PREDICTIONS CONCERNING INTERNAL PHOSPHORUS RELEASE IN COOTES PARADISE MARSH AND IMPLICATIONS FOR RESTORATION

PREDICTIONS CONCERNING INTERNAL PHOSPHORUS RELEASE IN COOTES PARADISE MARSH AND IMPLICATIONS FOR RESTORATION

By

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ABSTRACT

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To assess the relative contribution of phosphorus release from sediment in Cootes Paradise Marsh, I first examined spatial and temporal variability of laboratory-measured release rates from sediment samples collected from 12 sites within the wetland (Chapter 1). The microbial communities from these 12 sites were characterized on the basis of sole-carbon-source utilization using BIOLOG GN plates containing 95 substrates. Results from these experiments were entered into a principal component analysis and the release rates for all sites were regressed against corresponding PC1 and PC2 scores (Chapter 2). Differences in microbial physiology as defined by PC1 and PC2 scores accounted for 49% and 53%, respectively of the variation in phosphorus release rates. Using stepwise multiple regression, I concluded that the key determinants of laboratory-derived release rates from West Pond sediment (a highly eutrophic site within Cootes Paradise Marsh) are oxic state, ambient temperature and time of sediment collection (Chapter 3). Using this information I developed a multivariate model to predict release rates. This model, along with 2 alternative approaches, was used to estimate the extent of internal loading (kg d^{-1}) in Cootes Paradise Marsh (Chapter 4). Method 1 was a highly controlled, static approach and resulted in an internal load of 11.6 kg d^{-1}, which was attributed to diffusion. The remaining two methods were more dynamic in nature as they took into account temporal variation, ambient temperature and the development of anoxia. Using Method 2, I estimated an internal load of 7.8 to 8.5 kg d^{-1}, which was based on empirically-derived laboratory release rates. Method 3 consisted of the predictive model from which I estimated an internal load of 6.7 kg d^{-1}. I attributed the release from the latter two methods to microbial mineralization processes. Based on this work and a previous estimate of internal loading in Cootes Paradise Marsh (Prescott & Tsanis 1997), I concluded that mineralization accounts for 22.3% of total phosphorus loading to the system, preceded only by urban runoff (41%).

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GENERAL INTRODUCTION

Cootes Paradise

General information

Cootes Paradise is an 850-ha wildlife sanctuary owned and managed by the Royal Botanical Gardens (RBG) and is located at the western tip of Hamilton Harbour and Lake Ontario. It is an Area of National Significance as well as a Hamilton Region Conservation Environmentally Sensitive Area (Remedial Action Plan for Hamilton Harbour Stage 1 Report 1992). Within this sanctuary lies a 250-ha wetland, a receiving basin of four major hydrologic inputs: Borer's Creek, Chedoke Creek, Spencer's Creek and the Dundas Wastewater Treatment Plant (Remedial Action Plan for Hamilton Harbour Stage 1 Report 1992). Spencer's Creek drains 79% of the Cootes Paradise watershed (Chow-Fraser et al. 1998) and flows through mainly agricultural and forested land. This creek is considered a major source of organic and inorganic materials to the marsh. Chedoke and Borer's Creeks are much smaller tributaries. Chedoke is an urban creek, which passes through substantial residential and industrial developments and consists mostly of underground channels. The Dundas Wastewater Treatment Plant discharges effluent into the western end of the marsh.

Changes in Cootes Paradise Marsh

Morphological changes to Cootes Paradise Marsh date back to 1837 with the completion of the Desjardins Canal. The canal was constructed in order to provide a link between the town of Dundas and Lake Ontario as a trade route. A prominent logging industry existed in Dundas at that time it was hoped that the Desjardins Canal would be used to ship lumber. In 1852, the canal was dredged a second time to allow passage for larger ships; however in only five years, sedimentation was so great that dredging was yet again required to keep the canal navigable. The project was eventually abandoned. In 1854 a railway line was extended over the outlet of the marsh. As a result, the outlet was filled in as an embankment and a new outlet was dredged through Burlington Heights, a Lake Iroquois deposit which separated Cootes Paradise from Hamilton Harbour.

The Dundas Wastewater Treatment Plant (WWTP) was constructed in 1919 to provide primary treatment to the town's sewage before releasing the effluent into West Pond (CP5, Figure 1.1, Chapter 1). Prior to its construction, raw sewage was discharged directly into the marsh and although the facility now provides tertiary treatment, historically it has been the major contributor of nutrients and was primarily responsible for the eutrophication of Cootes Paradise (Remedial Action Plan for Hamilton Harbour Stage 1 Report 1992; Chow-Fraser et al. 1998). Algal blooms were a result of elevated phosphate levels that were a thousand times greater than that required to cause blooms (Lord 1993). Algal blooms settle to the bottom sediments and create such an oxygen demand as to result in complete anoxia (Bacchus 1974).

Carp (*Cyprinus carpio*) were first recorded in the marsh in 1911 (Holmes & Whillans 1984). The effects of carp on vegetation have been well documented: feeding and spawning activities uproot submergent macrophyte beds and increase turbidity levels (Lougheed et al. 1998). Elimination of vegetation increases wind-driven resuspension and inhibits light penetration for surviving submergents. The Cootes Paradise Fishway, which simultaneously prevents the access of large carp (>30 cm) and facilitates access and departure of other fish, was subsequently installed at the outlet to reduce carp disturbance to Cootes Paradise Marsh. With the installation and operation of this Fishway, the two remaining impediments to reestablishment of the vegetative community are deemed to be wind-driven resuspension and high nutrient levels.

Phosphorus Cycling

Phosphorus Cycling in Aquatic Systems

Phosphorus cycling processes in aquatic systems differs markedly from oxidized soils in forested or grassland systems. Phosphorus is introduced to aquatic systems via two main sources: tributaries or surface runoff (external loading) and mineralization or resuspension of sediments (internal loading). This nutrient has a negligible gaseous phase and is therefore, mainly cycled though liquid and solid compartments (Richardson 1999). Incoming phosphorus may be sequestered by living or non-living suspended particles and is eventually lost to the lake bottom. Once at the sediment surface, bacterial mineralization of organic matter liberates soluble phosphate (Gächter & Meyer 1993). Due to steep concentration gradients at the sediment/water interface, molecular diffusion is the primary means for transportation of phosphorus from the sediment to the water column (Wetzel 1999). However, newly liberated phosphate may also be adsorbed to inorganic surfaces or precipitated back out of solution. Adsorption involves ion (attraction of phosphate to positively charged sites) or ligand (phosphate replaces a surface hydroxyl that is coordinated with a metal cation) exchange, while precipitation involves the formation of a solid from the combination of two or more soluble components (Rhue & Harris 1999). Clay minerals, Fe and Al hydroxides and calcium components are the main inorganic P constituents. The flux of phosphate to and from the sediment is largely controlled by redox conditions (Gächter & Meyer 1993). These two processes, adsorption and precipitation, are the main reasons why phosphorus is generally low or limiting to growth in freshwater lakes.

In wetlands, sediment storage and vegetation become more important in nutrient cycling and consequently, natural wetlands are often considered nutrient sinks. Typically, functioning wetlands retain phosphorus; however, this depends on the type of wetland, the hydrologic conditions (flowing versus stagnant water), and the length of time the wetland has been subjected to external loading (Mitsch & Gosselink 1993). Prolonged cultural eutrophication results in alteration of biological community and the wetland ultimately can no longer protect downstream regions from nutrient enrichment (Kadlec & Walker 1999).

Factors Governing Phosphorus Release from Sediments

There are five main mechanisms controlling phosphorus release from sediments:

1. Diffusion

As previously mentioned, simple diffusion may be the primary means for transference of phosphorus to the overlying water since concentration gradients are typically much higher in the sediments.

2. Macrophytes

Rooted macrophytes obtain most of their nutrient requirements from the sediment and may subsequently excrete them into the surrounding water during growth and senescence (Richardson 1999).

3. Wind/bioturbation

In shallow systems wind and currents may resuspend the sediment: phosphorus complexes (Fe-, Al- or Ca-bound P) may disassociate before the sediment settles (Fabre 1988). A similar phenomenon can be caused by bioturbation due to benthic invertebrates and fish, enhancing nutrient release to the overlying water (Phillips et al. 1994; Goldsborough & Robinson 1996).

4. pH

Phosphate adsorption to Fe and Al hydroxides is based on exchange with hydroxyl groups (Danen-Louwerse et al. 1993). Results of Ostrofsky et al. (1989), Jacoby et al. (1982) and Boers (1991) showed that increases in pH result in higher sediment phosphorus release due to exchange of hydroxyl ions for phosphate on surfaces of metal oxide-hydroxides. Provided that calcium concentrations are sufficiently high, an increase in pH (due to photosynthesis, for example) may conversely result in increased precipitation of phosphate with calcite crystals (Carlton & Wetzel 1988).

5. Redox

The classical model of Mortimer (1941, 1942) states that Fe concentrations control the release of phosphorus from sediment. Under oxic conditions, phosphate will adsorb to ferric iron (oxidized) to form a solid complex in the sediment (Gächter et al. 1988); however, under reduced conditions, ferrous iron will release the phosphate to the overlying water.

Hamilton Harbour Remedial Action Plan

A remedial action plan (RAP) for Hamilton Harbour was formulated in 1992 to improve water quality in both Hamilton Harbour and Cootes Paradise Marsh, and to enhance sediment quality and fish and wildlife habitat under the Great Lakes Water Quality Agreement. One of the main concerns was eutrophication. Table i.1 indicates the sources of phosphorus to Cootes Paradise Marsh during the peak algal growing season in 1989 as estimated in the Remedial Action Plan for Hamilton Harbour Stage 2 Report (1992).

Table i.1 Phosphorus contributions to Cootes Paradise Marsh during the peak algal growing season in 1989.

Source	**Load ($kg \cdot d^{-1}$)**	**%**
Creeks	8.1	19.47
Dundas WWTP	3.6	8.65
Hamilton Harbour	7.7	18.51
Urban Runoff	7.4	17.79
Combined Sewer Overflow (CSO)	14.8	35.58
Total	**41.6**	**100**

The Regional Municipality of Hamilton-Wentworth constructed a retention basin at Main and King Streets to reduce CSO loading. This tank became operational in 1997 and has diminished CSO volumes by an estimated 50% and the phosphorus load has been reduced to 2.2 kg d^{-1} during the summer season (XCG Consultants Ltd. 1997).

The Hamilton Harbour RAP concluded that phosphorus release from the sediments was not significant in the Harbour. This document does not, however, recognize the potential of internal loading in Cootes Paradise despite the fact that the marsh has been implicated as a substantial source of nutrient enrichment to the Harbour (contributes 10.8% of total load). Previous studies have shown that internal phosphorus loading can play a substantial role in the recovery of small lakes following external load reductions (Phillips et al. 1994; Boström & Pettersson 1982) and thus delay restoration efforts (Danen-Louwerse et al. 1993; Marsden 1989). Due to the long history of external loading from the Dundas WWTP, it seems reasonable to hypothesize that the sediments have been saturated with phosphorus (particularly in the western end) and may therefore be a substantial nutrient source to both the marsh and the Harbour.

Prescott & Tsanis (1997) carried out a mass-balance model of phosphorus in Cootes Paradise Marsh. They estimated that approximately 57% of phosphorus came from sediment reflux. Subsequent to their study, substantial efforts have been made to reduce the impact of bioturbation through the construction of the Fishway and the elimination of carp (>30 cm) and the current estimate of sediment reflux should therefore be much lower. However, the extent to which the remaining two factors, bacterial mineralization and wind resuspension, contribute to internal loading has not yet been addressed.

Thesis Objectives

The primary objective of this thesis was to assess the magnitude of internal loading in Cootes Paradise Marsh that currently exists and thus provide needed information to guide the Hamilton Harbour RAP. A second but equally important objective was to increase our current knowledge of phosphorus cycling in wetlands, particularly as it pertains to internal loading.

I first determined spatial and temporal variability in phosphorus release rates from sediments collected throughout the marsh. Results were used to choose appropriate sites from which to sample for subsequent calculations of internal loading of the entire marsh. Because there were significant differences in release rates from site-to-site and month-to-month at the same site, I conducted a study to determine if these differences could be attributed to benthic microbial community structure (Chapter 2). Next, I investigated the use of key environmental variables to model sediment release rates from Cootes Paradise Marsh. Previous studies on lakes have attempted to model internal loading and have been met with mixed results. This is the first study on wetlands that aims to model phosphorus release from the following variables: chemical constituents, ambient temperature, redox conditions and time of sediment collection (Chapter 3). Finally, I used the predictive model in Chapter 3 to estimate the seasonal contribution of internal loading in Cootes Paradise and compared this to estimates of external sources (Chapter 4). This thesis concludes with a discussion of ecological implications of internal loading for the restoration of Cootes Paradise Marsh and an overview of various methods employed to reduce internal loading pressures in other systems.

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CHAPTER 1

Spatial and Temporal Variability in Sediment Phosphorus Release

ABSTRACT

Cootes Paradise is a hypereutrophic marsh located at the western end of Hamilton Harbour. Although actions have been taken to reduce external nutrient loading as part of the Hamilton Harbour Remedial Action Plan, the possible confounding effects of internal loading (phosphorus release from eutrophic sediments) which may persist for several decades, have not yet been properly assessed. We suspected that internal loading was a significant source of phosphorus to Cootes Paradise and should be considered if improvements in water quality are to be achieved. A preliminary study was carried out to evaluate spatial and temporal variability in phosphorus release rates from sediment. We collected samples from 12 sites within the marsh in June 1999 and conducted laboratory release experiments to assess spatial variability in phosphorus release rates. In addition, samples were collected May to August of the same year from a subset of four sites to assess temporal variability. A one-way ANOVA revealed significant spatial ($p<0.0001$) variability in sediment phosphorus release rates for the 12 sites under laboratory conditions. We also found significant spatial and temporal variability among the subset of 4 sites, and a significant interaction between site and time of sampling (two-way ANOVA; $p<0.0001$). We therefore conclude that an accurate calculation of internal

loading must be based on a sampling program that is both temporally and spatially representative of the whole marsh ecosystem.

INTRODUCTION

Sediment phosphorus release has been extensively studied over the past 20 years and has been identified as an important consideration in many aquatic systems that require ecological restoration (Jacoby et al. 1982; Premazzi & Provini 1985; Marsden 1989; Ostrofsky et al. 1989). A large proportion of this research however, has been focused on lakes while wetland systems have essentially been ignored. This absence of wetland-specific information is problematic because the eutrophication process in wetlands is fundamentally different from that in lakes. Unlike lakes where profundal sediments adsorb phosphorus more or less evenly throughout the system, eutrophication in wetlands is typically localized around a point of discharge (i.e. inflow of agricultural runoff or effluent from a sewage treatment plant). This usually creates a steep phosphorus gradient in which incoming phosphorus is either sequestered by available plant and microbial communities, or is adsorbed to sediment surrounding the discharge, leaving sites at further distances unaffected (Kadlec & Walker 1999). As sediments become saturated over time, however, sites downstream from the source may become progressively more affected by the moving front of phosphorus (Lowe & Keenan 1997). Consequently, the response of degraded wetlands to the pollutant gradient will vary depending on the duration of cultural eutrophication and the degree of sediment saturation. Alterations in species composition of the microbial, algal and macrophytic communities may also increase phosphorus availability (Kadlec & Walker 1999). This may explain why water-column concentrations of phosphorus in degraded wetlands (e.g. Cootes Paradise Marsh) are highly variable from site to site, and why wetland sediments are likely to be both spatially and temporally variable in its capacity to adsorb or release phosphorus (Lougheed & Chow-Fraser 1998; Chow-Fraser 1999).

Description of Study Site

Cootes Paradise Marsh is approximately 250 ha and lies within an 850 ha wildlife sanctuary located at the western end of Hamilton Harbour. It has been the receiving body of effluent from the Dundas Wastewater Treatment Plant (WWTP) since 1919 (Fig. 1.1). Until 1978, the effluent received only primary and secondary treatment, and represented the overwhelming proportion of external P to the ecosystem, the remaining phosphorus contributed by Spencer's Creek, the main tributary of the marsh which drains about 79% of the watershed (Chow-Fraser et al. 1998). At the peak of the cultural eutrophication during the early 1970s, phosphorus concentrations at the WWTP outfall reached 15.4 mg/L; despite these elevated levels, however, concentrations routinely dropped seven-fold to 2.2 mg/L within 600 m of the outfall (Chow-Fraser et al. 1998). The distribution of ammonia showed a similar gradient from the outfall to the marsh outflow (Chow-Fraser et al. 1998). This rapid removal of nutrients from the water column within a relatively short distance from the point of discharge exemplifies the localized response of wetlands to eutrophication (Kadlec & Walker 1999).

Prior to the early 1900s, Cootes Paradise was almost completely vegetated with emergent vegetation (Painter et al. 1989), but by the early part of the twentieth century, only 80% of the marsh remained. Over the century, the macrophyte community continued to decline to the extent that by 1993, only 10% of the marsh was covered with emergent vegetation (Chow-Fraser et al. 1998). The cause of the decline has been partly attributed to eutrophication resulting from historic nutrient loadings of the Dundas WWTP, and more recent loadings from agricultural and urban runoff (Remedial Action Plan for Hamilton Harbour Stage 2 Report 1992). A remedial action plan (RAP) was formulated in 1992 to improve water quality in both Hamilton Harbour and Cootes Paradise to enhance sediment quality and fish and wildlife habitat under the Great Lakes Water Quality Agreement. Cootes Paradise was included in the plan as it was considered a substantial source of suspended sediment and phosphorus to the Harbour and is the largest residual spawning and nursery habitat for the warm-water fish community of Hamilton Harbour (Remedial Action Plan for Hamilton Harbour Stage 2 Report 1992). To date, only external loading sources have been targeted for reduction in the RAP, while the contribution of phosphorus originating from biogeochemical interactions in the sediment have virtually been ignored. This is a notable omission since a previous mass-balance study indicated that approximately half of the annual phosphorus load to Cootes Paradise came from internal sources (Prescott & Tsanis 1997).

METHODS

Sampling

Twelve stations along a west-to-east gradient were selected to evaluate spatial variance in phosphorus release rates from sediment (Fig. 1.1). A basic description of these stations can be found in Table 1.1. Sediment samples were collected in June of 1999 with an Ekman Grab, which typically retrieved the top five centimetres of substrate. Excess water was drained and samples were stored in Freezer Ziploc™ bags and frozen prior to experimentation. We focused on four stations (CP1, CP8, CP23, CP24) to assess temporal variability. Samples were collected monthly (May to August) from these sites and were stored in a similar fashion.

Release Rate Determination

Phosphorus release experiments were conducted in the manner of Chow-Fraser et al. (1996): wet sediment (10 g) was spread over the bottom of 120 mL capacity acid-washed glass jars. Deionized water (100 mL) was added to each jar for a total of 24 jars for each station. Jars were covered with foil that received several puncture holes with a pencil tip to allow air exchange and were incubated in a dark growth chamber for up to 8 days at 25°C. Three jars from each site were removed from the growth chamber daily. Water samples were extracted from each jar using a pipette so as to limit disturbance at the sediment-water interface. Samples were analyzed for soluble reactive phosphorus

(SRP) using the molybdate blue method (Murphy & Riley 1962) to measure inorganic P in the extracts. The slope of the best-fit line relating phosphorus release (mgP m^{-2}) to time (d) was used to estimate release rate (mgP m^{-2} d^{-1}).

Statistical analyses were performed using SAS Jmp (SAS Institute Inc. 1982). One-way ANOVA and post-hoc Tukey-Kramer were used to determine significant differences in release rates among adjacent sites and a two-way ANOVA was used to determine differences from month-to-month and site-to-site for four stations.

RESULTS

SRP release from sediment generally increased linearly over time (Fig. 1.2). Rates ranged from 0.96 to 28.3 mgP m^{-2} d^{-1} (Table 1.2) and are consistent with those reported by Nürnberg (1988) for lakes (Fig. 1.3). Many of the observed release rates were below 5 mgP m^{-2} d^{-1}, suggesting a lower trophic state than hypereutrophic; however, Nürnberg reported TP release rates, not SRP, and therefore rates obtained in this investigations are lower than may otherwise be expected.

Results of the ANOVA (Table 1.3) revealed a significant difference in release rates among stations ($p<0.0001$). Release rates of adjacent stations were compared with a Tukey Kramer test (Fig. 1.4). Westdale Cut (CP17) had the highest release, with West Pond (CP5) second. The only remnant marsh site (CP8) had a release rate similar to that of West Pond. Open water sites were lower and generally had similar rates, although those clustered towards the east end (CP1, CP20, CP21) had higher rates than those in the middle of the marsh (CP19, CP24, CP10). In addition, the release rate of the outlet sediment (CP22) was higher than the adjacent open water stations.

A two-way ANOVA indicated that site location was a significant factor ($p<0.0001$) in the variability of sediment SRP release rates and there was also a significant effect of season, although only significant for certain stations (significant interaction term, p<0.0001) and there did not appear to be a consistent trend for all four stations (Fig. 1.5). A particularly large increase in release rate was observed for station CP8 from May to June.

DISCUSSION

Many of the release rates were lower than expected in comparison to those found in the literature for eutrophic systems by Nürnberg (1988). Nürnberg concluded that release rate is dependent of lake trophic status: release rates of up to 50 mgP m^{-2} d^{-1} are found in hypereutrophic systems, whereas those below 1 mgP m^{-2} d^{-1} are only found in oligotrophic systems. Release rates of the open water sites in Cootes Paradise were relatively low (mean of 1.48 mgP m^{-2} d^{-1} for CP19, CP24 and CP10), falling into the meso- or oligotrophic category. Rates for sites closer to point sources of phosphorus (i.e. CP5) were much higher (17.18 mgP m^{-2} d^{-1}) and were within hypereutrophic status. This high release is presumably related to the historic discharge of primary-treated sewage from the Dundas Wastewater Treatment Facility. Since the open-water stations are more remote from points of discharge, they may not been saturated by the moving front of phosphorus; consequently, these open water sites had lower release rates. Our results support the theory of Lowe & Keenan (1997) which states that eutrophication effects are localized to nutrient sources in wetlands, as well as that of Lijklema (1993) who stated that a spatial distinction must be made in such systems in order to accurately describe the extent of internal loading.

Nevertheless, we were surprised that CP17, rather than CP5, was associated with the highest release rate. We later discovered that a combined sewer overflow (CSO) empties directly into this portion of the marsh and is responsible for much of the external load (XCG Consultants Ltd. 1997). Another unexpected finding was that sediments obtained from CP8 (a remnant marsh site) yielded rates similar to that of CP5, the sewage lagoon. We speculate that this may reflect macrophyte excretion and senescence, which contributes organic phosphorus (OP) to the sediment since vegetation serves as a short-term sink (Richardson 1999). Bacterial mineralization of organic matter then converts OP to usable inorganic forms (Boström & Pettersson 1982), which can be sequestered by algae and higher plants. Finally sediment from CP22, the marsh outlet, was characterized by a relatively high release rate (9.31 mgP $m^{-2} d^{-1}$). There are two potential point sources of phosphorus influencing this area of Cootes Paradise: until quite recently, Chedoke Creek received effluent from CSO, which could enrich the sediments along its way out the marsh and into the Harbour.

Although there was significant temporal variation in release rates for all four sites, we could not detect any consistent seasonal trend. These differences appear to be associated with activity of different microbial communities that dominate the sediment from month to month (see Chapter 2). Further research is required to determine if the unexpectedly large increase in release rate from May to June at station CP8 is attributable to variation in precipitation pattern (i.e. additional phosphorus may have entered the marsh via runoff from the McMaster University campus).

Premazzi & Provini (1985) listed several environmental parameters that are important in controlling phosphorus release from sediments: oxidation-reduction status; ambient temperature; sediment composition; and concentration gradient from pore-free water. In this investigation, release rates were measured under constant laboratory conditions free of mixing, resuspension or changes in ambient temperature. The only differences that remain to be quantified are: microbial community structure, which we will examine in Chapter 2 and the amount of TP in sediment samples as well as sediment binding capabilities which we will examine in Chapter 3. Spatial variability in sediment phosphorus release rates of Cootes Paradise is probably a reflection of historical loadings. However, further study is required to uncover the causal mechanisms, whether biological or chemical in nature.

Table 1.1 Characteristics of Cootes Paradise Marsh sampling stations. TP concentrations correspond to ambient concentrations in the water column for the various sediment-sampling stations.

Station	Depth (cm)	TP (µg/L)	Description
CP5*	54.3	312.0	Sewage lagoon
CP3*	69.5	94.8	Spencer's Creek outfall
CP23	40	NA	Surrounded by *Typha sp.*, relatively sheltered
CP8*	36.3	217.4	Remnant marsh site, *Typha sp., Potemogeton pectinatus*
CP10*	65.5	148.9	Close to shoreline, relatively sheltered
CP24	40	NA	Embayment area
CP17*	30.6	377.2	Sheltered Cut
CP1*	95.6	162.1	Open water
CP19	50	NA	Open water
CP20	80	NA	Open water
CP21	70	NA	Open water
CP22	230	NA	Outlet to Hamilton Harbour

* Depth and TP values averaged from 1993-2000; all other stations correspond to 1999 values only (Chow-Fraser, unpub. data); NA = not available

Table 1.2 Soluble reactive phosphorus (SRP) release rates from sampled sediment of 12 stations within Cootes Paradise Marsh.

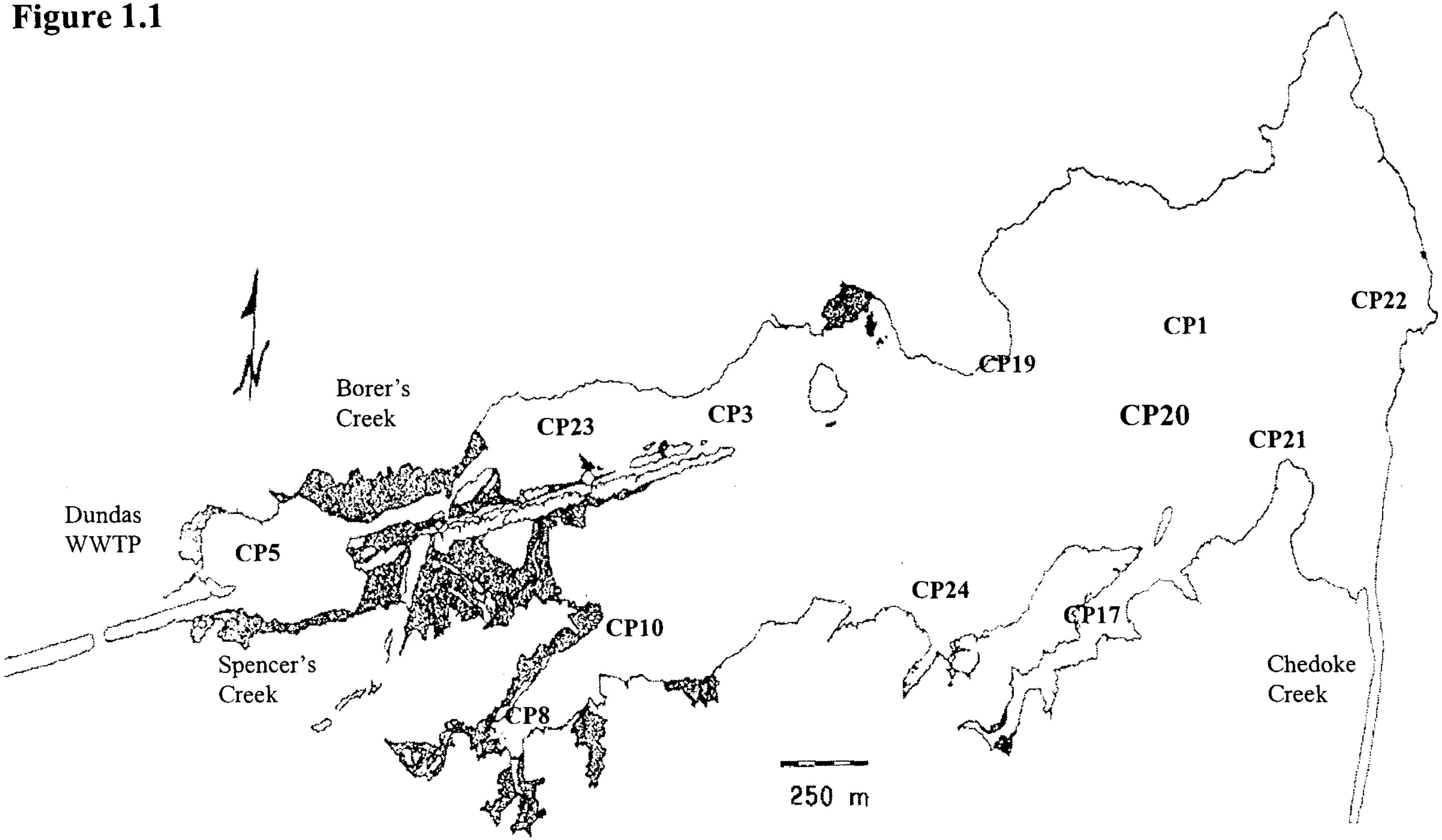
Station	SRP Release Rate ($mg\ m^{-2}\ d^{-1}$)	Standard Error
CP1	4.56	0.46
CP3	5.91	0.69
CP5	17.18	1.56
CP8	15.18	1.05
CP10	1.67	0.66
CP17	28.28	6.58
CP19	1.80	0.16
CP20	4.30	0.21
CP21	4.49	0.81
CP22	9.31	0.83
CP23	5.81	0.89
CP24	0.96	0.12

Table 1.3 Results of the one-way ANOVA to test for spatial variability in phosphorus release rates from Cootes Paradise Marsh sediment.

Source	**Degrees of Freedom**	**Sum of Squares**	**Mean Square**	**F Ratio**	**Prob>F**
Site	12	2344.4627	195.372	51.0692	<0.0001
Error	26	99.4664	3.826		
C. Total	38	2443.9291			

Figure 1.1 Map of Cootes Paradise Marsh. Approximate locations of sampling stations are indicated in bold.

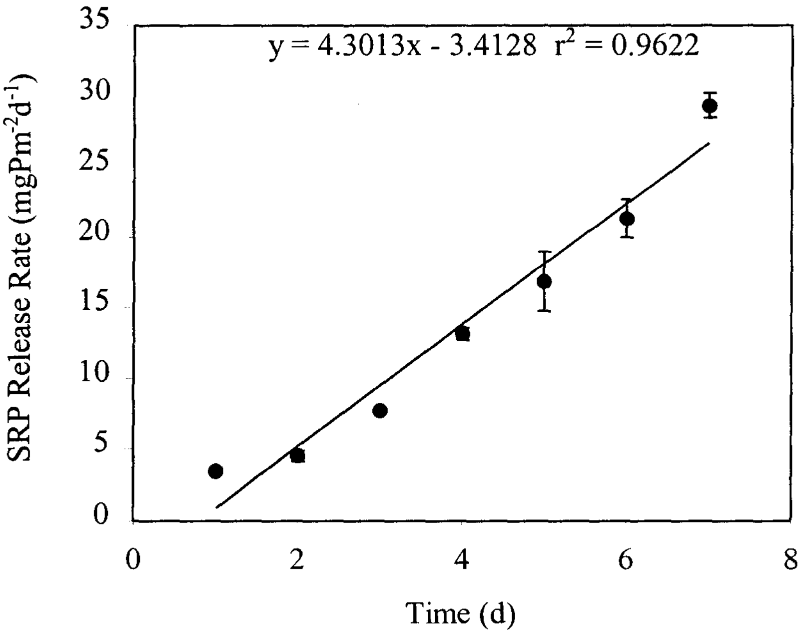
Figure 1.1



Borer's Creek
Dundas WWTP
CP5
CP23
CP3
CP19
CP1
CP22
CP20
CP21
CP10
CP24
CP17
CP8
Spencer's Creek
Chedoke Creek
250 m

Figure 1.2 Example of sediment soluble reactive phosphorus (SRP) release rate determination (for station CP20).

Figure 1.2



y = 4.3013x - 3.4128 $r^2 = 0.9622$
SRP Release Rate ($mgPm^{-2}d^{-1}$)
35
30
25
20
15
10
5
0
0
2
4
6
8
Time (d)

Figure 1.3 **Mean sediment release rates and lake trophy in worldwide lakes from the literature (hyper = hypereutrophic; eu = eutrophic; meso = mesotrophic; oligo = oligotrophic). Horizontal lines represent ranges. (From Nürnberg 1988)**

Figure 1.3

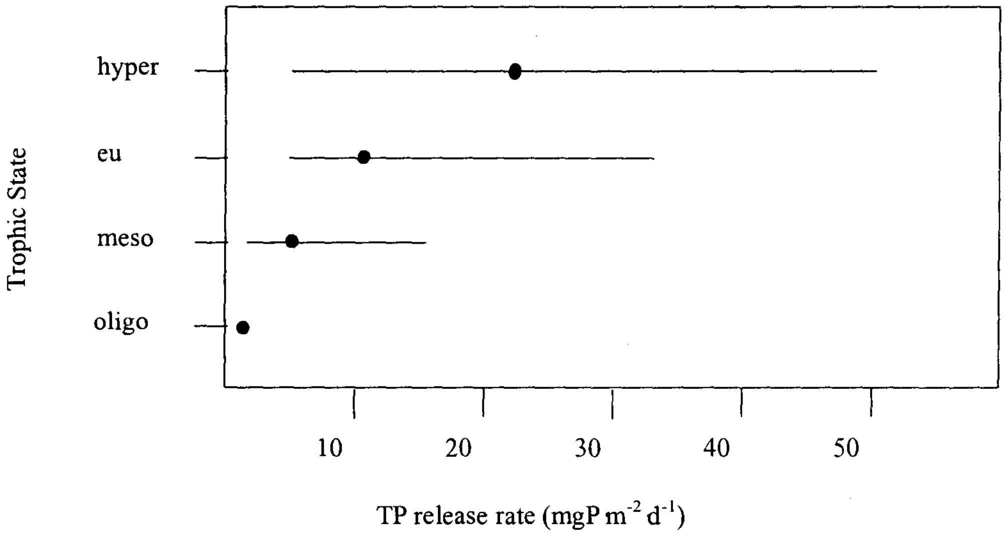

Trophic State
hyper
eu
meso
oligo
10
20
30
40
50
TP release rate (mgP m^{-2} d^{-1})

Figure 1.4 **Cootes Paradise Marsh sampling stations with statistically similar soluble reactive phosphorus (SRP) release rates from sediment.**

Figure 1.4

SRP Release Rates (mgP m^{-2} d^{-1})

CP19	1.48
CP1	4.45
CP23	5.86
CP22	9.31
CP8	15.18
CP5	17.18
CP17	28.28

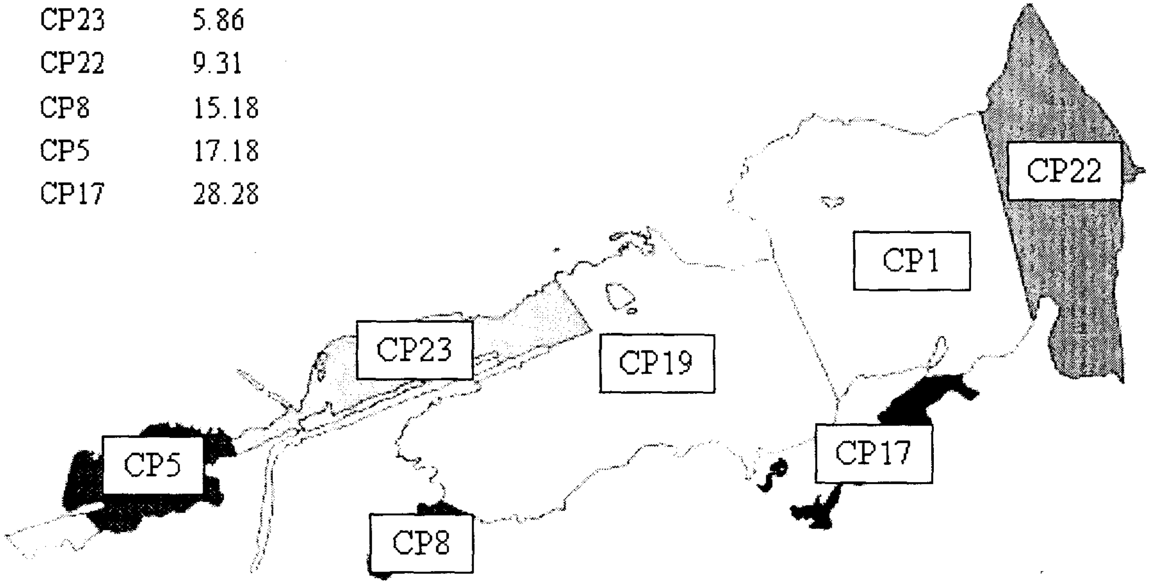
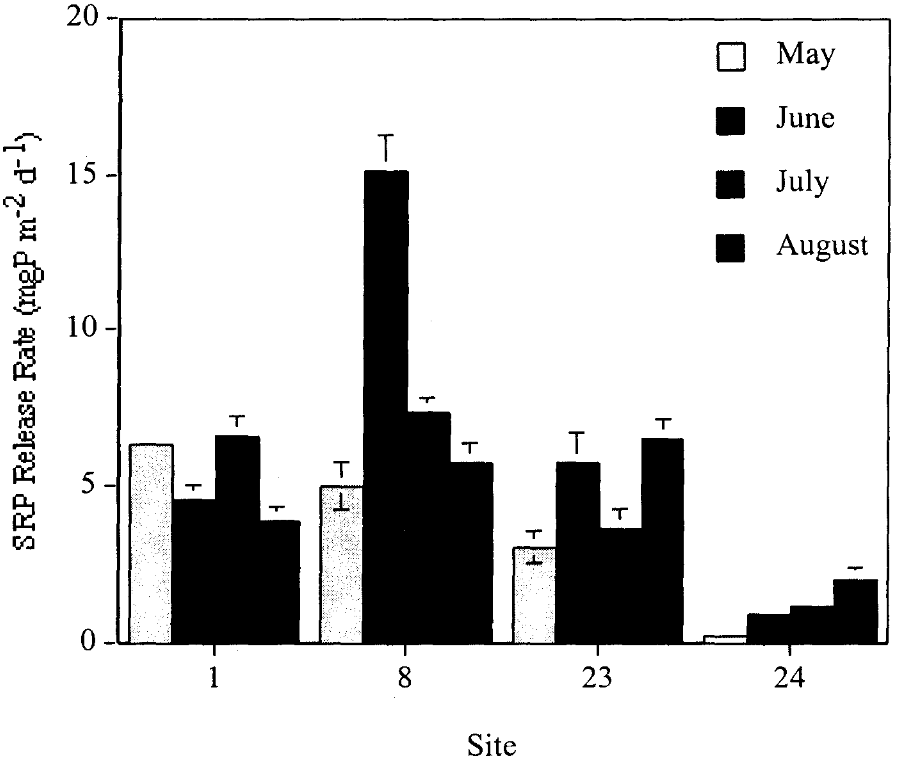


Figure 1.5 Temporal variability in sediment SRP release rates from four stations within Cootes Paradise. Vertical bars are standard errors.

Figure 1.5


20
15
10
5
0
SRP Release Rate (mgP m^{-2} d^{-1})
1
8
23
24
Site
☐ May
■ June
■ July
■ August

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CHAPTER 2

Characterization of the Benthic Microbial Community in Cootes Paradise Marsh and Investigating its use as a Predictor of Sediment Phosphorus Release

ABSTRACT

The role of microorganisms in phosphorus release from sediments is not well understood. It is typically assumed that bacteria play an indirect role through a lowering of the redox potential; however, more recent work has suggested that they play a more direct role. In an attempt to increase our basic understanding of bacterial function in controlling sediment release rates, we characterized the benthic microbial community for the twelve sites in Chapter 1 on the basis of sole-carbon-source utilization using BIOLOG GN plates. The relationships among samples were determined by PCA and linear regression was used to detect interactions between the bacterial community and rates of phosphorus release from the sediment. Separation of stations was essentially based on the microbial community's ability to use simple carbohydrates versus polymers as well as phosphorylated substrates. We found that 49% of the spatial variance in SRP release rates could be explained by PC1, while PC2 explained 53%.

activity as a possible factor. It has been assumed that microbes play only an indirect role in phosphorus release by producing reduced conditions through respiration (Gächter et al. 1988). However, Wetzel (1999) demonstrated that bacteria can also mineralize organic phosphorus (OP) to inorganic forms or release P upon cell death and thus directly contribute to the P-release process. In addition Gächter et al. (1988) and Sinke et al. (1993) demonstrated that bacteria may act as a sink for phosphorus through uptake. Gächter & Meyer (1993) speculated that the amount of phosphorus released from sediments is controlled in part by the bacterial demand and that many species are able to store phosphorus if it is in excess. In fact, it is well known that different bacterial species can sequester phosphorus under both aerobic and anaerobic conditions (de Montigny & Prairie 1993). Based on these findings we hypothesize that bacterial abundances would have some effect on the flux of phosphorus across the sediment/water interface since sediments of nutrient enriched areas have been found to yield 10^3 to 10^4 times more anaerobes than non-impacted sediments within the same system (Reddy et al. 1999). In addition, the microbial community may respond to detrimental environmental impact by species replacement and reduction in diversity (Reddy et al. 1999). Reddy et al. (1999) also found that the production of microbial enzymes can be altered by nutrient enrichment and suggested that changes in activity levels may be used as indicators of wetland eutrophication; alkaline phosphatase (APA) was found to decrease with phosphorus enrichment, while B-D glucosidase increased. However, the linkages between microbial abundances and/or species composition with phosphorus release rates remain to be formally tested.

Differences in taxonomic composition of the microbial community may be indicative of differences in environmental conditions (Reddy et al. 1999) and if a standardized methodology can be used to characterize taxonomic variability in sediment, classification may be a useful bioassessment tool. Garland & Mills (1991) developed an approach to characterize microbial communities based on a technique that identifies bacterial isolates which was developed by BIOLOG, Inc. (Hayward, CA). This approach is centred on sole-carbon-source utilization, which uses BIOLOG gram negative (GN) microplates containing 95 separate carbon sources and a control well, each of which includes the redox dye tetrazolium violet. Plates are inoculated with environmental samples, such as sediment solutions, and are incubated. The formation of colour indicates that microbes present can utilize the particular substrate, and utilization patterns are used to characterize the microbial communities.

In Chapter 1, we found that phosphorus release rates varied significantly from site-to-site as well as from month-to-month for samples collected from the same station. We speculated that at least part of these variations may be a function of differences in the bacterial communities, which may exhibit successional patterns and may be due to substrate availability. We therefore collected sediment samples to classify the microbial communities on the basis of carbon-utilization pattern and will relate these results to respective release rates.

METHODS

Sampling

Sediment samples used to characterize the microbial community were collected in June 2000 at approximately the same locations as those in Chapter 1 (with the exception of CP20). They were stored at 4°C for 24 hours until further analysis.

Characterization of the Microbial Community

A 1:10 soil solution for each site was prepared using 54 mL of 1% saline solution and 6 g of sediment in a 150 mL sterilized bottle. The solution was shaken, allowed to settle and poured into a petri plate. The sample was then aspirated with a pipette to obtain a clear solution. An 8-channel micropipettor was used to inoculate the wells of the GN BIOLOG plates with 150 L of each solution (BIOLOG plates inoculated in triplicate). The plates were incubated at room temperature in the dark. Colour development measurements were taken after 24 hours with a BIOLOG MicroStation plate reader using MicroLog 3N Software (Release 3.50 Version DE, (BIOLOG Inc. 1994). Raw difference data sets were obtained by subtracting the absorbance of each well from the absorbance of the control well. We then divided these values by the average well colour development (AWCD), the mean difference among absorbance values of the 95 C-source wells and the control well. This procedure reduces the influence of rate of colour development of the classification of samples (Garland & Mills 1991). The relationships among different samples were determined by PCA using SAS

Jmp (SAS Institute Inc. 1982). Multiple linear regression was used to test the predictive power of the PC scores with respect to SRP release rates.

RESULTS

Sediment samples from the 12 sites within Cootes Paradise did not have any immediate distinctive patterns on the basis of sole-carbon-source utilization (Fig. 2.1 ordination plot). Generally, separation along the first PC, which explained 19% of the variance in the data set, was based on the use of polymers for open water stations (i.e. CP1), whereas the communities of stations proximal to external nutrient sources (i.e. CP5) used only carbohydrates and phosphorylated substrates (Table 2.1). Separation along the second PC, which explained 11% of the variance, was based on the use of carboxylic acids and amino acids for stations on the positive end of the axis and carbohydrates on the negative end. No site-type trend could be extracted for the second axis. The third and fourth PCs explained 8.4 and 6.5% of the variance, respectively. Very few substrates were correlated with these axes (Table 2.2) and no site-type pattern was observed for either axis. We further performed a spatial graphical examination of PC1 scores (Fig. 2.2) for comparison to results obtained in Chapter 1 concerning differences among sites in phosphorus release rates from sediment. With the exception of a large discrepancy between CP1 and CP21, generally the spatial trend was remarkably similar: adjacent stations could be combined together. Open water sites tended to have negative PC1 scores, while those closer to external P sources had positive scores (with the exception of CP17 which had a negative score).

There was no significant relationship between SRP release rates and PC1 scores ($p = 0.24$) or PC2 scores ($p = 0.16$) when all data were pooled; however, when the release rate of CP17 (open circle), which was obviously an outlier (tested using Mahalanobis distance), was removed, we found a highly significant relationship ($r^2 = 0.771$, $p = 0.0057$).

DISCUSSION

The PCA was not able to distinguish among site types other than the open water stations, however this may have been a function of the small number of stations representing the marsh site and sewage lagoon. Had there been additional stations of the same site type, a more distinctive pattern may have emerged. The PCA plot revealed spatial separation of the sites along both axes. This separation is attributable to differences in the microbial community's ability to utilize a particular carbon source. Interpretation of the functional differences in these communities based on utilization of specific carbon sources would require a better understanding of microbial physiology, which is beyond the scope of this study. However, based on the findings of Chapter 1, we speculated that differences among stations may be somewhat attributable to the distance from the Dundas WWTP. Sewage is an exceptional medium for bacterial growth; it consists of carbohydrates, lignins, fats, and proteins (Bolton & Klein 1971). The variety of substrates facilitates the development of a wide variety of microorganisms able to break down these various substances (Gainey & Lord 1952). Prior to 1919, West Pond (CP5) received raw sewage, at which point primary treatment began. We theorize that the raw sewage had already been broken down into smaller carbohydrate molecules by the time it entered West Pond and that the microbial community has shifted from one with enzymes capable of mineralizing complex molecules into a community that uses smaller sugar molecules; this is probably the reason why we see a community that does not grow well on polymers and the separation along PC1.

The ability of microbes to use phosphorylated substrates was of particular interest to us because of the cultural eutrophication that has occurred in Cootes Paradise Marsh. Results for stations CP5, CP3, CP22, CP23 and CP8 were all associated with positive PC1 values, suggesting that microbes at these sites were better able to utilize phosphorylated substrates. These sites are all exposed to external phosphorus loading. For example, CP8 is a remnant marsh site which presumably supports an environment favourable for microorganisms that are able to mineralize organic matter (Kairesalo & Matilainen 1994) and decomposition of organic material can often result in the release of phosphate (Wetzel 1999). CP23 and CP3 support a substantial waterfowl population and are probably enriched through their excrement. In addition, CP3 is located at the outfall of the Desjardins Canal, which drains Spencer's Creek, a largely agricultural watershed, responsible for 30% of the annual external total phosphorus load into Cootes Paradise (XCG Consultants Ltd. 1997). CP22 is located adjacent to Hamilton Harbour, which is another source of P to the marsh, accounting for 22% of the annual external total phosphorus load (XCG Consultants Ltd. 1997). By comparison, results for stations CP1, CP20, CP10 and CP24 (open water sites) were associated with negative PC1 scores. We speculate that these open water sites are too far removed from external phosphorus sources.

On the other hand, stations CP17 and CP21 are unrelated to either PC1 or PC2. Even though CP17 is located at a combined sewer overflow (CSO) and was expected to have a similar microbial community to the lagoon site, bacteria at this sites were only able to grow to a limited extent on phosphorus-containing carbon substrates, the reason for which requires further study.

Separation of the sites along PC2 could not be explained given our results. The analysis indicated that carboxylic acids, a major component of fats and lipids in living organisms, were correlated with the positive end of PC2; however, we were unable to distinguish between site types on this basis.

Together PC1 and PC2 explained a significant portion of the variance in SRP release rates from sediment when CP17 was removed from the analysis. There was a positive relationship between PC1 and SRP release rate. The relationship between these two variables was also apparent from graphical examination of the PC1 scores (Fig. 2.2). A negative relationship between PC2 and sediment release rate was also observed, although the reasons underlying this relationship is not clear. The observation that CP17 was an outlier was in itself interesting. CSO contains sewage solids, human bacteria and viruses, metals, oil and grease. The bacteria at this site evidently use only specific substrates, substrates not accounted for in the BIOLOG plates. Future studies should identify these bacteria and their substrates and compare the findings with other CSO sites.

Several improvements can be made in future investigations. First, release rates were determined from sediment collected a year prior to microbial community characterization, and we have assumed that the monthly variation in the bacterial community overshadows year-to-year variation. Secondly, analyses were performed on plates from incubations after only 24 hours. This means that microorganisms with a longer growth period (those that require longer to produce a sufficient density of stained cells) were not included in this analysis. In addition, the characterizations do not necessarily reflect in situ conditions, but only the potential for particular species within the microbial community to utilize the substrates. Thirdly, release rates had been determined from frozen rather than fresh sediment; since the freezing should have destroyed the existing microbial population, the release rates represent diffusion alone, independent of any bacterial mineralization, which would take place under natural conditions.

The results of this study led us to conclude that bacterial processes play a substantial role in regulating phosphorus exchange across the sediment/water interface. This conclusion has been supported by many authors (Doremus & Clesceri 1982; Premazzi & Provini 1985; Gächter et al. 1988). For example, Gächter et al. (1988) found that bacteria grown on phosphorus-limited substrates under aerobic conditions sequestered large concentrations of this nutrient, however, when conditions became anoxic these microorganisms released between 14 to 25% of that stored. The microbial community, therefore, acts as a potentially large sink and source of phosphorus to the overlying water.

Table 2.1 Results of principal component analysis correlations of sole carbon sources to principal component (PC) axes 1 and 2 as indicators of microbial community structure.

PC1		**PC2**	
Carbon source	**r***	**Carbon source**	**r**
Polymers		**Carbohydrates**	
Dextrin	-0.843	D-melibiose	-0.618
glycogen	-0.857		
tween 40	-0.852	**Carboxylic acids**	
		α-hydroxybutyric acid	0.656
Carbohydrates		β-hydroxybutyric acid	0.629
N-acetyl-D-galactosamine	0.808	sebacic acid	0.638
N-acetyl-D-glucosamine	-0.817		
D-fructose	-0.793	**Amino acids**	
D-galactose	-0.729	D,L-carnitine	0.655
D-mannose	-0.844		
D-mannitol	-0.77		
D-raffinose	0.615		
L-rhamnose	0.661		
D-sorbitol	0.620		
Carboxylic acids			
citric acid	-0.787		
D-galactonic acid lactone	0.689		
D-galacturonic acid	0.700		
D-gluconic acid	-0.839		
D-glucuronic acid	0.768		
Phosphorylated chemicals			
glucose-1-phosphate	0.613		

* - regression coefficient

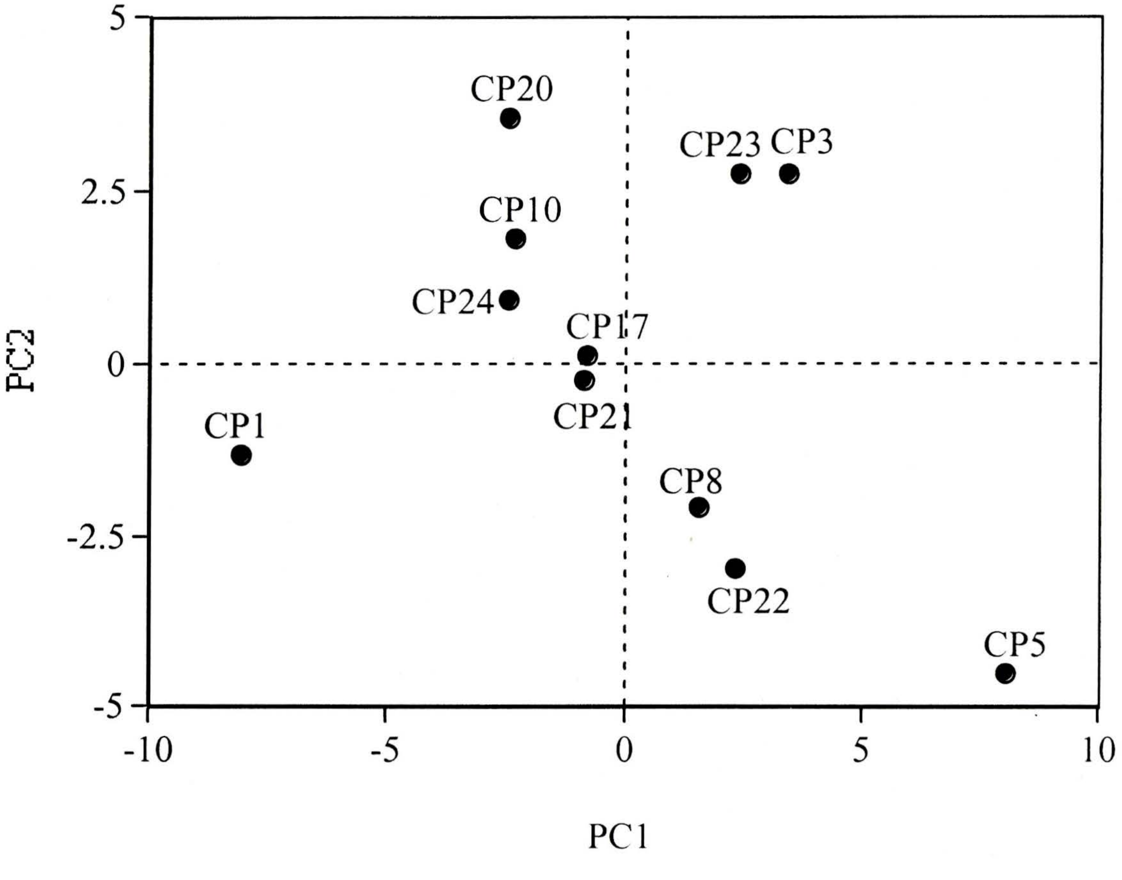
Table 2.2 Results of principal component analysis correlations of sole carbon sources to principal component (PC) axes 3 and 4 as indicators of microbial community structure.

PC3		**PC4**	
Carbon Source	**r***	**Carbon Source**	**r**
Carbohydrates		**Carbohydrates**	
xylitol	0.643	α-D-lactose	0.623
		sucrose	-0.698
Carboxylic acids		D-trehalose	-0.773
D-glucosaminic acid	0.747		

* - correlation coefficient

Figure 2.1 **Principal component analysis ordination plot of Cootes Paradise Marsh sampling stations based on differences in microbial community structure. Scores for the first two principal components are plotted.**

Figure 2.1



5
2.5
0
-2.5
-5
-10
-5
0
5
10
PC2
PC1
CP20
CP23
CP3
CP10
CP24
CP17
CP21
CP1
CP8
CP22
CP5

Figure 2.2 Site-to-site variation in PC1 scores (in bold).

Figure 2.2

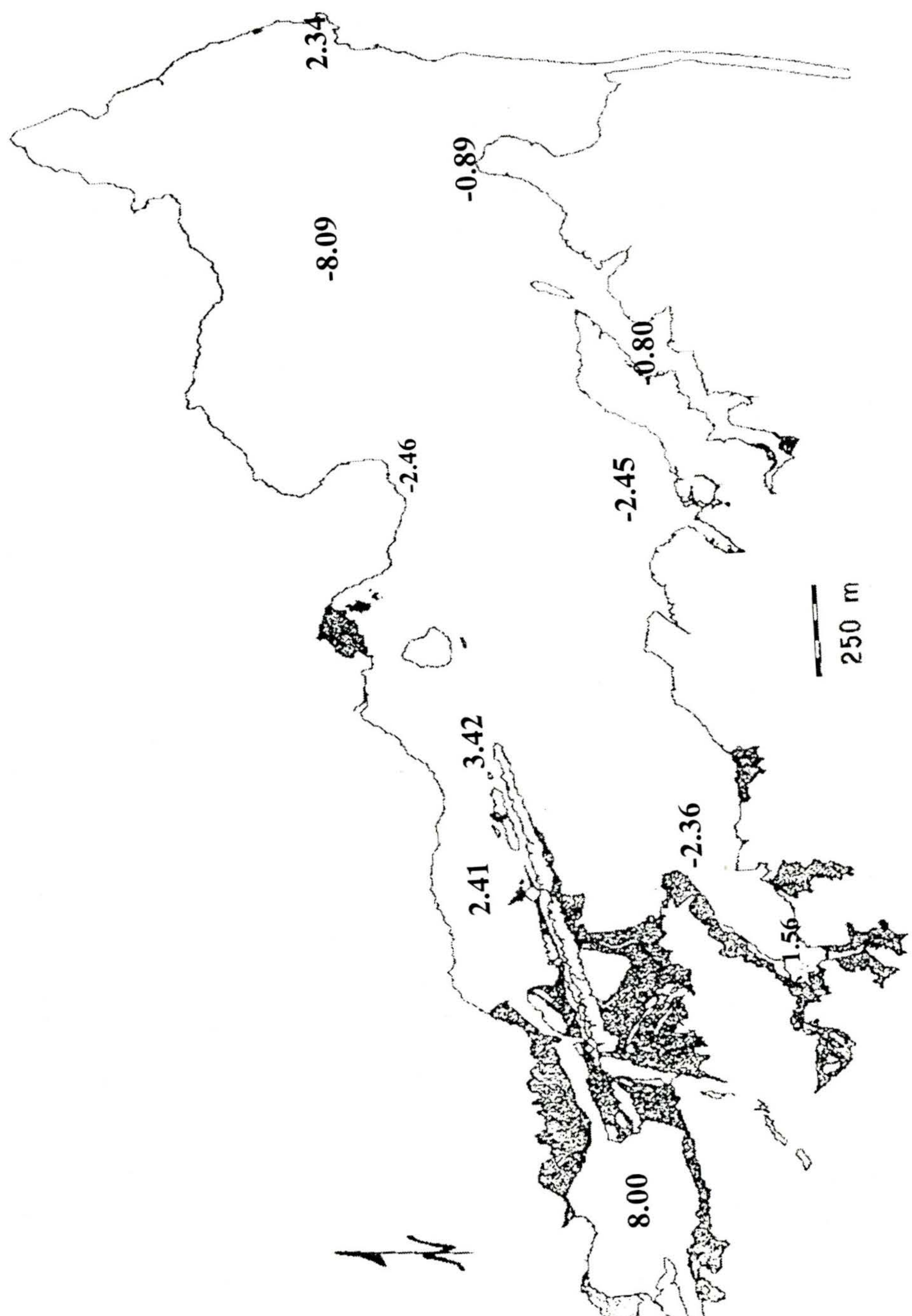
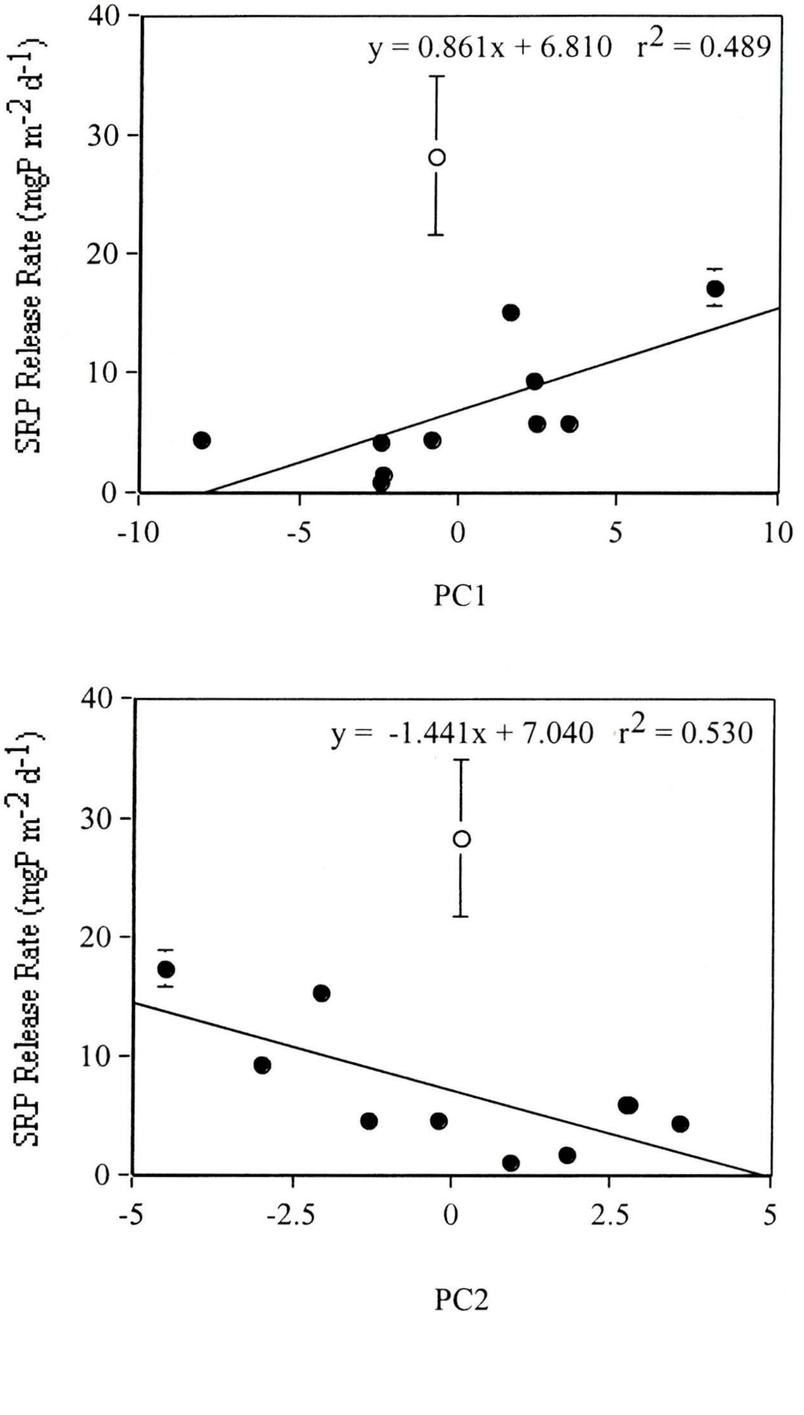


Figure 2.3 **Relationship between microbial sole-carbon-source utilization and SRP release rates from sediment. Vertical bars are standard errors.**

Figure 2.3


y = 0.861x + 6.810   r^2 = 0.489
SRP Release Rate (mgP m^{-2} d^{-1})
40
30
20
10
0
-10
-5
0
5
10
PC1
y = -1.441x + 7.040   r^2 = 0.530
SRP Release Rate (mgP m^{-2} d^{-1})
40
30
20
10
0
-5
-2.5
0
2.5
5
PC2

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CHAPTER 3:

Predictive Factors of Phosphorus Release Rates from Hypereutrophic Marsh Sediment

ABSTRACT

Previous work (Chapters 1 and 2) has suggested that the microbial community plays an important role in determining the extent of phosphorus release from sediments. In this study we evaluate the use of chemical and physical factors in addition to biological factors to develop a predictive model of phosphorus release rates from the hypereutrophic sediments of Cootes Paradise Marsh. Time of sampling, ambient temperature, oxic state and the distribution of phosphorus fractions in sediment were investigated. Although oxic state emerged as the primary influencing factor, confirming the probable role of Fe-P interactions in controlling P flux from the sediment, both ambient temperature and time of sampling had significant effects that masked any influence of iron content in the sediment. We attribute these latter effects to differences in the microbial community in sediment cores which may vary over time and whose activity may be moderated by the temperature of overlying waters. This finding reinforces our previous suggestion that the community structure of sediment microbes may play a more important role in regulating internal loading in eutrophic marsh sediment than has been previously acknowledged.

INTRODUCTION

Current methods used to estimate internal loading include measuring in vitro simulations of phosphorus release from sediment cores, measuring seasonal accumulation of phosphorus in anoxic hypolimnia, or calculating phosphorus release using a mass-balance approach. Although all of these methods are time-consuming and labour-intensive to carry out, measurements of in vitro release rates can be made more attractive to researchers if the key environmental factors that govern phosphorus release may be identified so that rates can be predicted from simple environmental variables.

There are several physical, biological and chemical factors known to influence the release of P from sediments (see Chapter 2, Introduction). Although conventional wisdom is that microorganisms only have an indirect role in phosphate release from the sediments, there is now speculation that they act more directly to control exchange across the sediment/water interface (Chapter 2; Jensen et al. 1992; Gächter & Meyer 1993). This is based on the premise that bacteria are capable of sequestering phosphates from the substrate or water, and can therefore reduce the release of phosphorus to the overlying water column (Gächter & Meyer 1993). Studies have yet to provide unequivocal evidence for this, but the unexplained spatial and temporal variance in measurements of release rates from sediment collected in the same wetland (i.e. Cootes Paradise Marsh, Chapter 1) may be attributable to difference in microbial communities.

According to a review of the literature, the most important environmental parameters that control phosphorus release from sediments should include the following: oxidation-reduction status; degree of mixing; resuspension and/or bioturbation activities; amount of bacterial activity; ambient temperature; sediment composition; and concentration gradients from pore-free water (Premazzi & Provini 1985). Even though it is beneficial to assess the predictive power of all factors simultaneously, it is logistically impossible to incorporate all the parameters listed above in a single study. Therefore, we have chosen to examine a subset of these parameters that are more readily manipulated under laboratory conditions: oxidation-reduction status, ambient temperature, and sedimentary P species. As a surrogate for changes in microbial community structure, and presumably bacterial activity, we have also chosen to examine temporal changes in activity by collecting sediments monthly over the summer season.

Description of Study Site

West Pond (CP5, Fig. 1.1, Chapter 1) is a sewage lagoon in the marsh, located just east of the Dundas WWTP outfall. Because of its proximity to the WWTP outfall, it has hypereutrophic sediments and water (Chow-Fraser 1999) compared with other long-term monitoring sites in residual marsh areas such as CP8. Based on the assumption that the highest phosphorus release rates are associated with the most eutrophic sediments, we feel that a study based on West Pond sediment would indicate the magnitude of potential internal loading. By collecting sediment from only one site, where there was no vegetation, we also eliminated any confounding effects of macrophyte uptake and/or secretion of nutrients. Finally, since West Pond currently has only a fringe of emergent vegetation, it is more characteristic of a shallow lake than a typical wetland and results from this study may be directly compared with data from published studies of shallow lakes.

METHODS

Sampling

Sediment samples were collected monthly (June-August 2000). Excess water was drained and samples were stored in Freezer Ziploc bags at 4°C in the dark for approximately 24 hours prior to experimentation.

Release Rate Determination

Phosphorus release experiments were conducted in the manner indicated in Chapter 1; however we included several treatment effects. Simulated oxic and anoxic conditions were created using methods described by Boström & Pettersson (1982) through the addition of nitrate (5 mg N l^{-1}) and acetate (10 mg C l^{-1}), respectively. Nitrate stabilizes the redox potential and prevents release while acetate stimulates microbial activity, increasing oxygen consumption and thus lowering the redox potential to a point where iron-phosphate complexes dissolve. Jars designated for "anaerobic" treatment were covered with parafilm and foil to prevent gas exchange. Jars designated for "aerobic" treatment were covered with foil that received several puncture holes. All jars were incubated in dark growth chambers for up to 8 days at 10, 15, 20 and 25°C. Three jars from each treatment were removed from their respective growth chamber daily. Water samples were extracted and analyzed for SRP and total phosphorus (TP).

Sediment Analysis

Water content was determined by drying pre-weighed wet samples at 51°C for 24 hours in a dehydrator. Sediment TP was quantified according to the ignition method described by Andersen (1975) from which organic matter was measured as loss on ignition (550°C for 1 hour). Sediment fractionation was accomplished using the sequential extraction scheme described by Hieltjes & Lijklema (1980). Although this method is commonly used, it does not identify organic phosphates originating from plant material, algae, zooplankton or benthos (Danen-Louwerse et al. 1993). This should not be of great consequence in this study since organic matter is typically very low in Cootes Paradise (~10%, Chow-Fraser 1999). This extraction scheme produced 4 fractions: NH_4Cl-extractable SRP (loosely sorbed P), NaOH-extractable SRP (P bound to Fe/Al), HCl-extractable SRP (P bound to Ca) and residual-P (calculated as the difference between sediment P concentration and the sum of the fractions).

Statistical analyses were performed with SAS Jmp (SAS Institute Inc. 1982); stepwise multiple regression was used to assess the predictive power of each factor.

RESULTS

Release Rates

Depending on temperature, SRP release rates ranged from 0 to 0.74 and from 3.71 to 18.89 mgP m^{-2} d^{-1}, respectively for aerobic and anaerobic treatments (Figures 3.1 and 3.2). As expected TP release rates were higher, ranging from 0 to 1.95 and 9.39 to 19.66 mgP m^{-2} d^{-1}, respectively (Figures 3.3 and 3.4). The "oxic" rates reported in this study are bracketed by published data, although our maximum values for anoxic conditions were twice that of the highest reported for lakes (see Table 3.1).

Generally, phosphorus concentrations in overlying water increased linearly with time for "anaerobic" treatments. Release rates measured in both TP and SRP increased from June to August, and from 10 to 25°C. By comparison, rates associated with the "aerobic" treatments were more variable and more difficult to summarize. In most treatments, concentrations declined in the initial few days of incubation, which we attributed to a consequence of phosphate adsorption processes or uptake by the microbial community.

There is a significant linear relationship between SRP and TP release rates (Fig. 3.5). Since there is a high correlation between SRP and biologically available P (Nürnberg & Peters 1984), it can be assumed that most of the P released from West Pond sediment is biologically available (Nürnberg 1988). The slope of the relationship is 0.74, indicating that the proportion of SRP declines predictably with magnitude of P release. The ratio of SRP:TP release rates dropped from 100% to approximately 75% at 20 mgP m^{-2} d^{-1}, and we suggest that this deviation from unity at high P release reflects phosphate adsorption on Fe hydroxides during oxic filtration in sample processing. Even though the slope of the SRP:TP relationship is less than 1.0, the relationship is predictable, and since determination of TP release rates is much more labour-intensive, we decided to focus the remainder of the investigation on SRP release rates.

Sediment composition

The composition of sediment fractions did not vary significantly from month to month (Table 3.2), indicating that the composition is conserved through the season for these fractions. We are confident that extractions were complete since the sum of all individual extractions was recovered via the TP determination. There was virtually no residual P.

Stepwise Multiple Regression

We entered all of the variables into a stepwise multiple regression analysis (Table 3.3). Oxic state was found to be the best predictor, explaining 77% of the variation, while temperature explained an additional 9% and time of sampling 0.9%. The corresponding multiple regression equation was:

$$\text{SRP RR (mgP m}^{-2}\text{ d}^{-1}) = 0.28\text{temperature} + 4.59\text{oxic state} - 0.52\text{month} + 0.27$$

Eq. 3.1

$(r^2 = 0.85)$ $(P<0.0001)$

Since the oxic state had such an overriding effect on release rates, we separated the data by oxic states and subjected the data to further analyses to determine if the importance of other factors may emerge. In the case of simulated aerobic conditions, temperature was the only other factor that influenced release rates (Table 3.4). The corresponding predictive equation was obtained:

$$\text{SRP release rate (mgP m}^{-2}\text{ d}^{-1}) = 0.051\text{temperature} - 0.54$$ **Eq. 3.2**

$(r^2=0.72)$ $(P<0.0001)$

By comparison, under anaerobic conditions, both temperature and time of sampling were shown to affect release rates (Table 3.5). The corresponding multiple regression equation was obtained:

$$\text{SRP release rate (mgP m}^{-2}\text{ d}^{-1}) = 0.50\text{temperature} - 1.05\text{month} + 1.1$$ **Eq.3.3**

$(r^2 = 0.66)$ $(P<0.0001)$

In neither case did any of the chemical fractions significantly explain more of the residual variation in the model. The relationships between SRP release rate and temperature as well as release rate and time of sampling (month) were each tested using an ANCOVA to determine if they differed depending on oxic state and ultimately if breakdown of the original predictive equation was warranted. Results indicated a significant interaction between temperature and oxic state (p<0.0001), revealing that the relationship between release rate and temperature was significantly different for oxic and anoxic conditions (i.e. the slopes were significantly different, Fig. 3.6). On the other hand, results indicated that the effect of time of sampling on release rate did not differ significantly (no interaction between time and oxic state, p=0.0651) between aerobic and anaerobic conditions (i.e. the slopes were not significantly different, Fig. 3.7).

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DISCUSSION

The total phosphorus (TP) concentration in sediment is not necessarily a good predictor of phosphate release as it does not reveal any information regarding the underlying cause(s) of release. By contrast, significant information can be obtained from sediment P fractionation schemes and this method has often been used to develop models to predict P release in lakes even though the classifications have been criticized by Graetz & Nair (1999) as being overly simplistic. Numerous schemes have been developed to identify different forms of P in sediment using acids, bases, reducing or oxidizing agents. Most of these methods target inorganic P (IP) fractions, although some have included organic estimates as well (Graetz & Nair 1999). Usually, organic phosphorus (OP) is calculated as the difference between sediment TP and IP since the extraction of organic fractions is time-consuming and expensive (Newman & Robinson 1999). In natural wetlands, however, OP can constitute >50% of the sediment TP (Newman & Robinson 1999) and should be directly measured in these systems. Assuming that OP does not constitute a large fraction of the TP in sediment, this approach is acceptable and in the case of Cootes Paradise, this assumption is valid (approximately 10% organic, Chow-Fraser 1999). We caution, however, that this assumption is not generally upheld in undisturbed wetlands where OP can typically account for >50% of the sediment TP (Crosbie & Chow-Fraser 1999; Newman & Robinson 1999).

There does not appear to be consensus in the literature on the usefulness of chemical species in sediment for predicting P-release (Table 3.6). Initially, Boström (1984) was even doubtful that a simple relationship between sediment composition and P-release would emerge. Subsequently, Nürnberg et al. (1986), Nürnberg (1988) and Ostrofsky et al. (1989) all found significant correlations between sediment TP and release; however, results in a later study by Jensen et al. (1992) discounted this relationship. Instead, they found that the ratio of Fe to P was negatively correlated with release rates, which appeared to be at least consistent with observations of Ostrofsky et al. (1989) who also found loosely-sorbed and Fe-bound P to be significantly correlated with release rates (Table 6). In general, our results agree with those of Jensen et al. (1992) and those of Premazzi & Provini (1985), who concluded that differences in P-release rates could not be attributed to variation in sediment composition.

Although the easiest explanation for the negative release rate in aerobic sediments is that phosphate became adsorbed to sediment, we speculate there are other reasons. First, had adsorption been the only cause, negative release rates would have been observed over the entire season and at all temperatures. This was not the case. Secondly, the concentrations of $CaCO_3$-, Fe-, Al-and Ca-bound phosphates in the sediment did not vary significantly over time in this study (Table 2), even though release rates varied significantly from month to month (Eq. 3). These observations suggest that a factor other than adsorption is responsible for the negative release, and we hypothesize that bacteria are largely responsible for removing P from the overlying water, and that temperature and temporal variability in P uptake is a result of seasonal succession of the microbial community.

Soil microbial communities respond rapidly to nutrient loadings, and enrichment can directly alter the size and diversity of that community (Reddy et al. 1999). Sinke et al. (1993) has proposed that bacterial processes have the potential to largely regulate the seasonal dynamics of phosphate concentration in the overlying water. One mechanism that may facilitate changes in the microbial communities in Cootes Paradise Marsh is the addition of organic carbon (acetates) and nitrates (resulting from aeration of ammonia) to West Pond from the Dundas WWTP effluent (Chow-Fraser et al. 1998). The addition of acetate as a carbon source results in anaerobic conditions through increased metabolism and oxygen consumption. This induced anoxia permits anaerobic bacteria, such as methanogens (for which acetate is the major substrate) to flourish (Levett 1990), and consequently alters the structure of the original community. On the other hand, the addition of nitrate promotes aerobic bacterial growth and hence, stimulates adsorption of phosphates. It is therefore probable that the bacterial community in Cootes Paradise is responding to variations in effluent-induced oxic and anoxic conditions, and is therefore playing a substantial role in regulating P flux across the sediment/water interface.

Increases in temperature accounted for a substantial proportion of the variability in sediment P release. Temperature is often overlooked as an environmental parameter to be included in predictive models. Jensen and Andersen (1992) examined SRP release in aerobic sediments and discovered that the effects of lake water temperature was more pronounced than that of nitrate and pH, accounting for greater than 70% of the seasonal variation in gross internal loading. Temperature has an indirect effect on P release by increasing microbial activity and hence, oxygen consumption, as well as increasing organic matter mineralization, which liberates inorganic phosphates (Boström & Pettersson 1982), although we acknowledge that sediment bacteria do not necessarily release P when they mineralize settled organic matter (Gächter & Meyer 1993). Although we expected little or no P release in the aerobic trials, due to the widely held notion that aerobic sediment surfaces act as a trap (Gächter & Meyer 1993), in many cases, we did observe significant P-release, especially at higher temperatures. This suggests that at sufficiently high temperature, even aerobic environments may experience lower redox potential due to increased activity of the microbial community in the sediment (Boström & Pettersson 1982).

Besides oxic state and temperature, we also found significant differences in release rates from June to August (Table 5). We could not attribute these differences to variation in the chemical composition of the sediments since sediment fractions did not vary significantly from month to month in this regard (Table 2). Gächter & Meyer (1993) speculated that seasonal changes of the bacterial P pool size can affect transport of P across the sediment/water interface. Sinke et al. (1993) also found that bacteria played a substantial role in uptake by aerobic sediments, although their methodology could not distinguish among the effects of different microbial communities. P-enriched soils tend to have higher numbers of anaerobes (10^3 to 10^4 times higher) compared with unimpacted soils (Drake et al. 1996), and these bacteria are capable of higher production of extracellular enzymes such as alkaline phosphatase and B-D glucosidase (Reddy et al. 1999). Based on these findings and our results from Chapters 1 and 2, we hypothesize that this temporal variability is a function of seasonal microbial succession.

Although oxic state and temperature were overwhelmingly responsible for much of the variation in P release from sediment in this study, the predictability of these factors may not be sufficient to forecast release from different sites within Cootes Paradise. We recommend that future studies be undertaken to establish a link between sediment P-release and the type of enzymatic activity and/or biomass of the microbial communities in aquatic sediments for use as an indicator of internal loading.

Table 3.1 Literature data on average P release rates (mgP m^{-2} d^{-1}), determined from laboratory incubations.

Lake	Condition	TP	SRP	Source
Varese	Oxic	3.39		Premazzi & Provini (1985)
Pusiano	Oxic	1.80-2.16		Premazzi & Provini (1985)
Alserio	Oxic	3.33-3.99		Premazzi & Provini (1985)
Red Chalk	Anoxic	0.05	0.09	Nürnberg (1988)
PT-10	Anoxic	0.04	0.14	Nürnberg (1988)
Chub	Anoxic	1.43	1.17	Nürnberg (1988)
Gravenhurst	Anoxic	5.27	5.89	Nürnberg (1988)
St. George	Anoxic	2.22	2.64	Nürnberg (1988)
Wononscopomuc, deep	Anoxic	7.30	6.51	Nürnberg (1988)
Wononscopomuc, shallow	Anoxic	2.10	2.21	Nürnberg (1988)
Waramaug	Anoxic	9.22	5.11	Nürnberg (1988)
Onondaga	Anoxic		13.3	Auer et al. (1993)
West Pond, Cootes Paradise	Oxic	0-1.95	0-0.74	This study
West Pond, Cootes Paradise	Anoxic	3.71-18.89	3.39-19.66	This study

Table 3.2 Average surface sediment composition for West Pond, Cootes Paradise (fractions are in mg/g d.w.). Values in parentheses are standard errors.

Month	(a) **Loosely sorbed P**	(b) **Fe/Al-P**	(c) **Ca-P**	(d) **TP (Σ(a-c))**	(e) **Measured TP**	(f) **%OM**
June	0.3036 (0.007)	0.9025 (0.025)	0.5223 (0.013)	1.7284 (0.040)	1.769 (0.071)	11.79 (0.076)
July	0.2755 (0.005)	0.9446 (0.031)	0.5164 (0.017)	1.7365 (0.035)	1.589 (0.092)	11.88 (0.249)
August	0.2531 (0.009)	0.9409 (0.055)	0.5604 (0.041)	1.7544 (0.037)	1.730 (0.086)	11.15 (0.010)

Table 3.3 Regressions of potential predictive factors against aerobic and anaerobic SRP release rates (mgP m^{-2} d^{-1}) from West Pond sediment collected June to August 2001.

Parameter	r^2	p
Oxic state	0.756	0.0000
Temperature	0.087	0.0000
Month (June-July)	0.009	0.0490
Month (June&July-Aug)	0.003	0.2676
Loosely sorbed P	0.000	0.6932
Ca-P	0.000	0.6507
Fe/Al-P	0.000	0.5742
TP_{sed}	0.000	0.5937
%OM	0.000	0.5433

Table 3.4 Regressions of potential predictive factors against aerobic SRP release rates (mgP m^{-2} d^{-1}) from West Pond sediment collected June to August 2001.

Parameter	r^2	p
Temperature	0.717	0.0000
Month (June-July)	0.000	0.9358
Month (June&July-Aug)	0.000	0.9907
Loosely sorbed P	0.002	0.7701
Fe/Al-P	0.002	0.7876
Ca-P	0.005	0.6695
TP_{sed}	0.000	0.9019
%OM	0.011	0.5420

Table 3.5 Regressions of potential predictive factors against anaerobic SRP release rates (mgP m^{-2} d^{-1}) from West Pond sediment collected June to August 2001.

Parameter	r^2	**p**
Temperature	0.588	0.0000
Month (June&July-Aug)	0.080	0.0122
Month (June-July)	0.015	0.1426
Loosely sorbed P	0.002	0.6381
Fe/Al-P	0.005	0.4989
Ca-P	0.003	0.5875
TP_{sed}	0.004	0.5404
%OM	0.005	0.4896

Table 3.6 Predictive relationships between lake sediment characteristics and sediment release rates (RR) from the literature.

Study	**Relationship**	r^2
Nürnberg et al. (1986)	$\mathrm{RR}\ (\mathrm{mgP\ m^{-2}\ d^{-1}}) = 12.116\log\mathrm{TP} - 9.708$	0.25
Nürnberg (1988)	$\mathrm{RR}\ (\mathrm{mgP\ m^{-2}\ d^{-1}}) = 3.88\mathrm{TP} - 4.30$	0.59
Ostrofsky et al. (1989)	$\mathrm{RR}\ (\mu\mathrm{gP\ 25cm^{-3}\ d^{-1}}) = 3.86\ln\mathrm{TP} - 1.70$	0.13
	$\mathrm{RR}\ (\mu\mathrm{gP\ 25cm^{-3}\ d^{-1}}) = \ln\mathrm{Fe} - 0.23\mathrm{LOI} - 47.51$	0.23
	$\mathrm{RR}\ (\mu\mathrm{gP\ 25cm^{-3}\ d^{-1}}) = 2.65\ln\mathrm{NH_4Cl\text{-}P} - 0.30\mathrm{LOI} + 8.14$	0.35
Jensen et al. (1992)	$\mathrm{RR}\ (\mathrm{mmolesP\ m^{-2}\ 21days^{-1}}) = 1.53\mathrm{NH_4Cl\text{-}P} + 3.17$	0.66
	$\mathrm{RR}\ (\mathrm{mmolesP\ m^{-2}\ 21days^{-1}}) = 12.7e^{-0.044\mathrm{Fe:P}}$	0.58

Figure 3.1 Soluble reactive phosphorus (SRP) release rates from CP5 sediment under simulated aerobic conditions. Vertical bars are standard errors.

Figure 3.1

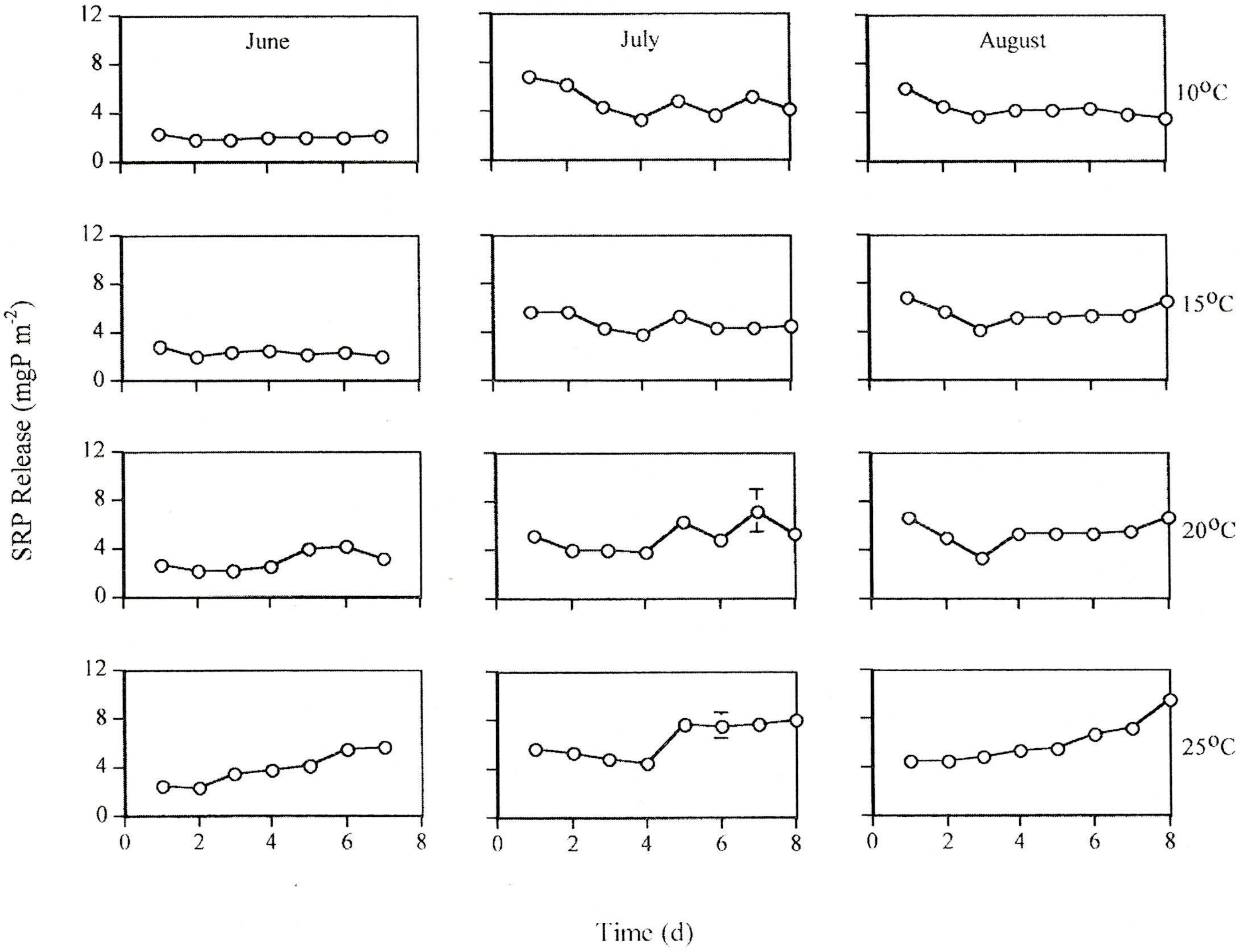
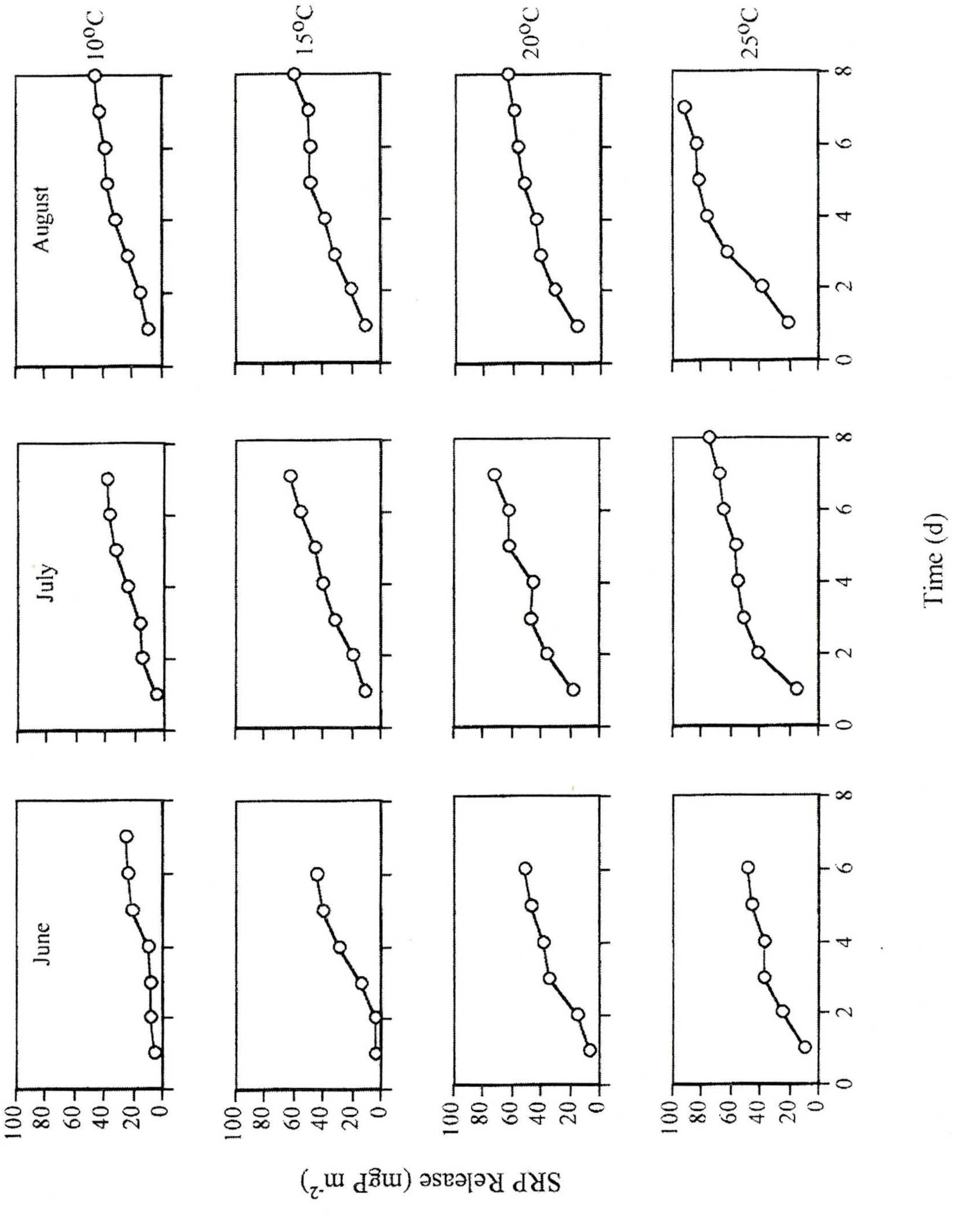

June
July
August
10°C
15°C
20°C
25°C
12
8
4
0
0
2
4
6
8
SRP Release (mgP m-2)
Time (d)

Figure 3.2 **Soluble reactive phosphorus (SRP) release rates from CP5 sediment under simulated anaerobic conditions. Vertical bars are standard errors.**

Figure 3.2



June
July
August
10°C
15°C
20°C
25°C
SRP Release (mgP m-2)
100
80
60
40
20
0
Time (d)
0
2
4
6
8

Figure 3.3 Total phosphorus (TP) release rates from CP5 sediment under simulated aerobic conditions. Vertical bars are standard errors.

Figure 3.3

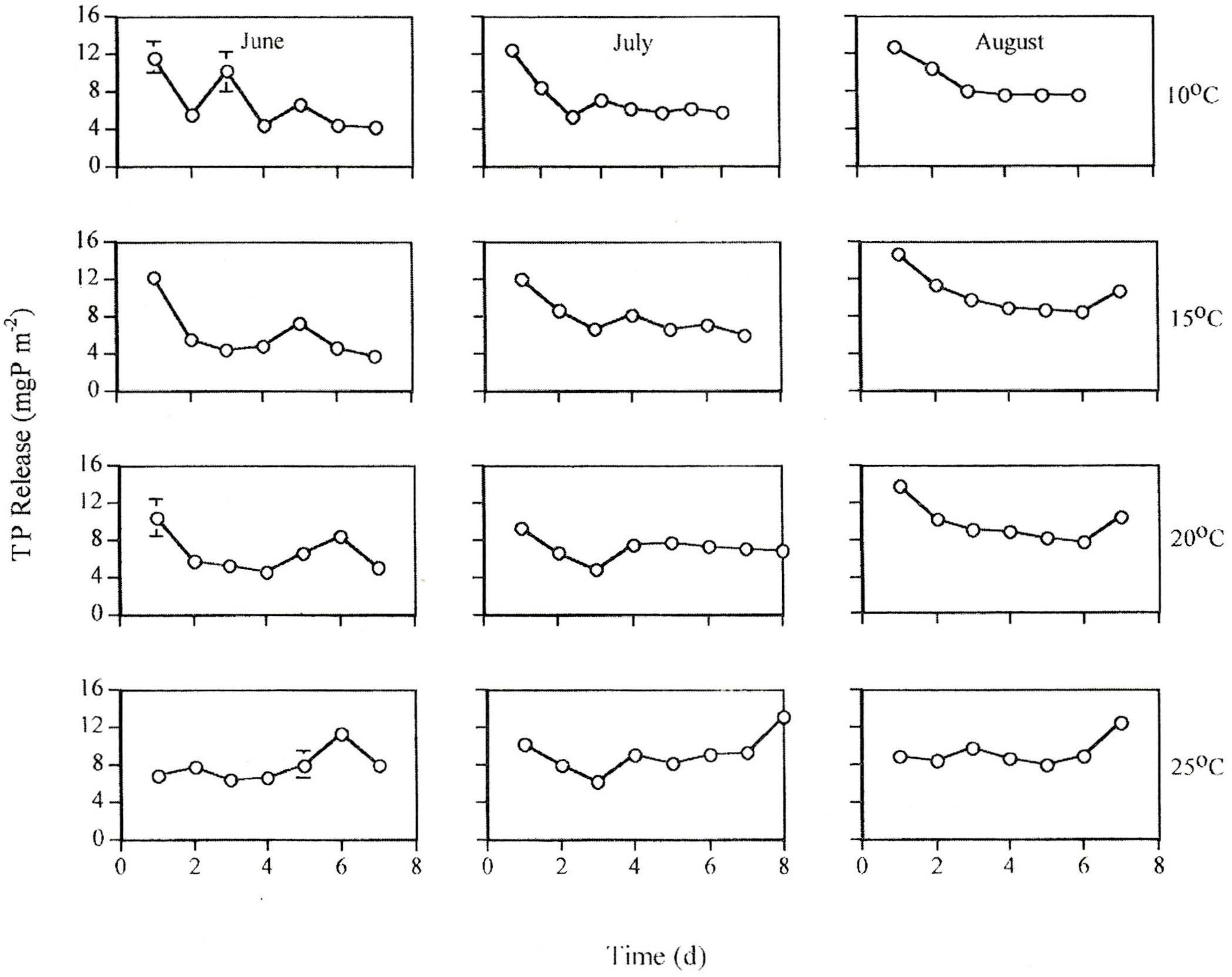

June
July
August
10°C
15°C
20°C
25°C
TP Release (mgP m⁻²)
0
4
8
12
16
0
2
4
6
8
Time (d)

Figure 3.4 Total phosphorus (TP) release rates from CP5 sediment under simulated anaerobic conditions. Vertical bars are standard errors.

Figure 3.4

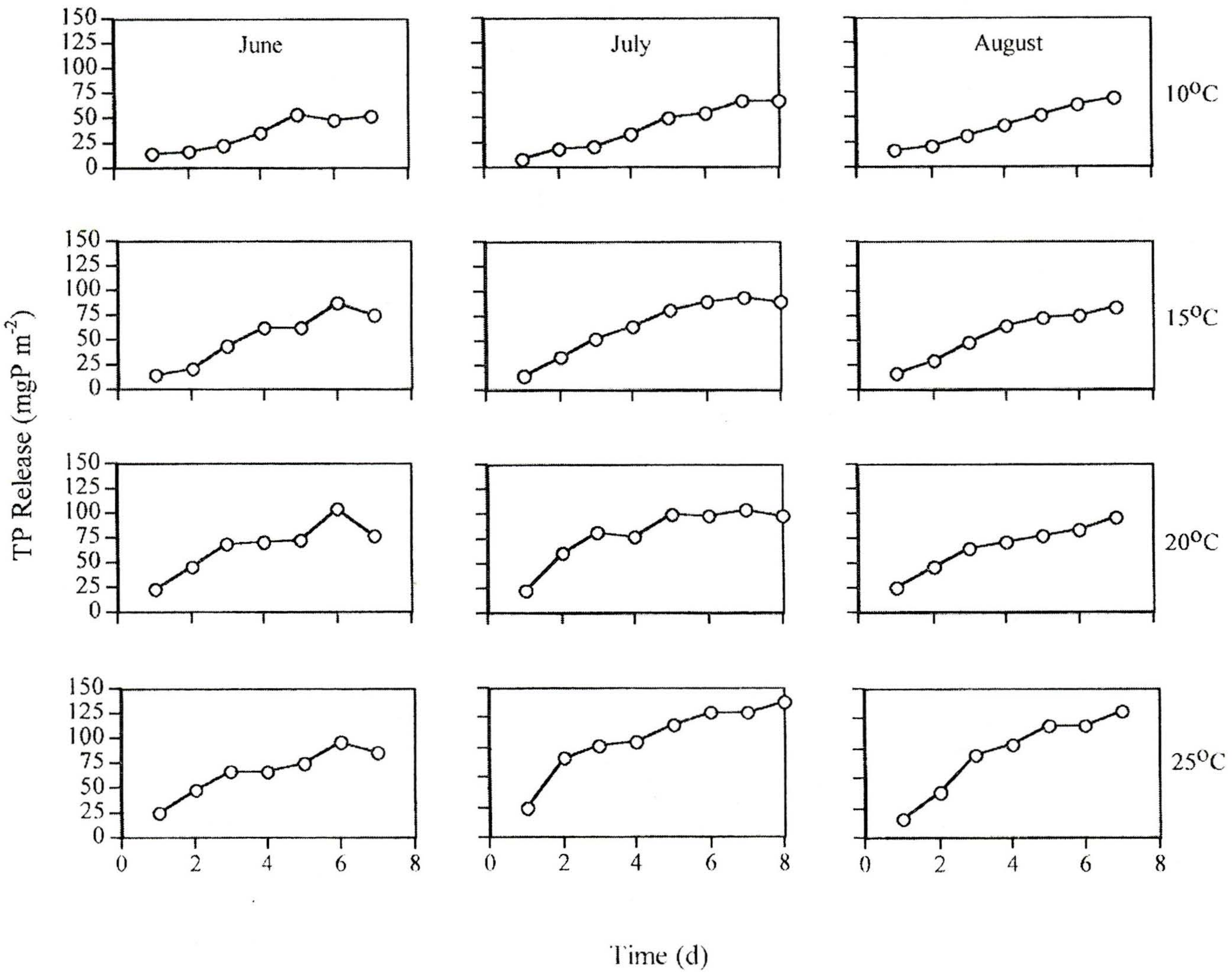
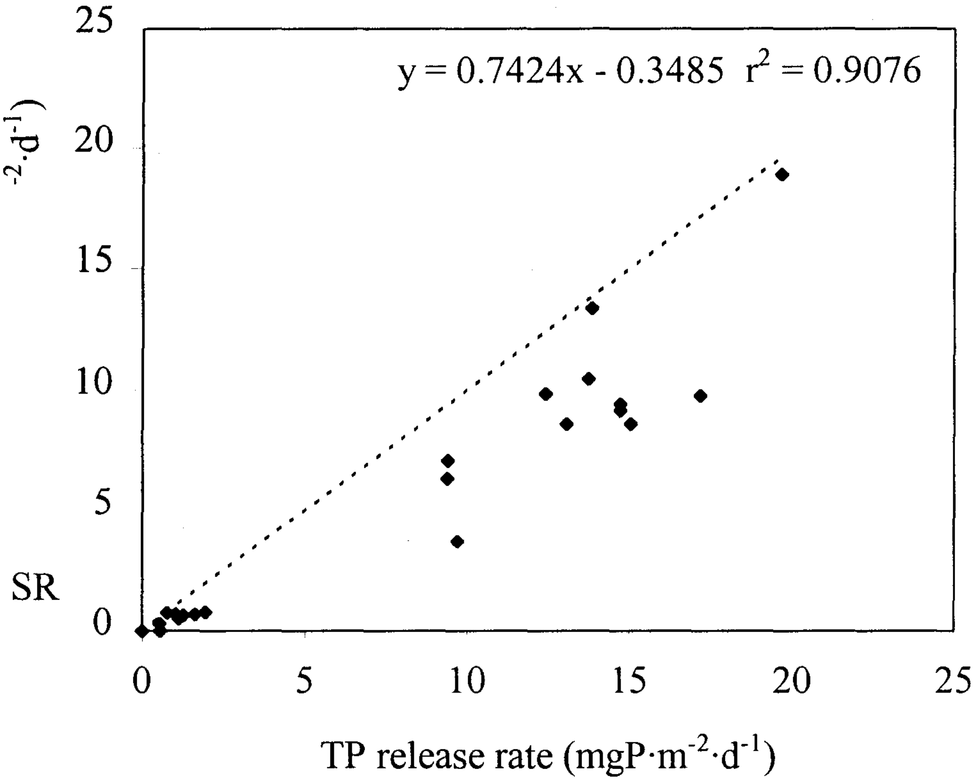

June
July
August
10°C
15°C
20°C
25°C
150
125
100
75
50
25
0
0
2
4
6
8
TP Release (mgP m^{-2})
Time (d)

Figure 3.5 **Comparison of SRP and TP release rates from West Pond (CP5) sediment [includes rates determined under both aerobic and anaerobic conditions and at varying temperatures (10, 15, 20 and 25°C)]. The 1:1 line is shown (dotted).**

Figure 3.5



y = 0.7424x - 0.3485 r^2 = 0.9076
25
20
15
10
5
0
SR
$^{-2}$·d^{-1})
0
5
10
15
20
25
TP release rate (mgP·m^{-2}·d^{-1})

Figure 3.6 **Relationship between SRP release rate and temperature under simulated oxic and anoxic conditions (oxic: SRP release rate = 0.0509temperature – 0.5364; anoxic: SRP release rate = 0.5029temperature + 0.7275).**

Figure 3.6

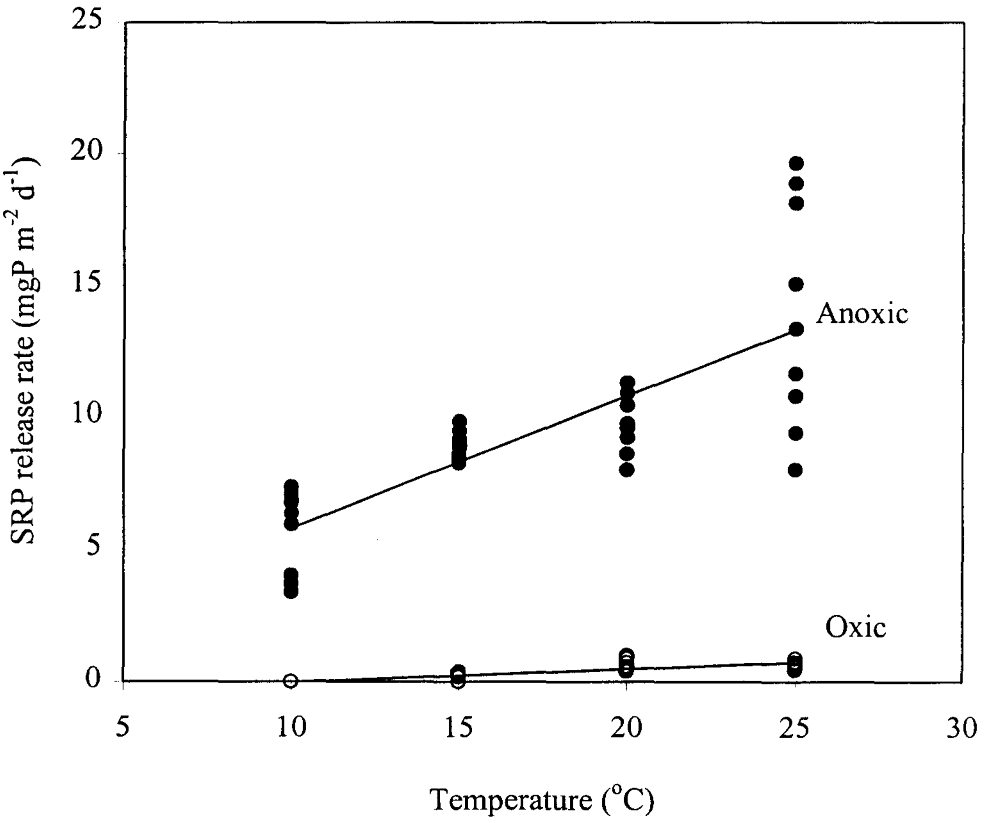
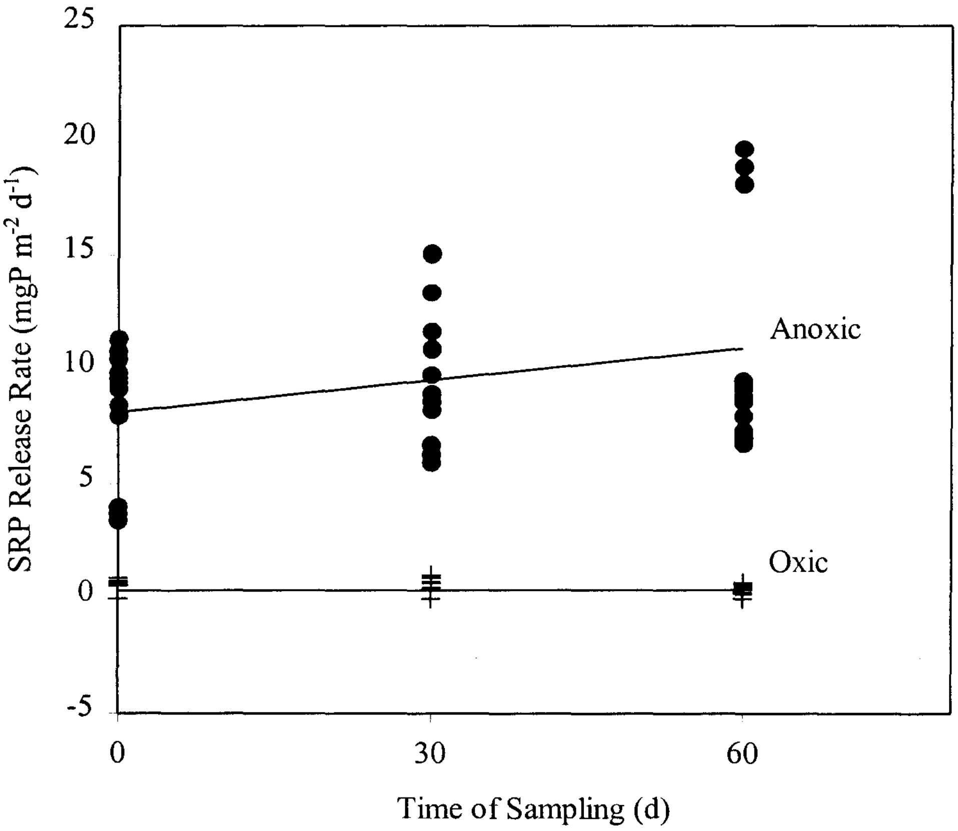

25
20
15
10
5
0
SRP release rate (mgP m^{-2} d^{-1})
Anoxic
Oxic
5
10
15
20
25
30
Temperature (°C)

Figure 3.7 **Relationship between SRP release rate and time of sampling under simulated oxic and anoxic conditions (oxic: SRP release rate = 0.0001day + 0.3512; anoxic: SRP release rate = 0.0461day + 8.1455)**

Figure 3.7



25
20
15
10
5
0
-5
SRP Release Rate (mgP m^{-2} d^{-1})
Anoxic
Oxic
0
30
60
Time of Sampling (d)

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<u>CHAPTER 4:</u>

An Estimate of the Internal Phosphorus Load in Cootes Paradise Marsh Based on Sediment Mineralization

ABSTRACT

Internal loading of phosphorus can severely delay the return of eutrophic systems to lower trophic states following reductions in external loading. Previous work involving mass balance modelling of phosphorus inputs into Cootes Paradise by Prescott & Tsanis (1997) estimated an internal component of approximately 57%. By comparison, this study uses three methods involving laboratory release rates from sediment collected from the marsh. The first method represented a snapshot of release from one month in which we sampled 7 representative segments of Cootes Paradise Marsh to estimate internal loading. We obtained an estimate of 11.9 kg d^{-1}, which represents 31% of the total P-budget. The second approach involved release rate measurements from sediment collected monthly from June to August at maximum and minimum ambient temperatures and assumed a 12-hour period of anoxia on a diurnal basis. We obtained an estimate ranging from 7.8 to 8.5 kg d^{-1}. Finally, our third approach modelled internal loading based on oxic state, ambient temperature and time of sampling. We determined the loading to be 6.7 kg d^{-1}. Assuming that the internal phosphorus load in Cootes Paradise Marsh is 57% (23.7 kg d^{-1}) of the total and that bioturbation has been reduced to negligible amounts, our estimates suggest that a substantial amount of the sediment

release is due to microbial mineralization (22.6% of total loading or 67% of internal loading).

INTRODUCTION

Control of cultural eutrophication usually concentrates on reducing external inputs of phosphorus. However, numerous researchers have found that internal loading can delay restoration measures by sustaining primary production even after external loadings have been reduced (Jacoby et al. 1982; Lijklema 1993; Sondergaard et al. 1993; Phillips et al. 1994). For example, no improvements were observed in Barton Broad for 12 years after a 90% reduction in external loading (Phillips et al. 1994). Similarly Shagawa Lake, Minnesota, continued to be highly productive particularly during July and August despite dramatic reductions in external phosphorus supplies (Larsen et al. 1981). In contrast, Lake Sammamish saw improved water quality after a decrease in phosphorus loading by one-third through wastewater diversion, however, the improvement did not occur as was predicted and showed a delayed response (Welch et al. 1986).

In highly eutrophic systems, such as those listed above, sediment can release phosphorus at levels comparable to external sources (from 30 to 100%) and is the controlling factor in maintaining elevated concentrations of phosphorus in the water column (Premazzi & Provini 1985; Nürnberg 1988; Auer et al. 1993). For example, internal loading almost equalled external sources in the summers of 1977 and 1978 in Long Lake, Washington (Jacoby et al. 1982). Increases in internal loading in shallow eutrophic lakes are usually prominent in the summer months when external loading is low (Riley & Prepas 1984). This is evidenced by increases in total phosphorus concentrations in the water column during these months.

Quantifying the internal load can be difficult, time-consuming and costly. Premazzi & Provini (1985) suggested that the best method involves detailing inputs, outputs and concentrations in the water column over time in order to determine fluxes to and from the sediment. Inputs may be difficult to determine and the length of the investigation determines the reliability of the mass balance (Premazzi & Provini 1985). An alternative approach involves laboratory measures to estimate release rates from sediment cores. Nürnberg (1987) found that laboratory release rates did not differ significantly from in situ hypolimnetic measurements and that these rates could predict internal loads as long as oxygen profiles were available.

Prescott & Tsanis (1997) applied a mass balance approach to estimating the phosphorus budget of Cootes Paradise. The results indicated that the primary source of phosphorus for the summer months was internal (approximately 57% of the total load). This internal load was assumed to be a result of wind-induced sediment resuspension as well as bioturbation due to high numbers of carp in the marsh; however, no mention was made concerning microbial mineralization. Since the development of this prediction, strong efforts have been put forth to reduce carp concentrations through the construction and operation of the Cootes Paradise Fishway, which as a result has reduced bioturbation.

Wind resuspension of the sediment is still of concern and the amount of release due to mineralization has not yet been assessed.

Based on the mass-balance study of Prescott & Tsanis (1997) and the continued high TP levels in Cootes Paradise Marsh following phosphorus-abatement programs, we hypothesize that the internal P load in the marsh must be substantial. Rather than estimating internal loading by mass balance because it does not provide a reasonable prediction for the short-term (Lijklema 1993), as on a seasonal basis, we sought to measure it by conducting laboratory release experiments with sediment samples collected at representative stations throughout the marsh.

METHODS

We used three methods to estimate the internal phosphorus budget of Cootes Paradise Marsh. The first method (Method 1) was based on a static representation, while Methods 2 and 3 were more dynamic in nature taking into consideration temporal changes as well as ambient temperature and oxic state.

Method 1

We calculated release rates under controlled laboratory conditions (i.e. oxic, at 25°C) corresponding to sediments from each marsh segment (Chapter 1). For logistical reasons, only site-to-site variation could be accounted for, and therefore, release rates were determined for the various sites for the month of June only.

A geographic information system (GIS) of Cootes Paradise (obtained from ECOWISE) was used to obtain areal information. The open water polygon was further divided to reflect significant differences in release rates (Chapter 1, Fig. 4.1). The total internal load was calculated using Equation 4.1 and was compared to the external loading data compiled by XCG Consultants Ltd. (1997). These estimates of external loading are more recent than those reported in the Hamilton Harbour RAP (see General Introduction, Hamilton Harbour Remedial Action Plan).

$$\text{Internal Load (kg d}^{-1}\text{)} = \Sigma[\text{area (m}^2\text{)} * \text{release rate (mg m}^{-2}\text{ d}^{-1}\text{)}]/1000 \qquad \textbf{Eq. 4.1}$$

Method 2

We sampled sediment from sites CP1 and CP5 monthly (June-August) and conducted laboratory release experiments to determine phosphorus release rates under both aerobic and anaerobic conditions and at the maximum and minimum monthly ambient temperatures recorded in the marsh. Temperature data was recorded every 30 minutes with an Onset StowAway TidbiT Temperature Logger® positioned at each site for the duration of the experiment. Anoxia was assessed by taking dissolved oxygen (DO) readings every hour over one 24-hour period in July with a Hydrolab MiniSonde®. In order to estimate internal loading at both the minimum and maximum monthly temperatures, we assumed a 12-hour period of anoxia.

Method 3

Using sediment from West Pond (CP5) only, we were able to develop predictive equations of release rates based on oxic state, ambient temperature and time of sampling (Chapter 3). We applied these equations using average daily ambient temperatures recorded by the Logger and again assumed a 12-hour period of anoxia in the marsh.

RESULTS

The greatest contribution to the internal load of Cootes Paradise Marsh was sediment found in the eastern end of the marsh towards the outlet and this is primarily because of the large area and the relatively high release rate of CP22 (Table 4.1). A comparison of the total internal load (11.9 kg d^{-1}) estimated from Method 1 with external sources revealed that internal loading is the second most important source of phosphorus to Cootes Paradise (31.6%, Table 4.2). However, the internal load was calculated as SRP whereas external sources reported by XCG Consultants Ltd. (1997) were given as TP. Therefore, we speculate that our estimate of internal loading may have been somewhat conservative, despite the fact that we demonstrated that a large majority of the TP released is SRP (Chapter 3).

Since Methods 2 and 3 were based on only 2 sites, we wanted to evaluate the error incurred by sampling only these sites (CP1 and CP5), which we believed were representative of the average release of hypereutrophic and mesotrophic sediments. The sediment surface area for CP1 was calculated as the sum of CP1, CP19, CP22 and CP23, while that for CP5 was the sum of CP5, CP17 and CP8. The internal load calculation based on the use of CP1 and CP5 indicates that these two sites can be used interchangeably for the purpose of budget calculations. The total internal load was determined as 11.6 kg d^{-1} (Table 4.3) compared to 11.9 kg d^{-1} indicated above. This extrapolated internal load represents 31.0% of all P sources (Table 4.4).

The above estimates were based on release rates presumably under oxic conditions (i.e. no addition of nitrate). We compared the results for all treatments (control, addition of nitrate, addition of acetate) to assess the relative effect of oxic state on release rate measurements. The use of acetate to simulate anaerobic conditions dramatically increased P release rates, while the use of nitrate did not appear to have any effect compared to the control (Figure 4.3 and Table 4.5). An ANCOVA indicated that release rates under the influence of nitrate did not differ significantly from the control ($p=0.4903$) and therefore, the results suggest that the addition of nitrate is not necessary to induce aerobic conditions.

The diurnal DO profile indicated a definite decline in concentration, which occurred between 6:00 and 10:00 am (Fig. 4.2), suggesting that conditions approaching anoxia do occur in the marsh. Correspondingly, total phosphorus concentrations rose, providing further evidence of the influence of reduced conditions on phosphorus release from sediments.

Based on the release rates obtained in the laboratory using the minimum and maximum monthly ambient temperatures (Table 4.6 and Table 4.7) and assuming that anaerobic conditions persist for 12 hours each night, we were able to determine a range of values that are likely to bracket the true internal phosphorus load due to mineralization. Our estimate suggests that sediment release contributes between 7.79 and 8.45 kg d^{-1} for the minimum and maximum temperatures, respectively.

Using the predictive equations from Chapter 3, daily ambient water temperature data (Fig. 4.4) and again, assuming that the sediment/water interface is anaerobic for half of each 24-hour period, the total phosphorus load is 6.66 kg d^{-1} (derived for CP5 sediment only). These calculations assume that release rates from CP1 are approximately one-third that of CP5 based on previously conducted release experiments (Table 4.8).

During the course of this experiment, we discovered that anaerobic release rates at temperatures above 25°C did not increase linearly (i.e. they were significantly lower than those at 25°C, Figure 4.5). Although temperatures greater than 25°C were observed in the field, average daily temperatures did not rise beyond 26°C and therefore, this discrepancy was not of great concern in our internal load estimates.

DISCUSSION

Our calculation of the internal load based on Method 1 was not very reliable because release rates were determined from previously frozen sediment. Chapter 3 demonstrated that the microbial community has an active role in controlling the flux of phosphorus across the sediment/water interface. Freezing of the sediment inactivates bacteria unable to produce spores and eliminates their influence on internal loading through phosphorus sequestering. In this case, release may be a function of chemical processes (diffusion, adsorption/desorption) alone and could potentially under- or overestimate internal loading. Another equally important consideration is that loss of a viable microbial community also eliminated the potential reduction in SRP in overlying waters attributable to phosphorus uptake by other microbes. For example, deMontigny & Prairie (1993) observed an increase in phosphorus release from sediment that had been sterilized (i.e. no viable microbial community). Our results support this finding: freezing, which presumably kills the microbial flora, can result in higher phosphorus release rates than when fresh samples (with presumably viable bacteria) are used.

Lower release rates were observed at temperatures greater than 25°C and may either reflect autolysis of cells and corresponding decline in phosphorus release or enhanced microbial growth and increased demand of phosphorus. It is also possible that lower release rates were observed due to microbial community succession. The predictive equations were based on release rates from sediments collected at the end of each respective month, while sediments collected for experiments at maximum and minimum ambient temperatures were sampled two weeks prior. Regardless of the causes, it is typically assumed that increases in temperature (as will occur with global warming) result in increased phosphorus release from sediment. Our findings suggest that depending on the magnitude of temperature increase with global warming climate change, internal loading may in fact subside in Cootes Paradise Marsh.

The internal phosphorus budget reported in this study is only a portion of the total internal load as it only takes into account mineralization and diffusion of the surface sediment and does not consider resuspension due to wind or bioturbation, horizontal transport of contaminated sediments, or vertical migration of phosphorus up through the deeper layers. Wind and/or bioturbation increases resuspension and turbulence, which may lower the gradient of redox potential, pH and phosphate across the sediment/water interface (DeGroot 1981; Premazzi & Provini 1985). The net result of resuspension depends on the sediment character: sediment particles may adsorb phosphate provided that there is sufficient iron, aluminum, calcium or clay particles present under aerobic conditions, or conversely phosphate may be released to the water column (DeGroot 1981). In experimental studies, Riley & Prepas (1984) demonstrated that total phosphorus concentrations in surface waters increased in 8 out of 9 mixing events; wind is therefore important in the phosphorus cycling in shallow systems.

Internal loading may also be enhanced by deeper layers of the sediment. Decomposition of organic matter in these layers mobilizes P, which migrates vertically to the sediment/water interface and accumulates (Carignan & Flett 1981; Doremus & Clesceri 1982; Sondergaard et al. 1993). Sondergaard et al. (1993) reported that net release primarily occurs from 10 to 23 cm below the sediment surface and therefore, these deeper layers are an important consideration when modeling phosphorus release. Since release experiments in this investigation were conducted with only the top 5 cm of sediment, future studies should be carried out to verify the extent to which our results have been biased by the sampling protocol.

The extent and duration of anoxia is very important in developing predictions since the amount of phosphorus released depends on these factors (Nürnberg 1988), although release can still be substantial in well aerated waters (Premazzi & Provini 1985). In shallow systems (i.e. Cootes Paradise Marsh), aerobic release is dominant (Marsden 1989) primarily due to wind and bioturbation processes. In these systems, epipelic algae are thought to mediate exchange across the sediment/water interface (Carlton & Wetzel 1988). During the day these organisms may assimilate nutrients from the sediments, and thereby preclude release; in addition, an aerobic microzone may be created through photosynthesis, which further prevents release (Goldsborough & Robinson 1996). When photosynthesis stops at night, however, the sediments are reduced and release may be substantial; redox conditions have been shown to vary on a distinctly diurnal basis (Reddy et al. 1999; Wetzel 1999). Our work does suggest that conditions approaching anoxia do occur in Cootes Paradise Marsh, but, further work is required to assess the duration and magnitude of the anoxia.

Prescott & Tsanis (1997) estimated an internal load of 23.7 kg d^{-1} (assuming 57% of 41.6 kg d^{-1}, their values), which could be apportioned among bioturbation, wind resuspension and mineralization (Table 4.9); however, this estimate was made prior to the construction of the Fishway. Using exclosure experiments, Lougheed et al. (1998) demonstrated that exclusion of carp may result in reductions of water turbidity by up to 45%. From these studies, we can estimate internal loading from wind resuspension and bioturbation to be 4.25 kg d^{-1} (based on our calculation of 8.79 kg d^{-1} from mineralization). This comparison indicates that urban runoff is the largest source of phosphorus load to the marsh (15.9 kg d^{-1}) , followed by mineralization (8.79 kg d^{-1}), then wind resuspension (4.25 kg d^{-1}). This assumes that the construction of the Cootes Paradise Fishway has in all likelihood reduced loading from bioturbation to negligible amounts. Regardless of the exact proportion, the importance of internal loading may be enhanced depending on its availability to algae. Nürnberg et al. (1986) suggested that external sources of phosphorus may only be partially available, but up to 80% of the internal load can be available. We have already demonstrated that approximately 70 to 100% of the TP released from Cootes Paradise sediment is in the form of SRP (Chapter 3) and it has been reported that SRP is likely a good estimate of biologically available P (BAP) (Nürnberg 1988). Therefore, further work is required to determine the extent of BAP from all sources.

One of the goals of this study was to confirm the magnitude of internal loading that has been estimated by the mass-balance approach (Prescott & Tsanis 1997). We have verified through direct measurements of release rates from sediments that the contribution from mineralization can be as high as 22.6% of the total phosphorus load, recognizing that this is likely a conservative estimate as previously discussed. In addition, part of our initial goal was to determine the extent to which Cootes Paradise Marsh would be affected by internal phosphorus supplies in the future; however, this requires a long-term study, involving full sediment mass balancing of phosphate as well as all potential adsorbents and absorbents (Lijklema 1993). Seasonal variations, such as those shown in Chapter 1, become less important as sediment composition changes over years (Lijklema 1993). Shallow systems, such as Cootes Paradise, are also highly unpredictable, that is, the roles of meteorological conditions are much more important and have not yet been assessed quantitatively in terms of their relationships with phosphorus release rates. In addition, difficulty in forecasting wind velocities, direction and ambient water temperatures will remain an obstacle in this endeavour should further studies be initiated to assess long-term effects.

Table 4.1 Estimated daily internal phosphorus loading from stations with significantly different release rates in Cootes Paradise Marsh (values in parentheses are standard errors).

Station	**Release Rate ($mg\,m^{-2}\,d^{-1}$)**	**Area (m^2)**	**Load ($kg\,d^{-1}$)**
CP1	4.45 (0.746)	585344	2.61
CP5	17.18 (1.564)	118138	2.03
CP8	15.18 (1.047)	39927	0.606
CP17	28.28 (6.58)	53367	1.51
CP19	1.48 (0.313)	688920	1.02
CP22	9.31 (0.831)	342614	3.20
CP23	5.86 (0.791)	166298	0.976
TOTAL			**11.94**

Table 4.2 Seasonal (June-August) phosphorus loading from various sources into Cootes Paradise Marsh. Internal loading calculated from all 7 significantly different segments.

Source	**Load (kg·d^{-1})**	**%**
Dundas WWTP	3.2	8.48
Spencer Creek	3.7	9.80
CSO	3	7.95
Urban runoff	15.9	42.13
Mean internal loading	11.94	31.64
Total	**37.74**	**100**

Table 4.3 Phosphorus loading from Cootes Paradise Marsh sediment using stations CP1 and CP5 (values in parentheses are standard errors).

Station	**Release Rate ($mg\,m^{-2}\,d^{-1}$)**	**Area (m^2)**	**Load ($kg\,d^{-1}$)**
CP1	4.45 (0.746)	1782177	7.94
CP5	17.18 (1.564)	211432	3.63
TOTAL			**11.57**

Table 4.4 Seasonal (June-August) phosphorus loading from various sources into Cootes Paradise Marsh. Internal loading calculated using stations CP1 and CP5 to represent release from the entire marsh.

Station	**Load ($kg\,d^{-1}$)**	**%**
Dundas WWTP	3.2	8.56
Spencer Creek	3.7	9.89
CSO	3	8.02
Urban runoff	15.9	42.51
Mean internal loading	11.6	31.02
Total	**37.4**	**100**

Table 4.5 Sediment release rates from May-sampled CP5 sediment under the 3 treatments: simulated anoxic (acetate), simulated oxic (nitrate) and control (no treatment). Values in parentheses are standard errors.

Treatment	**SRP Release Rate ($mg\ m^{-2}\ d^{-1}$)**
Acetate	10.94 (0.946)
Nitrate	0.903 (0.0821)
Control	1.076 (0.457)

Table 4.6 Anaerobic SRP and TP release rates (mg m^{-2} d^{-1}) for CP5 and CP1 determined using the maximum and minimum observed monthly temperatures. Values in parentheses are standard errors.

Month	**Temperature (°C)**	**CP1**		**CP5**	
		SRP RR	**TP RR**	**SRP RR**	**TP RR**
June	14	6.44 (0.364)	10.93 (1.03)	5.98 (0.458)	13.06 (0.987)
June	25	8.45 (0.473)	8.61 (0.904)	8.04 (0.456)	11.86 (0.941)
July	18	4.79 (0.390)	6.62 (0.357)	7.17 (0.594)	12.92 (1.51)
July	26	4.32 (0.209)	7.13 (0.335)	7.87 (0.969)	8.661 (0.676)
August	18	2.99 (0.125)	3.85 (0.281)	6.53 (0.295)	10.22 (0.423)
August	28	5.31 (0.479)	5.34 (0.277)	9.71 (0.530)	13.97 (1.06)

Table 4.7 Aerobic SRP and TP release rates (mg m^{-2} d^{-1}) for CP1 and CP5 determined using the maximum and minimum observed monthly temperatures. Values in parentheses are standard errors.

Month	**Temperature (°C)**	**CP1**		**CP5**	
		SRP RR	**TP RR**	**SRP RR**	**TP RR**
June	14	0	0	0.145 (0.053)	3.81 (1.18)
June	25	0.337 (0.048)	1.97 (0.624)	1.00 (0.078)	3.68 (0.866)
July	18	0	0	0	0
July	26	0	0	0.632 (0.046)	0.845 (0.131
August	18	0	0	0.437 (0.062)	0.595 (0.156)
August	28	0.546 (0.079)	0.659 (0.181)	1.06 (0.072)	1.02 (0.074)

Table 4.8 Comparison of SRP release rates for CP1 and CP5 sediment. Sediment was collected in the month of June and release experiments were conducted at 25°C, under aerobic conditions.

CP1	**CP5**	**Ratio**	**Source**
4.56	17.18	0.26	Chapter 1 (Table 1.2)
0.337	1.00	0.34	This chapter (Table 4.7)

Table 4.9 Seasonal (June-August) phosphorus loading in Cootes Paradise Marsh. A comparison of all estimates of internal loading.

Source	**Load (kg d^{-1})**	**% of All Sources**
External Loading		
Dundas WWTP	3.2	8.2
Spencer Creek	3.7	9.5
CSO	3	7.7
Urban Runoff	15.9	41.0
Subtotal of External	**25.8**	**66.4**
Internal Loading		
Bioturbation & Wind resuspension*	4.25	11.0
Net Mineralization/Diffusion		
Method 1 (diffusion only)	11.6	
Method 2	7.79-8.45	
Method 3	6.66	
Mean of 3 methods	*8.79*	*22.6*
Subtotal of Internal	**13.0**	**33.6**
Total of all sources	**38.8**	**100**

* Estimated as [(23.7 kg d^{-1} * 0.55) – 8.79 kg d^{-1}] based on Prescott & Tsanis' (1997) mass-balance estimate of total internal loading of 57% and Lougheed et al. (1998) estimate of a reduction in water turbidity (bioturbation) of up to 45% due to carp exclusion.

Figure 4.1 **Cootes Paradise Marsh sampling polygons used to determine areal information for internal load calculations.**

Figure 4.1

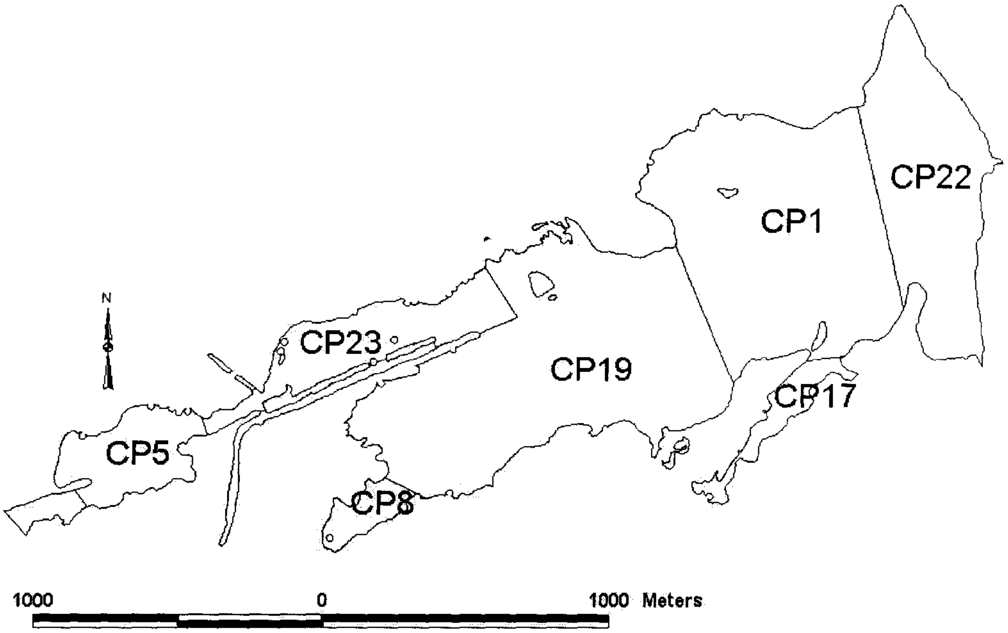


Figure 4.2 Diel changes in dissolved oxygen (DO) concentrations in West Pond at approximately 10 cm above the substrate.

Figure 4.2

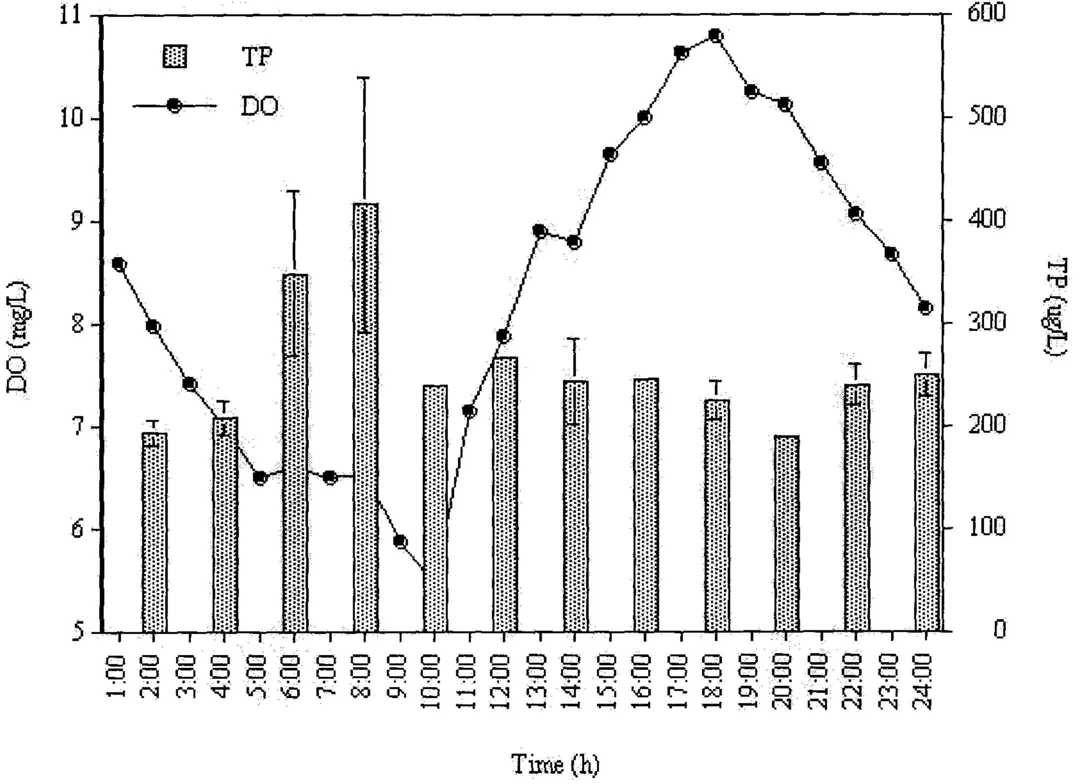
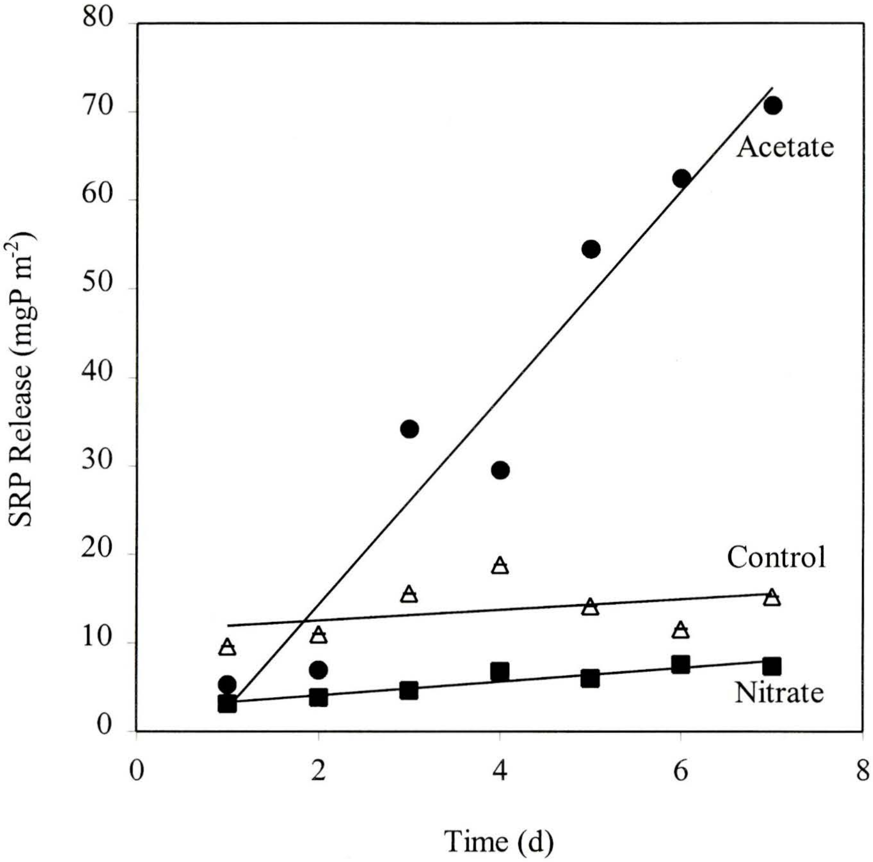

TP
DO
DO (mg/L)
11
10
9
8
7
6
5
TP (ug/L)
600
500
400
300
200
100
0
1:00
2:00
3:00
4:00
5:00
6:00
7:00
8:00
9:00
10:00
11:00
12:00
13:00
14:00
15:00
16:00
17:00
18:00
19:00
20:00
21:00
22:00
23:00
24:00
Time (h)

Figure 4.3 **Release rates from CP5 sediment under anaerobic (acetate), aerobic (nitrate) and controlled (no treatment) conditions.**

Figure 4.3



80
70
60
50
40
30
20
10
0
SRP Release (mgP m-2)
Acetate
Control
Nitrate
0
2
4
6
8
Time (d)

Figure 4.4 Average daily ambient temperatures at station CP5, June to August 2000.

GENERAL CONCLUSIONS AND IMPLICATIONS FOR RESTORATION

Overall, the results of this thesis have demonstrated two important findings: the first being the significance of the biological component of the sediment (i.e. the microbial community) with respect to internal loading, and the second being the relatively large component of sediment release in the overall phosphorus budget of Cootes Paradise Marsh. Chapter 1 revealed substantial spatial and temporal variance in release rates, which we later attributed to successional differences in benthic bacteria (Chapters 2 and 3). Further research is required to develop models relating either microbial species or specific enzymes to sediment phosphorus release. The fact that internal loading plays such a substantial role in the eutrophication of Cootes Paradise raises numerous questions concerning its control.

There are five main approaches that have typically been employed to control internal loading. Flushing the system with water of low phosphorus concentration would theoretically stimulate diffusion from the sediment (Moss et al. 1996) and wash it out into the Harbour. This approach is quite impractical and would be very slow.

A second method involves biomass removal (i.e. aquatic plants). There are three primary mechanisms by which plants can reduce internal loading: direct uptake of phosphorus from the sediment, anchorage of the sediment resulting in lowered wind and

Figure 4.4

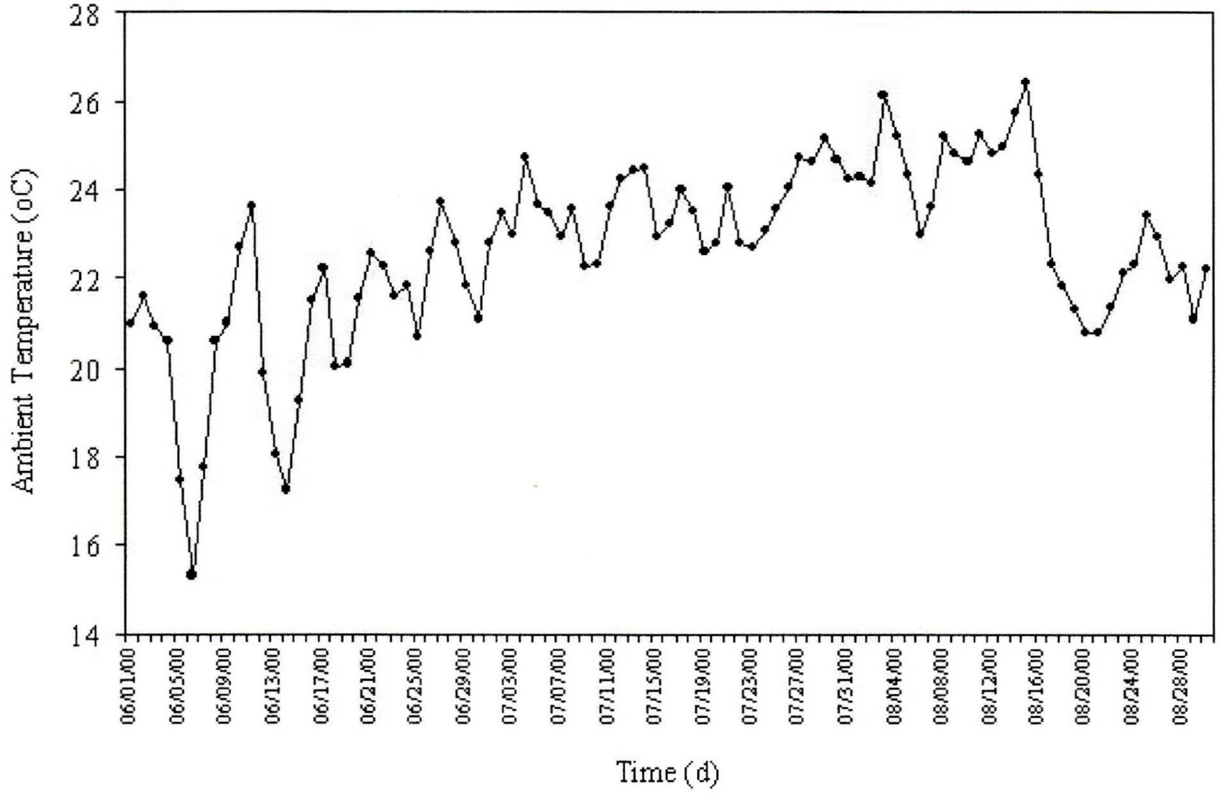
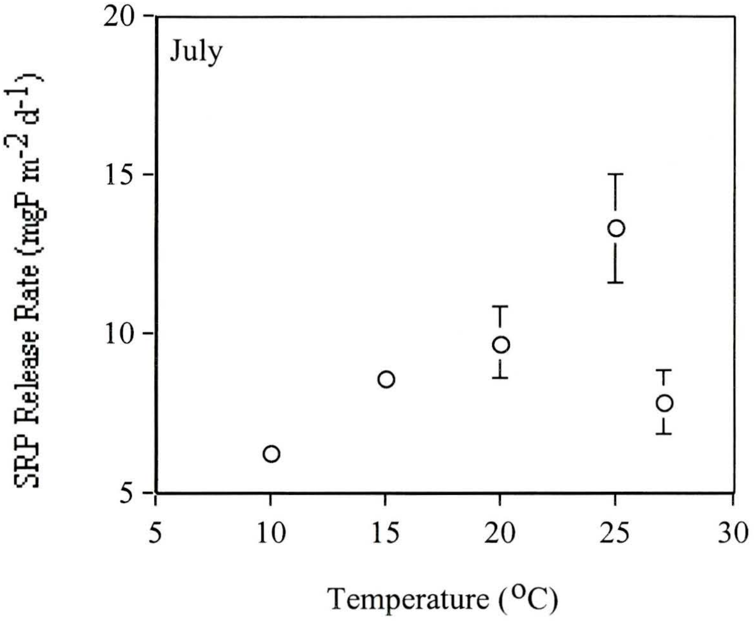
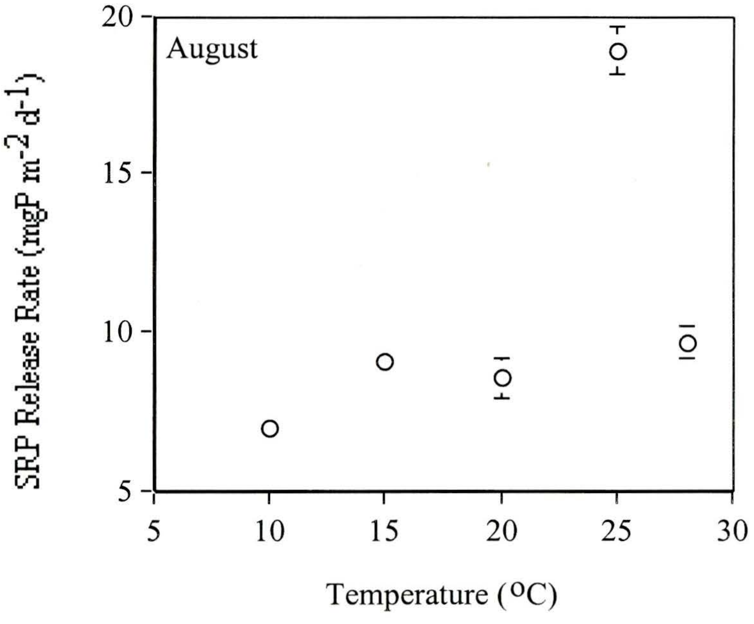

Ambient Temperature (oC)
28
26
24
22
20
18
16
14
06/01/00
06/05/00
06/09/00
06/13/00
06/17/00
06/21/00
06/25/00
06/29/00
07/03/00
07/07/00
07/11/00
07/15/00
07/19/00
07/23/00
07/27/00
07/31/00
08/04/00
08/08/00
08/12/00
08/16/00
08/20/00
08/24/00
08/28/00
Time (d)

Figure 4.5 Relationship between temperature and SRP release rate from CP5 sediment under anaerobic conditions in A) July and B) August.

Figure 4.5


July
SRP Release Rate (mgP m^{-2} d^{-1})
20
15
10
5
5
10
15
20
25
30
Temperature (°C)


August
SRP Release Rate (mgP m^{-2} d^{-1})
20
15
10
5
5
10
15
20
25
30
Temperature (°C)

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current resuspension, and finally the creation of an oxygenated microzone in the sediment.

Macrophytes obtain most of their phosphorus requirements from the sediment (Goldsborough & Robinson 1996); in fact, results of Carignan and Kalff (1980) showed that the sediments constitute the only significant source of phosphorus to rooted macrophytes in oligotrophic and mildly eutrophic lakes. For example, in Long Lake, Washington: a decline of *Elodea densa* biomass resulted in a sharp increase in phosphorus loading (Cooke et al. 1993). On the other hand, Cootes Paradise is a hypereutrophic marsh; Carignan and Kalff (1980) found that in hypereutrophic waters, macrophytes sequester a significant amount of phosphorus from the water in lieu of the sediments. In addition, the efficiency of nutrient uptake (the increase in plant P mass divided by the P mass available) declines with increasing eutrophication (Mitsch & Gosselink 1993). Therefore, as nutrient inputs increase, the proportion of phosphorus retained in vegetative biomass decreases (Mitsch & Gosselink 1993).

Macrophytes often act as nutrient pumps, further limiting their application to control internal loading. Although some of the phosphorus in the shoots is reallocated to the roots before death, most is released into the water column (Goldsborough & Robinson 1996; Mitsch & Gosselink 1993) and therefore, decomposition supplies a substantial quantity of P to the water column during winter months (Jacoby et al. 1982). Harvesting would provide a means to permanently remove some of the phosphorus; however this is

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counterproductive. Macrophytes help to stabilize the sediments as well as providing food and refuge (Vandermeulen & Gemza 1992) and therefore, biomass removal is not suitable as a restoration measure (Moss et al. 1996).

Removal of the enriched sediment, or dredging, is another option; however, it is highly disruptive and the sediment must be disposed of. It is generally very expensive and in the process removes existing macrophytes and the seed bank (Mitsch & Gosselink 1993). In addition, the underlying layers may have high organic and TP content, which may enhance release (Phillips et al. 1994).

Oxygenation of the sediment/water interface has been attempted in many instances to reduce the amount of nutrients released from sediments by increasing binding capabilities (Garrell et al. 1977; Ashley 1983; McQueen et al. 1986). However, these studies were conducted in stratified lakes where oxygen deficits occur over long periods. Cootes Paradise is a very shallow system; anaerobic conditions are thought to occur only on a diel basis. In addition, a long-term study by Gächter & Wehrli (1998) showed no lasting effect of oxygenation due to unchanged high sedimentation rates of organic matter.

The final approach to controlling internal loading is termed sediment sealing. This process typically involves the addition of aluminum, iron or calcium salts to the system to increase phosphate binding. Aluminum sulphate (alum) is one such salt that dissociates when added to water to form aluminum hydroxide. This hydroxide has high phosphate adsorption properties and settles through the water column under normal conditions with a pH of greater than 6.0 (Cooke et al. 1993). However in acidic waters soluble aluminum species dominate and at higher pH levels phosphate desorbs (Cooke et al. 1993). Sodium aluminate ($Na_2Al_2O_4$) will maintain a pH of 6-8, but is much more expensive. In addition, wind mixing may redistribute the alum floc to the centre of shallow systems, such as Cootes Paradise (Cooke et al. 1993).

The use of iron hydroxides is also questionable. Upon reduction, which occurs on a diurnal basis and during periods of high pH (i.e. due to photosynthesis), Fe^{3+} is reduced to Fe^{2+} releasing adsorbed phosphate (Cooke et al. 1993). In very eutrophic waters, most of the iron salt may become tied up as insoluble ferrous sulphide in the sediments (Moss et al. 1996).

The addition of calcite (calcium carbonate) leads to the coprecipitation of phosphate (Vandermeulen & Gemza 1992); however, this method has been shown to be ineffective in controlling internal loading. For example, Puslinch Lake was treated with calcite in 1988 and did not show any improvement in water quality. This was attributed to the fact that the solubility of calcium salts decreases with increasing pH (Vandermeulen & Gemza 1992).

Finally, an alternative approach was described by Ripl (1976). In this approach, nitrate is used to biochemically oxidize the sediment through denitrification (Foy 1986) and results have been positive in Danish lakes studied by Andersen (1982) and Jensen & Andersen (1992). This method was used in Chapter 3 to simulate aerobic conditions and did indeed substantially curb release of phosphate from the sediment.

Recommendations

One of the first steps in many restoration projects is to reduce nutrient loading. Since internal release is very difficult to manage, all efforts must be made to reduce external sources, the largest of which is urban runoff. Programs to educate the public concerning the effects of using fertilizers may alleviate some of this pressure. We question the suitability of diversion of the effluent from the Dundas WWTP. In the sewage treatment process, ammonium, which is toxic to fish, is oxidized to nitrate (Moss et al. 1996). If the effluent is diverted, nitrate will no longer be released into West Pond, which could potentially result in increased phosphorus release from the sediment. The use of chemical means to control internal release have been met with mixed results and dredging is very expensive and will remove any existing macrophytes as well as the seed bed. The best means for improvement may simply be time to allow the system to flush itself out, which can be assessed using the residence time, 2 to 40 days (Remedial Action Plan for Hamilton Harbour Stage 1 Report 1992), in Cootes Paradise Marsh. However, further study is required to determine if Cootes Paradise Marsh is a net source of phosphorus to Hamilton Harbour on a yearly, rather than seasonal, basis.

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