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Internal Phosphorus Loading in Spring Lake: Year 2

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Executive Summary

Experimental and desktop studies were conducted during 2004 to better understand the factors affecting internal phosphorus (P) loading in Spring Lake, Michigan. A prior study in 2003 resulted in estimates that between 55 and 65% of the total phosphorus entering the water column of Spring Lake, on an annual basis, originated via internal loading from the sediments. The results from the current study indicated the following:

- Phosphorus release rates were no different at alum concentrations of ≥ 15 mg alum/L compared to 24 mg/L. However, the higher alum concentration is likely to provide greater spatial coverage over the sediments in the lake, more protection from alum redistribution after sediment resuspension, and is still well below concentrations of environmental concern.
- Resuspension events, which would cause the sediments to become mixed, substantially increase total phosphorus concentrations in the water column, even at high alum concentrations, although the total soluble phosphorus concentrations remain low in the water column provided alum is present.
- Bioturbation has the potential to increase phosphorus release into the water column. However, several lines of evidence in our data suggest that this process does not play a significant role in Spring Lake sediments.
- Desktop analyses focusing on lake morphometry revealed that Spring Lake is very susceptible to internal loading but not very susceptible to resuspension events. In addition, calculations indicated that given the current concentration of P in the sediment, internal P loading can continue in Spring Lake for another 40 years, even if all new sources of phosphorus are eliminated.

Taken together, these results indicate that an alum application of 25 mg aluminum/L is likely to result in a substantial reduction in the phosphorus concentration in the water column of Spring Lake. The length of treatment effectiveness will depend on the degree to which 1) the alum remains in place on the sediment-water interface and 2) current and future external phosphorus loading to Spring Lake is reduced.

1.0 Introduction

1.1 Background

Internal loading of phosphorus refers to the release of phosphorus from lake sediments. This phosphorus pool accumulates in the sediments when external loading of phosphorus (from the watershed and atmosphere) is high. Internal loading is a frequent phenomenon in shallow, eutrophic lakes throughout the world, and may prevent lake water quality from recovering even after external loads are reduced (Sas 1989, Granéli 1999). Phosphorus release can occur via two different mechanisms (Selig 2003):

- release at the sediment-water interface during periods of anoxia or hypoxia (undetectable or very low levels of dissolved oxygen), and the subsequent diffusion of soluble phosphorus into the water column; and
- resuspension of bottom sediment, whereby phosphate in the pore water can be released into the water column or phosphorus adsorbed to sediment particles can desorb into the water column

In highly eutrophic lakes, internal loading can account for a substantial amount of the total phosphorus (TP) load entering the water column. Indeed, many studies have shown that reductions in external loading, to levels where water quality improvement should be detected, do not have the desired effect because of the counteracting release of phosphorus from sediments (Bjork 1985, Granéli 1999, Steinman et al. 1999).

Spring Lake has some of the highest phosphorus concentrations in west Michigan. For example, the USEPA has set a TP water quality goal of 15 ppb for the west Michigan ecoregion (USEPA 2000). However, during ice-free periods from 1999 through 2002, TP concentrations in Spring Lake averaged 100 ppb, and ranged from 6 to 631 ppb

(Progressive AE 2002), far in excess of USEPA standards. Given the high phosphorus concentrations in the lake, and especially the high concentrations measured near the sediment layer during summer months, it was believed that internal loading may be an important process in Spring Lake. As a consequence, research was conducted in 2003 by AWRI to evaluate: 1) the importance of internal loading; and 2) the effectiveness of alum in reducing internal loading in Spring Lake, Michigan. The results from our investigations indicated that internal loading accounted for between 55 and 65% of the total phosphorus entering the lake water column on an annual basis and that an alum application of 24 mg aluminum/L was extremely effective at reducing TP release from the sediments (Steinman et al. 2003, Steinman et al. 2004).

Although the results from Steinman et al. (2003) provided strong evidence that internal loading was important and that alum has the potential to effectively mitigate the process, a number of uncertainties emerged from the research (see Section 1.2). The goals of the current investigation were to address these uncertainties to the greatest extent practicable.

1.2 Project Objectives and Task Elements

The objective of this project was to address the uncertainties that emerged from the Year 1 (2003) Spring Lake study conducted by the Annis Water Resources Institute. This involved four distinct task elements:

- Determine the appropriate concentration of alum to be applied in Spring Lake (Experiment 1)
- Assess the influence of sediment resuspension on phosphorus release (Experiment 2)
- Assess the influence of bioturbation on phosphorus release (Experiment 3)
- Conduct a desktop study to examine others factors influencing internal phosphorus loading and the short-term and long-term effectiveness of alum in Spring Lake

2.0 Methods

2.1 Experiment 1: alum concentration

Field methods: Sediment cores were collected from all sites on April 20, 2004. Cores were collected from the same four sites in Spring Lake that were sampled during 2003 (Fig. 2.1.1). The sites were selected to provide spatial coverage of different geographic regions in the lake, and also to be as close as possible to sampling locations from other investigations (wherever practical) in order to take advantage of historical information.

Figure 2.1.1. Sampling sites for collection of cores for internal loading measurements.

Site 1 is at the southern end of Spring Lake just downstream of where Smith Bayou enters the mainstem of Spring Lake (Fig. 2.1.1). Site 2 is upstream of the confluence of Petty's Bayou and the mainstem of the lake. Site 3 is in Dunlevy Bay. Site 4 is at the northern end of Spring Lake. Sites 1, 2, and 4 corresponded to Stations 2, 3, and 7, respectively, in Progressive AE's 2002 report.

At each site, vertical profiles of dissolved oxygen, pH, temperature, specific conductance, chlorophyll *a*, and total dissolved solids were measured using a Hydrolab DataSonde 4a equipped with a Turner Designs fluorometer (Table 2.1.1). A secchi disk was used to measure water clarity and a Li-Cor quantum sensor and data logger were used to measure incident and underwater irradiance. Water samples for nutrient analysis were collected with a van Dorn bottle and maintained on ice until delivery to the laboratory. Nutrient analyses were performed on a BRAN+LUEBBE Autoanalyzer following standard methods (USEPA 1983).

Table 2.1.1. Methods used for water quality analyses in Spring Lake.

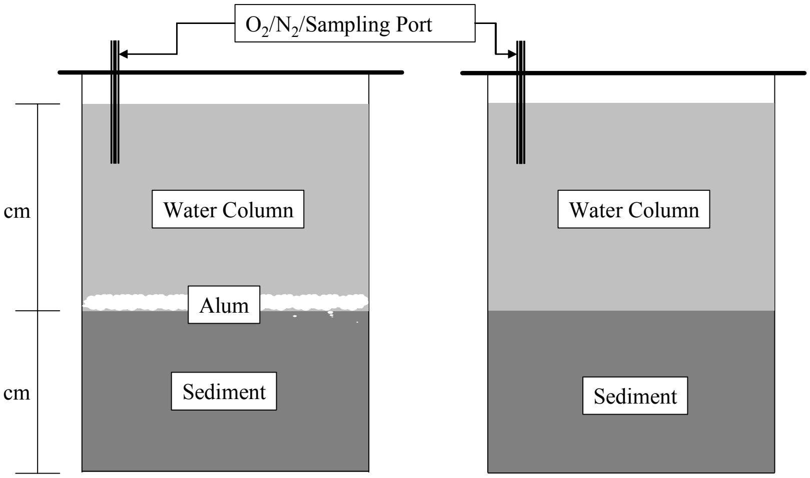
Parameter	Preparation	Preservation	Holding Time (d)	Reference* or method
DO, pH, temp, specific conductance, chl *a*, TDS, ORP	None	None	none	Hydrolab DataSonde 4a equipped with a Turner Designs fluorometer
Light transmittance	None	None	none	Li-Cor quantum sensor and Secchi disk
SRP/TSP	0.45 µm filter in field	Freeze –10°C	28	365.4*
TP	--	H_2SO_4 Cool to 4°C	28	365.4*

* USEPA (1983)

Sediment samples from Spring Lake were collected using a piston corer (Fisher et al. 1992, Steinman et al. 2004). Six cores were collected from each site. The coring device was constructed with a graduated 0.6 m long polycarbonate core tube (7 cm inner diameter), aluminum drive rods, and a PVC attachment assembly for coupling. The piston was advanced 20-25 cm prior to deployment to maintain a water layer on top of the core during collection. The corer was vertically positioned at the sediment/water interface and pushed downward with the piston cable remaining stationary. After collection, the core was brought to the surface and the bottom sealed with a rubber stopper prior to removal from the water, resulting in intact sediment cores that were approximately 20 cm in length, with a 25-cm overlying water column. The piston was then bolted to the top of the core tube to keep it stationary during transit. Core tubes were placed in a vertical rack and maintained at ambient temperature until being transported back to the laboratory (see Steinman et al. 2003 for illustration).

Laboratory methods: The 24 cores (six per site) were placed into a Revco environmental growth chamber, with the temperature maintained to match ambient conditions in Spring Lake at the time of collection. Cores from each site were exposed to one of six alum concentrations: 0, 5, 10, 15, 20, or 25 mg aluminum/L. This required the introduction of approximately 0, 114, 227, 341, 454, and 568 mg of alum (as aluminum sulfate solution), respectively, into the water columns of each treatment, which formed a floc at the water-sediment surface (Fig. 2.2.1). Time of alum introduction was considered time zero. Alum was obtained from General Chemical Corporation (River Rouge, MI). The water column of each core tube was bubbled with nitrogen (with 330 ppm CO_2) to create anaerobic conditions.

Figure 2.2.1. Schematic of experimental set-up. Core tubes consisted of a 25-cm overlying water layer and a 20-cm sediment core. Cores received alum treatments ranging from 0 (right tube) to 25 mg Al/L.



Internal load estimates were made using the methodology outlined in Moore et al. (1998), with minor modifications. Briefly, a 40 mL water sample was removed by syringe through the sampling port of each tube core at 2 h, 8 h, 1 d, 2 d, 4 d, 8d, 12 d, 16 d, 20 d, and 24 d after time zero, and replaced with an equal volume of filtered lake water. A 20 mL subsample was immediately refrigerated for analysis of TP, and a 20 mL subsample was filtered immediately through a 0.45 μm membrane filter and frozen for total soluble phosphorus (TSP) analysis. TP was analyzed using the methods described previously (Table 2.1.1); TSP was analyzed according to EPA methods (USEPA 1983).

Flux calculations were based on the increase in water column TP and TSP using the following equation:

$$P_{\mathrm{flux}} = (C_t - C_o) * V/A, \qquad (1)$$

where, P_{flux} is the net P flux or retention per unit surface area of sediments, C_t is the P concentration in the water column at time t, C_0 is the P concentration in the water column at time 0, V is the volume of water in the water column, and A is the surface area of the sediments. In addition to flux, net change in water column TP and TSP was calculated using the following equation:

$$P_{\text{net}} = C_t - C_o \qquad (2)$$

Statistical analysis: Differences in both total phosphorus and total soluble phosphorus concentrations were analyzed by repeated measures analysis of variance (RP-ANOVA). The overall model results were reported when there was no significant treatment X time interaction. If the interaction term was statistically significant, the ANOVA results were reported for each sampling time. Mean differences among dates were compared using Tukey's multiple comparison test.

2.2 Experiment 2: resuspension

Laboratory methods: At the end of experiment 1 (day 24), the cores were vigorously agitated by increasing the flow rate of gas. Flow rates were increased to the point where resuspension of sediments was visualized in all cores. Water samples were collected at 12, 24, and 48 h during resuspension; both TP and TSP were sampled, processed, and analyzed using the methods described above (Table 2.1.1).

Statistical analysis: Differences in both total phosphorus and total soluble phosphorus concentrations were analyzed by repeated measures analysis of variance (RP-ANOVA). The overall model results are reported when there was no significant treatment X time interaction (TP). If the interaction term is statistically significant, the ANOVA results are reported for individual times. Mean differences among dates were compared using Tukey’s multiple comparison test. Data transformation was not required.

2.3 Experiment 3: bioturbation

Field methods: Sediment cores were collected on May 22, 2004. The sampling sites and field methods followed those described for Experiment 1, with the following additions. An initial benthic invertebrate survey was conducted concurrent with the sediment cores. At each of the four Spring Lake coring sites, three replicate benthic samples were collected using a petite Ponar dredge. Upon collection, the benthic samples were washed through a 500-µm sieve under gentle pressure. Since living blood midge larvae are easily distinguished by their red color, which fades with preservation, samples were examined immediately for larvae of this subfamily and their presence was recorded prior to preservation. The community was saved in its entirety and preserved in 10% formalin. Rose Bengal stain was added to the formalin to aid in sorting invertebrates from organic debris, and samples were stored until identification in the laboratory (below).

Laboratory methods: Invertebrate analysis. In addition to the field survey, invertebrates were identified and counted at the end of the bioturbation experiment; each sediment core was analyzed for living and dead invertebrates. The top 10 cm of sediment were extracted from each core and washed gently through a 500-µm sieve.

Invertebrates from the both the ponars and cores were placed in a shallow white pan for sorting. All organisms found in the ponar dredges and extruded cores were identified to the lowest practicable level using a stereo microscope. Invertebrates that were not identified to lower taxonomic levels included: 1) worms of the class

Oligochaeta and phylum Nematomorpha, and 2) members of the family Chironomidae. All other organisms were identified to genus using conventional taxonomic keys.

Laboratory methods: Poisoning of cores. The six cores from each of the four sites were exposed to three alum levels (0, 5, and 10 mg/L) and 2 levels of poison (control vs poison), resulting in a total of 24 cores. Alum was applied as described previously (Experiment 1), concurrent with formaldehyde. Concentrated (40%) formaldehyde was applied to create a final concentration of 5% formaldehyde in the poison treatments. The initial intent was to measure TSP in the water column of all core tubes over time (0, 2 hr, 12 hr, 1 d, 2 d, 4 d, 8 d, 12 d, 16 d, 20 d, 24 d). However, the formaldehyde reacted with the reagents during the analysis on the autoanalyzer, reducing our confidence in the validity of the TSP results in the poison treatments. Instead, SRP was measured in the poison treatments. Unfortunately, there was insufficient volume of sample left to measure SRP in the control treatments as well, leaving us with TSP results for the control treatments and SRP results for the poison treatments. Nutrient samples were collected and analyzed as described above.

Statistical analysis. Invertebrate analysis. Data were analyzed with a one-way analysis of variance (ANOVA) to determine if there were statistically significant differences in site location for total densities and the major taxonomic units identified. All data were log-transformed prior to analysis to meet assumptions for the analysis.

Statistical analysis. Poisoning of cores. Differences in both TSP (for control treatments) and SRP (for poison treatments) concentrations were analyzed by repeated measures analysis of variance (RP-ANOVA). The sites served as replicates. The overall model results were reported when there was no significant treatment X time interaction. If the interaction term was statistically significant, the ANOVA results were reported for individual times. Mean differences among dates were compared using Tukey's multiple comparison test. Data transformation was not required.

2.4 Desktop analyses

Three calculations were conducted as part of this task: 1) Osgood Index; 2) Dynamic Ratio; and 3) phosphorus storage and release over time. Lake morphometry has an important influence on internal loading; phosphorus released from the sediments is more available to the algae in the photic zone in shallow lakes than in deep lakes. Hence, high rates of internal loading in stratified lakes may not always be important in managing epilimnetic water quality (Welch and Cooke 1995).

Osgood (1988) proposed an estimate of the probability that deep mixing events will occur in a lake during summer stratification, bringing P from bottom waters to the epilimnion (Cooke et al. 1993)---the ratio of mean depth to the square root of surface area:

$$\text{Osgood Index} = \overline{Z} / \sqrt{A_o} \qquad (3)$$

where: $\overline{Z}$ = mean depth (m)

$\sqrt{A_o}$ = square root of surface area (km^2)

The dynamic ratio (DR; Håkanson 1982) estimates the degree to which lake bottom areas are dominated by resuspension or sedimentation forces, and is essentially the inverse of the Osgood Index.

$$\text{Dynamic Ratio} = \sqrt{A} / \overline{D} \qquad (4)$$

where: $\sqrt{A}$ = square root of lake surface area

$\overline{D}$ = mean lake depth

A simple model was developed to estimate how long internal loading would remain a significant problem in Spring Lake. This estimated time (yr) is calculated based on the total mass of phosphorus in the top 10 cm of sediments (the active fraction; mg/m^2) divided by maximum average diffusive flux from the sediments during the portion of the year where the sediments are anaerobic.

$$T = P_{sed} / (\overline{P}_{flux} \times T_{anaer}) \qquad (5)$$

where: T = time (yr)

P_{sed} = phosphorus mass in sediments

$\overline{P}_{flux}$ = maximum average phosphorus flux from sediments (mg P/m^2/d)

T_{anaer} = portion of year where sediments are anaerobic (%)

3.0 Results

3.1 Experiment 1: alum concentration

Field Results: All sites showed very similar water quality patterns during the April cruise (Figs. 3.1.1, 3.1.2). Temperature and dissolved oxygen concentration were constant through the water column, with temperature about 12ºC and DO approximately 10 mg/L. It is unlikely that internal phosphorus loading was much of a factor at this time, given the aerobic conditions throughout the water column. Chlorophyll *a* concentrations generally increased with depth at all sites; this may have been due to photoinhibition near the water surface. Secchi disk (SD) values were 1.0 m at all sites except site 3, which was slightly less turbid (SD = 1.25 m). See Appendix (7.1) for additional field data.

Figure 3.1.1. Dissolved oxygen, temperature, and chlorophyll *a* values from Sites 1 and 2 in Spring Lake, sampled on April 20, 2004.

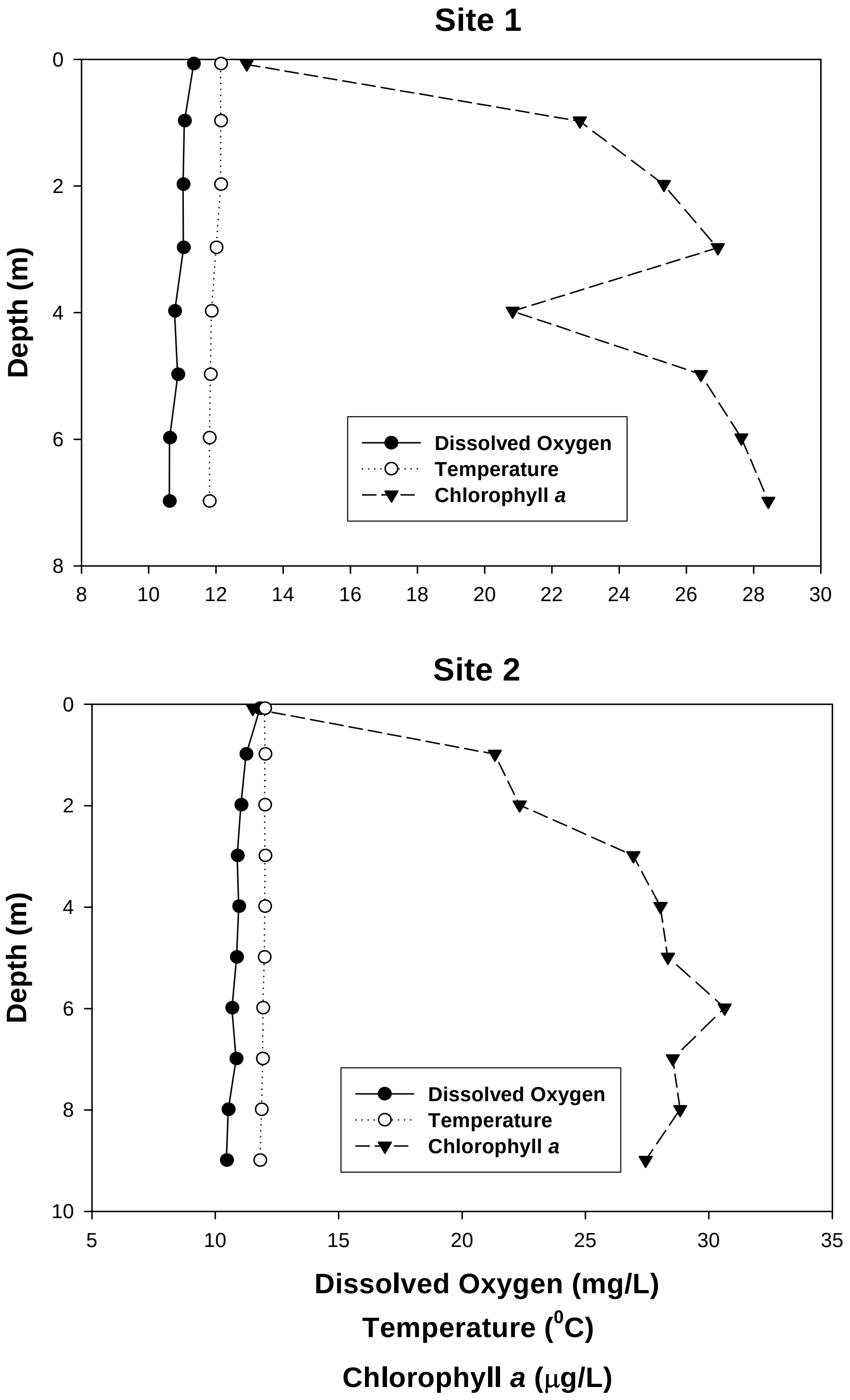
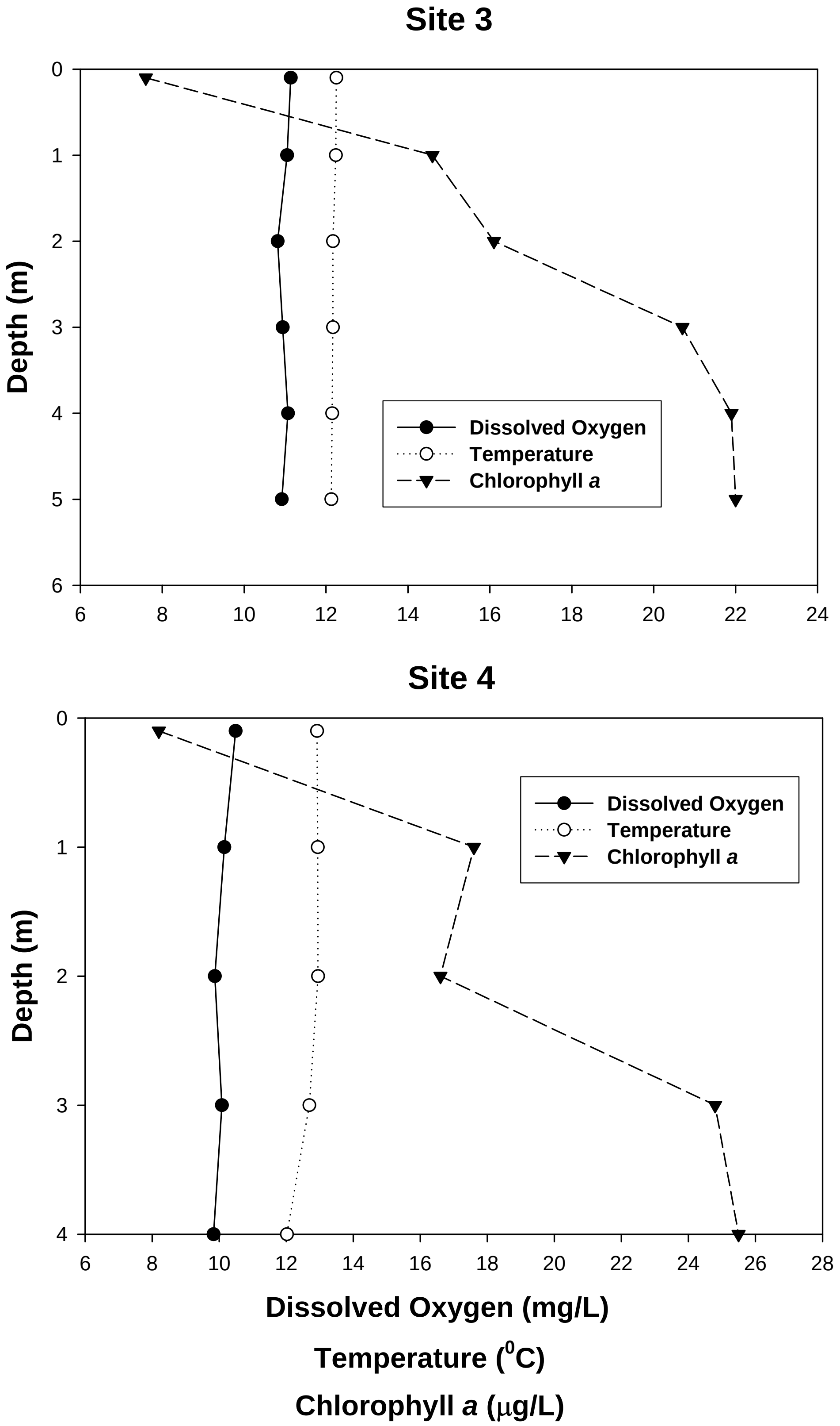


Figure 3.1.2. Dissolved oxygen, temperature, and chlorophyll *a* values from Sites 3 and 4 in Spring Lake, sampled on April 20, 2004.



Laboratory results-total phosphorus: Core water column total phosphorus concentrations showed a generally similar pattern at all sites, with the highest concentrations in the 0 alum treatment and much lower concentrations in all treatments with alum (Table 3.1.1; Fig. 3.2.1). Repeated measures ANOVA revealed a significant treatment X time interaction ($P < 0.001$), so separate ANOVAs were run on the individual sampling times. At all times except 2hr (when there was no difference between the 0 and 5 mg/L treatments), mean TP in the 0 alum treatment was statistically greater than mean TP in all other alum treatments (Table 3.1.1). After day 2, mean TP concentrations were not significantly different among any of the alum treatments ≥ 5 mg/L (Table 3.1.1).

Table 3.1.1. Mean total phosphorus concentrations (µg/L) exposed to different alum treatments (0-25 mg/L) sampled on days 1-24 (see text for more detail). TP means with different letters within a row indicate statistically significant differences (Tukey's multiple comparison test; p < 0.05).

Time (d)	Alum concentration (mg/L)					
	0	5	10	15	20	25
1	31.5^{A}	15.5^{B}	$13.5^{B,C}$	$13.0^{B,C}$	10.0^{C}	10.0^{C}
2	33.5^{A}	18.5^{B}	$15.0^{B,C}$	$12.5^{C,D}$	$12.5^{C,D}$	10.0^{D}
4	30.5^{A}	10.0^{B}	10.0^{B}	10.0^{B}	10.0^{B}	10.0^{B}
8	50.0^{A}	12.5^{B}	10.0^{B}	10.0^{B}	10.0^{B}	10.0^{B}
16	166.0^{A}	25.5^{B}	13.5^{B}	10.0^{B}	10.0^{B}	10.0^{B}
24	132.0^{A}	28.0^{B}	13.0^{B}	10.0^{B}	10.0^{B}	10.0^{B}

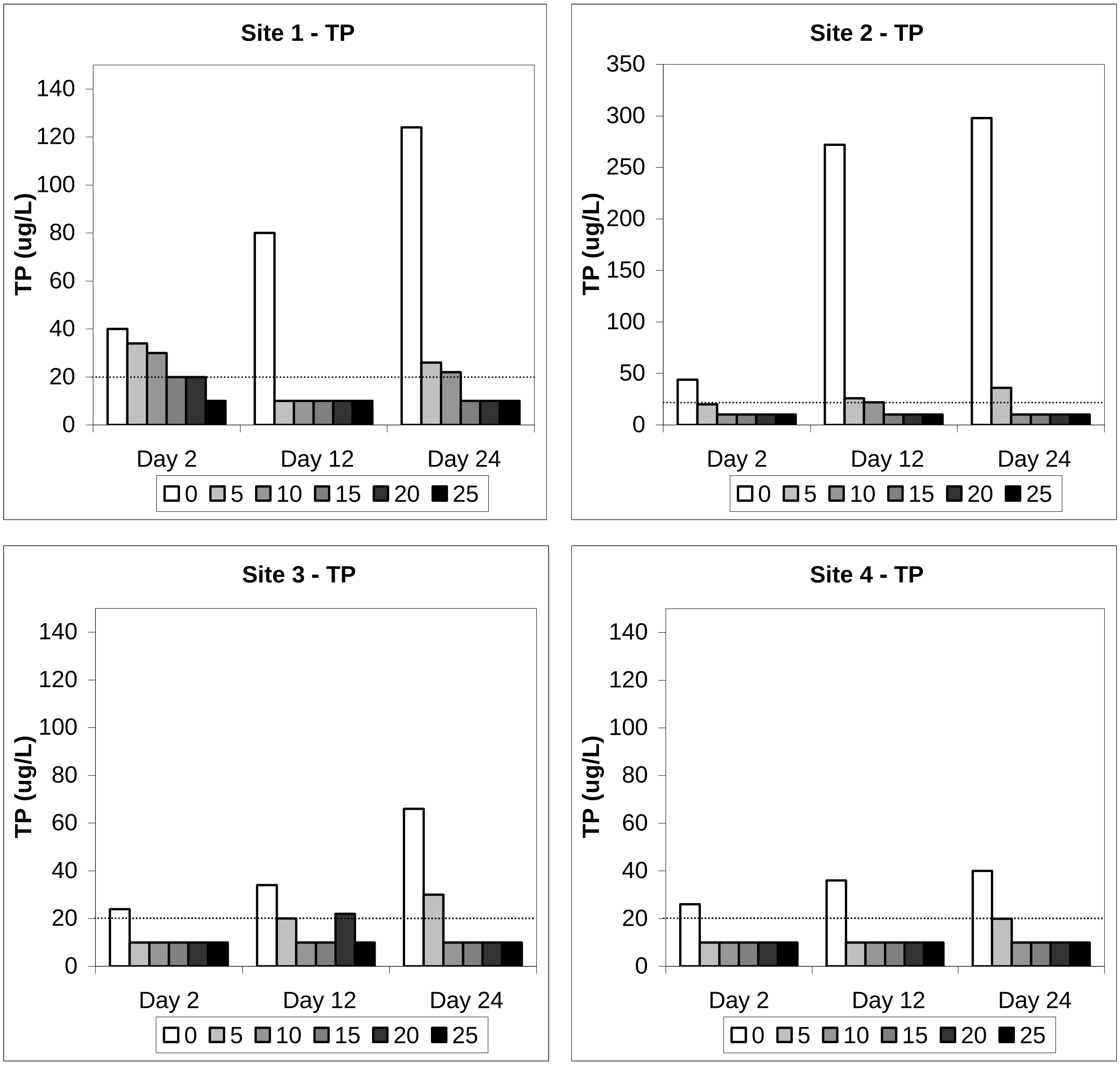
Even the 5 mg/L alum treatment was effective at reducing TP diffusion into the water column (Table 3.1.1). On days 1 and 2, mean TP concentrations declined with increasing alum. After day 4, there were no statistically significant differences in TP among the ≥ 5 mg/L alum treatments, although mean TP concentrations did increase on days 16 and 24 in the 5 mg/L alum treatment (Table 3.1.1).

TP concentrations varied with site (Fig. 3.1.3). Although TP concentrations in the 0 alum treatments tended to increase over time at all sites, higher concentrations were measured at sites 1 and 2 (the deeper sites) than sites 3 and 4. This is consistent with the observations from the 2003 experiments (Steinman et al. 2003). With the exception of site 2, the TP concentrations in 2004 were much lower than those measured in 2003 (Table 3.1.2). This is probably because this year's cores were collected in April, when lower water temperatures and higher DO levels constrained mineralization and TP diffusion (cf. Jensen and Andersen 1992, James and Barko 2004).

Table 3.1.2. Maximum TP concentrations in water column overlying sediment cores (2003 vs. 2004 data).

Site	2003 TP data (µg/L)	2004 TP data (µg/L)
1	780 (day 20)	194 (day 16)
2	170 (day 12)	374 (day 16)
3	330 (day 8)	66 (day 20)
4	390 (day 20)	48 (day 20)

Figure 3.1.3. Total phosphorus concentrations (µg/L; y-axis) measured in water columns at select times (days 2, 12, and 24). Sediments cores were exposed to alum concentrations of 0, 5, 10, 15, 20, or 25 mg/L. Dotted line shows detection limit of 20 µg/L; all measurements below detection are shown as ½ detection limit.

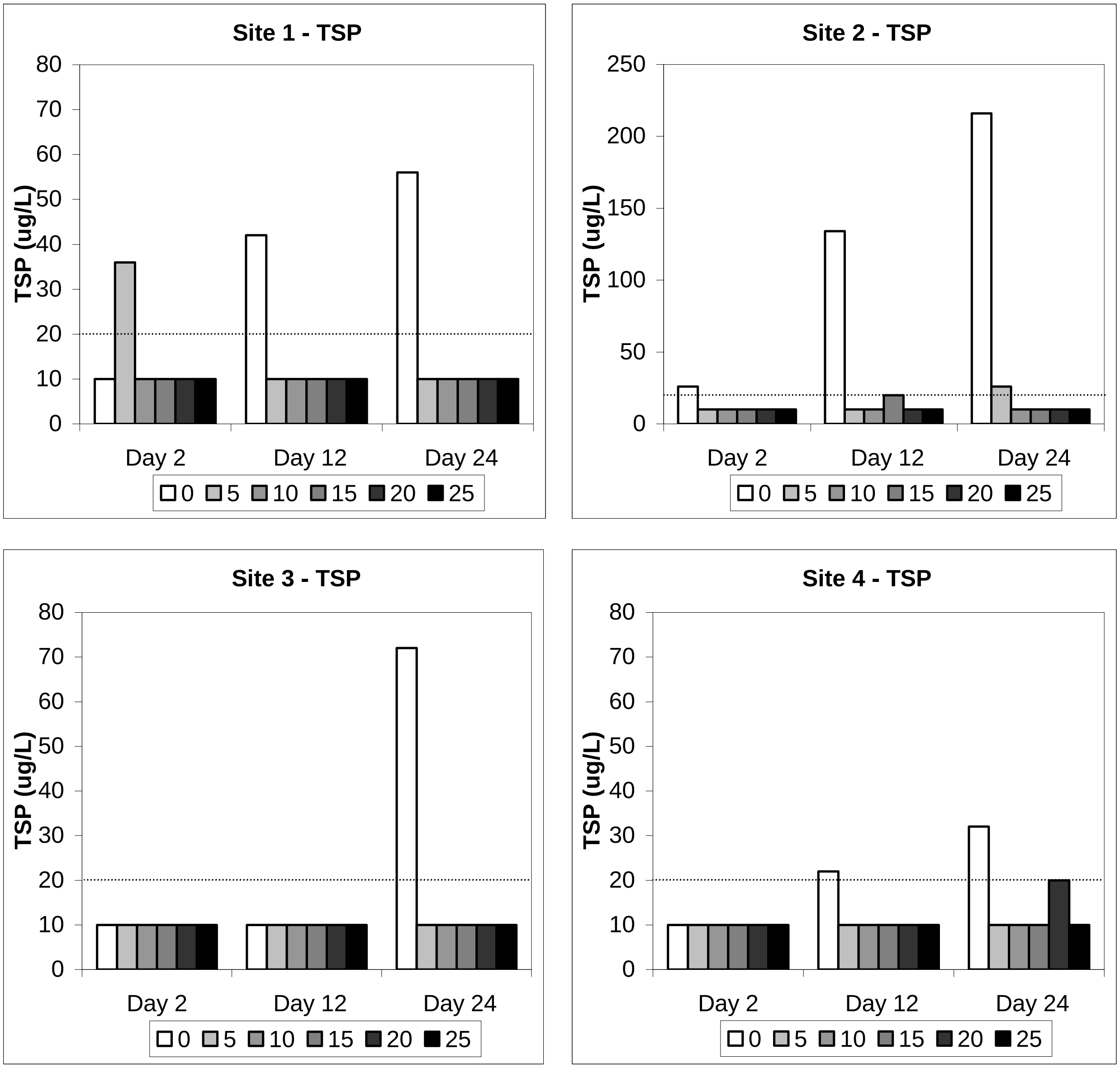


Laboratory results—total soluble phosphorus. Water column TSP concentrations were rarely above detection (20 µg/L) in treatments with alum, regardless of the concentration applied. Even in the 0 alum treatment, detectable concentrations of TSP were not measured until day 12 at all sites except Site 2, where detectable TSP was measured on day 1 (Fig. 3.1.4). Repeated measures ANOVA revealed a significant treatment X time interaction ($P < 0.05$); there were no statistically significant differences in TSP up through 16 d. On days 20 and 24 however, TSP in the 0 alum treatment was significantly greater than all other treatments (Table 3.1.3).

Table 3.1.3. Mean total soluble phosphorus concentrations (µg/L) exposed to different alum treatments (0-25 mg/L) sampled on days 1-24 (see text for more detail). TSP means with different letters within a row indicate statistically significant differences (Tukey's multiple comparison test; p < 0.05).

Time (d)	**Alum concentration (mg/L)**					
	0	**5**	**10**	**15**	**20**	**25**
1	15.5^{A}	10.0^{A}	10.0^{A}	10.0^{A}	10.0^{A}	10.0^{A}
2	16.5^{A}	14.0^{A}	10.0^{A}	10.0^{A}	10.0^{A}	10.0^{A}
4	19.5^{A}	10.0^{A}	10.0^{A}	10.0^{A}	10.0^{A}	10.0^{A}
8	27.0^{A}	10.0^{A}	10.0^{A}	10.0^{A}	10.0^{A}	10.0^{A}
16	56.5^{A}	13.0^{A}	10.0^{A}	10.0^{A}	10.0^{A}	10.0^{A}
20	81.5^{A}	20.0^{B}	10.0^{B}	10.0^{B}	12.5^{B}	10.0^{B}
24	94.0^{A}	14.0^{B}	10.0^{B}	10.0^{B}	12.5^{B}	10.0^{B}

Figure 3.1.4. Total soluble phosphorus concentrations (µg/L; y-axis) measured in water columns at select times (days 2, 12, and 24). Sediments cores were exposed to alum concentrations of 0, 5, 10, 15, 20, or 25 mg/L. Dotted line shows detection limit of 20 µg/L; all measurements below detection are shown as ½ detection limit.



The maximum concentrations of both TP and TSP released from the sediments to the water column decreased as alum concentrations increased for both TP and TSP (Table 3.1.4). TP concentrations usually declined to below detection after 2-8 hours for all alum treatments that were ≥ 10 mg/L (Appendix 7.2). At 5 mg/L, TP remained at measurable concentrations through day 2 (sites 1 and 2) or 8 hours (sites 3 and 4), became undetectable through approximately day 20, and then was again measurable, albeit at relatively low concentrations (< 40 µg/L) on day 24 (Appendix 7.2). TSP was usually below detection with alum concentrations of ≥ 10 mg/L.

Table 3.1.4. Maximum water column total phosphorus (TP) and total soluble phosphorus (TSP) concentrations, and respective date of measurement, from the sediment cores collected on April 20, 2004 from Spring Lake. BD = below detection (< 20 µg/L).

Site	Alum Treatment (mg/L)	TP concentration (µg/L)	TSP concentration (µg/L)
Site 1	0	194 (day 16)	64 (day 20)
	5	52 (hr 2)	36 (day 2)
	10	46 (hr 2)	BD
	15	26 (hr 2)	BD
	20	28 (hr 2)	BD
	25	26 (hr 2)	BD
Site 2	0	374 (day 16)	216 (day 24)
	5	50 (hr 2)	26 (day 24)
	10	26 (hr 2)	BD
	15	20 (hr 2)	20 (day 12)
	20	20 (hr 2)	BD
	25	BD (hr 2)	BD
Site 3	0	66 (day 20)	72 (day 24)
	5	46 (hr 2)	30 (day 20)

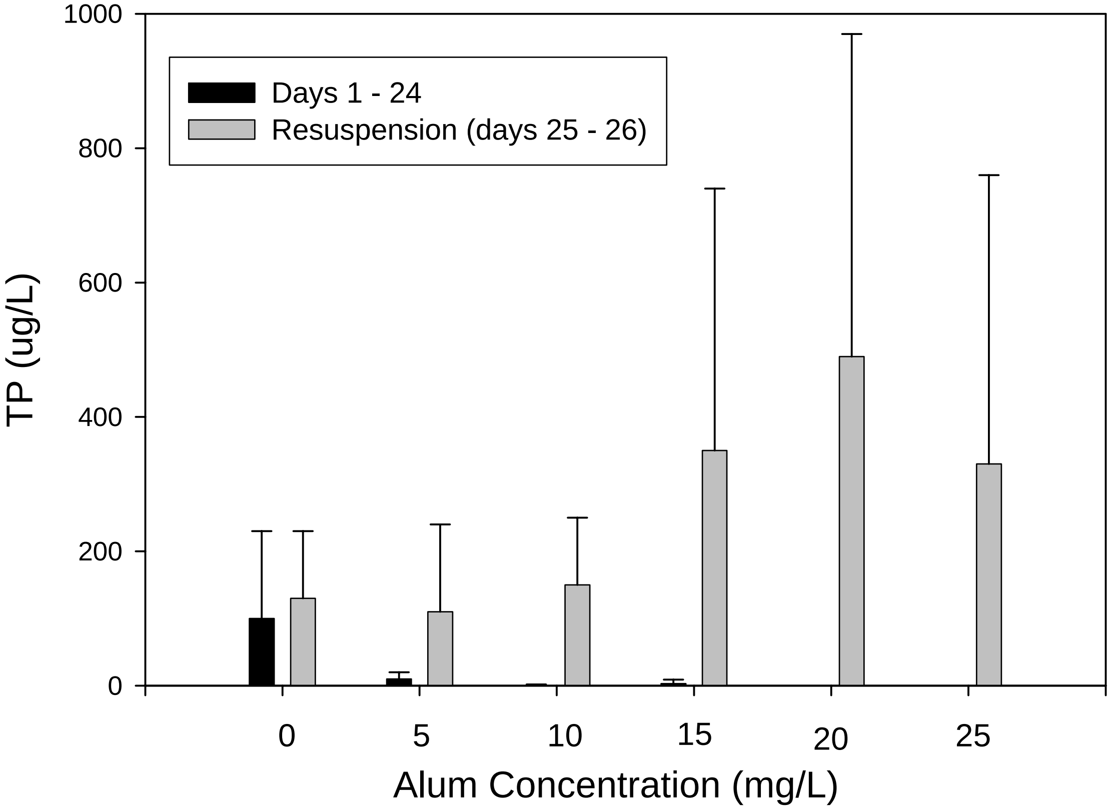
	10	30 (hr 2)	BD
	15	26 (hr 2)	BD
	20	28 (hr 2)	BD
	25	26 (hr 2)	BD
Site 4	0	48 (day 20)	32 (day 20)
	5	48 (hr 2)	BD
	10	60 (hr 2)	BD
	15	20 (hr 8)	BD
	20	26 (hr 8)	20 (day 24)
	25	38 (hr 2)	BD

These data suggest that an alum application of 15 mg/L would be as effective at reducing TP concentrations in the water column as an application of 25 mg/L, and an alum application of 5 mg/L would be as effective at reducing TSP concentrations as one of 25 mg/L. However, these data were generated under static laboratory conditions, where the alum remains in place and presumably has maximum efficiency at phosphorus binding. In a natural lake, where wind-wave action results in sediment resuspension and mixing, it is likely that the ability of alum to bind phosphorus would be greatly reduced.

3.2 Experiment 2: resuspension

Total phosphorus: The mean net TP concentrations (i.e. TP at 48 hrs minus TP at time 0) after 48 hr of resuspension ranged from 129 (0 alum) to 487 µg/L (20 mg/L alum; Fig. 3.2.1). A repeated measures ANOVA showed no significant treatment X time interaction, so ANOVA was performed on combined sampling times. Although mean net TP concentrations clearly increased with alum concentration (Fig. 3.2.1), there was no significant treatment effect. High variances in the 15, 20, and 25 mg/L alum treatments accounted for the lack of statistically significant differences. TP was much greater after the 48 hrs of resuspension than after the 24-day diffusive flux study in all alum treatments (Fig. 3.2.1).

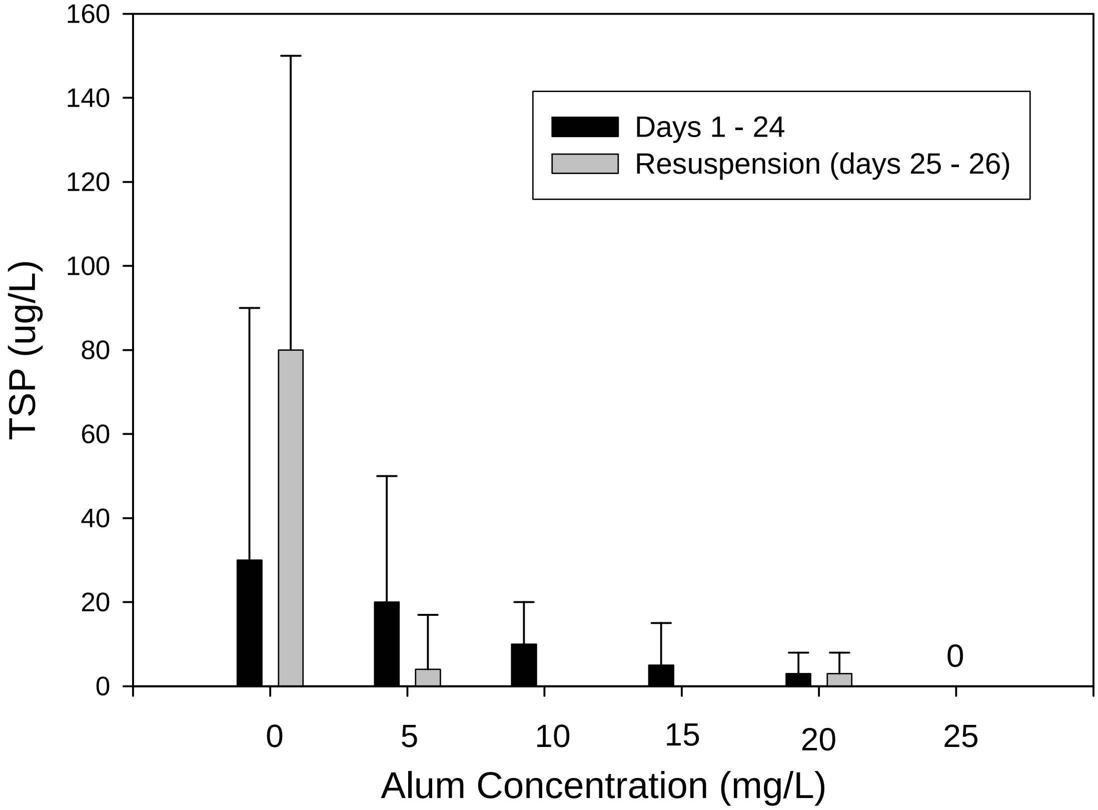
Figure 3.2.1. Mean (±1 SD) total phosphorus concentrations (µg/L) in water column, averaged across all 4 sites, after the 24-day incubation under static conditions (black bars: experiment 1) and after a 48-hr resuspension event (gray bars).



The mean net TSP concentrations after 48 hr of resuspension showed a very different pattern than that of TP (Fig. 3.2.2), with values ranging from 0 (20 and 25 mg/L) to 34 µg/L (no alum; Fig. 3.2.2). A repeated measures ANOVA showed no significant treatment X time interaction, so ANOVA was performed on combined sampling times. This analysis did reveal a significant treatment effect ($P < 0.001$);

Tukey's multiple comparison test revealed that the no alum-treatment had significantly greater net TSP concentrations than all alum treatments, while none of the alum treatments were significantly different from one another.

Figure 3.2.2. Mean (±1 SD) total soluble phosphorus concentrations (μg/L) in water column, averaged across all 4 sites, after the 24-day incubation under static conditions (black bars: experiment 1) and after a 48-hr resuspension event (gray bars).



3.3 Experiment 3: bioturbation

Field results: Sites 1 and 2 showed similar water quality patterns during the May cruise (Fig. 3.3.1) with temperature relatively constant at 18ºC until about 6m, and then declining to about 14.5ºC indicating that stratified conditions were beginning to form. Dissolved oxygen concentrations mimicked temperature with relatively constant levels of approximately 7 mg/L until a depth of 6 m, when it declined to ≤ 2 mg/L. Chlorophyll *a* concentrations were low and varied between 2 and 4 µg/L at both sites (Fig. 3.3.1). The onset of thermal stratification was less evident at sites 3 and 4 (Fig. 3.3.2) than at sites 1 and 2. DO concentrations showed a small but steady decline with depth at sites 3 and 4 but it is likely that the shallower depths at these sites resulted in greater mixing of the water column, which in turn precluded the rapid DO drops observed at sites 1 and 2. Chlorophyll *a* concentrations were again relatively low, ranging between 3 and 6 µg/L at both sites (Fig. 3.3.2). It is possible that measurable internal phosphorus loading was occurring at sites 1 and 2 at this time in late May, given the low DO levels at these sites, although TP concentrations were very similar between surface and bottom collections at those sites (Appendix 7.4). Secchi disk values ranged from a high of 1.75 m at site 1 to a low of 1.0 m at site 4. Additional field data collected from each site are included in the Appendix (7.4).

Figure 3.3.1. Dissolved oxygen, temperature, and chlorophyll *a* values from Sites 1 and 2 in Spring Lake, sampled on May 22, 2004.

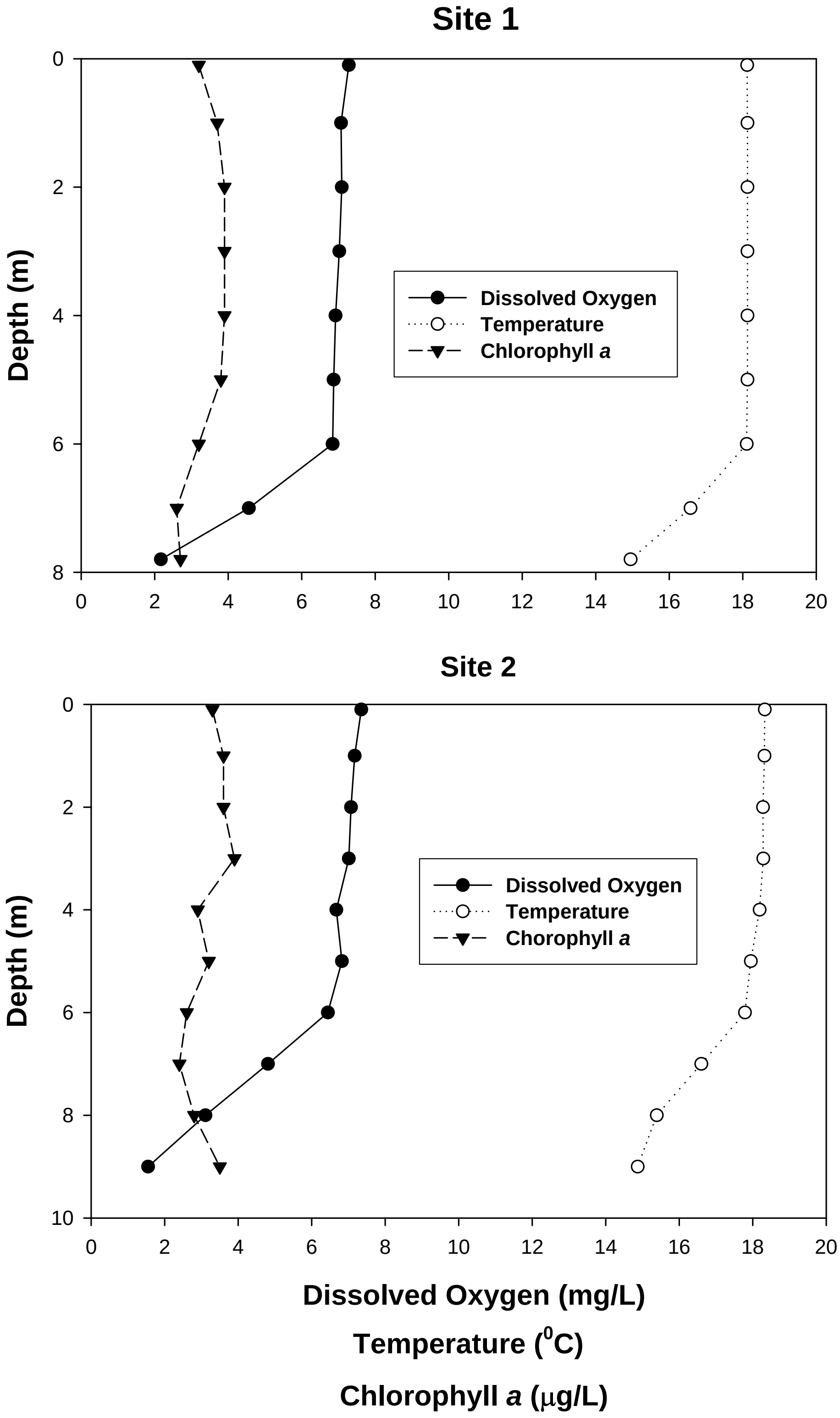
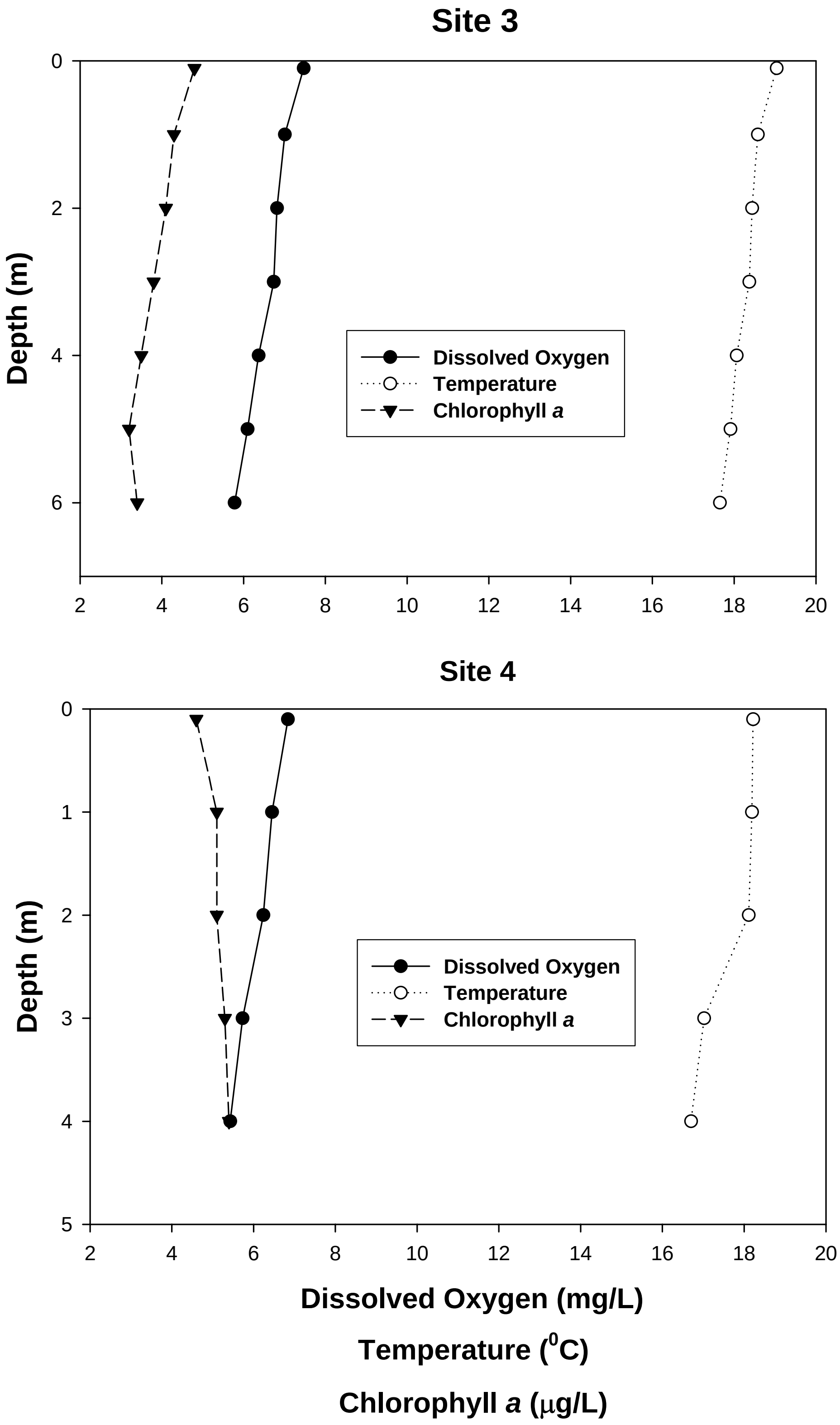


Figure 3.3.2. Dissolved oxygen, temperature, and chlorophyll *a* values from Sites 3 and 4 in Spring Lake, sampled on May 22, 2004.



Benthic invertebrate survey. Mean benthic invertebrate densities were significantly greater at site 4 than at sites 1 and 2 (Table 3.3.1).

Table 3.3.1. Mean (± 1 SD) benthic invertebrate densities (#/m^2) collected by ponar dredge on May 22, 2004 from Spring Lake. N = 3 per site.

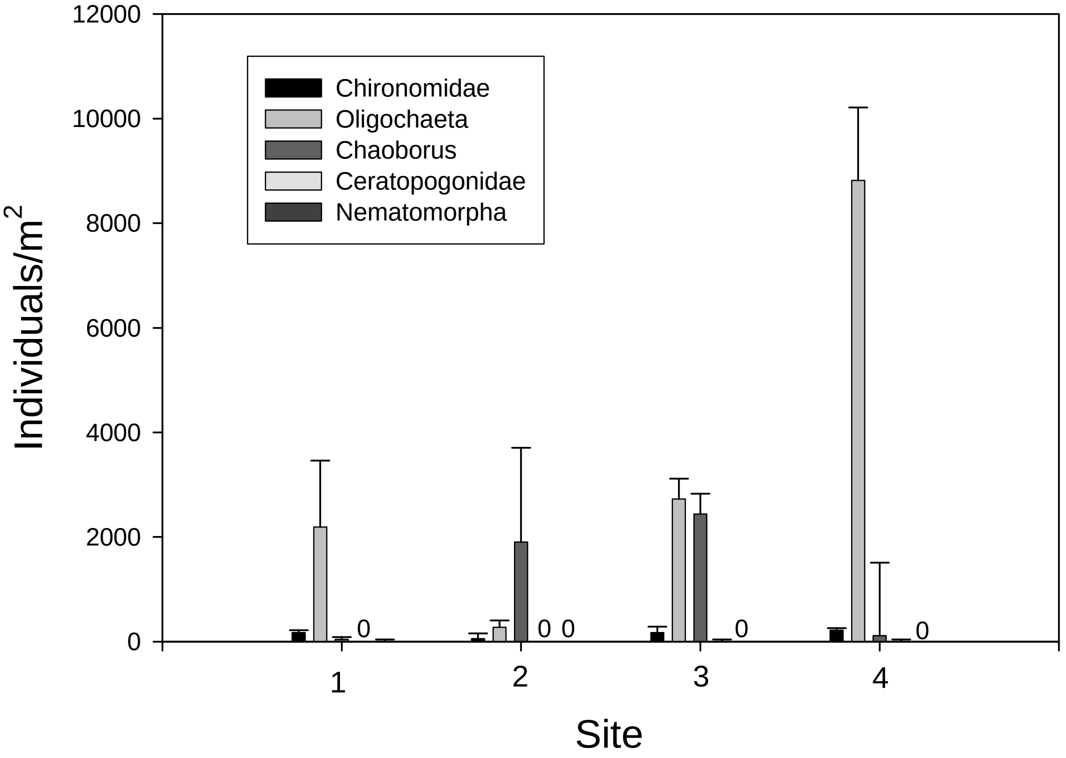
Site	Mean (± 1 SD)	Tukey Grouping
1	2424 (± 1269)	B
2	2236 (± 1932)	B
3	5353 (± 909)	A,B
4	9161 (± 1407)	A

The most abundant taxa found in Spring Lake were chironomids, oligochaetes, and *Chaoborus* (Table 3.3.2). Oligochaetes had the greatest abundance at site 4 and lowest abundance at site 2, whereas *Chaoborus* was most abundant at sites 2 and 3 (Table 3.3.2; Fig. 3.3.3). The oligichaete to chironomid ratio was greatest at site 4, suggesting a

Table 3.3.2. Mean (± 1 SD) densities (#/m^2) of most abundant benthic invertebrate taxa collected by ponar dredge on May 22, 2004 from Spring Lake. N = 3 per site. Taxa with different letters within a column indicate statistically significant differences among sites (Tukey's multiple comparison test; P < 0.05).

Site	Taxa			
	Chironomidae	Oligochaetes	*Chaoborus*	Oliogochaete/ Chironomidae Ratio
1	173.1±43.3^A	2193.0±1266.6^B	43.3±43.3^B	17
2	57.7±99.9^A	274.1±132.2^C	1904.4±1799.9^A	2
3	173.3±115.51^A	2726.8±389.5^B	2438.5±390.3^A	10
4	216.4±43.3^A	8815.2±1396.0^A	105.4±25.0^B	50

Figure 3.3.3. Densities (#/m^2) of all benthic invertebrate taxa collected by ponar dredge on May 22, 2004 from Spring Lake. Data from each replicate ponar are shown.



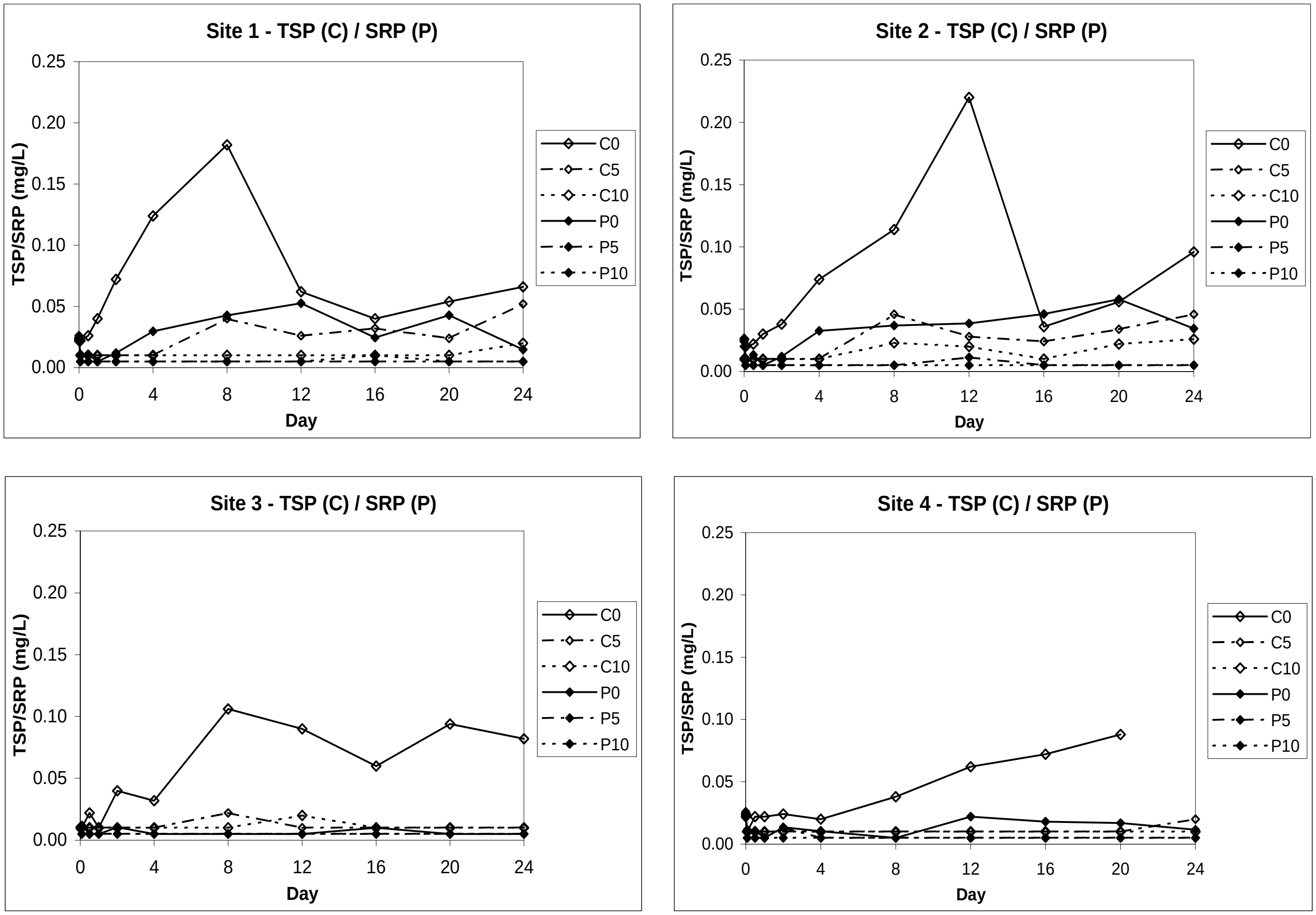
level of enrichment at this site.

Overall, the mean density per unit area of benthic invertebrates in Spring Lake (4794 individuals/m^2) was about 40% and 60% lower than densities measured in White Lake (8068/m^2) and Muskegon Lake (11,922/m^2), respectively (Rediske, unpubl. data).

Poisoning of cores. Our inability to measure TSP in the poisoned treatments, because of interference with reagents, forced us to measure SRP in the poisoned cores, and therefore confounded our attempt to attribute different phosphorus concentrations to specific treatments. The highest phosphorus concentrations at all sites were measured in the control, 0 alum treatments (Fig. 3.3.4). TSP declined after day 8 at site 1 and after day 12 at site 2, whereas no declines were observed in this treatment at sites 3 and 4 (Fig. 3.3.4).

A repeated measures ANOVA revealed that the control, 0 alum treatment resulted in significantly greater P levels (as TSP) by 12 hr and remained significantly greater than all other treatments except the poisoned, 0 alum treatment on day 4. On all sampling times after 12 hr, there were no statistically significant differences among the five remaining treatments. Further interpretation of these results is presented in the discussion.

Figure. 3.3.4. Phosphorus release data from control (open symbols) and poisoned (filled symbols) cores exposed to either 0 (solid line), 5 (long dashes), or 10 (short dashes) mg/L alum. Cores were collected from 4 sites in Spring Lake. TSP was measured in control cores; SRP was measured in poisoned cores.



3.4 Desktop Analyses

Two indices were used to evaluate the influence of lake morphometry on the importance of internal loading and resuspension. The Osgood Index (OI; Osgood 1988), which is the ratio of mean depth to the square root of lake surface area, provides an estimate of the probability that deep mixing events will occur in a lake during summer stratification, bringing P from bottom waters to the epilimnion (Cooke et al. 1993). Osgood classified lakes into two categories: those with an OI > 8 were relatively deep and the importance of internal loading was considered limited, and those with an OI < 6-7 were shallower and the potential importance of internal loading was relatively high. The OI for the entirety of Spring Lake, assuming a mean depth of 6.1 m and a surface area of 5.25 km^2 is 2.66, suggesting that internal loading is an important process in the lake.

The dynamic ratio (DR) estimates the degree to which lake bottom areas are dominated by resuspension or sedimentation forces, and hence can provide useful information on how likely an alum treatment will stay in place over time. Håkanson (1982) proposed 4 classes of lakes based on their DR:

- 0.1 < DR < 1.1 = resuspension limited
- 1.1 < DR < 2.45 = resuspension rather important
- 2.45 < DR < 3.8 = resuspension important
- DR > 3.8 = resuspension dominates

The DR for Spring Lake, again using the same assumptions as were used for the OI, is 0.47. This suggests that naturally occurring sediment resuspension is limited in Spring Lake.

Finally, we estimated the length of time that internal phosphorus loading might remain a significant problem in Spring Lake. For this computation, it was assumed that external phosphorus loading to Spring Lake was zero. This assumption helped simplify the model, although it is recognized that this is not a realistic scenario. Hence, the time estimates represent a "best case" analysis; any level of external loading will increase the length of time that internal loading will last because some fraction of the new P that enters the lake ultimately will reach the sediments (e.g. as settling algal cells) and lead to more internal loading. Based on our analysis of P content in the sediments, we estimated a total mass of 118 tons of P in the upper 10 cm of sediments (the portion of the sediment that actively exchanges phosphorus with the overlying water) throughout the lake. We then used our empirically determined mean TP diffusive flux rates (Steinman et al. 2003) under low, medium, and high release conditions to estimate TP loss from sediments (Table 3.4.1). This rate was weighted against the percentage of time during the year the lake was anaerobic (20%; Steinman et al. 2003) because release was most evident under no or low oxygen conditions. Assuming no new phosphorus enters Spring Lake, there is sufficient TP in the sediments to sustain internal loading anywhere from 15.5 y to 37 y (Table 3.4.1).

Table 3.4.1. Estimated number of years required to deplete TP in upper 10 cm of Spring Lake sediments, assuming zero external phosphorus entering the lake, under low, moderate, and high conditions of internal loading.

Parameter	**Internal Loading Condition**		
	Low	**Moderate**	**High**
Mean TP Release Rate (mg $P/m^2/d$)	7.5	15.6	18.0
Years to deplete TP	37.0	17.0	15.5

4.0 Discussion

The studies conducted in 2003 clearly indicated that internal phosphorus loading is a significant source of P in Spring Lake, potentially accounting for somewhere between 55 to 65% of the total P load to the system (Steinman et al. 2003, 2004). This is consistent with other studies from shallow, eutrophic lakes that have shown internal loading can be a significant source of nutrients and result in serious impairment to water quality (Welch and Cooke 1995, Steinman et al. 1999, Søndergaard et al. 2001). The experiments conducted as part of the current study were an effort to address some of the uncertainties that arose during the 2003 study.

Alum concentration: The alum concentration applied in the 2003 study was patterned after the approach developed by Kennedy and Cooke (1982), which is based on applying as much aluminum to the sediments as environmental safety allows, via additions to the lake's hypolimnion (Cooke et al. 1993). This dose determination is based on lake water alkalinity. Kennedy and Cooke (1982) adopted 50 µg Al/L as an environmentally safe upper limit for a post-treatment dissolved aluminum concentration based on data that showed rainbow trout could tolerate chronic aluminum exposure of up to 52 µg/L dissolved aluminum (Everhart and Freeman 1973). As long as lake water remains within a pH range of 6 to 8, aluminum concentrations should remain less than 50 µg/L. In addition, the maximum formation of $Al(OH)_3$ occurs in this pH range, which should result in maximum deposition and removal of aluminum from the water, as well as maximum formation of the P-retaining floc at the sediment-water interface (Cooke et al. 1993).

The 24 mg Al/L dose that we applied was extremely effective at inactivating P in the Spring Lake sediment cores (Steinman et al. 2003). However, it was unclear if a lower concentration could result in an equal level of P inactivation, and thereby provide an even greater margin of environmental safety to the biota. The results from the sediment cores clearly indicated that doses as low as 15 mg Al/L, and even lower in some cases, could be applied to achieve similar levels of P inactivation as 24 mg/L. However, it is likely that these levels of P inactivation are much higher than what would take place in Spring Lake itself, where wind-wave action, bioturbation, and boat-related disturbances could disturb the alum layer at the sediment surface. Instead of a stationary layer of alum floc, as was observed in the core tubes, it is likely that lake sediments would have variable coverages, with some areas dominated by thick floc layers and other areas having thin or no layers of alum. The more alum applied, the greater the likelihood that lake sediments would have at least some coverage by alum. In addition to providing greater physical coverage of the lake sediments, surplus aluminum has been shown to adsorb mobilized SRP from sediments (Gächter et al. 1988, Gächter and Meyer 1993).

Regarding the fate of alum, Rydin et al. (2000) found that alum-treated lakes formed a stable Al-P complex in sediments, and that P adsorbed by aluminum was transformed from mobile P to permanently bound P. In addition, Welch and Cooke (1999) reviewed alum applications from 21 lakes worldwide, and found that aluminum in the sediment of alum-treated lakes was generally not distinguishable from background concentrations. They attributed this to downward distribution of the floc in the low-density sediment of the lakes. Hence, although an alum concentration of 15 mg/L provides a similar degree of P-inactivation as 24 mg/L, the latter concentration is still well within the environmental margin of safety and provides a greater probability of P inactivation in the lake setting, but will cost more to apply.

Resuspension: Previous studies have shown that P release from sediments is enhanced by physical mixing (e.g. Ahlgren 1980, Andersen 1974, Holdren and Armstrong 1980, Søndergaard et al. 1992, Reddy et al. 1996). Boström et al. (1982) identified several mechanisms that could increase P concentrations due to wind-induced turbulence: 1) diffusive flux will increase because dissolved P leaving the sediments will rapidly disperse, maintaining a steep concentration gradient; 2) rapid dispersion of dissolved P from the sediments should accelerate equilibrium-regulated processes; and 3) resuspension of particles may occur, increasing TP in the water column. In addition to TP, SRP concentration in the water column also has been shown to increase after experimental resuspension events; Søndergaard et al. (1992) measured an SRP increase of 20-80 µg/L. The increase in water column SRP concentration typically occurs due to liberation of dissolved phosphorus in the sediment porewater or its desorption from suspended particles, assuming the equilibrium concentration is driving the adsorbed P off the particle and into the water column (see below).

The data from the current study indicated that resuspension of the Spring Lake sediments can introduce a substantial amount of TP into the water column. The trend of increasing TP with increasing alum application, albeit not statistically significant, is provocative. It may be that the greater amount of alum results in greater phosphorus binding, which is then captured and measured during resuspension. However, the high variability in the data suggests that if this is the case, it is spatially variable. The significant increase in TSP after resuspension in the 0 alum treatment clearly indicated that much of liberated phosphorus was in a bioavailable form, consistent with results from previous studies that showed increases in SRP after resuspension (Søndergaard et al. 1992, Reddy et al. 1996). The desorption of bioavailable phosphorus from bound forms depends on the equilibrium phosphorus concentrations in the system. A recent study in the Mississippi River (James and Barko 2004) suggests that P desorption from total suspended solids is an important source of bioavailable P in that system.

The results from the current study indicate that alum is very effective at reducing TSP, but not TP, during resuspension events. This suggests that at least in the short term, the alum treatment should result in relatively little bioavailable phosphorus for algal uptake even during resuspension events.

Bioturbation/Poison treatment: Bioturbation can affect P release rate from sediments in several ways. In sediments low in iron, where P-binding capacity is low to begin with, bioturbation can enhance P release rates (e.g. Phillips et al. 1994). On the other hand, bioirrigation by benthic invertebrates can result in oxygen moving into the sediments, thereby inhibiting P release due to oxidation of iron-phosphorus complex (Böstrom et al. 1982). Both invertebrate density and taxonomic composition can influence particle mixing rate and transport of solutes across the sediment-water interface. A linear relationship between P release and midge density has been shown in some sediments (Edwards and Rolley 1965, Gallepp 1979). However, other studies have shown a nonlinear response of P flux as benthic invertebrate densities increased, suggesting that factors such as density dependence may play a role (Matisoff et al. 1985, Matisoff and Wang 1998). Tubicifid oligochaetes are especially implicated in reworking benthic sediments and acting as bioirrigators (e.g. Fisher et al. 1980, Matisoff et al.

1999), although many other invertebrates including mayfly (Stief et al. 2004) and *Chaoborus* (Gosselin and Hare 2003) larvae are also known to influence sediment structure. Matisoff and Wang (1998) suggested that burrow irrigating organisms, such as *Coelotanypus*, *Chironomus plumosus*, and *Hexagenia limbata*, may be more important in influencing solute transport across the water-sediment interface than deposit-feeding oligochaete worms.

The benthic macroinvertebrate densities in the sediment at the sites sampled in Spring Lake ranged from about 2000 to 9000 individuals/m^2. Densities of 4000 individuals/m^2 have been shown to effectively move solutes across the water-sediment interface (Matisoff and Wang 1998). Given the densities measured in Spring Lake, and that the dominant organisms in Spring Lake included both burrow irrigators (Chironomidae) and deposit-feeders (oligochaetes), it was expected that bioturbation-mediated solute transport would occur in Spring Lake sediments.

The influence of bioturbation on alum-treated sediments has received less attention but we hypothesized that the reworking of the sediments by burrowing organisms would disrupt the alum coverage over the sediments, and thereby result in reduced P inactivation compared to sediments without bioturbation. Unfortunately, the chemical interference that we encountered in measuring TSP in the poisoned treatments confounds our ability to assign direct causality to our treatments. We have dealt with the data using several approaches.

First, comparing just the TSP concentrations within the control treatments, the data reaffirm the ability of alum, even at low concentrations, to limit phosphorus release into the water column under these laboratory conditions. At all sites, the water column

TSP concentrations in the unpoisoned, 0 alum treatments were significantly greater than in the unpoisoned, alum treatments. This was to be expected, and was consistent with all our previous findings.

Second, comparing just the 0 alum cores, the significantly greater P concentration in the unpoisoned vs. the poisoned treatment suggests, at least superficially, that bioturbation is a potentially important process affecting P release in these sediments. If bioturbation was not important, we would expect the P concentrations to be the same in unpoisoned and poisoned treatments. However, this contrast is potentially confounded because of two reasons: 1) the poison also kills the bacterial community in the sediments; and 2) different forms of phosphorus were measured in the two treatments: TSP in the unpoisoned treatment vs. SRP in the poisoned treatment.

We did not quantify the affect of the poison on bacteria, so it impossible to know to what degree the community was killed or metabolism reduced. These impacts could result in P release and a change in redox reactions, such that reduced respiration may lead to a less reduced environment. Regarding the second potentially confounding factor, SRP comprises some variable fraction of the TSP in the water column, so the higher (TSP) concentrations in the unpoisoned treatments reflect, at least to some degree, a natural phenomenon. To address this confounding factor, we measured the ratio of TSP: SRP in the water column of Spring Lake to establish a natural benchmark, and compared that to the ratio we measured in the water from core tubes. The TSP:SRP in the Spring Lake water column was 4.5 ± 1.5 (n=4), establishing a benchmark expectation of 0.22 µg of SRP for every µg of TSP in the water column. If bioturbation *was* playing a significant role in P release, we would expect the poisoned, 0 alum treatments to have reduced SRP concentrations, and the amount of SRP to be much lower than 0.22 per µg of TSP.

Our experimental data showed that the mean TSP:SRP ratio across all sites and times in the 0 alum cores was 5.19 (± 2.11; n=9), or 0.19 µg of SRP for every µg of TSP. This difference is not statistically different from the ratio in Spring Lake water, suggesting that in these treatments without alum, bioturbation was not having a major impact on P release.

We used the same approach for assessing the role of bioturbation in the treatments with alum. The mean TSP:SRP ratio across all sites and times in the 5 mg/L alum cores was 3.40 (± 1.76; n=9), or 0.29 µg of SRP for every µg of TSP. In the 10 mg/L alum cores, the mean TSP:SRP ratio across all sites and times was 2.37 (± 0.54; n=9), or 0.42 µg of SRP for every µg of TSP. This compares to the expected amount of 0.22 µg of SRP for every µg of TSP; it is possible that a less reduced environment, on account of poisoning, accounted for the *relative* amount of SRP in these treatments with alum. Regardless, the data suggest that bioturbation was not important in these cores. It is also important to recognize that even though the relative amount of SRP increased in the poisoned treatments with alum, the *absolute* amount of P released was very low.

In total, these data suggest that whatever bioturbation-related activity might have been occurring, regardless of whether the cores were poisoned or unpoisoned, it was insufficient to overcome the influence of alum, at least for the 24 d that the experiment lasted. The concentrations of P being released to the water column under all treatments with alum rarely exceeded 30 µg/L, and usually were at our detection limit. One final but important caveat is that invertebrate densities were lower in our sediment cores (analyzed after the incubations were completed) than in our field ponars. Densities were usually < 1000 individuals/m^2, and we observed no invertebrates in 3 of the 8 cores with 0 alum. Hence, bioturbation may have a stronger influence on P release under field conditions, where invertebrate densities are greater.

Desktop analyses: The morphometry of a lake will influence the importance of phosphorus released by internal loading. Deep lakes, with strong thermal stratification, often result in anoxic sediment layers, which help promote diffusive flux of P from the sediments (Mortimer 1941, 1942). However, this P being released into the hypolimnion may not reach the photic zone during the peak algal growth period because of the persistent thermocline. Rather, internal loading may be far more important in shallow lakes, where the P released from the sediments has a greater opportunity to reach the photic zone during peak algal growth periods. We calculated the Osgood Index and Dynamic Ratio to assess the influence of lake morphometry on the potential importance of internal P loading.

The calculated Osgood Index for Spring Lake strongly suggested that its morphometry makes it a good candidate for internal loading to affect the nutrient status of the lake's photic zone. Our empirical data supported this analysis, where over 50% of the annual phosphorus load to Spring Lake appears to come from the sediments (Steinman et al. 2004). Given the complex morphometry of Spring Lake, with its bayous and variable depths, it is likely that the importance of internal phosphorus loading varies throughout the lake. Indeed, our results from 2003 found much higher rates of internal loading at the deeper sites (1 and 2) than the shallower sites (3 and 4). It is possible that greater mixing at the shallower sites restricted stratification, resulting in more aerobic conditions at the bottom layers and a greater tendency for the phosphorus to remain bound to minerals (cf. Mortimer 1971). Alternatively, different concentrations of sediment P may result in the spatial patterns of P release. Phosphorus content did vary among sites, with the mean P content at Sites 1 and 2 exceeding that at Sites 3 and 4 (Table 4.0.1). Our data do not allow us to determine if the greater concentrations at Sites 1 and 2 are due to naturally more enriched sediments or due to lake hydrodynamics, which may focus the P-enriched sediments away from Sites 3 and 4 toward Sites 1 and 2.

Table 4.0.1. Total phosphorus content of Spring Lake sediments (mg/kg dry weight); n=6. Mean values with different letters indicate statistically significant differences among sites (Tukey's multiple comparison test; P < 0.05).

Site	Mean ± SD
1	1512 ± 179[a]
2	1592 ± 84[a]
3	995 ± 353[b]
4	1171 ± 87[b]

Field data were not available to directly measure sediment resuspension and hydrodynamic variables in Spring Lake. Calculation of the dynamic ratio, while clearly not as rigorous as empirical data, did allow us to estimate the importance of resuspension events in Spring Lake. The low dynamic ratio suggested that resuspension, particularly in the deeper main channel of the lake, would be a limited phenomenon. This is the region of the lake where the primary alum application would take place. Hence, this calculation provides circumstantial evidence that resuspension of the alum is not particularly likely. Of course, this calculation does not address wind-wave action in Spring Lake, which also can move and focus lake sediments.

Welch and Cooke (1999) evaluated the effectiveness and longevity of alum applications in 21 lakes across the United States. Their analysis revealed that in polymictic (unstratified) lakes, internal loading rate was reduced in 6 out of 9 cases, with average reduction of about 67%, which lasted for 5-11 years. In dimictic (stratified) lakes, internal loading rate was reduced in 7 out of 7 cases, with average reduction of about 80%, and which lasted for 4 to 21 years. The major constraint in polymictic lake application was interference by macrophytes. They concluded that a reasonable expectation of longevity for alum treatments is 10 years in polymictic and 15 years in dimictic lakes. Because each lake is unique, it is impossible to pre-determine how long an alum treatment may remain effective in Spring Lake. However, our data did allow us to calculate a *minimum* estimate of how long internal loading will remain in this system. Assuming no new phosphorus entered Spring Lake, which of course is unrealistic, the lake sediments will continue to yield phosphorus for another 15 to 40 years. If the external loads from nonpoint source pollution (e.g. lawn runoff, storm drain effluent, atmospheric deposition, tributary inflow) are taken into account, this time estimate will increase substantially. The sooner that controls or incentives are put in place to limit these external sources of phosphorus, the sooner the internal loading problem in the lake will go away.

5.0. Conclusions and Recommendations

Based on the data generated in this study, we conclude the following:

- An alum application of 15 mg aluminum/L appears sufficient to inactivate P diffusive flux from Spring Lake sediments. However, an application of up to 25 mg/L, assuming appropriate pH levels are maintained in the lake, will still be environmentally safe and provide a greater margin of error for ensuring P inactivation, although likely cost more to apply.

- Resuspension of sediments at the conclusion of the alum concentration experiment resulted in significant increases in TP compared to the pre-resuspension concentrations, irrespective of alum concentration. However, TSP concentrations remained very low in the water column during resuspension, suggesting bioavailable P will be in short supply, even during sediment mixing events, provided alum is present.
- Benthic macroinvertebrate densities in Spring Lake were sufficient to influence solute transport across the sediment-water interface. Our ability to assess the influence of bioturbation was limited by analytical interference of phosphorus by the poison we used and low densities in the cores. Once we accounted for the measurement of the different forms of phosphorus, our data suggested that in treatments both with and without alum, bioturbation had little or no detectable impact on P release. When alum was present, it appeared to sorb P very effectively, irrespective of the role of bioturbation.
- Spring Lake's morphometry is well-suited for internal loading processes, with resuspension events relatively limited. This suggests that the alum floc, once applied, will not be subject to as much movement as in other lakes that are shallower and wider.
- There is sufficient phosphorus in the Spring Lake sediments to maintain high internal phosphorus loading rates for a minimum of 15 to 40 years. However, this is an unrealistically low estimate, given that it does not take into account current

external sources of phosphorus that continue to contribute to the P in the sediments. The sooner that external P loading is reduced into Spring Lake, the sooner the internal loading problem will decrease in importance.

We recommend the following:

- Assuming that the alum application is conducted, continue monitoring of: 1) TP and SRP concentrations in the epilimnion and hypolimnion (biweekly); 2) chlorophyll *a* concentrations (biweekly); 3) aluminum concentrations in the sediments and water column (monthly during summer); and 4) internal loading rates (once per year).
- A vital prerequisite for restoring lake water quality is the removal of the underlying reasons for the impairment. Thus, regardless of the long-term effectiveness of an alum treatment, it is critical that external load reduction complement the chemical additions (Hansson et al. 1998). Continued efforts at reducing stormwater discharge, conversion of septic systems to sewers, use of low-P fertilizer, and implementation of other best management practices should be emphasized and incentivized wherever possible.

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7.0 Appendix

7.1. Experiment 1: Field data

7.1A. Site 1.

Spring Lake Site 1 **Date:** 4/20/2004

Depth m	**Temp** C	**% DO** % sat	**DO** mg/L	**Cond** µS/cm	**TDS** g/L	**Turb** NTUs	**pH**	**ORP** mV	**Chl a** µg/L
0.1	12.14	106.9	11.33	558.8	0.3577	7.9	8.43	377	12.9
1	12.14	104.3	11.06	557.5	0.3569	8.1	8.52	369	22.8
2	12.14	103.4	11.02	557.7	0.3573	7.7	8.60	362	25.3
3	12.00	104.0	11.03	558.6	0.3566	7.7	8.63	359	26.9
4	11.86	101.1	10.77	543.7	0.3473	7.8	8.58	360	20.8
5	11.83	101.7	10.86	542.5	0.3465	7.7	8.57	360	26.4
6	11.80	99.4	10.62	537.0	0.3439	7.2	8.55	359	27.6
7	11.80	99.2	10.61	537.7	0.3446	7.8	8.54	359	28.4

Depth m	**Light** Umol/m2/s	**%** of surf.
Surface	861	
0.1	364	42.276
0.5	189	21.951
1.0	83	9.640
1.5	39.44	4.581
2.0	19.91	2.312
2.5	9.54	1.108
3.0	5.983	0.695
3.5	3.055	0.355
4	1.579	0.183
5	0.444	0.052
6	0.096	0.011
7	0	0.000

Extinction Coefficient 1.597

Secchi: 1.0 m **Bottom:** 27.8 ft / 8.2 m

Conditions:

Time	3:30 pm - 4:45 pm
Air temp	13.9 C
Wind	
Sky	mostly cloudy
Water	slight waves

Field Crew:

Lori
Eric
Mary
Rick
Bob, Tony, Jim

Other notes:

43° 04.97'
86° 12.18'

7.1B. Site 2.

Depth m	**Temp** C	**% DO** % sat	**DO** mg/L	**Cond** µS/cm	**TDS** g/L	**Turb** NTUs	**pH**	**ORP** mV	**Chl a** µg/L
0.1	12.00	110.9	11.80	498.9	0.3191	8.0	8.50	379	11.5
1	12.01	105.4	11.24	496.6	0.3180	7.9	8.59	370	21.3
2	12.00	104.0	11.04	495.8	0.3175	7.9	8.64	364	22.3
3	12.01	102.4	10.89	496.6	0.3179	7.3	8.66	361	26.9
4	12.00	102.7	10.95	496.7	0.3184	7.8	8.64	360	28.0
5	11.98	101.9	10.86	499.9	0.3196	7.6	8.62	360	28.3
6	11.92	100.1	10.67	499.7	0.3195	7.8	8.59	359	30.6
7	11.91	101.3	10.84	501.9	0.3216	7.7	8.58	359	28.5
8	11.89	98.7	10.52	500.4	0.3205	7.6	8.56	360	28.8
9	11.80	97.7	10.45	499.0	0.3191	8.3	8.53	360	27.4

Depth m	**Light** Umol/m2/s	**%** of surf.
Surface	2717	
0.1	1589	58.484
0.5	839	30.880
1.0	365	13.434
1.5	198	7.287
2.0	106	3.901
2.5	55	2.024
3.0	31.25	1.150
3.5	15.47	0.569
4	8.146	0.300
5	2.103	0.077
6	0.618	0.023
7	0.111	0.004
8	0.01	4E-04

Secchi: 1.0 m

Bottom: 35.6 ft / 10.67 m

Conditions:

Time	1:30 pm - 3:00 pm
Air temp	12.6 C
Wind	
Sky	mostly cloudy
Water	choppy

Field Crew:

Lori
Eric
Mary
Rick
Bob, Tony, Jim

Extinction Coefficient

1.487

Other Notes:

43° 05.28'
86° 11.13'

7.1C. Site 3.

Spring Lake Site 3 **Date:** 4/20/2004

Depth m	**Temp** C	**% DO** % sat	**DO** mg/L	**Cond** µS/cm	**TDS** g/L	**Turb** NTUs	**pH**	**ORP** mV	**Chl a** µg/L
0.1	12.25	105.2	11.14	473.0	0.3028	7.3	8.42	380	7.6
1	12.24	104.3	11.05	471.7	0.3023	7.7	8.52	370	14.6
2	12.17	102.3	10.82	472.2	0.3022	7.4	8.58	365	16.1
3	12.17	103.4	10.94	471.2	0.3018	6.8	8.59	362	20.7
4	12.15	104.4	11.07	470.9	0.3021	6.9	8.57	360	21.9
5	12.13	103.0	10.92	470.9	0.3013	7.4	8.57	359	22.0

Depth m	**Light** Umol/m2/s	**%** of surf.
Surface	1771	
0.1	822	46.414
0.5	348	19.650
1.0	181	10.220
1.5	116	6.550
2.0	60.2	3.399
2.5	29.6	1.671
3.0	18.44	1.041
3.5	10.26	0.579
4	5.24	0.296
5	1.46	0.082

Secchi: 1.25 m

Bottom: 22.4 ft
6.71 m

Extinction Coefficient
1.510

Conditions:

Time 11:15 am - 1:00 pm
Air temp 12.0 C
Wind
Sky mostly clear, slightly hazy
Water slightly choppy

Field Crew:

Lori
Eric
Mary
Rick
Bob, Tony, Jim

Other Notes:

43° 06.23'
86° 11.12'

7.1D. Site 4.

Spring Lake Site 4 **Date:** 4/20/2004

Depth m	**Temp** C	**% DO** % sat	**DO** mg/L	**Cond** µS/cm	**TDS** g/L	**Turb** NTUs	**pH**	**ORP** mV	**Chl a** µg/L
0.1	12.92	100.6	10.49	447.8	0.2867	9.9	8.30	386	8.2
1	12.94	97.4	10.15	446.6	0.2855	9.3	8.36	378	17.6
2	12.95	94.8	9.87	445.5	0.2850	9.0	8.40	374	16.6
3	12.69	96.5	10.08	456.1	0.2935	9.1	8.41	370	24.8
4	12.02	93.0	9.83	475.2	0.3037	10.2	8.42	368	25.5

Depth m	**Light** Umol/m2/s	**%** of surf.
Surface	2154	
0.1	1006	46.704
0.5	425	19.731
1.0	115	5.339
1.5	63	2.925
2.0	27	1.253
2.5	14.5	0.673
3.0	6.69	0.311
3.5	2.28	0.106
4	1.63	0.076

Extinction Coefficient: 1.969

Secchi: 1.0 m

Bottom: 17 ft / 5.18 m

Conditions:

Time	9:00 am - 11:00 am
Air temp	4.4 C
Wind	
Sky	mostly clear & sunny
Water	slightly choppy

Field Crew:

Lori
Eric
Mary
Rick
Bob, Tony, Jim

Other Notes:

43° 07.05'
86° 10.02'

7.2. Experiment 1: Laboratory data

Total Phosphorus:

Site	TP-P mg/L										
	T=0	T=H2	T=H8	T=D1	T=D2	T=D4	T=D8	T=D12	T=D16	T=D20	T=D24
1A	0.086	0.084	0.068	0.040	0.040	0.034	0.036	0.080	0.194	0.186	0.124
1B	0.058	0.052	0.034	0.032	0.034	0.010	0.010	0.010	0.020	0.022	0.026
1C	0.110	0.046	0.024	0.024	0.030	0.010	0.010	0.010	0.010	0.022	0.022
1D	0.126	0.026	0.010	0.010	0.020	0.010	0.010	0.010	0.010	0.020	0.010
1E	0.114	0.028	0.010	0.010	0.020	0.010	0.010	0.010	0.010	0.010	0.010
1F	0.130	0.026	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010
2A	0.072	0.060	0.048	0.040	0.044	0.058	0.118	0.272	0.374	0.348	0.298
2B	0.080	0.050	0.010	0.010	0.020	0.010	0.020	0.026	0.034	0.030	0.036
2C	0.092	0.026	0.010	0.010	0.010	0.010	0.010	0.022	0.010	0.020	0.010
2D	0.068	0.020	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010
2E	0.090	0.020	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010
2F	0.080	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.024	0.010
3A	0.050	0.040	0.030	0.024	0.024	0.020	0.020	0.034	0.054	0.066	0.066
3B	0.086	0.046	0.028	0.010	0.010	0.010	0.010	0.020	0.038	0.040	0.030
3C	0.054	0.030	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010
3D	0.046	0.026	0.010	0.022	0.010	0.010	0.010	0.010	0.010	0.010	0.010
3E	0.040	0.028	0.010	0.010	0.010	0.010	0.010	0.022	0.024	0.010	0.010
3F	0.060	0.026	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010
4A	0.048	0.036	0.030	0.022	0.026	0.010	0.026	0.036	0.042	0.048	0.040
4B	0.046	0.048	0.020	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.020
4C	0.056	0.060	0.020	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010
4D	0.036	0.010	0.020	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010
4E	0.046	0.010	0.026	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010
4F	0.038	0.038	0.028	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010

Total Soluble Phosphorus:

Site	TSP-P mg/L										
	T=0	T=H2	T=H8	T=D1	T=D2	T=D4	T=D8	T=D12	T=D16	T=D20	T=D24
1A	0.010	0.020	0.010	0.010	0.010	0.010	0.010	0.042	0.058	0.064	0.056
1B	0.010	0.010	0.010	0.010	0.036	0.010	0.010	0.010	0.010	0.020	0.010
1C	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010
1D	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010
1E	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010
1F	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010
2A	0.010	0.010	0.010	0.032	0.026	0.048	0.060	0.134	0.136	0.172	0.216
2B	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.020	0.026
2C	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010
2D	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.020	0.010	0.010	0.010
2E	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010
2F	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010
3A	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.022	0.058	0.072
3B	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.022	0.030	0.010
3C	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010
3D	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010
3E	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010
3F	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010
4A	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.022	0.010	0.032	0.032
4B	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010
4C	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010
4D	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010
4E	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.020
4F	0.024	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010

7.3. Experiment 2.

Total Phosphorus:

Site	TP-P mg/L			
	T=0 (D24)	T=H12	T=H24	T=H48
1A	0.124	0.126	0.172	0.190
1B	0.026	0.050	0.158	0.082
1C	0.022	0.212	0.362	0.258
1D	0.010	0.096	0.258	0.328
1E	0.010	0.474	0.750	0.890
1F	0.010	0.052	0.034	0.088
2A	0.298	0.358	0.514	0.526
2B	0.036	0.158	0.206	0.340
2C	0.010	0.126	0.088	0.228
2D	0.010	0.588	0.854	0.922
2E	0.010	0.064	0.518	0.940
2F	0.010	0.400	0.550	0.964
3A	0.066	0.144	0.232	0.256
3B	0.030	0.070	0.068	0.088
3C	0.010	0.162	0.170	0.120
3D	0.010	0.064	0.120	0.150
3E	0.010	0.186	0.124	0.106
3F	0.010	0.286	0.272	0.236
4A	0.040	0.056	0.062	0.070
4B	0.020	0.032	0.030	0.034
4C	0.010	0.040	0.040	0.036
4D	0.010	0.064	0.028	0.054
4E	0.010	0.054	0.054	0.052
4F	0.010	0.028	0.044	0.052

Total Soluble Phosphorus:

Site	TSP-P mg/L			
	T=0 (D24)	T=H12	T=H24	T=H48
1A	0.056	0.066	0.050	0.036
1B	0.010	0.024	0.010	0.010
1C	0.010	0.028	0.024	0.020
1D	0.010	0.010	0.010	0.010
1E	0.010	0.024	0.010	0.010
1F	0.010	0.010	0.010	0.010
2A	0.216	0.282	0.288	0.270
2B	0.026	0.082	0.080	0.096
2C	0.010	0.032	0.028	0.026
2D	0.010	0.032	0.032	0.030
2E	0.010	0.010	0.010	0.010
2F	0.010	0.010	0.010	0.010
3A	0.072	0.190	0.150	0.184
3B	0.010	0.024	0.020	< 0.02
3C	0.010	0.024	0.024	0.020
3D	0.010	0.010	0.010	0.010
3E	0.010	0.010	0.010	0.010
3F	0.010	0.010	0.010	0.010
4A	0.032	0.026	0.022	0.022
4B	0.010	0.010	0.010	0.010
4C	0.010	0.010	0.010	0.010
4D	0.010	0.010	0.010	0.010
4E	0.020	0.010	0.010	0.010
4F	0.010	0.010	0.010	0.010

7.4. Experiment 1: Field data

7.4A. Site 1.

Spring Lake Site 1 **Date:** 5/22/2004

Depth m	**Temp** C	**% DO** % sat	**DO** mg/L	**Cond** µS/cm	**TDS** g/L	**Turb** NTUs	**pH**	**ORP** mV	**Chl a** µg/L
0.1	18.12	78.7	7.28	489.7	0.3134	6.2	8.34	356	3.2
1	18.13	76.0	7.07	490.3	0.3138	6.1	8.56	340	3.7
2	18.13	76.3	7.09	490.3	0.3137	5.7	8.60	336	3.9
3	18.13	75.5	7.02	490.4	0.3139	5.7	8.58	335	3.9
4	18.13	74.4	6.92	490.4	0.3139	5.5	8.55	336	3.9
5	18.13	73.8	6.87	490.3	0.3138	5.6	8.50	336	3.8
6	18.11	73.5	6.84	491.6	0.3150	5.5	8.46	339	3.2
7	16.58	48.1	4.56	512.2	0.3278	7.2	8.04	352	2.6
7.8	14.95	21.8	2.17	524.9	0.3356	10.6	7.84	359	2.7

Depth m	**Light** Umol/m2/s	**%** of surf.
Surface	186	
0.1	98	52.688
0.5	53	28.495
1.0	32	17.204
1.5	18.5	9.946
2.0	11.4	6.129
2.5	6.11	3.285
3.0	3.01	1.618
3.5	1.663	0.894
4	1.039	0.559
5	0.378	0.203
6	0.1406	0.076
7	0.063	0.034

Secchi: 1.75 m **Bottom:** 29 ft 8.80 m

Extinction Coefficient
1.240

Conditions:

Time	8:30 am - 10:00 am
Air temp	15.4 C
Wind	calm
Sky	100% cloudy, foggy
Water	calm

Field Crew:

Lori, Eric, Mary, Jeremy
Rick
Jim Winks - captain
Bob Pennell - deckhand

Other Notes:

43° 04.96'
86° 12.18'

Blood Midges:

Rep A - 4
Rep B - 0
Rep C - 1

7.4B. Site 2.

Spring Lake Site 2 **Date:** 5/22/2004

Depth m	**Temp** C	**% DO** % sat	**DO** mg/L	**Cond** µS/cm	**TDS** g/L	**Turb** NTUs	**pH**	**ORP** mV	**Chl a** µg/L
0.1	18.33	79.3	7.35	455.6	0.2914	5.1	8.34	350	3.3
1	18.32	77.3	7.17	459.5	0.2938	5.0	8.45	342	3.6
2	18.28	76.5	7.07	459.9	0.2938	5.1	8.51	338	3.6
3	18.29	75.9	7.01	459.1	0.2932	5.1	8.53	336	3.9
4	18.19	71.5	6.67	468.1	0.3003	4.8	8.52	336	2.9
5	17.95	73.3	6.82	484.7	0.3106	5.5	8.48	337	3.2
6	17.79	68.8	6.44	493.0	0.3153	5.5	8.41	338	2.6
7	16.60	50.2	4.81	508.5	0.3255	6.0	8.16	346	2.4
8	15.39	31.6	3.11	516.1	0.3300	6.6	7.93	354	2.8
9	14.87	15.0	1.55	517.7	0.3310	8.8	7.76	360	3.5

Depth m	**Light** Umol/m2/s	**%** of surf.
Surface	1890	
0.1	798	42.222
0.5	458	24.233
1.0	257	13.598
1.5	162	8.571
2.0	96	5.079
2.5	53.7	2.841
3.0	29.9	1.582
3.5	18.37	0.972
4	11.33	0.599
5	4.135	0.219
6	1.56	0.083
7	0.588	0.031
8	0.209	0.011
9	0.053	0.003

Extinction Coefficient

1.201

34.5 ft

Secchi: 1.5 m **Bottom:** 10.30 m

Conditions:

Time	10:15 am/3:15pm
Air temp	16.5 C
Wind	SW at 10 mph
Sky	100% clouds, some rain & sun
Water	calm

Field Crew:

Lori, Eric, Mary, Jeremy
Rick
Jim Winks - captain
Bob Pennell - deckhand

Other Notes:

43° 05.32'
86° 11.16'
* water quality done in am, back in pm to collect cores

Blood Midges:

Rep A - 2
Rep B - 0
Rep C - 0

7.4C. Site 3.

Spring Lake Site 3 **Date:** 5/22/2004

Depth m	**Temp** C	**% DO** % sat	**DO** mg/L	**Cond** µS/cm	**TDS** g/L	**Turb** NTUs	**pH**	**ORP** mV	**Chl a** µg/L
0.1	19.04	81.8	7.47	433.1	0.2775	6.1	8.27	344	4.8
1	18.58	76.1	7.01	440.0	0.2816	6.1	8.33	338	4.3
2	18.44	73.8	6.82	445.4	0.2855	6.1	8.41	333	4.1
3	18.37	72.8	6.74	452.7	0.2891	5.6	8.45	330	3.8
4	18.06	68.6	6.37	454.1	0.2902	6.0	8.41	330	3.5
5	17.91	65.2	6.10	439.2	0.2819	7.3	8.32	333	3.2
6	17.65	61.5	5.78	448.1	0.2895	7.3	8.27	334	3.4

Depth m	**Light** Umol/m2/s	**%** of surf.
Surface	1327	
0.1	539	40.618
0.5	239	18.011
1.0	127	9.570
1.5	66.1	4.981
2.0	32.2	2.427
2.5	14.6	1.100
3.0	8.55	0.644
3.5	4.59	0.346
4	2.546	0.192
5	0.72	0.054
6	0.192	0.014

Extinction Coefficient
1.581

25 ft

Secchi: 1.4 m **Bottom:** 7.60 m

Conditions:

Time	2:00 pm - 3:00 pm
Air temp	18.5 C
Wind	calm
Sky	100% clouds/some sun later
Water	calm

Field Crew:
Lori, Eric, Mary, Jeremy
Rick
Jim Winks - captain
Bob Pennell - deckhand

Other Notes:
43° 06.22'
86° 11.09'
*site moved away from shore slightly
*cores/ponars done first

Blood Midges:
Rep A - 4
Rep B - 1
Rep C - 2

7.4D. Site 4.

Spring Lake Site 4 **Date:** 5/2/2004

Depth m	**Temp** C	**% DO** % sat	**DO** mg/L	**Cond** µS/cm	**TDS** g/L	**Turb** NTUs	**pH**	**ORP** mV	**Chl a** µg/L
0.1	18.22	73.7	6.84	399.6	0.2558	10.4	7.96	359	4.6
1	18.19	69.4	6.45	400.6	0.2560	10.2	8.03	353	5.1
2	18.11	67.0	6.24	397.1	0.2537	10.3	8.07	347	5.1
3	17.02	60.3	5.73	325.7	0.2006	19.5	7.82	351	5.3
4	16.70	56.5	5.43	289.4	0.1817	30.0	7.70	354	5.4

Depth m	**Light** Umol/m2/s	**%** of surf.
Surface	1172	
0.1	530	45.222
0.5	215	18.345
1.0	91	7.765
1.5	38.5	3.285
2.0	15.7	1.340
2.5	5.54	0.473
3.0	1.697	0.145
3.5	0.332	0.028
4	0.053	0.005

Extinction Coefficient
2.330

18.4 ft

Secchi: 1.0 m **Bottom:** 5.50 m

Conditions:

Time	12:15 PM
Air temp	24.5 C
Wind	
Sky	partly sunny
Water	small waves

Field Crew:
Lori, Eric, Mary, Jeremy
Rick
Jim Winks - captain
Bob Pennell - deckhand

Other Notes:
43° 07.04'
86° 10.02'
*cores/ponars collected first

Blood Midges:
Rep A - 1
Rep B - 3
Rep C - 1

7.4E. Phosphorus concentrations measured from surface and bottom depths on May 22, 2004.

AWRI #	Site	TSP-P	TP-P
		mg/L	mg/L
12253	Site 1 Top	< 0.02	0.050
12254	Site 1 Bottom	< 0.02	0.032
12255	Site 2 Top	< 0.02	0.032
12256	Site 2 Bottom	< 0.02	0.034
12257	Site 3 Top	< 0.02	0.037
12258	Site 3 Bottom	< 0.02	0.036
12259	Site 4 Top	< 0.02	0.036
12260	Site 4 Bottom	0.030	0.083