.0	91.5	425.5	**8.4**
	3-4	194.8	1.8	0.0	1.8	0.0	12.7	6.2	33.6	52.5	6.0	1.8	7.8	196.6	62.1	258.7	**6.7**
	4-6	356.6	3.3	3.3	0.0	0.0	20.7	7.1	66.0	93.8	7.1	7.1	14.1	363.2	107.9	471.1	**6.7**

Table 11: Organic P forms and concentrations (µg/g dry sed.) from 2008 cores.

Date	Depth	Inorganic P				Organic P								Totals			
						Monoesters					Diesters						
		Ortho P	Pyro P	Poly P	Phosph-onates	mono gen	IHP	Scyllo P	Other Mono	Total Mono	DNA	OthDi	Total Diesters	Pi	Po	Total P	M:D
May	0-1	230.5	6.3	0.0	2.1	0.0	19.6	4.2	32.6	56.4	4.2	2.1	6.3	236.8	64.9	301.7	**9.0**
	1-2	232.3	4.6	0.0	0.0	0.0	16.6	5.5	41.2	63.3	7.1	0.0	7.1	236.9	70.4	307.2	**8.9**
	2-3	252.3	2.6	0.0	0.0	65.1	0.0	2.6	0.0	67.8	7.9	0.0	7.9	254.9	75.7	330.6	**8.5**
	3-4	346.6	3.5	0.0	0.0	0.0	24.9	3.5	51.1	79.4	3.5	3.5	7.0	350.1	86.4	436.5	**11.3**
	4-6	281.8	2.9	0.0	0.0	60.7	0.0	2.9	0.0	63.6	6.1	2.9	8.9	284.6	72.5	357.1	**7.1**
	6-10	267.9	2.6	0.0	0.0	50.3	0.0	2.6	0.0	52.9	2.6	2.6	5.3	270.5	58.2	328.7	**10.0**
July	0-1	133.6	1.4	0.0	1.4	0.0	11.4	2.4	19.1	32.9	2.6	1.4	4.0	135.0	38.3	173.3	**8.2**
	1-2	118.5	1.3	0.0	1.3	0.0	7.5	2.6	24.9	35.0	2.4	1.3	3.7	119.8	39.9	159.8	**9.5**
Aug	0-1	124.7	1.3	0.0	0.0	0.0	7.2	3.7	20.6	31.5	2.4	0.0	2.4	126.0	33.9	159.9	**13.1**
	1-2	238.8	2.5	0.0	0.0	0.0	14.9	7.4	36.6	58.9	7.1	2.5	9.6	241.3	68.5	309.8	**6.1**
	2-3	278.7	2.9	0.0	2.9	0.0	17.0	5.8	42.6	65.3	8.3	2.9	11.2	281.6	76.5	361.0	**5.8**
	3-4	249.6	2.6	0.0	0.0	60.9	0.0	7.6	0.0	68.5	7.6	2.6	10.3	252.2	78.8	331.0	**6.7**
	4-6	355.9	3.6	0.0	0.0	61.4	0.0	10.2	0.0	71.6	10.2	3.6	13.8	359.4	85.4	444.8	**5.2**
	6-10	190.0	1.7	0.0	1.7	34.0	0.0	3.3	0.0	37.3	5.2	1.7	6.9	191.6	44.2	237.5	**5.4**
Oct	0-1	235.7	2.5	0.0	0.0	0.0	15.1	4.9	40.1	60.2	7.7	2.5	10.2	238.2	70.3	308.5	**5.9**
	1-2	201.4	2.0	0.0	0.0	0.0	13.0	2.0	26.6	41.6	3.8	2.0	5.8	203.4	47.4	250.8	**7.2**
	2-3	187.5	1.6	0.0	0.0	0.0	11.5	1.6	27.2	40.4	3.5	1.6	5.2	189.1	45.5	234.7	**7.0**
	3-4	184.7	0.0	0.0	0.0	0.0	19.5	4.4	28.3	52.3	3.7	3.7	7.3	184.7	59.6	244.3	**7.2**
	4-6	177.2	0.0	0.0	1.8	0.0	14.0	3.2	24.8	42.1	5.3	3.4	8.7	177.2	52.6	229.9	**4.8**
	6-10	205.6	0.0	0.0	1.7	0.0	10.4	1.7	22.2	34.3	3.5	1.7	5.2	205.6	41.2	246.8	**6.6**

Organic P Trends with Depth and Season

Total P_{org} concentrations trends with respect to depth varied for each core collected at different times during the summers (Figure 12, a-f). In May 2008, P_{org} concentrations tended to increase with depth in the top 4 cm but then decreased in the 4-6 and 6-10 cm segments. In August 2008, concentrations increased with depth except in the 6-10 cm segment which contained about half as much as measured in the 2-6 cm region of the core (although it was slightly higher than in the 0-1 cm segment). The 0-1 cm segment of the October 2008 core contained the largest amount of P_{org} compared to any other segment of that core as well as any other core from 2008. The P_{org} concentrations for October 2008 tended to decrease with depth, although concentrations in the 3-4 cm section increased relative to the 2-3 cm segment. In 2007, total P_{org} concentrations were generally higher compared to values from 2008 and no obvious trends with respect to depth were observed. The ratio of P_i to P_{org} did not display an obvious trend with respect to depth or season.

The seasonal TP_{org} variation is most noticeable in the 0-1 cm segment from the 2008 cores. August and July TP_{org} concentrations in the 0-1 cm segment were close to half as much as measured in that segment of the May core (38 and 33 vs 65 µg/g dry sed., respectively). In October, TP_{org} increased to concentrations slightly higher than were measured in May (70 µg/g dry sed.). This is most likely due to the onset of the bloom die off, although %C was not significantly higher (3% compared an average of $2.8 \pm 0.3\%$). In 2007, total P_{org} in the 1-2 and 2-3 cm section decreased slightly from June to October whereas the 0-1 cm P_{org} content increased slightly (87 to 92 µg/g dry sed.).

Figure 12 a-f: Concentrations of organic P species in sediments with respect to depth from cores collected in June (a) and October (b), 2007 and May (c), July (d), August (e), and October (f) 2008. Total OP concentrations tend to be lower in 2008 compared to 2007. Monoesters were the dominant OP species in all sediment segments.
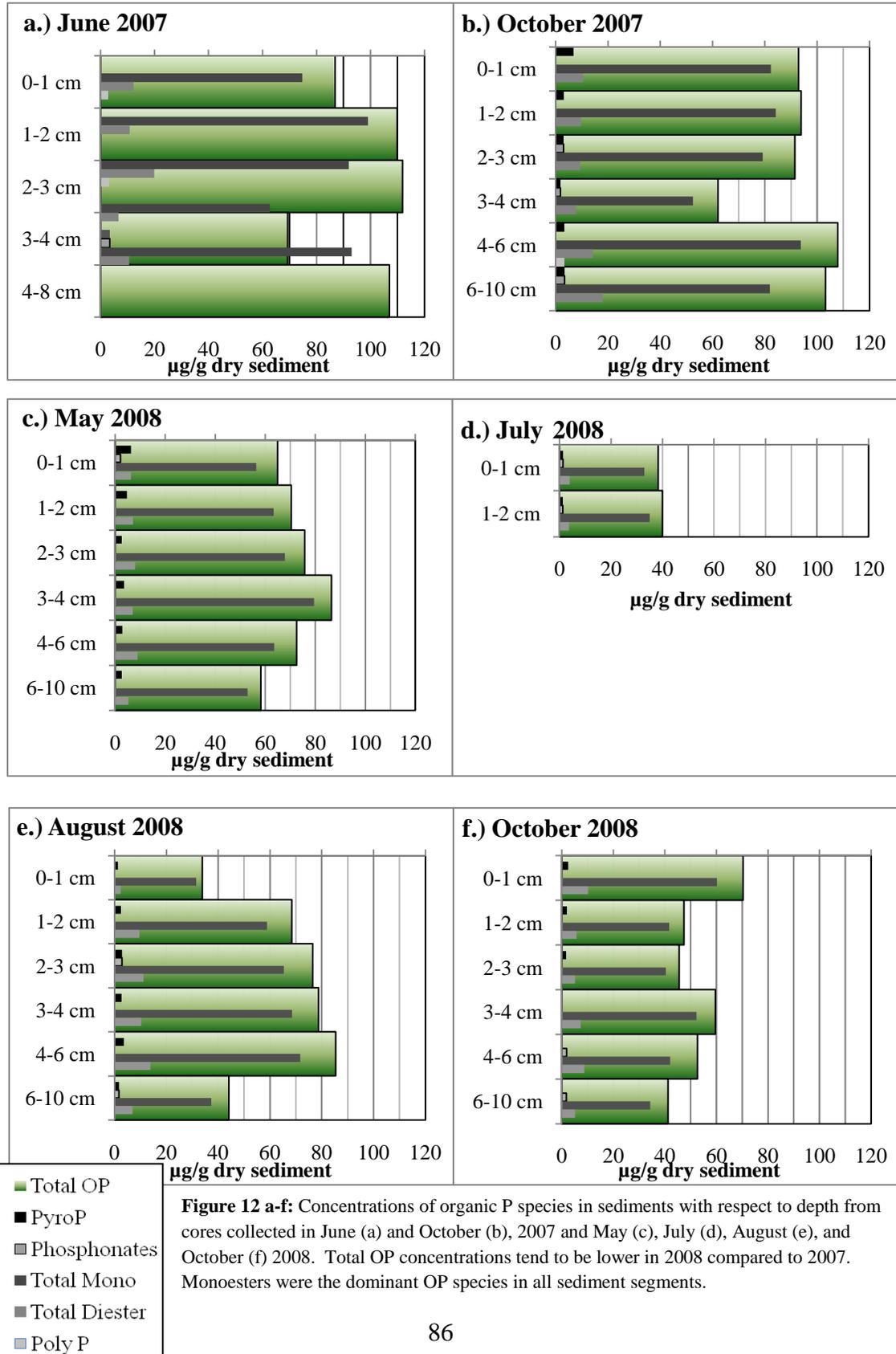
Monoesters and Diesters

Scyllo-inositol phosphate (a 6 carbon ring with 6 monoester phosphate groups) was detected in all samples. These inositol phosphates are of microbical origin – most likely a product of epimerization of myo-inositol phosphates or hexakisphosphate (phytic acid) (McKelivie, 2007; L'Annunziata, 1975). Inositol hexakisphosphate (IHP), or phytic acid, was detected in most of the samples where the monoester peak could be clearly identified and ranged from 29-46% of the monoesters. Inositol hexakisphosphates are of plant origin and most commonly found in crop seeds and fruits (McKelvie, 2007; Lott et al. 2000). It has been suggested that IHP sources in aquatic sediments are largely from terrigenous production rather than from aquatic plants (Weimer & Armstrong, 1979). DNA was the only discernable diester from the spectra and comprised 50-100% of the total diester concentrations. Other diesters include phospholipids and teichoic acids. A distinct trend in diester concentration with depth and seasonal was not observed.

Monoester:Diester ratios

Inositol hexaphosphates are more recalcitrant in soils and sediments than most diesters because of their high charge density and propensity to react with oxides (Turner, et al. 2002). Although diester phosphate inputs into soils and sediments are generally higher than monoester phosphates (Makarov et al. 2005; Turner, Cade-Menun, & Westermann, 2003), diesters are more subject to degradation because they sorb weakly (e.g., DNA) or not at all to sediments, especially in pH >5 (Greaves and Wilson, 1969). The ratio of total monoesters to diesters (M:D) in cores from May of 2007 and June of 2008 showed little to no trend with depth, although the ratios were generally higher in

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June 2008 (ratios ranged from 6.1 - 9.5 and 7.1 - 11.4, respectively, Figure 13). In August 2008, the M:D ratio in the surface sediments reached 13, the highest of all samples. The M:D ratio in the cores from both October 2007 and 2008 show a general decrease with depth but the ratios for 2008 were slightly lower than 2007. By October of 2008, this ratio decreased to about 6 in the surface sediments (0-1 cm).

The higher M:D ratio in the 0-1 cm segment in August 2008 corresponded with lower total P concentrations (from EDTA-NaOH extraction), indicating selective degradation or increased mobility of diesters compared to monoesters, or a combination of both. DNA comprised 100% of the detected diester phosphates from this segment, indicating that other diesters, such as phospholipids, were the likely P compounds mobilized or degraded. The sediments were more reduced in August, increasing the likelihood of P release from redox reactive mineral surfaces. Additionally, the RP concentration in the surface sediments was lower in August compared to May and June of 2008 supporting the idea of increased P mobility at this time. Sediment redox conditions were found to affect the mineralization rate of some P_{org} compounds but no studies directly focusing on diester sorption and release to iron oxides are known to the authors at this time.

Carman et al. (2000) found that diester phosphates were more abundant in anoxic lake sediments, concluding that diester degradation was more efficient in oxic conditions. Our results contradict their findings although the samples analyzed by Carman et al. (2000) were collected at one point in time and the top 5 cm of each core were combined

for analysis. Differences in lake morphology, biological structure, sediment type, and bacterial metabolism between the studies could have contributed to the opposing trend.
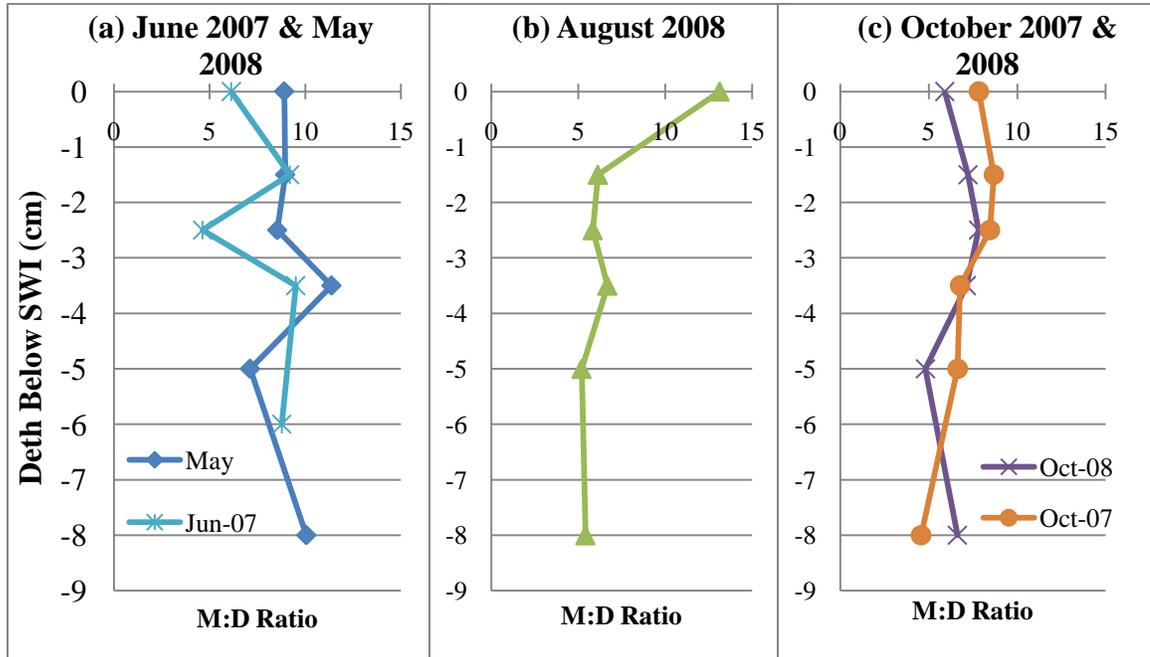
Figure 13 (a), (b), and (c): Monoester to Diester Ratios with Depth and Season. (a) June 2007 and May 2007 show similar trends with depth. (b) The M:D ratio was much higher in the surface sediments of August compared to any other sample. (c) M:D ratios in October 2007 and 2008 were similar; ratios were generally lower in 2008.

The surface sediments were still reduced in October 2008 and the M:D ratio decreased to near 6, which was lower than the ratio observed in May 2008 (close to 9). Ortho-P concentrations in the 0-1 cm segment were also lower in August. The cyanobacteria bloom density was the highest in August meaning that a large amount of P would have to be incorporated into biomass. The fact that RP and NaOH-EDTA P were lowest in the surface sediments at this time suggests that a portion of the P needed to fuel the bloom may have originated from the sediments. The enormous biomass in the water column could have consumed the more bioavailable P_{org} that could have diffused from

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the sediments, while a lack of ortho-P could have activated phytase activity in sediment microbes, assisting in the hydrolysis of monoesters phosphates. Additionally, Suzumura and Kamatani (1995) found that IHPs are mineralized to inorganic P at a faster rate in reducing conditions than in oxic conditions in marine sediments. The redox conditions of Missisquoi Bay sediments may not only drive P release but may also influence sediment microorganism metabolism and transformation rates of $P_{org.}$

Pyrophosphate

Pyrophosphate (pyro-P) was detected in most samples except in the deeper sediments of the October 2008 core. The highest concentrations of pyro-P were detected in May 2008 and tended to decrease across the 2008 season (Figure 14). Pyrophosphates ($P_2O_7^-$, e.g. AMP) are formed by the hydrolysis of polyphosphates (Madigan, Martinko, and Parker, 2003). It has been observed that microbes store excess P in the form of poly-P under aerobic conditions and release them under anaerobic conditions (Hupfer, Gächter, and Ruegger, 1995; Deinnema et al. 1985). Also, poly-P is stored under luxury consumption (when ortho-P is high), or under nitrogen stress. Any poly-P extracted from sediments were likely part of living organisms because once released, they are subjected to rapid hydrolysis (Comeau, 1986; Hupfer, Gächter, and Ruegger, 1995); the release of PO_4^{3-} has been documented when poly-P storing microbes are changed from an aerobic to an anaerobic environment. Furthermore, Hupfer, Gächter, and Ruegger (1995) extracted polyphosphate only from sediment layers in which bacteria that contained polyphosphate granules were present, as evidenced by transmission electron microscopy. It is also

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important to note that some poly-P may degrade to pyro-P during lyophilization of samples (Cade-Menun and Preston, 1996).

Other studies have only detected pyro-P or poly-P in the top few cm of sediment (Gachter, 1988; Hupfer, Gächter, and Ruegger, 1995; Alghren et al. 2007) but pyro-P was detected at least 6 cm below the surface in our samples. Pyro-P made up a higher proportion of total organic P in the surface sediments of 2008 compared to deeper sediments. Poly-P was only detected in the 0-1 and 2-3 cm segments of June 2007 and 4-6 cm segment in October 2007. Iron reduction was not detected voltammetrically until 2 cm and 4 cm below the SWI in June and October 2007, respectively. Oxygen was not present and Mn(II) was detected at shallower depths; indicating that poly-P storing organisms also exist in suboxic conditions. The pyro-P trend for 2008 demonstrates that pyro-P decreased as sediments became more reducing in July, August, and October. By October, no pyro-P was detected in the deeper sediments.
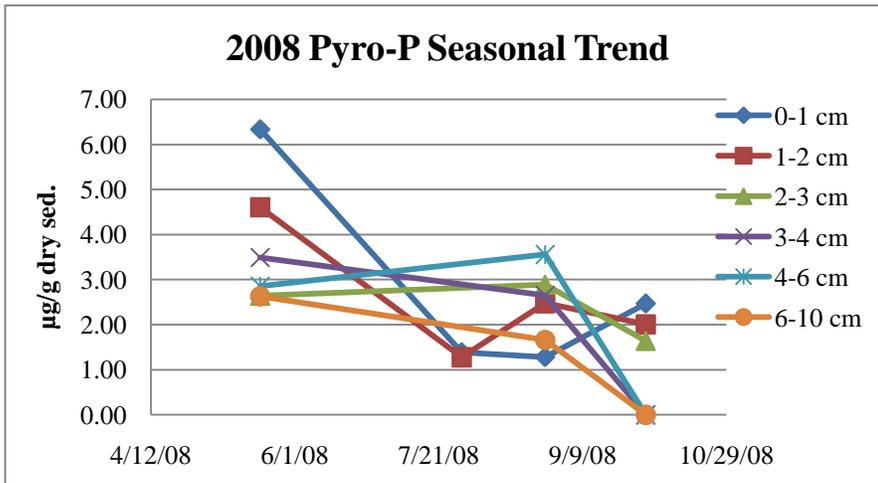
Figure 14: Seasonal and Depth Variation of Pyrophosphate to Orthophosphate Ratios. The ratio is the highest in the top sediments of the early summer. A decreasing ratio could indicate that pyrophosphate was hydrolyzed to orthophosphate over time. The ratio was the lowest in October when the sediments were highly reduced.

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P_{org} Associations with Other Sediment Constituents

A correlation table tested for significant relationships between organic P species and sediment Fe, Mn, Al, and Ca from all of the extraction types and Mn(II) and Fe(II) from electrochemical analysis. Although a few relationships were significant ($p < 0.050$), the R^2 values indicated that relationships were not highly correlated (R^2 was below 0.50). Total P_{org} correlated well with porewater Fe(II) in the May and August cores with the exception of the 6-10 cm segment – for this segment P_{org} values tended to be low but Fe(II) values were high (Figure 15a). A similar relationship comparing ortho-P and Fe(II) is evident but the R^2 value is lower. Relationships with P_{org} content and electrochemical data were not evident in the 2007 cores, but the analysis was limited because only the top 30 mm of the cores was profiled.
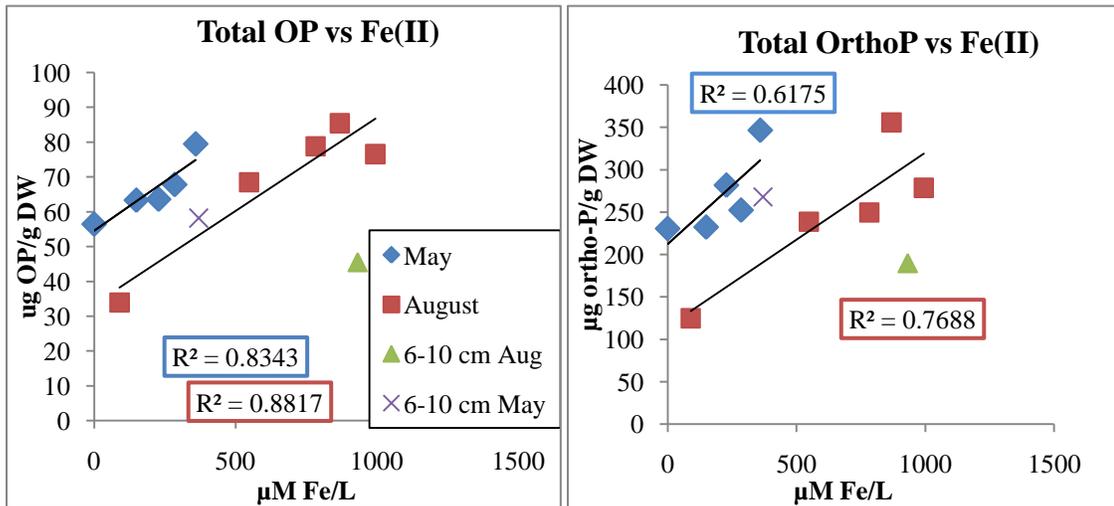
Figure 15 a,b. (**a**) Relationship between Total Organic P and Porewater Fe(II), p<0.05. Values were well correlated except in the 6-10 cm segment. Fe(II) concentrations were relatively high but TOP values tended to decrease. (b) Relationship between Total OrthoP and Porewater Fe(II). Values were correlated but not as strongly compared to Organic P.

The data analysis suggests that P_{org} is somewhat associated with other chemical constituents, mostly porewater Fe(II) with ortho-P demonstrating a weaker, though

significant relationship to Fe(II) (Figure 15 a and b). Fe(II) in the porewater suggests conditions are reducing enough to dissolve at least some Fe(III) (oxy)hydroxides, and the correlation suggests that higher concentrations of organic P is present in more reduced conditions, except for in the 6-10 cm segment. As sediments become more reduced over the course of a summer, orthophosphate will be liberated from Fe and Mn oxyhydroxides before P_{org} because some forms of P_{org} preferentially sorb to Fe and Mn oxyhydroxides over ortho-P (i.e. under more reducing conditions and greater amounts of FeOOH dissolution, more P_{org} will remain associated with these minerals relative to ortho-P) (Wang et al. 2007; Rodel et al. 1977).

The relatively low TP_{org} concentrations in the 6-10 cm segments of Missisquoi Bay samples suggest that P_{org} is degrading over time in more reduced sediments. This could be due to the release of P_{org} molecules from mineral surfaces under more reducing conditions and subsequent enzymatic hydrolysis. Additionally, as P_i concentrations decrease, the microbial cycling rate between organic and inorganic forms tends to increase as phosphatase (enzyme) activity is activated (Wetzel, 1999). P_i is liberated in reducing conditions and either migrates vertically or is consumed in the sediment, in which case phosphatase activity will be activated and P_{org} degradation rates will theoretically increase.

CONCLUSIONS

The data obtained from this study demonstrates the degree of mobility, variability, and complexity of P_{org} forms in the sediments of a eutrophic shallow fresh water body. The lack of a definite, consistent trend of P_{org} fractionation with respect to depth in sediment cores taken at different intensities of an algae bloom suggests that there is variable mobility and degradation of these complex organic compounds over small temporal and spatial timescales. Organic P is strongly correlated to porewater Fe(II), which is a measure of Fe(OOH) reduction, indicating that Fe(OOH) reduction may play an important role in P_{org} mobility and degradation in sediments. A relative decrease of monoesters in the surface sediments from August to October suggests that more reducing conditions may have promoted the degradation of this typically more recalcitrant P_{org} species. Because P_{org} is mostly likely bound to Fe(III)OOH in the sediments, reducing conditions will solubilize a portion of this Fe, release and therefore expose phosphate groups on the P_{org} molecule which will increase the ease of hydrolysis. This study demonstrated the multifaceted relationships between redox conditions, other chemical constituents, and biological factors associated with P_{org} cycling in sediments.

REFERENCES

Ahlgren, J., Tranvik, L., Gogoll, A., Waldeback, M., Markides, K., & Rydin, E. (2005). Sediment depth attenuation of biogenic phosphorus compounds measured by P-31 NMR. *Environmental Science & Technology, 39*(3), 867-872.

Andersen, F. O., & Jensen, H. S. (1992). Regeneration of inorganic phosphorus and nitrogen from decomposition of seston in a fresh-water sediment. *Hydrobiologia, 228*(1), 71-81.

Anschutz, P., Zhong, S. J., Sundby, B., Mucci, A., & Gobeil, C. (1998). Burial efficiency of phosphorus and the geochemistry of iron in continental margin sediments. *Limnology and Oceanography, 43*(1), 53-64.

Bentzen, E., Taylor, W. D., & Millard, E. S. (1992). The importance of dissolved organic phosphorus to phosphorus uptake by limnetic plankton. *Limnology and Oceanography, 37*(2), 217-231.

Bostrom, B., Andersen, J. M., Fleischer, S., & Jansson, M. (1988). Exchange of phosphorus across the sediment - water interface. *Hydrobiologia, 170*, 229-244.

Brendel, P. J., & Luther, G. W. (1995). Development of a gold amalgam voltammetric microelectrode for the determination of dissolved Fe, Mn, O_2 and $S^{(-ii)}$ in porewaters of marine and fresh-water sediments. *Environmental Science & Technology, 29*(3), 751-761.

Burgess, H. (2007). *Geochemical indicators of productivity change in Lake Champlain, USA-CANADA*. MS Thesis. The University of Vermont, Burlington, VT.

Cade-Menun, B. J. (2005). Characterizing phosphorus in environmental and agricultural samples by P-31 nuclear magnetic resonance spectroscopy. *Talanta, 66*(2), 359-371.

Cade-Menun, B. J., & Preston, C. M. (1996). A comparison of soil extraction procedures for P-31 NMR spectroscopy. *Soil Science, 161*(11), 770-785.

Cai, W. J., Zhao, P., Theberge, S.M., Witter, A., Wang, Y., Luther, G.W.(III) (2002). Porewater redox species, pH, pCO2, in aquatic sediments: Electrochemical sensor studies in Lake Champlain and Sapelo Island. In M. a. R. Taillefert, T.F. (Ed.), *Environmental Electrochemistry Analyses of Trace Element Biogeochemistry* (pp. 188-209). Washington, D.C.: American Chemical Society.

Carignan, R., & Lean, D. R. S. (1991). Regeneration of dissolved substances in a seasonally anoxic lake - the relative importance of processes occurring in the

water column and in the sediments. *Limnology and Oceanography, 36*(4), 683-707.

Carman, R., Edlund, G., & Damberg, C. (2000). Distribution of organic and inorganic phosphorus compounds in marine and lacustrine sediments: a P-31 NMR study. *Chemical Geology, 163*(1-4), 101-114.

Cembella, A. D., Antia, N. J., & Harrison, P. J. (1984a). The utilization of inorganic and organic phosphorus-compounds as nutrients by eukaryotic microalgae - a multidisciplinary perspective . *Crc Critical Reviews in Microbiology, 10*(4), 317-391.

Cembella, A. D., Antia, N. J., & Harrison, P. J. (1984b). The utilization of inorganic and organic phosphorus-compounds as nutrients by eukaryotic microalgae - a multidisciplinary perspective .2. *Crc Critical Reviews in Microbiology, 11*(1), 13-81.

Cotner, J. B., & Wetzel, R. G. (1992). Uptake of dissolved inorganic and organic phosphorus-compounds by phytoplankton and bacterioplankton. *Limnology and Oceanography, 37*(2), 232-243.

Druschel, G. K. H., A., Lomonaco R. and Oldrid, K. (2005). Determination of sediment phosphorus concentrations in St. Albans Bay, Lake Champlain: Assessment of internal loading and seasonal variations of phosphorus sediment-water column cycling. Report to the VT Agency of Natural Resources.

Gachter, R., Meyer, J. S., & Mares, A. (1988). Contribution of bacteria to release and fixation of phosphorus in lake-sediments. *Limnology and Oceanography, 33*(6), 1542-1558.

Greaves, M. P. a. W., M.J. (1969). The adsorption of nucleic acids by montmorillonite. *Soil Biol. Biochem., 1*, 317-323.

Hegmen, W., Wang, D., & Borer, C. (1999). Estimation of Lake Champlain basinwide nonpoint source. Report to the Lake Champlain Basin Program, Grand Isle, VT.

Hupfer, M., & Gachter, R. (1995). Polyphosphate in lake-sediments - p-31 nmr-spectroscopy as a tool for its identification. *Limnology and Oceanography, 40*(3), 610-617.

Jeppesen, E., Jensen, J. P., Sondergaard, M., Lauridsen, T., Pedersen, L. J., & Jensen, L. (1997). Top-down control in freshwater lakes: The role of nutrient state, submerged macrophytes and water depth.

Kostka, J. E., & Luther, G. W. (1995). Seasonal cycling of fe in salt-marsh sediments. *Biogeochemistry, 29*(2), 159-181.

L'Annunziata, M. F. (1975). *The origin and transformation of the soil inositol phosphate isomers.* Paper presented at the Soil Science Society of America Proceedings.

Lott, J. N. A., Ockenden, I., Raboy, V., & Batten, G. D. (2000). Phytic acid and phosphorus in crop seeds and fruits: a global estimate. *Seed Science Research, 10*(1), 11-33.

Luther, G. W., & Church, T. M. (1988). Seasonal cycling of sulfur and iron in porewaters of a delaware salt-marsh. *Marine Chemistry, 23*(3-4), 295-309.

Luther, G. W., & Rickard, D. (2005). Metal sulfide cluster complexes: Structure, reactivity and environmental impact.

Makarov, M. I., Haumaier, L., Zech, W., Marfenina, O. E., & Lysak, L. V. (2005). Can P-31 NMR spectroscopy be used to indicate the origins of soil organic phosphates? *Soil Biology & Biochemistry, 37*(1), 15-25.

McKelive, I. D. (2007). Inositol Phosphates in Aquatic Systems. In B. L. Turner, Richardson, A.E., and Mullaney, E.J. (Ed.), *Inositol Phosphates Linking Agriculture and the Environment* (pp. 261-277). Cambridge, MA: CABI.

Mortimer, C. H. (1941). The exchange of dissolved substances between mud and water in lakes. *J. Ecol., 29*, 280-329.

Penn, M. R., Auer, M. T., Doerr, S. M., Driscoll, C. T., Brooks, C. M., & Effler, S. W. (2000). Seasonality in phosphorus release rates from the sediments of a hypereutrophic lake under a matrix of pH and redox conditions. [Article]. *Canadian Journal of Fisheries and Aquatic Sciences, 57*(5), 1033-1041.

Reitzel, K., Ahlgren, J., DeBrabandere, H., Waldeback, M., Gogoll, A., Tranvik, L., et al. (2007). Degradation rates of organic phosphorus in lake sediment. *Biogeochemistry, 82*(1), 15-28.

Rickard, D. (2006). The solubility of FeS. *Geochimica Et Cosmochimica Acta, 70*(23), 5779-5789.

Rodel, M.G., Armstrong, D.E., Harris, R.F. Sorption and hydrolysis of added organic phosphorus compounds in lake sediments. *Limnology and Oceanography.* 22(3), 415-422.

Roden, E. E. a. E., J.W. (1997). Phosphate mobilization in iron-rich anaerobic sediments: microbial Fe(III) oxide reduction versus iron-sulfide formation. *Arch. Hydrobiol.,*

139(3), 347-378.

Roesler, A. J., Gammons, C. H., Druschel, G. K., Oduro, H., & Poulson, S. R. (2007). Geochemistry of flooded underground mine workings influenced by bacterial sulfate reduction. *Aquatic Geochemistry, 13*(3), 211-235.

Rozan, T. F., Taillefert, M., Trouwborst, R. E., Glazer, B. T., Ma, S. F., Herszage, J., et al. (2002). Iron-sulfur-phosphorus cycling in the sediments of a shallow coastal bay: Implications for sediment nutrient release and benthic macroalgal blooms. *Limnology and Oceanography, 47*(5), 1346-1354.

Sannigrahi, P., & Ingall, E. (2005). Polyphosphates as a source of enhanced P fluxes in marine sediments overlain by anoxic waters: Evidence from P-31 NMR. *Geochemical Transactions, 6*(3), 52-59.

Smith, V. H., Tilman, G. D., & Nekola, J. C. (1999). Eutrophication: impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems. *Environmental Pollution*. 100, 179-196.

Smernik, R.J. and Dougherty, W.J. (2007). Identification of phytat in phosphorus-31 nuclear magnetic resonance spectra: The need for spiking. *Soil Science Society of America Journal*. 71(3), 1045-1050.

Sondergaard, M., Jensen, J. P., & Jeppesen, E. (2003). Role of sediment and internal loading of phosphorus in shallow lakes. *Hydrobiologia*. 506-509: 135-145.

Sondergaard, M., Kristensen, P., & Jeppesen, E. (1993). 8 years of internal phosphorus loading and changes in the sediment phosphorus profile of lake sobygaard, denmark. *Hydrobiologia, 253*(1-3), 345-356.

Sundareshwar, P.V. and Morris, J.T. (2001). Phosphorus sorption characteristics of intertidal marsh sediments along an estuarine salinity gradient.

Sundby, B., Gobeil, C., Silverberg, N., & Mucci, A. (1992). The phosphorus cycle in coastal marine-sediments. *Limnology and Oceanography, 37*(6), 1129-1145.

Suzumura and Kamatani (1995). Mineralization of inositol hexaphosphate in aerobic and anaerobic marine sediments: Implications for the phosphorus cycle. *Geochemica et Cosmochimica Acta*. 59(5), 1021-1026.

Taillefert, M., Luther, G. W., & Nuzzio, D. B. (2000). The application of electrochemical tools for in situ measurements in aquatic systems. *Electroanalysis, 12*(6), 401-412.

Troy, A. (2007). Updating the Lake Champlain basin land use data to improve prediction of phosphorus loading. In D. Wang (Ed.), *Report to the Lake Champlain Basin Program*. Grand Isle, Vermont: LCBP Technical Report.

Turner, B. L., Cade-Menun, B. J., & Westermann, D. T. (2003). Organic phosphorus composition and potential bioavailability in semi-arid arable soils of the western United States. *Soil Science Society of America Journal, 67*(4), 1168-1179.

Turner, B. L., Mahieu, N., & Condron, L. M. (2003). Phosphorus-31 nuclear magnetic resonance spectral assignments of phosphorus compounds in soil NaOH-EDTA extracts. *Soil Science Society of America Journal, 67*(2), 497-510.

Van der Molen, D. T., & Boers, P. C. M. (1994). Influence of internal loading on phosphorus concentration in shallow lakes before and after reduction of the external loading. *Hydrobiologia, 275*, 379-389.

VTDEC (2008). Lake Champlain long-term monitoring. Vermont Department of Environmental Conservation. http://www.vtwaterquality.org/cfm/champlain/lp_longterm-lakes.cfm

Wang, S. R., Jin, X. C., Pang, Y., Zhao, H. C., & Zhou, X. N. (2005). The study of the effect of pH on phosphate sorption by different trophic lake sediments. [Article]. *Journal of Colloid and Interface Science, 285*(2), 448-457.

Weimer, W. C. a. A., D.E. (1979). Naturally occurring organic phosphorus compounds in aquatic plants. *Environmental Science & Technology, 13*(7), 826-829.

Wetzel, R. G. (1999). Organic phosphorus mineralization in soils and sediments. In K. R. Reddy, O'Connor, G.A., Schelske, C.L. (Ed.), *Phosphorus Biogeochemistry in Subtropical Ecosystems* (pp. 225-245). Boca Raton, LA: Lewis Publishers.

Williams, J. D. H., Mayer, J. (1972). Effects of sediment diagenesis and regeneration of phosphorus with special reference to lakes Erie and Ontario. In H. E. a. K. Allen, J.R. (Ed.), *Nutrients in Natural Waters* (pp. 281-315). New York: J. Wiley & Sons.

CHAPTER 5: CONCLUSIONS

The goals of this study were accomplished by 1) developing a better understanding of the relationship between redox chemistry at the SWI, nutrient mobility, and the presence of a cyanobacteria bloom on seasonal and diel timescales and 2) determining the degree of P_{org} mobility across a season compared to P_i. The timing of this study also proved to be advantageous; the absence of a bloom in 2007 and the presence of an intense bloom 2008 allowed for direct comparison of sediment chemistry and nutrient mobility.

Seasonal and diel redox cycling at the SWI is influenced by the presence of a cyanobacteria bloom which has important implications for nutrient mobility, specifically P, in the sediments. Reactive P and RFe are strongly correlated in the sediments; these components comprise about 6-22% and 30-40% of the total Fe and P pool, suggesting that a large portion of P mobility is associated with Fe oxyhydroxide minerals. This mobile P pool is therefore subject to liberation into the water column as sediments become more reduced over the season. A net loss in RP in the surface sediments was observed in 2008 when the sediments were anoxic, but not in 2007 when they were oxic, indicating that P was released into the water column in 2008. Ammonium in sediment pore water is also influenced by late summer reducing conditions as concentrations in the surface sediments were much higher in August 2008 (when conditions were most reducing at the SWI) compared to any other month. The results of this study demonstrate that sediment chemistry is significantly influenced by changes in the water column associated with seasonal and diel biogeochemical characteristics. Additionally, diel

redox fluctuations at the SWI imply that P flux between the sediment and water column changes on hourly timescales, which could be important for the success and dominance of cyanobacteria species with the ability to vertically migrate.

The P speciation (P_i and P_{org}) data obtained from this study demonstrates the degree of mobility, variability, and complexity of P_{org} forms in the sediments of a eutrophic, shallow, freshwater lake. The lack of a definite, consistent trend of P_{org} fractionation with respect to depth in sediment cores taken at different intensities of an algae bloom suggests that there is variable mobility and degradation of these complex organic compounds over small timescales. Organic P is strongly correlated to porewater Fe(II), which is a measure of Fe(OOH) reduction, indicating that Fe(OOH) reduction may play an important role in P_{org} mobility and degradation in sediments.   A relative decrease of monoesters in the surface sediments from August to October suggests that more reducing conditions may have promoted the degradation of this typically more recalcitrant P_{org} species. Because P_{org} is mostly likely bound to Fe(III)OOH in the sediments, reducing conditions will solubilize a portion of this Fe, therefore releasing and exposing phosphate groups on the P_{org} molecule resulting in increased ease of hydrolysis by sediment microorganisms.

This study demonstrated the complex relationships between redox conditions, other chemical constituents (Fe, Mn, Al, and Ca), and biological factors associated with P_{org} and P_i cycling in sediments. Phosphorus (both P_i and P_{org}) mobility in the sediments is most dependent on reactive Fe and is susceptible to liberation from Fe(III)OOH under reducing conditions. The presence of a bloom was related to more reducing conditions

suggesting the occurrence of a "feedback mechanism" in which redox influenced nutrient release from the sediment provided more nutrients for the proliferation and sustainability of the bloom. Despite the fact that internal loading is occurring in Missisquoi Bay, it is still imperative that external loading is minimized. This study suggests that excessive water column nutrients initiate the bloom while sediment nutrient diffusion sustains it and allows for the dominance of one cyanobacteria species. Combining and synthesizing the data obtained from this study with cyanobacteria population data (species and density) obtained from our collaborating group will provide better insight on the diel and seasonal relationships between the bloom dynamics and sediment and water chemistry.

COMPREHENSIVE BIBLIOGRAPHY

Ahlgren, J., Tranvik, L., Gogoll, A., Waldeback, M., Markides, K., & Rydin, E. (2005). Sediment depth attenuation of biogenic phosphorus compounds measured by P-31 NMR. *Environmental Science & Technology, 39*(3), 867-872.

Andersen, F. O., & Jensen, H. S. (1992). Regeneration of inorganic phosphorus and nitrogen from decomposition of seston in a fresh-water sediment. [article]. *Hydrobiologia, 228*(1), 71-81.

Andersen, j. M. (1982). Effect of nitrate concentration in lake water on phosphate release from the sediment. [Article]. *Water Research, 16*(7), 1119-1126.

Anschutz, P., Sundby, B., Lefrancois, L., Luther, G. W., & Mucci, A. (2000). Interactions between metal oxides and species of nitrogen and iodine in bioturbated marine sediments. *Geochimica Et Cosmochimica Acta, 64*(16), 2751-2763.

Anschutz, P., Zhong, S. J., Sundby, B., Mucci, A., & Gobeil, C. (1998). Burial efficiency of phosphorus and the geochemistry of iron in continental margin sediments. *Limnology and Oceanography, 43*(1), 53-64.

Ault, A. (1998). *Techniques and Experiments for Organic Chemistry* (6 ed.). Sausalito: University Science Books.

Bainfield, J.F., Welch, S.A., Zhang, H., Thomsen Ebert,T., Penn, R.Lee. (2000). Aggregation-based crystal growth and microstructure development in natural iron oxyhydroxide biomineralization products. *Science*, 289, 751-753.

Barbiero, R. P., & Welch, E. B. (1992). Contribution of benthic blue-green-algal recruitment to lake populations and phosphorus translocation. [Article]. *Freshwater Biology, 27*(2), 249-260.

Bebie, J., Schoonen, M. A. A., Fuhrmann, M., & Strongin, D. R. (1998). Surface charge development on transition metal sulfides: An electrokinetic study. *Geochimica Et Cosmochimica Acta, 62*(4), 633-642.

Beck, N. G., & Bruland, K. W. (2000). Diel biogeochemical cycling in a hyperventilating shallow estuarine environment. *Estuaries, 23*(2), 177-187.

Bentzen, E., Taylor, W. D., & Millard, E. S. (1992). The importance of dissolved organic phosphorus to phosphorus uptake by limnetic plankton. *Limnology and Oceanography, 37*(2), 217-231.

Boers, P. C. M. (1991). The influence of ph on phosphate release from lake-sediments. [Article]. *Water Research, 25*(3), 309-311.

Bostrom, B., Andersen, J. M., Fleischer, S., & Jansson, M. (1988). EXCHANGE OF PHOSPHORUS ACROSS THE SEDIMENT - WATER INTERFACE. [Article]. *Hydrobiologia, 170*, 229-244.

Bostrom, B., & Pettersson, K. (1982). Different patterns of phosphorus release from lake-sediments in laboratory experiments. *Hydrobiologia, 91-2*(jul), 415-429.

Breitburg, D. (2002). Effects of hypoxia, and the balance between hypoxia and enrichment, on coastal fishes and fisheries. *Estuaries, 25*(4B), 767-781.

Brendel, P. J., & Luther, G. W. (1995). Development of a gold amalgam voltammetric microelectrode for the determination of dissolved fe, mn, o-2, and s(-ii) in porewaters of marine and fresh-water sediments. *Environmental Science & Technology, 29*(3), 751-761.

Burgess, H. (2007). *Geochemical indicators of productivity change in Lake Champlain, USA-CANADA.* The University of Vermont, Burlington, VT.

Cade-Menun, B. J. (2005). Characterizing phosphorus in environmental and agricultural samples by P-31 nuclear magnetic resonance spectroscopy. *Talanta, 66*(2), 359-371.

Cade-Menun, B. J., & Preston, C. M. (1996). A comparison of soil extraction procedures for P-31 NMR spectroscopy. *Soil Science, 161*(11), 770-785.

Cai, W. J., Zhao, P., Theberge, S.M., Witter, A., Wang, Y., Luther, G.W.(III) (2002). Porewater redox species, pH, pCO2, in aquatic sediments: Electrochemical sensor studies in Lake Champlain and Sapelo Island. In M. a. R. Taillefert, T.F. (Ed.), *Environmental Electrochemistry Analyses of Trace Element Biogeochemistry* (pp. 188-209). Washington, D.C.: American Chemical Society.

Cainfield, D. E., Kristensen, E., and Thamdrup, B. (2005). *Aquatic Geomicrobiology.* Amsterdam: Elsevier.

Carignan, R., & Lean, D. R. S. (1991). Regeneration of dissolved substances in a seasonally anoxic lake - the relative importance of processes occurring in the water column and in the sediments. *Limnology and Oceanography, 36*(4), 683-707.

Carman, R., Edlund, G., & Damberg, C. (2000). Distribution of organic and inorganic phosphorus compounds in marine and lacustrine sediments: a P-31 NMR study. *Chemical Geology, 163*(1-4), 101-114.

Cembella, A. D., Antia, N. J., & Harrison, P. J. (1984a). The utilization of inorganic and organic phosphorus-compounds as nutrients by eukaryotic microalgae - a multidisciplinary perspective .1. *Crc Critical Reviews in Microbiology, 10*(4), 317-391.

104

Cembella, A. D., Antia, N. J., & Harrison, P. J. (1984b). The utilization of inorganic and organic phosphorus-compounds as nutrients by eukaryotic microalgae - a multidisciplinary perspective .2. *Crc Critical Reviews in Microbiology, 11*(1), 13-81.

Christophoridis, C., & Fytianos, K. (2006). Conditions affecting the release of phosphorus from surface lake sediments. *Journal of Environmental Quality, 35*(4), 1181-1192.

Cotner, J. B., & Wetzel, R. G. (1992). Uptake of dissolved inorganic and organic phosphorus-compounds by phytoplankton and bacterioplankton. *Limnology and Oceanography, 37*(2), 232-243.

Cornell, R.M., Schwertmann, U., 2003. The Iron Oxides: Structure, Properties, Reactions, Occurrences and Uses, 2nd, Completely Revised and Extended Edition. Wiley-VCH Verlag, Weinheim, Germany, p. 703.

Currie, D. J., & Kalff, J. (1984). The relative importance of bacterioplankton and phytoplankton in phosphorus uptake in fresh-water. [Article]. *Limnology and Oceanography, 29*(2), 311-321.

Davanzo, C., & Kremer, J. N. (1994). Diel oxygen dynamics and anoxic events in an eutrophic estuary of waquoit bay, massachusetts. [Article]. *Estuaries, 17*(1B), 131-139.

Drake, J. C., & Heaney, S. I. (1987). Occurrence of phosphorus and its potential remobilization in the littoral sediments of a productive english lake. [Article]. *Freshwater Biology, 17*(3), 513-523.

Druschel, G. K., Hamers, R. J., Luther, G. W., & Banfield, J. F. (2003). Kinetics and mechanism of trithionate and tetrathionate oxidation at low pH by hydroxyl radicals. *Aquatic Geochemistry, 9*(2), 145-164.

Druschel, G. K. H., A., Lomonaco R. and Oldrid, K. (2005). *Determination of sediment phosphorus concentrations in St. Albans Bay, Lake Champlain: Assessment of internal loading and seasonal variations of phosphorus sediment-water column cycling*.

Einsele, W. Uber die Beziehungen der Eisenkreislaufes zum Phosphorkreislauf im eutrophen. See Arch. Hydrobiol. 29: 664-686.

Ekholm, P., Malve, O., & Kirkkala, T. (1997). Internal and external loading as regulators of nutrient concentrations in the agriculturally loaded Lake Pyhajarvi (southwest Finland). *Hydrobiologia, 345*, 3-14.

Ferris, F.G. (2005). Biogeochemical properties of bacteriogenic iron oxides. *Geomicrobiology*, 22, 79-85.

Fortin, D. and Langley, S. (2005). Formation and occurrence of biogenic iron-rich minerals. *Earth-Science Reviews*, 72, 1-19.

Frausto da Silva, J. J. R. W., R. J. P (1991). Manganese: dioxygen evolution and glycosylation *The Biological Chemistry of the Elements/The Inorganic Chemistry of Life*. Oxford: Clarendon Press.

Fukuhara, H., & Sakamoto, M. (1987). Enhancement of inorganic nitrogen and phosphate release from lake sediment by tubificid worms and chironomid larvae. *Oikos, 48*(3), 312-320.

Gächter, R., Meyer, J. S., & Mares, A. (1988). Contribution of bacteria to release and fixation of phosphorus in lake-sediments. [Article]. *Limnology and Oceanography, 33*(6), 1542-1558.

Gorenstein, D. G. (1984). *Phosphorus-31 NMR Principals and Applications*. Orlando: Academic Press, Inc.

Greaves, M. P. a. W., M.J. (1969). The adsorption of nucleic acids by montmorillonite. *Soil Biol. Biochem., 1*, 317-323.

Gunnars, A., & Blomqvist, S. (1997). Phosphate exchange across the sediment-water interface when shifting from anoxic to oxic conditions - an experimental comparison of freshwater and brackish-marine systems. *Biogeochemistry, 37*(3), 203-226.

HARRNESS (2005). *Harmful algal research and response: a national environmental science strategy 2005-2015*. Washington, DC: Ecological Society of America.

Hecky, R. E., & Kilham, P. (1988). Nutrient limitation of phytoplankton in fresh-water and marine environments - a review of recent-evidence on the effects of enrichment. [Review]. *Limnology and Oceanography, 33*(4), 796-822.

Hegmen, W., Wang, D., & Borer, C. (1999). *Estimation of Lake Champlain basinwide nonpoint source*. Grand Isle, VT: Lake Champlain Basin Program.

Hesslein, R. H. (1976). An in situ sampler for close interval pore water. *Limnology and Oceanography, 21*, 912-914.

Horppila, J., & Niemisto, J. (2008). Horizontal and vertical variations in sedimentation and resuspension rates in a stratifying lake - effects of internal seiches. *Sedimentology, 55*(5), 1135-1144.

Horppila, J., & Nurminen, L. (2001). The effect of an emergent macrophyte (Typha angustifolia) on sediment resuspension in a shallow north temperate lake. *Freshwater Biology, 46*(11), 1447-1455.

106

Hosomi, M., & Sudo, R. (1992). Development of the phosphorus dynamic-model in sediment-water system and assessment of eutrophication control programs. *Water Science and Technology, 26*(7-8), 1981-1990.

Hupfer, M., & Gachter, R. (1995). Polyphosphate in lake-sediments - p-31 nmr-spectroscopy as a tool for its identification. *Limnology and Oceanography, 40*(3), 610-617.

Hupfer, M., Gachter, R., & Giovanoli, R. (1995). Transformation of phosphorus species in settling seston and during early sediment diagenesis. [Article]. *Aquatic Sciences, 57*(4), 305-324.

Hupfer, M., & Lewandowski, J. (2008). Oxygen Controls the Phosphorus Release from Lake Sediments - a Long-Lasting Paradigm in Limnology. [Review]. *International Review of Hydrobiology, 93*(4-5), 415-432.

Istvanovics, V. (1988). Seasonal-variation of phosphorus release from the sediments of shallow lake balaton (hungary). [Article]. *Water Research, 22*(12), 1473-1481.

Jahnke, R. A., Alexander, C. R., & Kostka, J. E. (2003). Advective pore water input of nutrients to the Satilla River Estuary, Georgia, USA. *Estuarine Coastal and Shelf Science, 56*(3-4), 641-653.

Jansson, M. (1987). Anaerobic dissolution of iron phosphorus complexes in sediment due to the activity of nitrate-reducing bacteria. [Article]. *Microbial Ecology, 14*(1), 81-89.

Jensen, H. S., & Andersen, F. O. (1992). Importance of temperature, nitrate, and ph for phosphate release from aerobic sediments of 4 shallow, eutrophic lakes. [Article]. *Limnology and Oceanography, 37*(3), 577-589.

Jensen, H. S., Kristensen, P., Jeppesen, E., & Skytthe, A. (1992). *Iron-phosphorus ratio in surface sediment as an indicator of phosphate release from aerobic sediments in shallow lakes*.

Jeppesen, E., Jensen, J. P., Sondergaard, M., Lauridsen, T., Pedersen, L. J., & Jensen, L. (1997). *Top-down control in freshwater lakes: The role of nutrient state, submerged macrophytes and water depth*.

Jones, C. A., & Welch, E. B. (1990). Internal phosphorus loading related to mixing and dilution in a dendritic, shallow prairie lake. *Research Journal of the Water Pollution Control Federation, 62*(7), 847-852.

Kleeberg, A., & Schubert, H. (2000). Vertical gradients in particle distribution and its elemental composition under oxic and anoxic conditions in a eutrophic lake, Scharmutzelsee, NE Germany. *Archiv Fur Hydrobiologie, 148*(2), 187-207.

Koski-Vahala, J., & Hartikainen, H. (2001). Assessment of the risk of phosphorus loading due to resuspended sediment. *Journal of Environmental Quality, 30*(3), 960-966.

Kostka, J. E., & Luther, G. W. (1994). Partitioning and speciation of solid-phase iron in salt-marsh sediments. *Geochimica Et Cosmochimica Acta, 58*(7), 1701-1710.

Kostka, J. E., & Luther, G. W. (1995). Seasonal cycling of fe in salt-marsh sediments. *Biogeochemistry, 29*(2), 159-181.

Kristensen, P., Sondergaard, M., & Jeppesen, E. (1992). Resuspension in a shallow eutrophic lake. *Hydrobiologia, 228*(1), 101-109.

L'Annunziata, M. F. (1975). *The origin and transformation of the soil inositol phosphate isomers.* Paper presented at the Soil Science Society of America Proceedings.

Lijklema, L. (1980). Interaction of orthophosphate with iron(III) and aluminum hydroxides. *Environmental Science and Technology, 14*(5), 537-541.

Lorenson, G. (2006). Application of in situ au-amalgam microelectrodes in yellowstone national park to guide microbial sampling: an investigation into arsenite and polysulfide detection to define microbial habitats. The University of Vermont, Burlington.

Lott, J. N. A., Ockenden, I., Raboy, V., & Batten, G. D. (2000). Phytic acid and phosphorus in crop seeds and fruits: a global estimate. *Seed Science Research, 10*(1), 11-33.

Lovley, D. R. (1991). Dissimilatory fe(iii) and mn(iv) reduction. *Microbiological Reviews, 55*(2), 259-287.

Lovley, D. R., & Phillips, E. J. P. (1988). Manganese inhibition of microbial iron reduction in anaerobic sediments. *Geomicrobiology Journal, 6*(3-4), 145-155.

Luther, G. W., & Church, T. M. (1988). Seasonal cycling of sulfur and iron in porewaters of a delaware salt-marsh. *Marine Chemistry, 23*(3-4), 295-309.

Luther, G. W., Ferdelman, T., & Tsamakis, E. (1988). Evidence suggesting anaerobic oxidation of the bisulfide ion in chesapeake bay. *Estuaries, 11*(4), 281-285.

Luther, G. W., Glazer, B., Ma, S. F., Trouwborst, R., Shultz, B. R., Druschel, G., et al. (2003). Iron and sulfur chemistry in a stratified lake: Evidence for iron-rich sulfide complexes. *Aquatic Geochemistry, 9*(2), 87-110.

Luther, G. W., Reimers, C. E., Nuzzio, D. B., & Lovalvo, D. (1999). In situ deployment of voltammetric, potentiometric. and amperometric microelectrodes from a ROV to determine dissolved O-2, Mn, Fe, S(-2), and pH in porewaters. *Environmental*

Science & Technology, 33(23), 4352-4356.

Luther, G. W., & Rickard, D. (2005). *Metal sulfide cluster complexes: Structure, reactivity and environmental impact*.

Makarov, M. I., Haumaier, L., Zech, W., Marfenina, O. E., & Lysak, L. V. (2005). Can P-31 NMR spectroscopy be used to indicate the origins of soil organic phosphates? *Soil Biology & Biochemistry, 37*(1), 15-25.

McKelive, I. D. (2007). Inositol Phosphates in Aquatic Systems. In B. L. Turner, Richardson, A.E., and Mullaney, E.J. (Ed.), *Inositol Phosphates Linking Agriculture and the Environment* (pp. 261-277). Cambridge, MA: CABI.

Michaud, E., Desrosiers, G., Mermillod-Blondin, F., Sundby, B., & Stora, G. (2005). The functional group approach to bioturbation: The effects of biodiffusers and gallery-diffusers of the Macoma balthica community on sediment oxygen uptake. *Journal of Experimental Marine Biology and Ecology, 326*(1), 77-88.

Michaud, E., Desrosiers, G., Mermillod-Blondin, F., Sundby, B., & Stora, G. (2006). The functional group approach to bioturbation: II. The effects of the Macoma balthica community on fluxes of nutrients and dissolved organic carbon across the sediment-water interface. *Journal of Experimental Marine Biology and Ecology, 337*(2), 178-189.

Mortimer, C. H. (1941). The exchange of dissolved substances between mud and water in lakes. *J. Ecol., 29*, 280-329.

NRD State of Vermont Natrual Resources Board: Water Resources Panel. Vermont Water Quality Standards**.** Vt. Code R. 12 004 052 January 2008. http://www.nrb.state.vt.us/wrp/publications/wqs.pdf

Olila, O. G., & Reddy, K. R. (1997). Influence of redox potential on phosphate-uptake by sediments in two sub-tropical eutrophic lakes. *Hydrobiologia, 345*, 45-57.

Penn, M. R., Auer, M. T., Doerr, S. M., Driscoll, C. T., Brooks, C. M., & Effler, S. W. (2000). Seasonality in phosphorus release rates from the sediments of a hypereutrophic lake under a matrix of pH and redox conditions. [Article]. *Canadian Journal of Fisheries and Aquatic Sciences, 57*(5), 1033-1041.

Perkins, R. G., & Underwood, G. J. C. (2001). The potential for phosphorus release across the sediment-water interface in an eutrophic reservoir dosed with ferric sulphate. *Water Research, 35*(6), 1399-1406.

Petticrew, E. L., & Arocena, J. M. (2001). *Evaluation of iron-phosphate as a source of internal lake phosphorus loadings*.

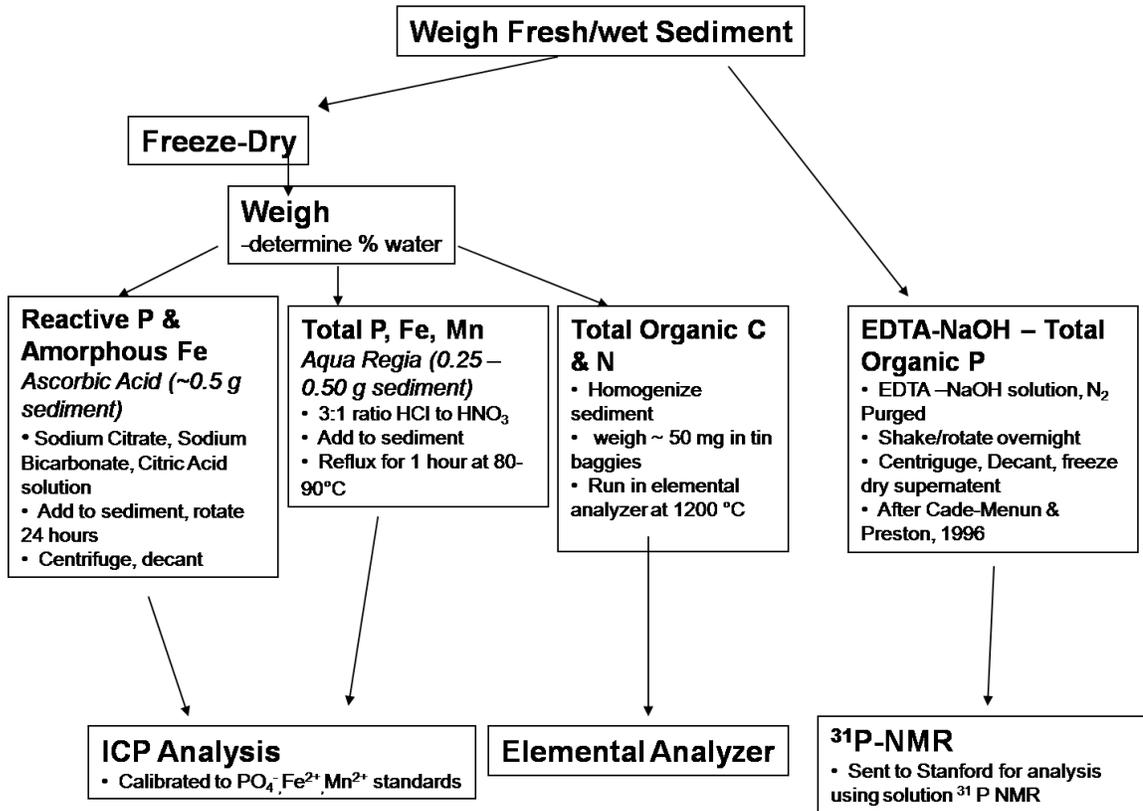Reitzel, K., Ahlgren, J., DeBrabandere, H., Waldeback, M., Gogoll, A., Tranvik, L., et al.

(2007). Degradation rates of organic phosphorus in lake sediment. *Biogeochemistry, 82*(1), 15-28.

Rickard, D. (2006a). The solubility of FeS. *Geochimica Et Cosmochimica Acta, 70*(23), 5779-5789.

Roden, E. E. a. E., J.W. (1997). Phosphate mobilization in iron-rich anaerobic sediments: microbial Fe(III) oxide reduction versus iron-sulfide formation. *Arch. Hydrobiol., 139*(3), 347-378.

Rodel, M.G., Armstrong, D.E., Harris, R.F. Sorption and hydrolysis of added organic phosphorus compounds in lake sediments. *Limnology and Oceanography*. 22(3), 415-422.

Roesler, A. J., Gammons, C. H., Druschel, G. K., Oduro, H., & Poulson, S. R. (2007). Geochemistry of flooded underground mine workings influenced by bacterial sulfate reduction. *Aquatic Geochemistry, 13*(3), 211-235.

Roitz, J. S., Flegal, A. R., & Bruland, K. W. (2002). The biogeochemical cycling of manganese in San Francisco Bay: Temporal and spatial variations in surface water concentrations. *Estuarine Coastal and Shelf Science, 54*(2), 227-239.

Rozan, T. F., Taillefert, M., Trouwborst, R. E., Glazer, B. T., Ma, S. F., Herszage, J., et al. (2002). Iron-sulfur-phosphorus cycling in the sediments of a shallow coastal bay: Implications for sediment nutrient release and benthic macroalgal blooms. *Limnology and Oceanography, 47*(5), 1346-1354.

Ryding, S. O. (1985). Chemical and microbiological processes as regulators of the exchange of substances between sediments and water in shallow eutrophic lakes. *Internationale Revue Der Gesamten Hydrobiologie, 70*(5), 657-702.

Sannigrahi, P., & Ingall, E. (2005). Polyphosphates as a source of enhanced P fluxes in marine sediments overlain by anoxic waters: Evidence from P-31 NMR. *Geochemical Transactions, 6*(3), 52-59.

Schindler, D. W. (1974). Eutrophication and Recovery in Experimental Lakes: Implications for Lake Management. *Science, 184*(4139).

Schindler, D. W., Hecky, R. E., Findlay, D. L., Stainton, M. P., Parker, B. R., Paterson, M. J., et al. (2008). Eutrophication of lakes cannot be controlled by reducing nitrogen input: Results of a 37-year whole-ecosystem experiment. [Article]. *Proceedings of the National Academy of Sciences of the United States of America, 105*(32), 11254-11258.

Schlesinger, W. H., & Hartley, A. E. (1992). A global budget for atmospheric nh3. *Biogeochemistry, 15*(3), 191-211.

Skoog, D. A., Holler, F.J., Crouch, S.R. (2007). *Principles of Instrumental Analysis* (6 ed.). Belmont, CA: Thomson Brooks Cole.

Skoog, P. L. L., J.J. (1998). *Principles of Instrumental Analysis* (5 ed.). TX: Saunders College Publishing.

Smeltzer et al. (2008, unpublished) Environmental factors associated with the severity of algae blooms in Missisquoi Bay, Lake Champlain. Vermont Agency of Natural Resources.

Smernik, R.J. and Dougherty, W.J. (2007). Identification of phytat in phosphorus-31 nuclear magnetic resonance spectra: The need for spiking. *Soil Science Society of America Journal*. 71(3), 1045-1050.

Smith, V.H. (1998). Cultural eutrophication of inland, estuarine, and coastal waters. In: Pace, M.L, Groffman, P.M. (Eds.), Successes, Limitations and Frontiers in Ecosystem Science. Springer, New York, pp. 7-49.

Smith, V. H., Tilman, G. D., & Nekola, J. C. (1999). Eutrophication: impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems. *Environmental Pollution*. 100, 179-196.

Søndergaard, M., Jensen, J. P., & Jeppesen, E. (2003). *Role of sediment and internal loading of phosphorus in shallow lakes*.

Søndergaard, M., Kristensen, P., & Jeppesen, E. (1992). Phosphorus release from resuspended sediment in the shallow and wind-exposed lake arreso, denmark. [Article]. *Hydrobiologia, 228*(1), 91-99.

Søndergaard, M., Kristensen, P., & Jeppesen, E. (1993). 8 years of internal phosphorus loading and changes in the sediment phosphorus profile of lake sobygaard, denmark. *Hydrobiologia, 253*(1-3), 345-356.

Spears, B. M., Carvalho, L., Perkins, R., & Paterson, D. M. (2008). Effects of light on sediment nutrient flux and water column nutrient stoichiometry in a shallow lake. *Water Research, 42*(4-5), 977-986.

Sundareshwar, P.V. and Morris, J.T. (2001). Phosphorus sorption characteristics of intertidal marsh sediments along an estuarine salinity gradient.

Sundby, B., Anderson, L. G., Hall, P. O. J., Iverfeldt, A., Vanderloeff, M. M. R., & Westerlund, S. F. G. (1986). The effect of oxygen on release and uptake of cobalt, manganese, iron and phosphate at the sediment-water interface. *Geochimica Et Cosmochimica Acta, 50*(6), 1281-1288.

Sundby, B., Gobeil, C., Silverberg, N., & Mucci, A. (1992). The phosphorus cycle in

coastal marine-sediments. *Limnology and Oceanography, 37*(6), 1129-1145.

Suzumura and Kamatani (1995). Mineralization of inositol hexaphosphate in aerobic and anaerobic marine sediments: Implications for the phosphorus cycle. *Geochemica et Cosmochimica Acta*. 59(5), 1021-1026.

Taillefert, M., Luther, G. W., & Nuzzio, D. B. (2000). The application of electrochemical tools for in situ measurements in aquatic systems. *Electroanalysis, 12*(6), 401-412.

Taillefert, M., Rozan, T. F., Glazer, B. T., Herszage, J., Trouwborst, R. E., & Luther, G. W. (2000). Seasonal variations of soluble Fe(III) in sediment porewaters as revealed by voltammetric microelectrodes. *Abstracts of Papers of the American Chemical Society, 220*, 31-ENVR.

Troy, A. (2007). Updating the Lake Champlain basin land use data to improve prediction of phosphorus loading. In D. Wang (Ed.), *Report to the Lake Champlain Basin Program*. Grand Isle, Vermont: LCBP Technical Report.

Turner, B. L., Cade-Menun, B. J., & Westermann, D. T. (2003). Organic phosphorus composition and potential bioavailability in semi-arid arable soils of the western United States. *Soil Science Society of America Journal, 67*(4), 1168-1179.

Turner, B. L., Mahieu, N., & Condron, L. M. (2003). Phosphorus-31 nuclear magnetic resonance spectral assignments of phosphorus compounds in soil NaOH-EDTA extracts. *Soil Science Society of America Journal, 67*(2), 497-510.

Tyler, R. M., Brady, D. C., & Targett, T. E. (2009). Temporal and Spatial Dynamics of Diel-Cycling Hypoxia in Estuarine Tributaries. *Estuaries and Coasts, 32*(1), 123-145.

van der Zee, C., Roberts, D. R., Rancourt, D. G., & Slomp, C. P. (2003). Nanogoethite is the dominant reactive oxyhydroxide phase in lake and marine sediments. *Geology, 31*(11), 993-996.

Vandermolen, D. T., & Boers, P. C. M. (1994). Influence of internal loading on phosphorus concentration in shallow lakes before and after reduction of the external loading. *Hydrobiologia, 275*, 379-389.

Visser, P. M., Ibelings, B.W., Mur, L.R., and Walsby, A.E. (2005). The ecophysiology of the harmful cyanobacterium Microcystis. In J. Huisman, Matthijis, H.C.P., Visser, P.M. (Ed.), *Harmful Cyanobacteria* (pp. 109-142): Springer.

Vitousek, P. M., & Howarth, R. W. (1991). Nitrogen limitation on land and in the sea - how can it occur. *Biogeochemistry, 13*(2), 87-115.

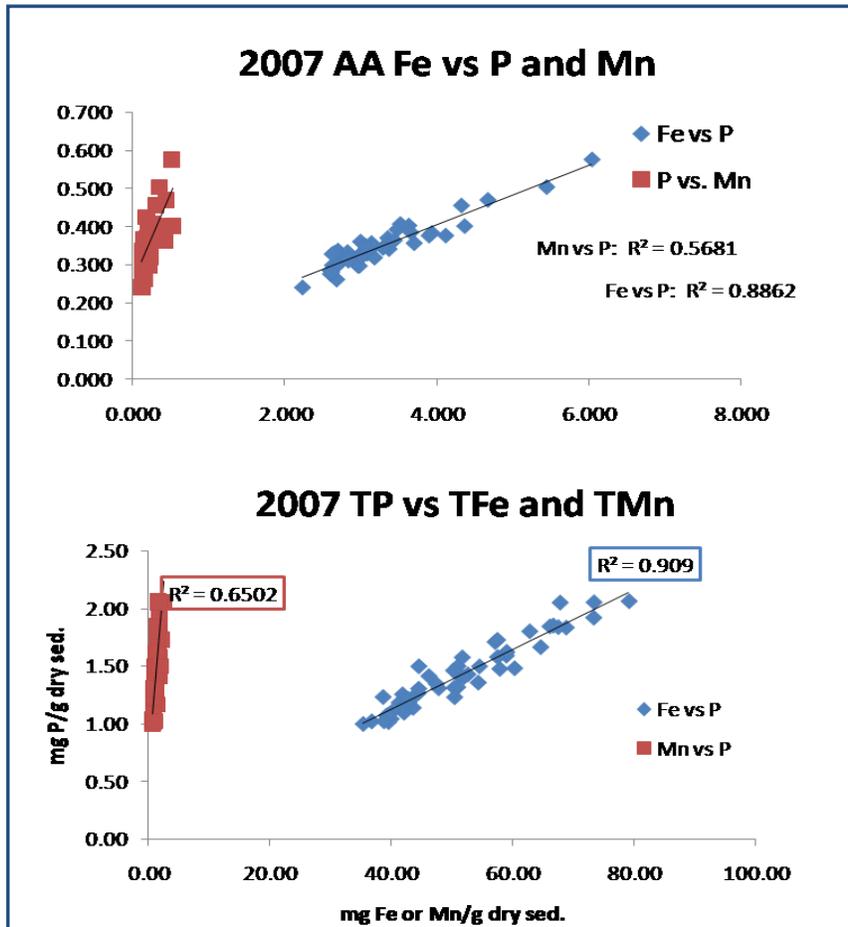von Liebig, J. (1855). Principals of agricultural chemistry with special reference to the

late researchs made in England. In L. R. Pomery (Ed.), *Cycles of Essential Elements* (Vol. 1, pp. 11-28). Amsterdam: Dowden, Hutchinson, and Ross.

Wang, S. R., Jin, X. C., Pang, Y., Zhao, H. C., & Zhou, X. N. (2005). The study of the effect of pH on phosphate sorption by different trophic lake sediments. [Article]. *Journal of Colloid and Interface Science, 285*(2), 448-457.

Watzin, M.C., E.K. Brines, M. Kreider and M. Levine. 2005. Monitoring and evaluation of cyanobacteria in Lake Champlain: Summer 2004. Report to the Lake Champlain Basin Program, Grand Isle, Vermont.

Watzin, M.C, A.d. Shambaugh and E. Brines. 2003. Monitoring and evaluation of cyanobacteria in Burlington Bay, Lake Champlain: Summer 2002. Report prepared for Lake Champlain Basin Program, Grand Isle, Vermont.

Weimer, W. C. a. A., D.E. (1979). Naturally occurring organic phosphorus compounds in aquatic plants. *Environmental Science & Technology, 13*(7), 826-829.

Wetzel, R. G. (1999). Organic phosphorus mineralization in soils and sediments. In K. R. Reddy, O'Connor, G.A., Schelske, C.L. (Ed.), *Phosphorus Biogeochemistry in Subtropical Ecosystems* (pp. 225-245). Boca Raton, LA: Lewis Publishers.

Williams, J. D. H., Mayer, J. (1972). Effects of sediment diagenesis and regeneration of phosphorus with special reference to lakes Erie and Ontario. In H. E. a. K. Allen, J.R. (Ed.), *Nutrients in Natural Waters* (pp. 281-315). New York: J. Wiley & Sons.

Wright, S. and Larsen, F. (2004). Surficial Geology of the Barre-Montpelier Region. www.anr.state.vt.us/dec/geo/pdfdocs/BarreWestwright.pdf

VGS. 1971. Doll, C.G., Stewart, D.P., and MacClintock, Paul, 1970, Surficial geologic map of Vermont: Vermont Department of Water Resources, Map , scale 1:250000.

VGS. 1961. Doll, C.G., Cady, W.M., Thompson, J.B., and Billings, M.P., 1961, Centennial geologic map of Vermont: Vermont Geological Survey, Miscellaneous Map MISCMAP-01, scale 1:250000.

Xie, L. Q., Xie, P., & Tang, H. J. (2003). Enhancement of dissolved phosphorus release from sediment to lake water by Microcystis blooms - an enclosure experiment in a hyper-eutrophic, subtropical Chinese lake. *Environmental Pollution, 122*(3), 391-399.

Zhang, J. Z., & Huang, X. L. (2007). Relative importance of solid-phase phosphorus and iron on the sorption behavior of sediments. [Article]. *Environmental Science & Technology, 41*(8), 2789-2795.
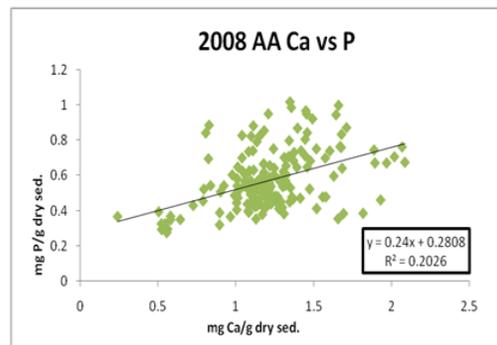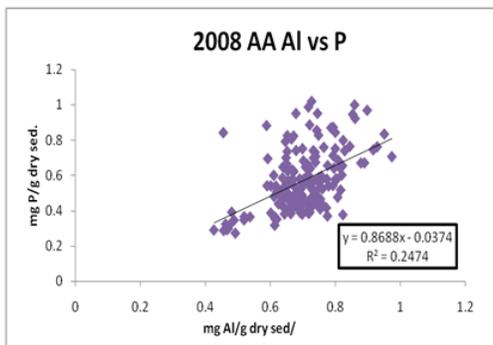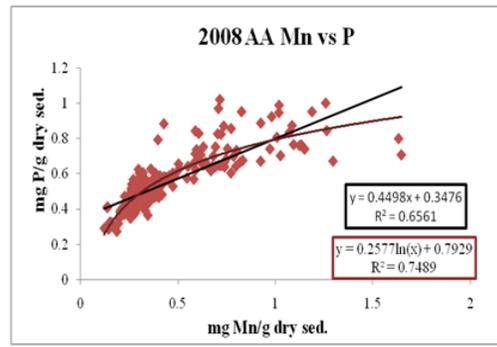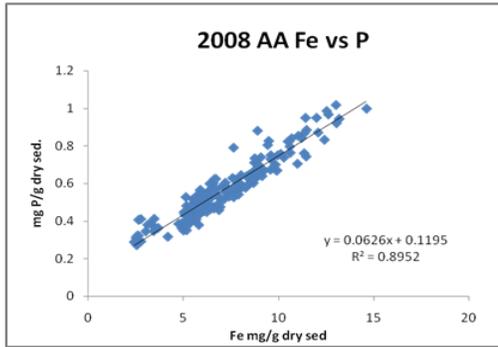
APPENDIX A: Sediment Extraction Flow Diagram

Weigh Fresh/wet Sediment

Freeze-Dry

Weigh
–determine % water

Reactive P & Amorphous Fe
Ascorbic Acid (~0.5 g sediment)
• Sodium Citrate, Sodium Bicarbonate, Citric Acid solution
• Add to sediment, rotate 24 hours
• Centrifuge, decant

Total P, Fe, Mn
Aqua Regia (0.25 – 0.50 g sediment)
• 3:1 ratio HCl to HNO_3
• Add to sediment
• Reflux for 1 hour at 80-90°C

Total Organic C & N
• Homogenize sediment
• weigh ~ 50 mg in tin baggies
• Run in elemental analyzer at 1200 °C

EDTA-NaOH – Total Organic P
• EDTA –NaOH solution, N_2 Purged
• Shake/rotate overnight
• Centriguge, Decant, freeze dry supernatent
• After Cade-Menun & Preston, 1996

ICP Analysis
• Calibrated to PO_4^-, Fe^{2+}, Mn^{2+} standards

Elemental Analyzer

³¹P-NMR
• Sent to Stanford for analysis using solution ³¹P NMR
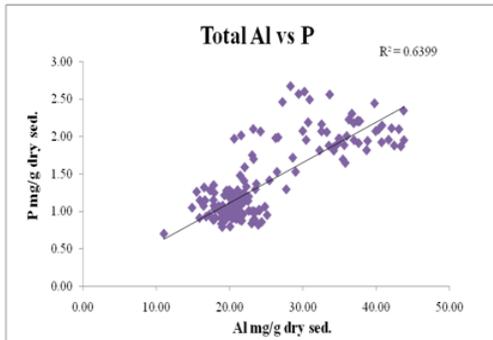
APPENDIX B: Sediment Extraction Correlations
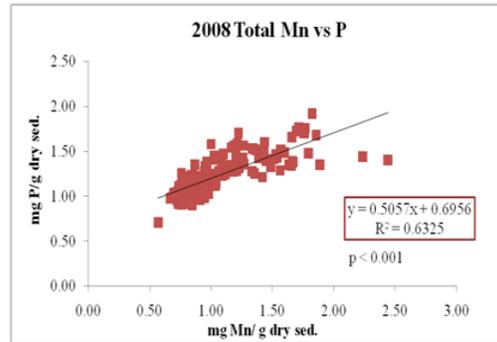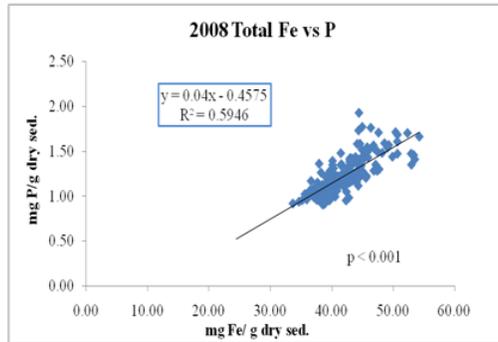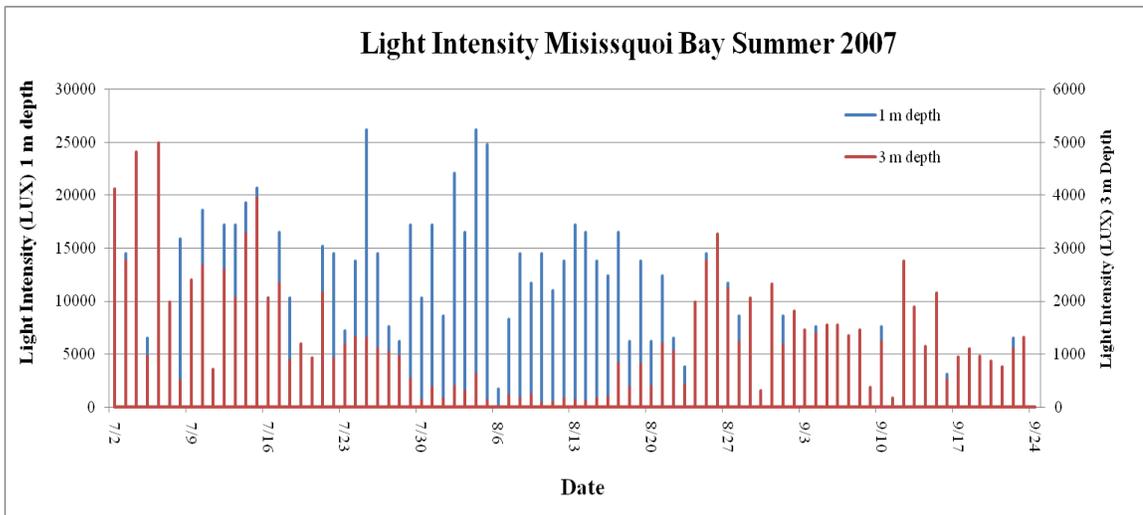
2007 Sediment Extraction Correlations

2008 Sediment Extraction Correlations

Reactive – Ascorbic Acid Extraction

2008 AA Fe vs P
$y = 0.0626x + 0.1195$
$R^2 = 0.8952$

2008 AA Mn vs P
$y = 0.4498x + 0.3476$
$R^2 = 0.6561$
$y = 0.2577\ln(x) + 0.7929$
$R^2 = 0.7489$

2008 AA Al vs P
$y = 0.8688x - 0.0374$
$R^2 = 0.2474$

2008 AA Ca vs P
$y = 0.24x + 0.2808$
$R^2 = 0.2026$

Total – Aqua Regia Digest

2008 Total Fe vs P
$y = 0.04x - 0.4575$
$R^2 = 0.5946$
$p < 0.001$

2008 Total Mn vs P
$y = 0.5057x + 0.6956$
$R^2 = 0.6325$
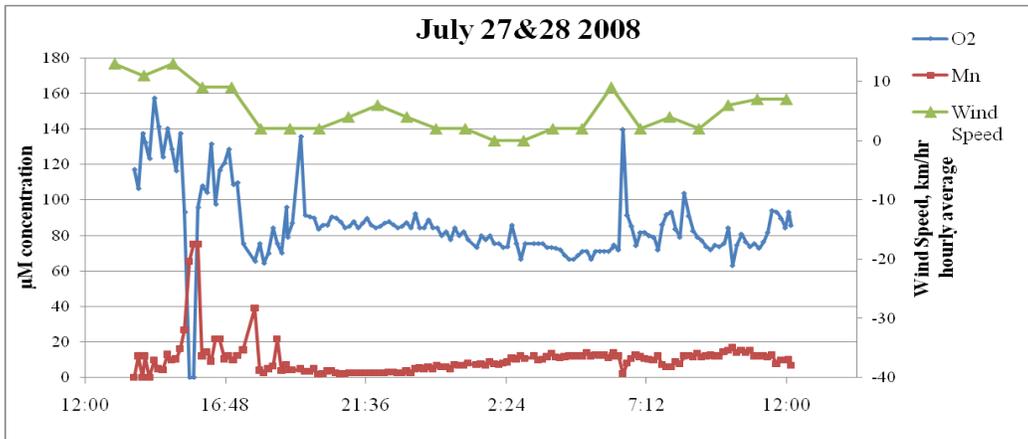$p < 0.001$

Total Al vs P
$R^2 = 0.6399$

Total Ca vs P Aq Reg
$R^2 = 0.07$
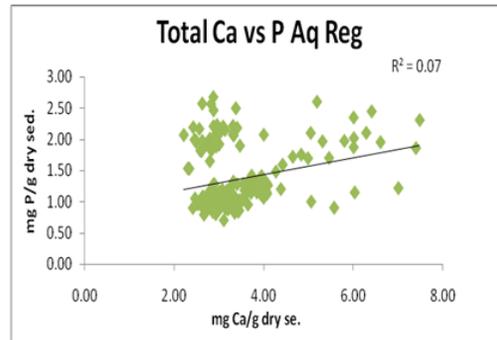
APPENDIX C: Sediment and Water Temperature and Light Intensity Measurements
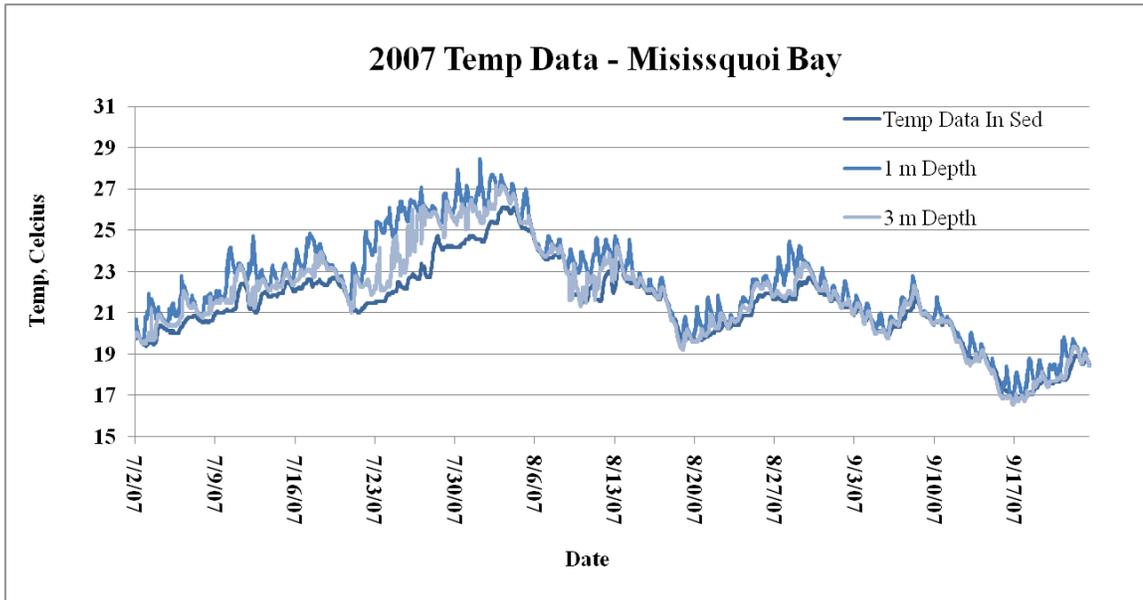
APPENDIX D: Hourly Average Surface Wind Data (km/hr) Plotted with Diel Redox
Measurements at the SWI
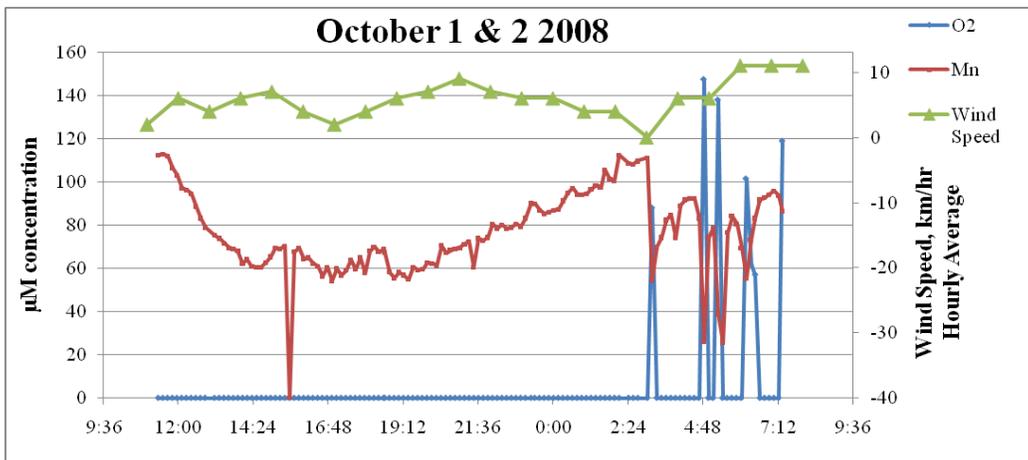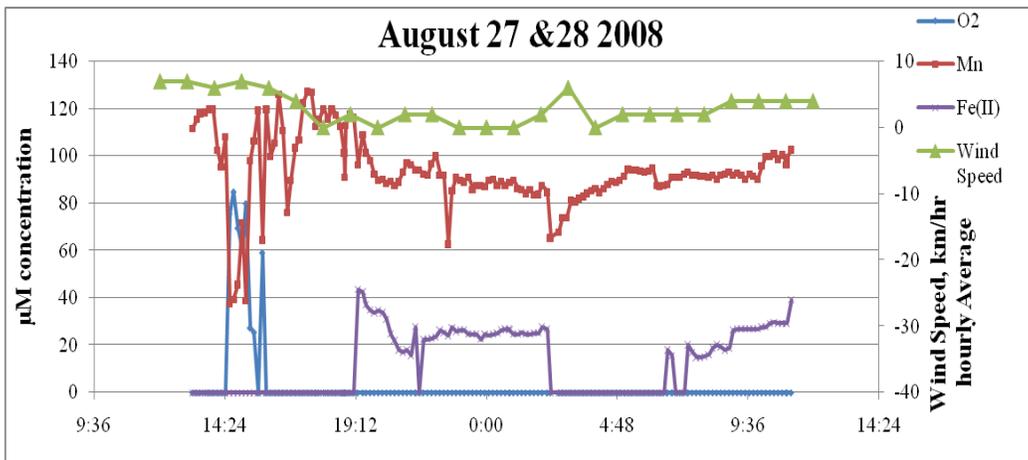
Wind speed (km/hr, hourly average) obtained from the Freighlisberg, QUE weather station is
plotted on the diel cycle figures. Wind, resulting in water column mixing, appears to have some
influence on some of the variability in redox species concentrations at the SWI.
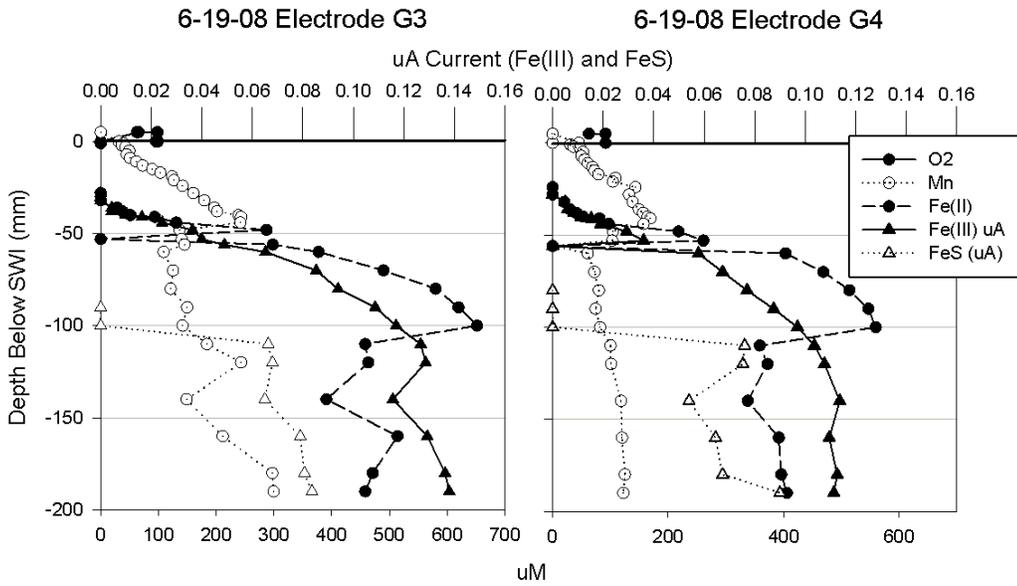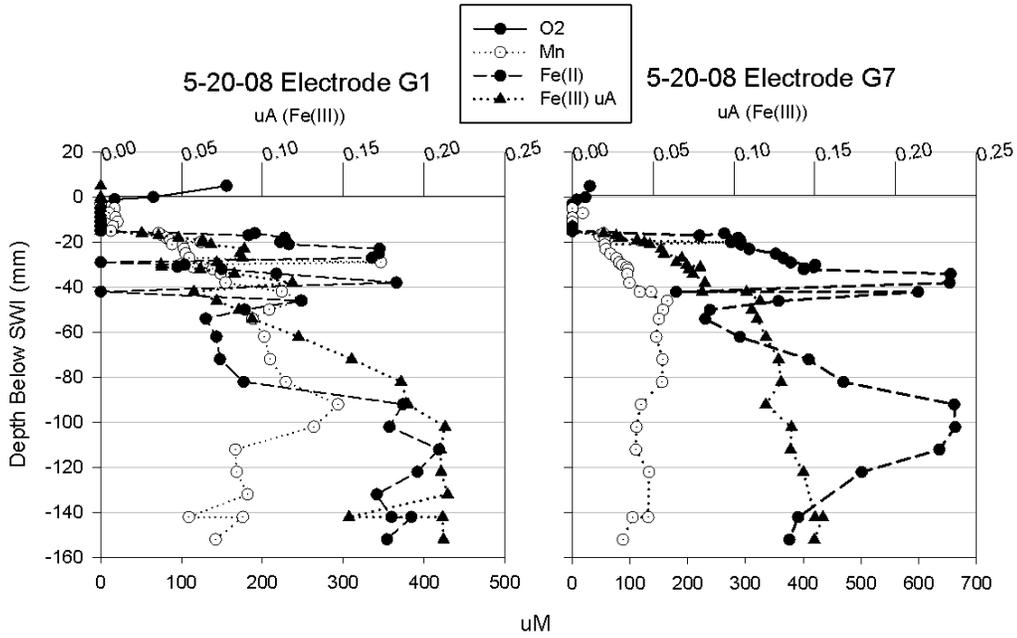
2007 Core Redox Profiles

June 2007 Core 1

June 2007 Core 2

July 2007

August 2007

October 2007
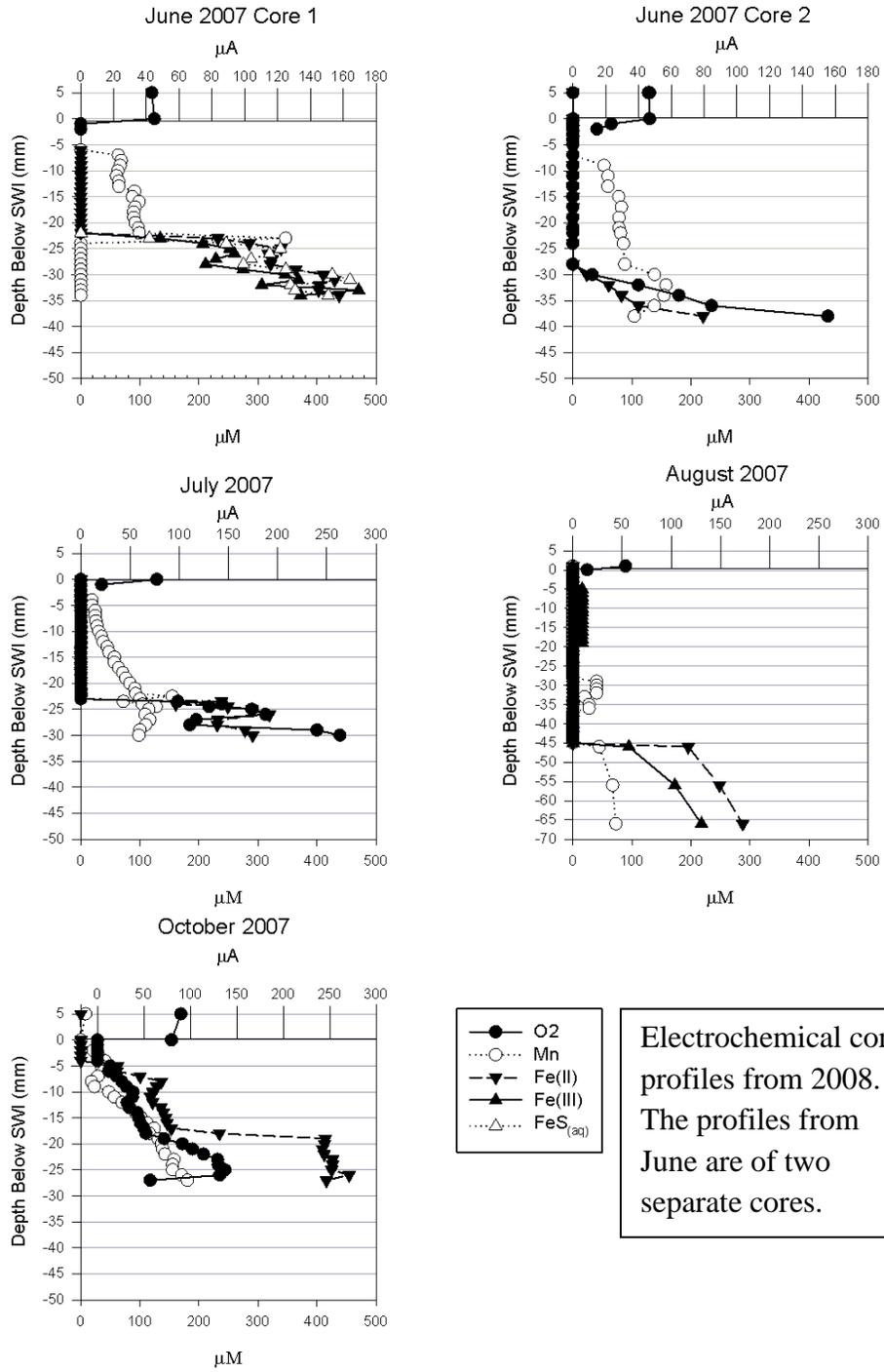
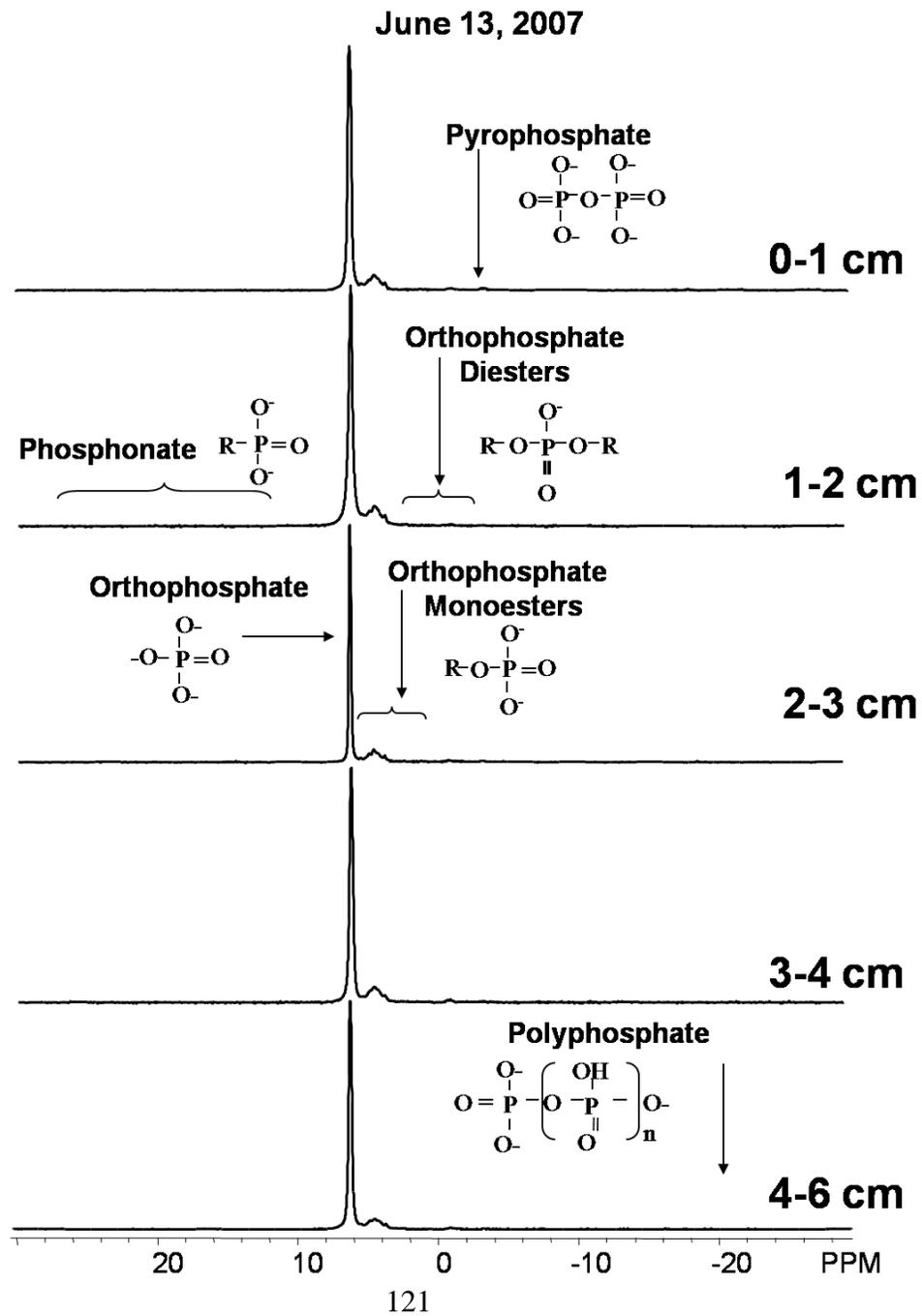Electrochemical core profiles from 2008. The profiles from June are of two separate cores.

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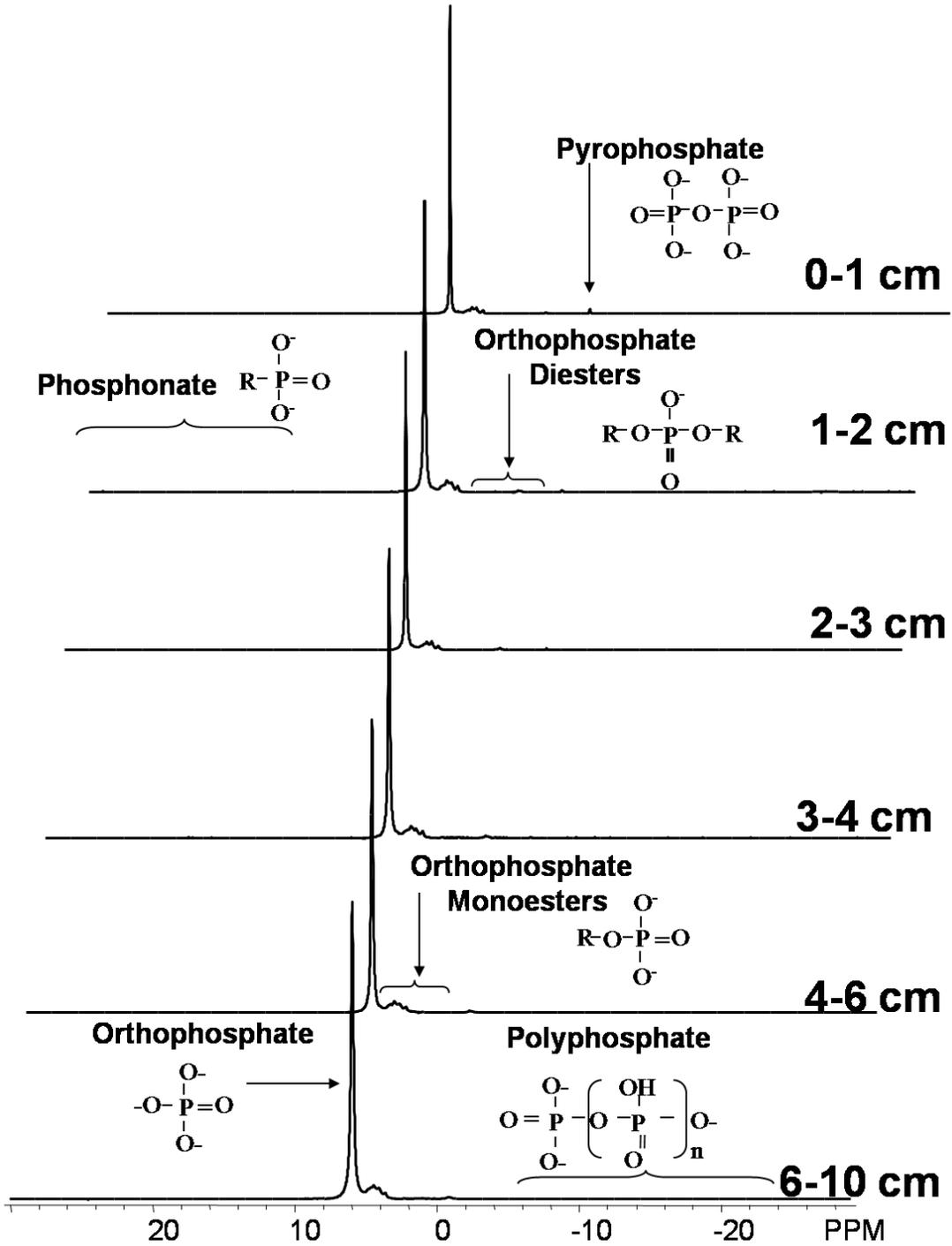
2008 Core Redox Profiles

Core Profiles from May and June 2008 (that were not in main text). These profiles are within the same core using two electrodes at the same time. The electrochemical profiles appear to be homogenous within one core.

APPENDIX F: 31P-NMR Spectra

^{31}P NMR spectra are presented in the following pages with the chemical shift (in PPM relative to an 85% H_3PO_4 standard) of P_{org} compounds identified in the spectra.

June 13, 2007

Pyrophosphate

0-1 cm

Orthophosphate Diesters

Phosphonate

1-2 cm

Orthophosphate

Orthophosphate Monoesters

2-3 cm

3-4 cm

Polyphosphate

4-6 cm

20   10   0   -10   -20   PPM

October 11, 2007

Pyrophosphate

0-1 cm

Phosphonate

Orthophosphate Diesters

1-2 cm

2-3 cm

3-4 cm

Orthophosphate Monoesters

4-6 cm

Orthophosphate

Polyphosphate

6-10 cm

20   10   0   -10   -20   PPM
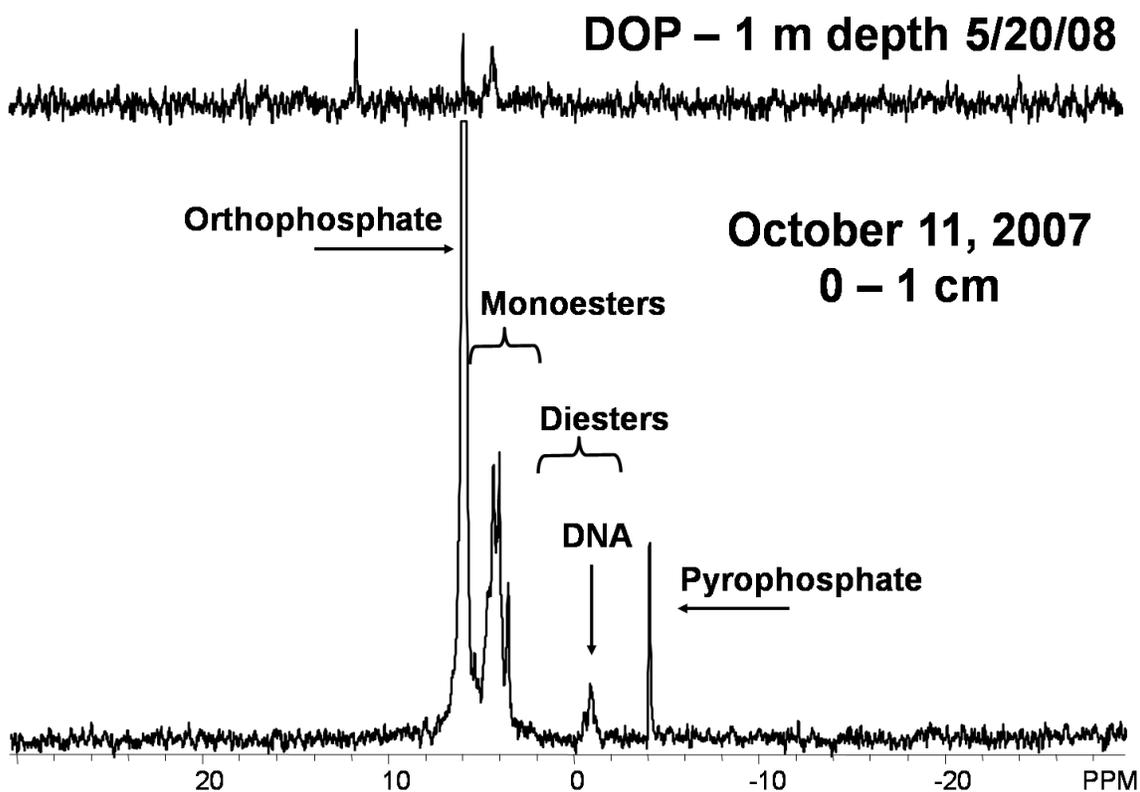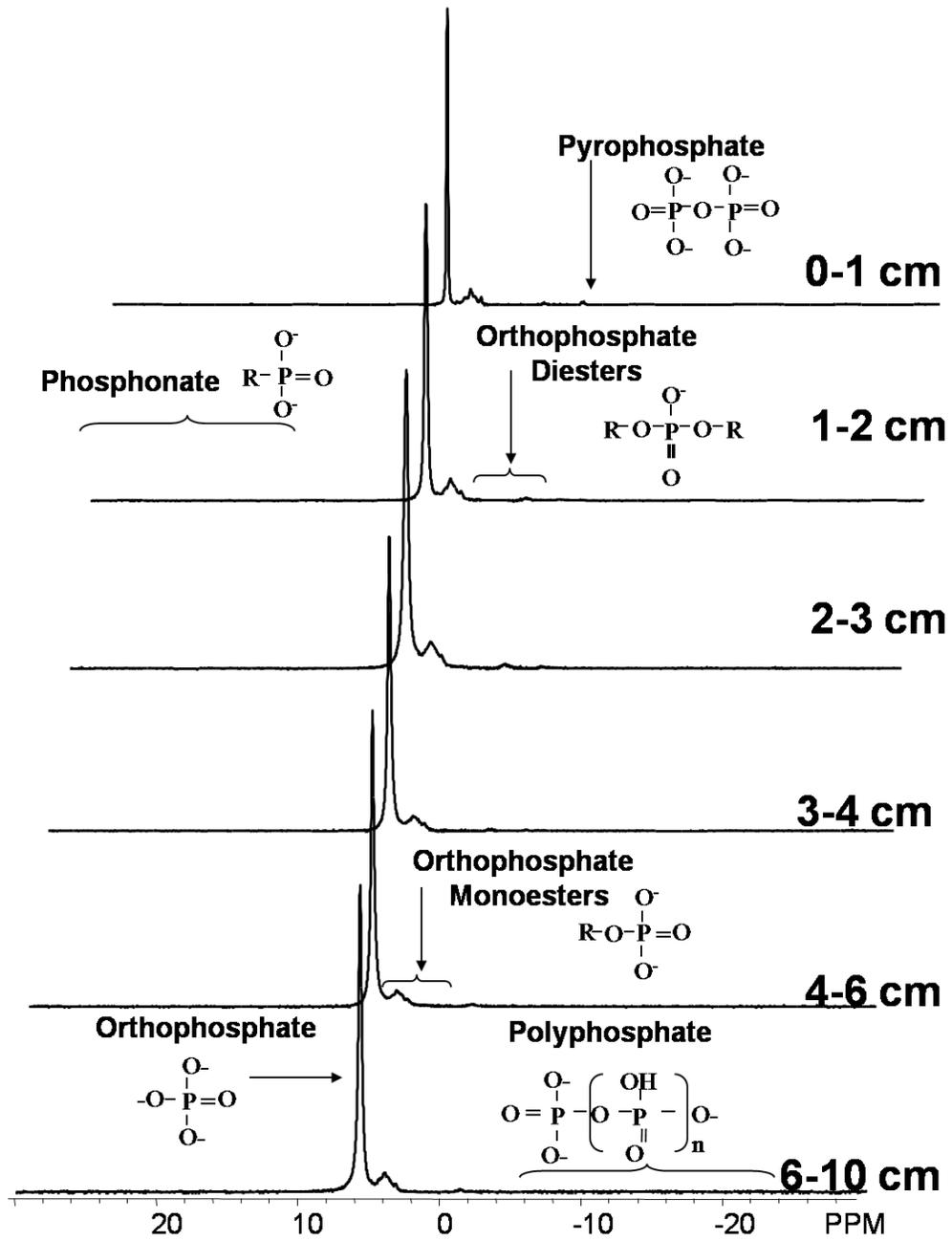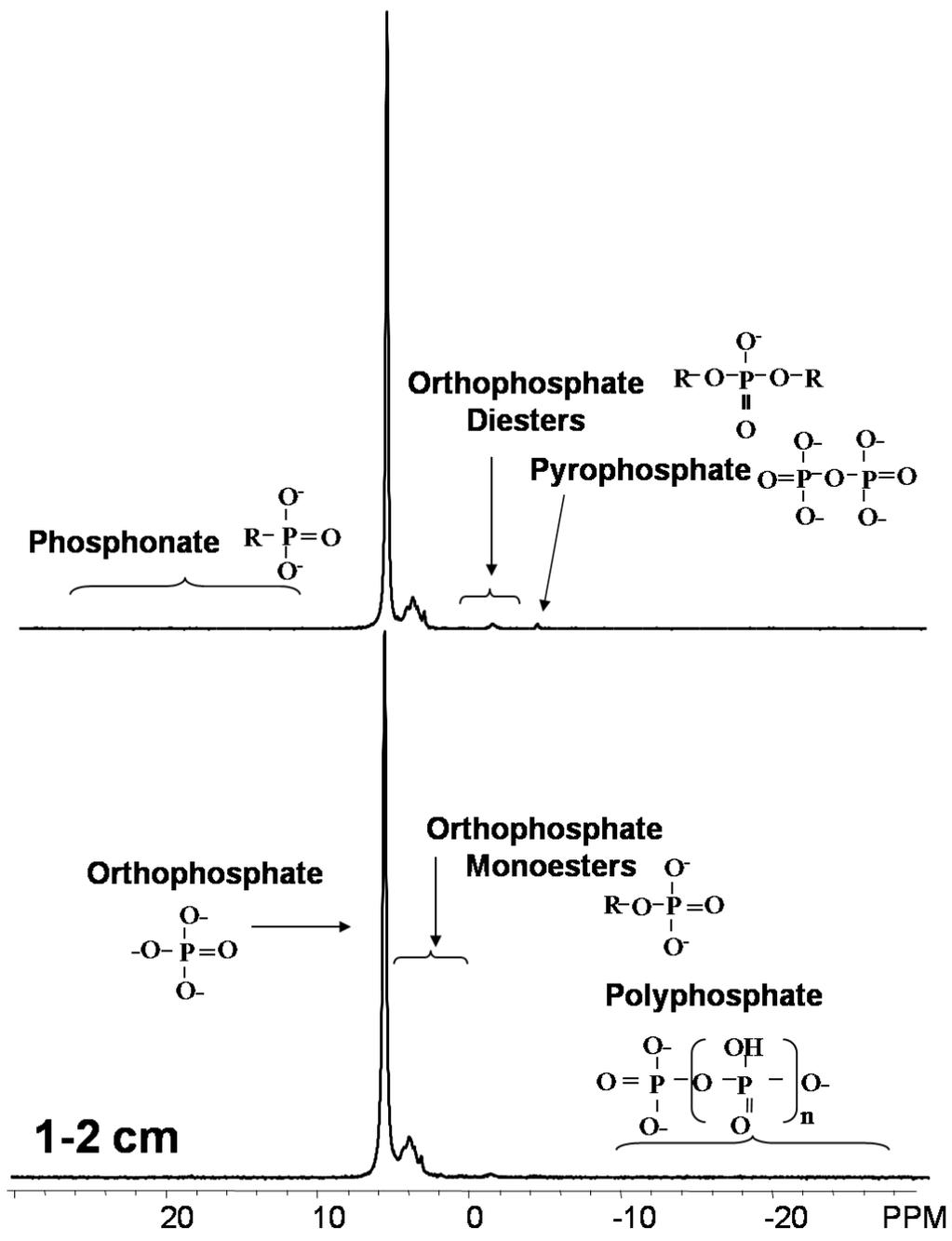
A water sample collected from 1 m depth on 5/20/08 was extracted and analyzed according to Cade-Menun et al. (2006) and the ^{31}P NMR spectra is shown above in comparison to a sample with higher concentrations. Integrations of the peaks in this sample showed that P_{org} comprised about 60% of the total P.

MB Core 1A, May 2008

MB Core D1, July 27, 2008

Phosphonate

Orthophosphate Diesters

Pyrophosphate

Orthophosphate

Orthophosphate Monoesters

Polyphosphate

1-2 cm

20   10   0   -10   -20   PPM

MB Core E4, August 27, 2008

Pyrophosphate

Phosphonate

Orthophosphate Diesters

Orthophosphate Monoesters

Orthophosphate

Polyphosphate

0-1 cm
1-2 cm
2-3 cm
3-4 cm
4-6 cm
6-10 cm

20   10   0   -10   -20   PPM

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MB Core F1, October 1, 2008

Pyrophosphate

Phosphonate

Orthophosphate
Diesters

0-1 cm

1-2 cm

2-3 cm

3-4 cm

Orthophosphate
Monoesters

4-6 cm

Orthophosphate

Polyphosphate

6-10 cm

A phytic acid spiking experiment was conducted to identify phytate in the monoester region for some samples. The experiment resulted in the identification of inositol hexakisphosphate (IHP) and *scyllo*-inositol phosphate.

APPENDIX G: Average Total and Reactive Fe, P, Mn, Al, and Ca Concentrations by depth segment for 2007 and 2008

2007 & 2008 Mean Fe, Mn, P, Al, and Ca concentrations by Depth										
	Total 2007					**Total 2008**				
	Fe	Mn	P	Al	Ca	Fe	Mn	P	Al	Ca
0-1	47.75	1.60	1.44	19.91	3.86	43.01	1.44	1.36	22.94	3.88
1-2	53.43	1.19	1.48	20.37	3.16	44.05	1.18	1.40	24.20	3.56
2-3	43.93	0.93	1.22	19.16	2.92	41.01	1.00	1.19	24.28	3.38
3-4	48.89	1.01	1.33	21.3	3.06	42.14	0.98	1.20	24.40	3.13
4-6	40.50	0.80	1.12	20.68	3.03	40.90	0.85	1.12	25.07	3.22
6-10	50.65	0.99	1.33	20.93	3.00	40.68	0.83	1.13	26.10	3.42
	Reactive 2007					**Reactive 2008**				
	Fe	Mn	P	Al	Ca	Fe	Mn	P	Al	Ca
0-1	6.79	0.672	0.592	0.61	0.81	9.34	0.86	0.69	0.76	1.58
1-2	3.38	0.215	0.360	0.54	0.20	8.86	0.59	0.68	0.75	1.29
2-3	2.89	0.170	0.321	0.54	0.29	6.90	0.44	0.56	0.68	1.09
3-4	3.08	0.161	0.340	0.55	0.27	6.05	0.37	0.51	0.68	1.08
4-6	2.92	0.156	0.326	0.53	0.16	5.42	0.28	0.47	0.64	1.04
6-10	3.20	0.169	0.356	0.53	0.29	5.60	0.29	0.46	0.64	1.06

APPENDIX H: Descriptions of Electronic Database for Data Relevant to this Thesis

The Following Data are Included in the Accompanying CD:

Redox Data

This folder contains raw electrochemical data (voltammetric scans) that were collected in the field during summers of 2007 and 2008 (both in raw and analysis format). The files are in a format that must be read using AIS Systems DLK-60 Analysis Program. Spreadsheets of reduced data, including current peak measurements and calibrations, are also included in this folder, separated by SWI or Core. Data for 2007 and 2008 were compiled into the same workbook but separated by month.

Sediment Extraction Data

Spreadsheets for sediment extractions are included in this file, Aqua Regia and ascorbic acid extraction data for 2007 and 2008 are combined into one work book but are separated by spreadsheet. A key for the naming system of the cores are also included in this spreadsheet. CN data for 2007 and 2008 are combined into a workbook separated by spreadsheet.

Porewater Data

Ion concentrations for sediment porewater for 2007 and 2008 are included in this file as separate spreadsheets.

^{31}P NMR Data

An excel spreadsheet containing the percentage of specific P compounds as deteremined by ^{31}P-NMR spectra are included in this folder. The total P, Fe, Mn, Al, and Ca concentrations as determined by ICP-OES for the sediment samples used for ^{31}PNMR analysis are included in the spreadsheet "EDTA TP values" and the corresponding concentration of each P species is included in the OP concentration" worksheet.

^{31}P NMR spectra images can be found in the folder entitled 31PNMR spectra.

Light Intensity and Temperature Data

2007 and 2008 LUX and Temperature data obtained from HOBO data loggers are included as a workbook entitled "2007&2008_HOBO_compiled_seasonal_data."