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TABLE OF CONTENTS

TABLE OF CONTENTS ... (cont.)

ABSTRACT

The residual variability surrounding chlorophyll *a* - total phosphorus (Chl-TP) relationships decreases the predictive ability of phosphorus loading models to forecast algal biomass production in lakes. Although lakes, rivers and estuaries all show strong Chl-TP relationships, the chlorophyll to total phosphorus ratio (chlorophyll yield) decreases from lakes > rivers > estuaries. When data from these three classes of aquatic systems (i.e. aquatic biomes) are pooled, the total amount of variability in chlorophyll *a* concentration increases considerably.

In the first section of this thesis I attempt to account for the residual variation in chlorophyll *a* concentration by examining how the sequestration of phosphorus into non-algal compartments of the water column varies among aquatic biomes. This is achieved by examining the relationships between chlorophyll *a* and various chemical forms (particulate, soluble, organic and inorganic) and size classes (total, >64µm, 30-64µm, 3-30µm, 0.2-3µm) of phosphorus. In the second section, I examine if the much lower than expected chlorophyll yield of Prince Edward Island (PEI) estuaries is the result of mussel grazing and/or increased turbidity or if it is the result of a general regional effect caused by the Island's geological characteristics. This latter hypothesis implies that PEI lakes and rivers would also possess lower chlorophyll yields. This question is examined by comparing the data from each of the PEI biomes (lakes, rivers, estuaries) with that of reference set possessing the expected chlorophyll *a*

to total phosphorus ratio.

The results of the first section suggest that the residual variability surrounding the chlorophyll *a* - total phosphorus relationship of aquatic biomes is related to varying amounts of soluble phosphorus (which correlate to differences in nutrient limitation) and to small particulate phosphorus (possibly indicating bacterial competition). Therefore, the best fraction with which to predict chlorophyll *a* concentration among most aquatic biomes is that of particulate phosphorus larger than 3µm, a fraction that best represents the phosphorus directly in algae. In the second section, it is discovered that all aquatic systems on PEI possess lower chlorophyll *a* yields than predicted by Chl-TP relationships derived for similar biomes elsewhere in North America. Turbidity and mussel grazing do not account for the lower chlorophyll yield between the Island and reference relationships, although the presence of mussel aquacultures is correlated with a lower chlorophyll yield in the Island's estuaries compared to that of the Island's lakes and rivers. The results suggest that the lower chlorophyll *a* to total phosphorus ratios on Prince Edward Island is a regional effect and may be related to the iron-oxide rich soils.

RÉSUMÉ

La variance résiduelle qui entoure la relation entre la chlorophylle *a* et le phosphore total (Chl-TP), décroît l'habilité des models de chargement en phosphore de prédire la production en biomasse des algues. Bien que les lacs, rivières et estuaires possèdent tous des relations Chl-TP fortes, la proportion entre la concentration de chlorophylle et le phosphore (rendement en chlorophylle) décroît de lac, en rivière, en estuaire. Quand les données pour ces trois classes de systèmes aquatiques (biomes aquatiques) sont regroupées, la quantité totale de variabilité en concentration de chlorophylle augmente considérablement.

Au cours de la première section de ce mémoire, je tente de tenir compte de la variance en concentration de chlorophylle en vérifiant comment varie, selon les biomes aquatiques, la séquestration du phosphore dans les composantes non-alguales de la colonne d'eau. Ceci est accompli en étudiant les relations entre la chlorophylle et des classes de grandeurs (total, >64µm, 30-64µm, 3-30µm, 0.2-3µm) et formes chimiques (soluble, particulière, organique et inorganique) variées du phosphore. Dans la deuxième section du mémoire, je vérifie si le rendement inférieur en chlorophylle des estuaires de l'Ile du Prince Edouard (IPE) est le résultat de broutage par des moules et/ou par la turbidité ou si c'est le résultat d'un effet régional causé par les caractéristiques géologiques de l'Ile. Cette dernière hypothèse implique que les lacs et les rivières de l'IPE posséderont aussi

des rendements inférieurs à la norme. Cette question est étudiée en comparant les données de chaque biome de l'IPE (lacs, rivières et estuaires) avec ceux d'une série de référence possédant le rapport Chl-TP attendu.

Les résultats de la première section suggèrent que la variance résiduelle de la relation chlorophylle - phosphore des biomes aquatiques est apparentée à des quantités variées de phosphore soluble (qui correspond à des différences en limitation en nutriments) et au phosphore à taille réduite (ce qui indique possiblement la compétition bactérienne). Ceci dit, la meilleure fraction avec laquelle prédire la concentration en chlorophylle *a* parmi la plupart les biomes aquatiques est celle de particules de phosphore plus grandes que 3µm. Cette fraction est la meilleure représentation du phosphore retrouvé directement dans les algues. Dans la deuxième section, il est découvert que tous les systèmes aquatiques de l'Ile du Prince Edouard possèdent un rendement en chlorophylle inférieur à celui prédit par les relations Chl-TP dérivés pour les biomes similaires ailleurs en Amérique du Nord. La turbidité et le broutage par les moules ne sont pas responsables pour ce rendement inférieur, bien que la présence de fermes de moules dans les estuaires de IPE correspond avec une baisse du rendement, comparé aux lacs et rivières de l'Ile. Les résultats suggèrent que la proportion inférieure entre la concentration de chlorophylle et le phosphore sur L'Ile du Prince Edouard est un effet régional et pourrait être relié aux sols riches en oxydes de fer.

LIST OF TABLES

LIST OF TABLES ... (cont.)

LIST OF FIGURES

LIST OF FIGURES ... (cont.)

GENERAL INTRODUCTION

The control of algal abundance is of concern not only to freshwater ecologists but also to the general public. The success in controlling eutrophication of aquatic systems rests on phosphorus loading models. These management models are based on the strong relationship found between chlorophyll *a* (an estimate of algal biomass) and total phosphorus (an estimate of the phosphorus available to algae for growth). However, there is much unaccounted-for variation surrounding these relationships, therefore limiting the predictability of the loading models.

Chlorophyll *a* - total phosphorus (Chl-TP) relationships have now been derived for every imaginable aquatic system (i.e. lakes (Dillon and Rigler 1974), impoundments (Soballe and Kimmel 1987), saline lakes (Bierhuizen and Prepas 1985), rivers (Basu and Pick 1996), streams (Van Nieuwenhuyse and Jones 1996), coastal lagoons (Contreras and Kerekes 1993), estuaries (Meeuwig et al. 1998)). A comparison of these various relationships finds that there is typically less chlorophyll *a* produced per unit total phosphorus in rivers than in lakes, and even less in estuaries (see Chapter 1). The goal of the present thesis is to attempt to determine the source of some of the residual variability surrounding Chl-TP relationships, in general, by examining the relationships of lakes, rivers and estuaries more closely.

In Chapter 1, I examine if the amount of variability in chlorophyll *a* yield among aquatic systems can be reduced by accounting for differences in the

segregation of phosphorus into non-algal compartments of the water column. In particular, I test if the high variability in the chlorophyll to phosphorus ratio of lakes, rivers and estuaries on Prince Edward Island and lakes in the Outaouais region (Ottawa River Valley) of central Canada are due to varying levels of phosphorus present in soluble forms, in particulate inorganic forms, in zooplankton (larger than 30µm particulate forms) or in bacteria (smaller than 3µm particulate forms).

Among the results presented in Chapter 1, I discover that there is very little variation in chlorophyll *a* concentration among the lakes, rivers and estuaries on Prince Edward Island, and that these, as a group, differ from the Outaouais region lakes. Chapter 2 takes this idea further by examining if the lower chlorophyll *a* yield reported for Prince Edward Island estuaries is a phenomenon influencing only this class of aquatic systems, or if it is a regional effect due to geological characteristics of the Island.

CHAPTER 1

Predicting Chlorophyll from Total Phosphorus in Lakes, Rivers and Estuaries: What Causes the Residual Variation?

ABSTRACT

The ability of phosphorus loading models to predict algal biomass in lakes is limited by the level of residual variability in the chlorophyll *a* - total phosphorus relationship. The amount of variability surrounding this relationship increases dramatically when data from lakes, rivers and estuaries (i.e. aquatic biomes) are pooled. We attempt to account for the residual variation in chlorophyll *a* concentration by examining how phosphorus sequestration into non-algal compartments of the water column varies among aquatic biomes. This is done by examining the relationships between chlorophyll *a* and various chemical forms (particulate, soluble, organic and inorganic) and size classes (total, >64µm, 30-64µm, 3-30µm, 0.2-3µm) of phosphorus. Results show that particulate phosphorus larger than 3µm (the algal fraction) best predicts chlorophyll *a* concentration in most biomes. Estuaries are the exception since they have a greater proportion of very small chlorophyll particles. The residual variability surrounding the chlorophyll *a* - total phosphorus relationship is therefore related 1) to varying amounts of soluble phosphorus (which correlate to differences in nutrient limitation) and 2) to variations in small particulate phosphorus (possibly indicating bacterial competition).

INTRODUCTION

One of the most consistent properties of temperate-zone lakes worldwide is the relationship between plankton chlorophyll *a* concentration, an estimate of algal biomass, and total phosphorus levels (Dillon and Rigler 1974, Prepas and Trew 1983, Prairie et al. 1989, McCauley et al. 1989, Currie 1990, Quirós 1990). In some data sets, total phosphorus can account for 90% or more of the variability in the chlorophyll *a* concentrations among lakes (Dillon and Rigler 1974).

Chlorophyll *a* - total phosphorus (Chl-TP) relationships have been incorporated into models used to predict phytoplankton responses to nutrient enrichment (Vollenweider and Kerekes 1980). The resulting phosphorus loading models have become useful tools for managing water quality in lakes (e.g. Dillon 1975). However, as successful as lake management models may be, their predictive power is limited by the level of residual variance surrounding the Chl-TP relationship. The amount of chlorophyll predicted at a given level of phosphorus can vary up to an order of magnitude. While this residual variability may not be large relative to the total variability in chlorophyll *a* concentration among lakes, it is problematic for practical lake management.

The variability in the ratio of plankton chlorophyll *a*: total phosphorus concentration (which we will call chlorophyll yield) is even greater if one considers other classes of aquatic systems. Chl-TP relationships can account for up to 76 percent of the variation in chlorophyll *a* among temperate streams and rivers

worldwide (Søballe and Kimmel 1987, Van Nieuwenhuyse and Jones 1996, Basu and Pick 1996). However, the yield of chlorophyll produced is significantly lower than that observed in lakes having the same amount of phosphorus (Søballe and Kimmel 1987, Van Nieuwenhuyse and Jones 1996). In coastal brackish waters, total phosphorus can account for as much as 65 percent of the variability in chlorophyll *a* (Contreras and Kerekes 1993, Meeuwig et al. 1998). As with rivers, the chlorophyll yield is also lower than that of lakes (Meeuwig et al. 1998).

We compared published chlorophyll *a* - total phosphorus relationships derived for eastern Canadian lakes (Currie 1990, Currie et al. 1999, Wilson 1996), rivers (Basu and Pick 1996) and estuaries (Meeuwig et al. 1998). Each of these Chl-TP relationships is significant and accounts for 65 - 76 % of the variability in chlorophyll *a* concentrations among water bodies of the same class (Table 1.1a). Yet, the chlorophyll-phosphorus relationships for rivers and estuaries differ significantly in both slope and intercept from that of lakes, as well as from one another (Table 1.1b; also Champion and Currie 1999 - Appendix E). On average, there is less chlorophyll *a* per unit total phosphorus in rivers and still less in estuaries (Figure 1.1). A Chl-TP relationship based on the pooled data explains only 29% of the total variability (Table 1.1c). In other words, variability in chlorophyll yield is even greater if one considers a broader range of types of aquatic systems (which we will call aquatic biomes).

Many studies have attempted to explain the chlorophyll - phosphorus residual variability among lakes by relating it to other lake characteristics such as lake morphology (Paloheimo and Zimmerman 1983), water chemistry (Bierhuizen and Prepas 1985) or nitrogen limitation (Smith 1982). Others have suggested that the variability is due to predatory interactions from higher levels of the food web (Pace 1984, Carpenter et al. 1985) or to competition with bacteria (Currie 1990). To date, none of these factors have been shown to account for large amounts of the unexplained variability, nor have consistent trends been identified. Neither, to our knowledge, have the differences in chlorophyll yield among aquatic biomes been satisfactorily explained.

In the present study, we address the question of what causes residual variance in the Chl-TP relationships by using a different approach. The variation among water bodies in the yield of chlorophyll per unit total phosphorus must be due to one or both of the following phenomena. First, it is possible that the proportion of total phosphorus actually in algae differs among systems. In other words, variation in chlorophyll yield results from phosphorus sequestered in non-algal components of the total phosphorus. These may include the soluble fraction, particulate inorganic phosphorus (e.g. flocculated particles in estuaries), zooplankton (Prepas and Rigler 1982), bacteria (Currie 1990) or detritus. The second possibility is that the amount of chlorophyll produced per unit of algal phosphorus differs among water bodies. This situation could reflect differences in

nutrient limitation among the sites. In cases where phosphorus is less limiting, algal cells may take up the excess phosphorus in the environment and store it for later use (Harris 1986). Light availability or turbidity can also potentially affect chlorophyll *a* content per unit algal biomass (Nicholls and Dillon 1978, LaBaugh 1995).

To test these hypotheses, we sampled a series of lakes, rivers and estuaries. Various chemical forms (particulate, soluble, organic and inorganic) and size classes (total, >64µm, 30-64µm, 3-30µm, 0.2-3µm) of phosphorus concentrations were measured, as well as chlorophyll *a* concentration. The relationships between chlorophyll yield and particular phosphorus fractions were then examined. To detect variation in phosphorus limitation among systems, alkaline phosphatase activity levels were determined. These enzymes can break down organic phosphates, liberating phosphorus for use by algae. The more severe the nutrient limitation, the greater should be the activity (Healey and Hendzel 1980, Pettersson 1980).

METHODS

Study area

Most of the sites sampled were in the province of Prince Edward Island on Canada's Atlantic coast (Figure 1.2a). This 5 660 km^2 island is well known for its red soils, rich in iron-oxides. The highest point in the province is only 150 metres

above sea level. Consequently, the many lakes and rivers are small and shallow, and the coastline incorporates many tidal inlets. Estuaries on the southern shore have broad openings to the Gulf of St-Lawrence whereas northern shore estuaries connect to the Gulf via narrow channels through systems of sand-dunes. Several of the estuaries support commercial aqua-cultures of mussels.

A total of 13 lakes, 15 rivers and 17 estuaries (45 sites) were sampled in July and August 1997 (Appendix A). Sites were chosen to maximize trophic variability and to be independent of one another (i.e. we avoided sampling more than one water body in a given drainage area). Since we were primarily interested in inter-site variation rather than within-site variation, each site was only visited once.

In the summer of 1998, we sampled 8 additional lakes in the Outaouais region (Ottawa River Valley) of eastern Ontario and south-western Québec (Figure 1.2b; Appendix A) in order to compare Prince Edward Island lakes with the better studied central Canadian lakes.

Sampling

Each site was sampled at 4 stations along a given transect. In lakes, the transect crossed the lake as close to the middle point as possible, whereas longitudinal transects were used for rivers and for estuaries. Estuaries were sampled within 3 hours of high tide.

At each station, total depth and Secchi depth were recorded. Temperature, salinity and conductivity profiles were determined using a YSI S-C-T meter. Light extinction was measured (on all but the shallowest sites) with a LiCor 185B 4II underwater photometer. Finally, 4 Litres of water were drawn at each station using a length of 2.5 cm diameter Tygon tubing. This produced an integrated sample over the epilimnion to a maximum depth of 7m in deep sites or to 50 cm from the bottom in shallower sites. In the few cases where total depth was less than 50 cm, the samples were taken by lowering the collecting bottle directly into the water and moving it around in the water column, taking care not to disturb the sediment.

Within 6 hours of collection, water samples were returned to the lab. Alkaline phosphatase activity (Prince Edward Island sites only) was measured in 4 ml sub-samples from each station, using 3-0 methylfluorescein phosphate in 10mM TRIS buffer (pH 8.7), as substrate (modified from Pick 1987). Fluorescence was measured in a Turner model 111 fluorometer equipped with a Wratten 47 B primary filter, a 2A-12 secondary filter, and with the window set at 1x. Activity was measured as the slope of fluorescence as a function of time.

The remaining water was filtered through a series of various size Nitex screens (pore size; 64µm and 30µm) and Nucleopore filters (pore size; 3µm, 1µm and 0.2µm) in order to measure the amounts of phosphorus and chlorophyll *a* in particles of varying size.

Filtering protocol

The filtering protocol was developed in order to keep the various size fractions as independent of one another as possible. Only in the case of the two smallest fractions were they not. The procedure was repeated separately for each of the four station replicates. The full approach, described below, is represented schematically in Figure 1.3.

First, unfiltered water was saved for total phosphorus analysis. Next, unfiltered water was passed through a 64µm filter. We retained the filter for analysis of the >64µm particulate fraction. To yield a 30-64µm fraction, a sub-sample of water was first filtered through a 64µm screen before being passed through a 30µm filter. Similarly, the procedure was repeated to produce a 3-30µm fraction. The resulting filtrate was then passed through a 0.2µm filter to produce a 0.2-3µm fraction. Finally, the filtrate was collected as the soluble fraction (<0.2µm). Two sets of filters were produced for each step mentioned above; one to be used for phosphorus analysis and one for chlorophyll *a* analysis. Total chlorophyll *a* was calculated as the sum of the chlorophyll in these fractions. The filtrates of the two replicates were pooled when they were needed for following steps of the procedure. Unfiltered water was also passed through a 1µm filter to produce the >1µm fraction needed for particulate inorganic phosphorus analysis.

Total and soluble phosphorus samples were preserved sealed in pre-

cleaned, phosphorus-free glass tubes and all filters were wrapped in foil and frozen until analysis.

Laboratory analyses

Total phosphorus in each size fraction (excluding those bound for inorganic particulate analysis) was determined spectrophotometrically using the molybdenum blue method after potassium persulfate digestion (Menzel and Corwin 1965). To measure inorganic particulate phosphorus, the 1µm filters were placed in 100 ml double-distilled water in quartz tubes to which was added a few drops of H_2O_2 (30%). These were subjected to 500 watt UV radiation for 1.5 hours, a process that solubilizes the organic phosphorus. The samples were then divided into two portions of 50 ml. To solubilize inorganic phosphorus 1 ml of HCl was added to one of the portions (fraction B). The second portion (fraction A) remained intact. Both portions were then placed in a boiling water bath for 2 hours. The tubes were cooled to room temperature after which 1 ml of HCl was added to fraction A in order to equalize the pH in the two portions (modified from Solórzano and Strickland 1968). Phosphorus in each fraction was then measured using the molybdenum blue method. The phosphorus measured in fraction A (P_A) represents particulate organic phosphorus. Particulate inorganic phosphorus (PIP) content of the samples was then determined with the equation;

$$PIP = 2(P_B - P_A)$$

where P_B is the phosphorus in fraction B .

Chlorophyll *a* concentrations were analysed by spectrophotometer after extraction in 95% ethanol for 24 hours (Ostrofsky and Rigler 1987). Chlorophyll *a* concentrations were then calculated using the calibration equation of Wintermans and Demots (1965).

Particulate and particulate organic phosphorus calculations

Our data allowed particulate phosphorus to be calculated in two ways; 1) by subtracting soluble phosphorus from total phosphorus and 2) by taking the sum of the phosphorus found on the 64, 30, 3 and 0.2µm filters for a given site. Similarly, we also had two ways of measuring particulate organic phosphorus; 1) as directly measured on the 1µm filters and 2) as total phosphorus less the soluble fraction, less the inorganic fraction measured in the greater than 1µm fraction. If the statistical analyses (see below) did not distinguish between the two measurements, results were only shown for one of the two calculations.

Statistical analyses

General linear models were used to examine the relationships between chlorophyll *a* concentration and various phosphorus variables. To test whether systematic differences among aquatic biomes remain in these relationships, aquatic biome was used as a categorical variable in the models. Only significant

terms were retained as the best model. Goodness-of-fit was determined by the proportion of the variance accounted for by the model (i.e. r^2 value).

Unexplained variation

We also attempted to estimate what proportion of the residual variability in the chlorophyll - phosphorus relationships represents within-water body variation (i.e. experimental error, in the context of this study). These sources of experimental error include temporal variation in chlorophyll *a* concentration, within-site spatial variation in chlorophyll *a* concentration and analytical or measurement error. Our replicate samples from multiple stations within individual sites enable us to estimate the variability due to the sum of spatial variance and measurement error. We have no measure of the actual temporal variability of chlorophyll concentration for our sites since each was only visited once. To estimate the temporal variance in our sites we used the following equation from Marshall et al. (1988):

$\log (s^2) = -0.53 + 2.10 (\log X)$

where s^2 is the annual temporal variance and X the mean chlorophyll *a* concentration. Since this equation was derived for total chlorophyll concentrations, we were not able to estimate the temporal variance associated with the various chlorophyll size fractions used in our models.

RESULTS

Site characteristics

The morphology of sites on Prince Edward Island differs markedly from that of central Canadian lakes (Table 1.2). Prince Edward Island lakes, rivers and estuaries are shallower and cooler than Outaouais lakes. Estuaries have an average salinity of 23‰. However, even freshwater systems on Prince Edward Island show traces of salt spray: many have salinities slightly higher than zero and conductivity levels 10-12 times higher than those in Outaouais lakes. Estuarine conductivity is over 25 times that of the Island's lakes and rivers. The Outaouais lakes sampled are mesotrophic (TP = 12 - 29 $\mu g \cdot L^{-1}$) whereas Prince Edward Island systems are generally eutrophic (TP = 15 - 127 $\mu g \cdot L^{-1}$). On average, alkaline phosphatase activity levels were greater in lakes than either rivers or estuaries.

Chlorophyll *a* - total phosphorus relationships

Significant Chl-TP relationships were developed for each of the biomes studied (Table 1.3a). The r^2 values are low relative to many other published relationships. This may be partly due to sampling each system only once. However, it also reflects the fact that the range of total phosphorus concentrations observed in this study was not wide. The residual mean squared errors (RMSE) observed here are actually comparable to those observed in other studies (e.g.

this study, Outaouais lakes RMSE = 0.031, in Currie 1990 RMSE = 0.055 and in Currie et al. 1999 RMSE = 0.019).

Relationships between chlorophyll *a* and total phosphorus differed significantly among systems studied (Table1.3b, Figure 1.4). *A posteriori* comparisons show that lakes had significantly more chlorophyll per unit total phosphorus than did Prince Edward Island rivers and estuaries. Although less chlorophyll per unit total phosphorus was observed in PEI lakes than in Outaouais lakes, the difference was not significant.

When the data from all biomes are pooled, only 10% of the total variation in log chlorophyll *a* can be accounted for by log total phosphorus. An additional 23% is related to differences among biomes (Figure 1.5, A).

How much of the total variation in chlorophyll *a* concentration is experimental error? The variability in chlorophyll due to experimental error was calculated for each of the chlorophyll *a* fractions used in the linear models developed. Together, spatial variance and measurement error accounted for 3 to 5% of the total variability in chlorophyll *a* yield. Temporal variance accounted for 7% of this same variability (Figure 1.5, top of bars).

The phosphorus sequestration hypothesis

Chlorophyll yield may vary among systems because differing amounts of phosphorus are sequestered in non-algal components of the water column. This

hypothesis predicts that the chlorophyll - phosphorus relationship would be stronger if non-algal component(s) were excluded. We test this by progressively removing non-algal phosphorus components from the total phosphorus pool.

Is the residual variability in the Chl-TP relationship due to differences in soluble phosphorus among aquatic biomes? To test this possibility, we examined the relationship between chlorophyll *a* concentration and particulate phosphorus (PP). When soluble phosphorus was removed from the total phosphorus concentration, the total amount of variance relatable to phosphorus more than doubles (Figure 1.5, B vs A). In other words, variation in chlorophyll yields among water bodies of a given biome are at least partly due to differences in the proportion of total phosphorus that is particulate versus soluble. Yet differences in chlorophyll yield among aquatic biomes remain after excluding the soluble phosphorus.

When the analysis was repeated with the Prince Edward Island data only, the total variability explained is similar to that of the model with all four biomes together, but the biome term disappears (Figure 1.6, B). In other words, Prince Edward Island water bodies as a group have less chlorophyll per unit phosphorus than do Outaouais lakes, and the difference cannot be attributed to soluble phosphorus. In contrast, the average differences in chlorophyll yield among PEI lakes, rivers and estuaries can be attributed to differing amounts of soluble phosphorus.

Does variation in chlorophyll yields among aquatic systems also reflect varying amounts of non-algal particulate phosphorus? For example, phosphorus associated with inorganic particles is thought to be largely unavailable to the algae. In the case of PEI, iron from the soil may bind with phosphorus, leaving less available for algae. Is the residual variability in the Chl-TP relationship related to differing amounts of particulate inorganic phosphorus (PIP) in different systems? To answer this question, we related chlorophyll *a* concentrations to particulate organic phosphorus (POP) concentrations. We found that, when particulate inorganic phosphorus is removed from the particulate phosphorus model, no additional variability is explained. On the contrary, explained variation is smaller (Figure 1.5, C and Figure 1.6, C). In other words, some of the phosphorus extracted in the analysis of particulate inorganic phosphorus is actually algal.

Is the residual variability in the Chl-TP relationship due to differences in amounts of large particulate phosphorus? Prepas and Rigler (1982) found that the variance among replicate measurements of total phosphorus concentration decreased when particles larger than 250μm were removed from the samples first. This procedure removes large crustacean zooplankton which are typically present in sufficiently low numbers so that one individual more or fewer may influence the amount of phosphorus measured in a given sample.

We found that removing the particulate matter (i.e. both the phosphorus

and the chlorophyll) larger than 64µm from the samples did not lead to stronger chlorophyll - phosphorus relationships (Figure 1.5, D). Similar results were obtained when particulate matter larger than 30µm was removed (Figure 1.5, E). As was the case for the particulate phosphorous models, the effect of biome was non-significant when only PEI systems are included in the analysis (Figure 1.6 D, E).

Might the residual variability in the Chl-TP relationship be due to differences in bacterial use of phosphorus? Bacteria can compete with algae for phosphorus (Currie and Kalff 1984a, 1984c) and differences in bacterial abundance among water bodies might influence the amount of phosphorus in the small particulate fractions just as zooplankton could for the large fractions. Currie and Kalff (1984b) found that, in central Canadian lakes, most bacteria passed through 3.0µm filters, while most chlorophyll was retained by these same filters. We therefore examined the effect of bacteria by removing the particulate matter (i.e. both phosphorus and chlorophyll) in the 0.2-3µm size fraction from the chlorophyll - phosphorus relationship.

Slightly more of the variability in chlorophyll *a* concentration was related to phosphorus in this model, although large, systematic differences among biomes remain (Figure 1.5, F). For this particulate fraction, the differences among biomes cannot be accounted for by Outaouais lakes. Contrary to the other particulate models derived for PEI data, biome remained a significant term in the model

(Figure 1.6, F). By rerunning the analysis, systematically removing one biome from the others, we found that the biome effect in the >3µm fraction was due to PEI estuaries. When the estuarine data are removed, phosphorus accounts for 65 percent of the variability in chlorophyll *a* concentration among Outaouais lakes and PEI lakes and rivers (Figure 1.5, I and Figure 1.7).

Why do estuaries have a lower chlorophyll *a* yield than the other biomes for the >3µm fraction? A closer look at the distribution of chlorophyll *a* in the different size fractions shows that estuaries have considerably more small chlorophyll particles than the other biomes. Almost 60% of the total chlorophyll in estuaries is found in the 0.2-3µm fraction (Table 1.4). Removing this fraction from the model removes much of the chlorophyll *a* in estuaries, resulting in the differences among biomes. Therefore, a good predictor of chlorophyll *a* for estuaries must include particles in this 0.2-3µm fraction. We found that the best model predicting chlorophyll *a* in estuaries was the 0.2-30µm particulate fraction (i.e. <30µm fraction), where phosphorus accounted for 69% of the variability in chlorophyll *a* (Figure 1.5, II).

Because of the large increase in the predictability of the chlorophyll - phosphorus model when particles less than 3µm were removed, we investigated the relationship between these two variables in this fraction. We found that there is no correlation between chlorophyll *a* and phosphorus in the 0.2µm - 3µm fraction in our biomes (Figure 1.8).

The varying algal phosphorus hypothesis

A second general hypothesis to explain variation in chlorophyll yield among water bodies is that the amount of chlorophyll produced per unit of algal phosphorus may differ among systems. As nutrients other than phosphorus begin to limit algal growth, algae may store increasing amounts of phosphorus (Harris 1986). Thus lower chlorophyll yields might be stored in water bodies in which phosphorus is less strongly limiting.

To test if varying levels of phosphorus limitation accounted for any of the remaining variation in chlorophyll *a* yield, alkaline phosphatase activity (APA) was added as a independent variable to each of the above mentioned models. The only model in which the APA term was significant was the total phosphorus model. In that case, the effect of APA removes that of biome. Together, TP and APA explain 46% of the variability in chlorophyll *a* among the sites, an amount significantly better than TP and biome (33%). APA on its own was not significantly related to any of the variability in chlorophyll.

Since the effect of APA levels disappears with the removal of the soluble phosphorus fraction, the relationship between these two variables was examined. We found that APA and soluble phosphorus were negatively correlated (Figure 1.9a). This correlation was not present between APA and TP (Figure 1.9b).

Decreased light availability has also been found to increase the chlorophyll *a* content of algal cells (Nicholls and Dillon 1978). To test if any remaining

residual variability in the Chl-TP relationship is due to light limitation we added the ratio of euphotic depth : mixed depth as an additional independent variable to the above phosphorus models. Euphotic depth was calculated as the depth of 1% surface light intensity. The ratio was not significant in any model.

DISCUSSION

Total phosphorus concentration has been traditionally used to represent phosphorus available to algal growth since it includes the phosphorus concentrations already incorporated into algal cells as well as all the potentially available phosphorus found in either soluble or suspended form (Nicholls and Dillon 1978). The assumption is that the phosphorus unavailable to algae is a negligible amount of the total phosphorus hydrolysed.

By comparing the chlorophyll - phosphorus relationships of different aquatic biomes (lakes, rivers, estuaries) for various phosphorus chemical forms and size classes, the present work shows that phosphorus in compartments other than algae is responsible for much of the variability of chlorophyll - total phosphorus relationships.

The converse to our first hypothesis, that proportion of phosphorus unavailable or unused by algae differs among biome, is that the proportion of phosphorus utilised by algae does not differ among biome. In other words, the chlorophyll *a* - algal phosphorus (the phosphorus concentration genuinely in

algae) relationships of the biomes should be identical. Consequently, better predictions of chlorophyll *a* concentration should be given by the algal phosphorus fraction.

Algal phosphorus cannot be easily measured by any conventional method (Nicholls and Dillon 1978). Yet, the larger than 3µm size fraction represents that in which most or all particles are of algal nature (Currie et al. 1986). This fraction is our closest, and therefore best, approximation of the true algal phosphorus concentration. Our results show that this fraction is indeed the best predictor of chlorophyll *a* concentration among three of the biomes studied, explaining 30 % more of the variability in chlorophyll concentration than the chlorophyll - total phosphorus relationship.

In the one biome where this was not the case (i.e. PEI estuaries), the best approximation of algal phosphorus was not the >3µm particulate fraction but the 0.2 - 30µm particulate fraction. We found that the best predictor of chlorophyll *a* concentration in estuaries was indeed this <30µm particulate fraction. The amount of variability in chlorophyll *a* explained by phosphorus in this model was similar to that explained by the >3µm fraction for the other biomes.

These findings uphold our first hypothesis that predicts that the variation in chlorophyll yield of the biomes is the result of phosphorus segregated in non-algal components of the total phosphorus pool. This result also corresponds to those of Chow-Fraser et al. (1994) that showed that total biologically active phosphorus (a

measure of the amount of phosphorus used by algae in a test culture) is a better predictor of chlorophyll *a* in lakes then is total phosphorus.

We find that the predictive ability of the chlorophyll - phosphorus relationship among biomes increases by 30% when soluble phosphorus is removed from the total phosphorus concentration. Smith (1982) hypothesised that as nitrogen limitation increases, the concentration of total dissolved phosphorus (orthophosphate and dissolved organic phosphate) in the water column also increases, due to the lessened phosphate demand from algae. Therefore, soluble phosphorus would represent a greater proportion of the total measured phosphorus in nitrogen limited systems. Consequently, total phosphorus would no longer be a good estimation of the phosphorus utilizable for algal growth in these systems. The negative correlation found between alkaline phosphatase activity levels, our estimate of phosphorus limitation, and soluble phosphorus concentration are consistent with this hypothesis. Many studies support nitrogen limitation as a cause of variation in Chl-TP relationships (Elser et al. 1990, Prairie et al. 1989, McCauley et al. 1989, Chow-Fraser et al. 1994). Perhaps some of the effects of nitrogen limitation on algal abundance can be remedied by using particulate phosphorus instead of total phosphorus as a variable.

Phytoplankton are not thought to have a way of liberating phosphorus from inorganic particles and as a result, particulate inorganic phosphorus is considered to be of no biological use to algae (Wetzel 1983). The predictive ability of

particulate organic phosphorus to account for chlorophyll *a* concentration should therefore be similar or greater than that of particulate phosphorus. However, we find that for our data set, the amount of variability in chlorophyll concentration explained after the removal of the particulate inorganic phosphorus from the total particulate phosphorus pool is much lower.

It is possible, although unlikely, that phosphorus in inorganic particles can influence algal abundance. Interactions between various ions and inorganic compounds in the water could potentially liberate phosphorus for algal uptake or algae might have yet undiscovered mechanisms enabling them to liberate phosphorus on the outer surface of inorganic molecules. However, the most plausible explanation for our result is that of measurement error. An improper or incomplete solubilization of organic material in the sample would result in an overestimation of inorganic phosphorus.

Removal of the large particulate fraction that could contain zooplankton phosphorus did not alter the amount of variability explained from that accounted for in the total particulate phosphorus model. This result was not completely unexpected as zooplankters can easily avoid the tube sampler used to retrieve our water samples (Patalas 1951, Clutter and Anraku 1968). Consequently, their presence in our samples would be highly unusual to start with. However, the non significant result also establishes that there are no other large particles (e.g. large flocced complexes in estuaries) influencing the variability in chlorophyll yield.

The result of removing particulate matter smaller than 3µm from the total particulate phosphorus fraction is a significant increase in the amount of chlorophyll *a* accounted for by phosphorus. The fraction removed (0.2-3µm), represents the fraction in which most bacteria are found (Currie and Kalff 1984b). The increase in the variability explained may be explained by varying bacterial abundance in the biomes. Currie (1990) examined various possible relationships between phosphorus, algal abundance and bacterial abundance in lakes and found that bacterial abundance could explain 18-65% of the residual variance in Chl-TP relationships. Currie (1990) also found that the best model accounting for the variation in abundance of both phytoplankton and bacteria is one in which phosphorus influences both groups and that they also influence each other. This set of interactions may account for the fact that we did not find any relationship between the chlorophyll and phosphorus found in the 0.2-3µm fraction.

To conclude, sequestration of phosphorus into non-algal compartments of the total phosphorus does account for differences in the chlorophyll yield among aquatic biomes, and thus accounts for the residual variability surrounding the Chl-TP relationship. We found that the best predictor of chlorophyll *a* concentration was the size fraction that best approximates the phosphorus actually found in algae. For most aquatic bodies, the algal fraction is represented by particulate phosphorus larger than 3µm. The residual variability surrounding the chlorophyll *a* - total phosphorus relationship is therefore related to varying amounts of soluble

phosphorus (which correlate to differences in nutrient limitation) and of small particulate phosphorus (which may indicate bacterial competition).

Table 1.1: Summary of regression equations and ANCOVA models predicting log chlorophyll *a* (Chl *a*) concentrations from log total phosphorus (TP) concentrations in three aquatic biomes (lakes, rivers and estuaries). The data presented are taken from the literature - Lakes (Currie 1990, Currie et al. 1999, Wilson 1996), Rivers (Basu and Pick 1996) and Estuaries (Meeuwig et al. 1998). Indep.Var. = independent variable; SS = sum of squares; RMSE = residual mean square error.

A) Chlorophyll *a* - total phosphorus relationships (individual data)

Biome	Equation	N	r^2	RMSE
Lakes	log(Chl *a*) = -0.57 + 1.08 log(TP)	95	0.75	0.035
Rivers	log(Chl *a*) = -0.26 + 0.73 log(TP)	31	0.76	0.027
Estuaries	log(Chl *a*) = -2.89 + 1.76 log(TP)	15	0.65	0.019

B) ANCOVA model

Indep.Var.	SS	d.f.	F	P	N	r^2	RMSE
log(TP)	1.75	1	55.64	<0.001	141	0.76	0.03
Biome	0.45	2	7.07	0.001			
log(TP)*Biome	0.47	2	7.39	0.001			

C) Chlorophyll *a* - total phosphorus relationship (pooled data)

Biome	Equation	N	r^2	RMSE
All biomes	log(Chl *a*) = -0.31 + 0.499 log(TP)	95	0.29	0.091

Table 1.2: The mean and range of morphometric and physico-chemical variables of the 53 sites analysed. The sites were distributed into 13 Prince Edward Island (PEI) lakes, 15 PEI rivers, 17 PEI estuaries and 8 Outaouais lakes. Temp. = temperature; Sal. = salinity; Cond. = conductivity; K = light extinction coefficient; APA = alkaline phosphatase activity (in fluorescence units minute^{-1}); TP = total phosphorus; Chl = chlorophyll *a*; Max. = maximum; Min = minimum.

		Max. Depth (m)	Mean Depth (m)	Secchi Depth (m)	Temp. (°C)	Sal. (‰)	Cond. (µmhos cm^2)	K (m^{-1})	APA (flu/min)	TP (µg·L^{-1})	Chl (µg·L^{-1})
PEI	Mean	2.4	1.7	2.4	18.6	0.4	1345.8	1.1	1.5	69.1	10.7
Lakes	Max.	3.5	3.1	3.5	24.0	3.5	7835.4	2.0	7.6	127.7	35.9
	Min.	1.3	0.9	1.3	13.7	0.0	27.4	0.6	0.1	15.0	0.3
PEI	Mean	1.4	1.0	1.4	15.5	0.9	1747.5	1.4	0.3	66.5	3.6
Rivers	Max.	3.4	2.5	3.4	21.5	9.2	14562.5	2.2	1.3	110.5	14.4
	Min.	0.2	0.2	0.2	10.1	0.0	160.9	0.7	0.1	18.8	0.7
PEI	Mean	5.2	4.2	3.1	20.7	23.2	35170.6	0.7	0.3	63.8	3.2
Estuaries	Max.	8.0	6.6	4.9	23.6	28.5	43000.0	1.1	1.7	105.4	7.6
	Min.	2.3	1.8	1.2	17.7	17.2	27104.2	0.1	0.0	29.3	0.9
Outaouais	Mean	6.3	5.3	3.71	24.5	0.0	139.40	0.7	n/a	17.5	3.2
Lakes	Max.	13.0	13.0	7.2	26.5	0.0	255.2	0.9	n/a	29.9	5.7
	Min.	2.3	1.9	2.3	18.0	0.0	75.0	0.5	n/a	11.6	1.7

Table 1.3: Summary of regression equations and ANCOVA model predicting log chlorophyll *a* concentrations from log total phosphorus (TP) concentrations in the four aquatic biomes studied - Prince Edward Island (PEI) lakes, PEI rivers, PEI estuaries and Outaouais lakes. The interaction between log total phosphorus (TP) and the categorical variable (Biome) was not significant. Indep.Var. = independent variable; SS = sum of squares; RMSE = residual mean square error.

A) Chlorophyll *a* - total phosphorus relationships (individual data)

Biome	Equation	N	r^2	RMSE
PEI Lakes	log(Chl *a*) = -1.79 + 1.40 log(TP)	13	0.41	0.19
PEI Rivers	log(Chl *a*) = -0.35 + 0.40 log(TP)	15	0.09	0.093
PEI Estuaries	log(Chl *a*) = -1.11 + 0.84 log(TP)	17	0.15	0.066
Outaouais Lakes	log(Chl *a*) = -0.49 + 0.78 log(TP)	8	0.32	0.031

B) ANCOVA model

Indep.Var.	SS	d.f.	F	P	N	r^2	RMSE
log(TP)	1.50	1	14.73	<0.001	53	0.33	0.10
Biome	1.679	3	5.49	0.003			

Table 1.4: Mean percentage of total chlorophyll *a* concentration ($\mu g \cdot L^{-1}$) measured in each size fraction for the four biomes studied.

Biome	N	Size Fraction			
		>64µm	30-64µm	3-30µm	0.2-3µm
PEI Lakes	13	24.5	10.3	41.1	28.2
PEI Rivers	15	16.3	10.8	40.5	33.4
PEI Estuaries	17	4.1	3.1	35.0	58.1
Outaouais Lakes	8	13.3	11.5	40.2	35.0

Figure 1.1: The relationship between log chlorophyll *a* and log total phosphorus for lakes, rivers and estuaries. The lakes data (n = 95) come from Currie (1990), Currie et al. (1999) and Wilson (1996); river data (n = 31) from Basu and Pick (1996); and estuary data (n = 15) from Meeuwig et al (1998). The lines represent the regression models fitted to the data of each system.

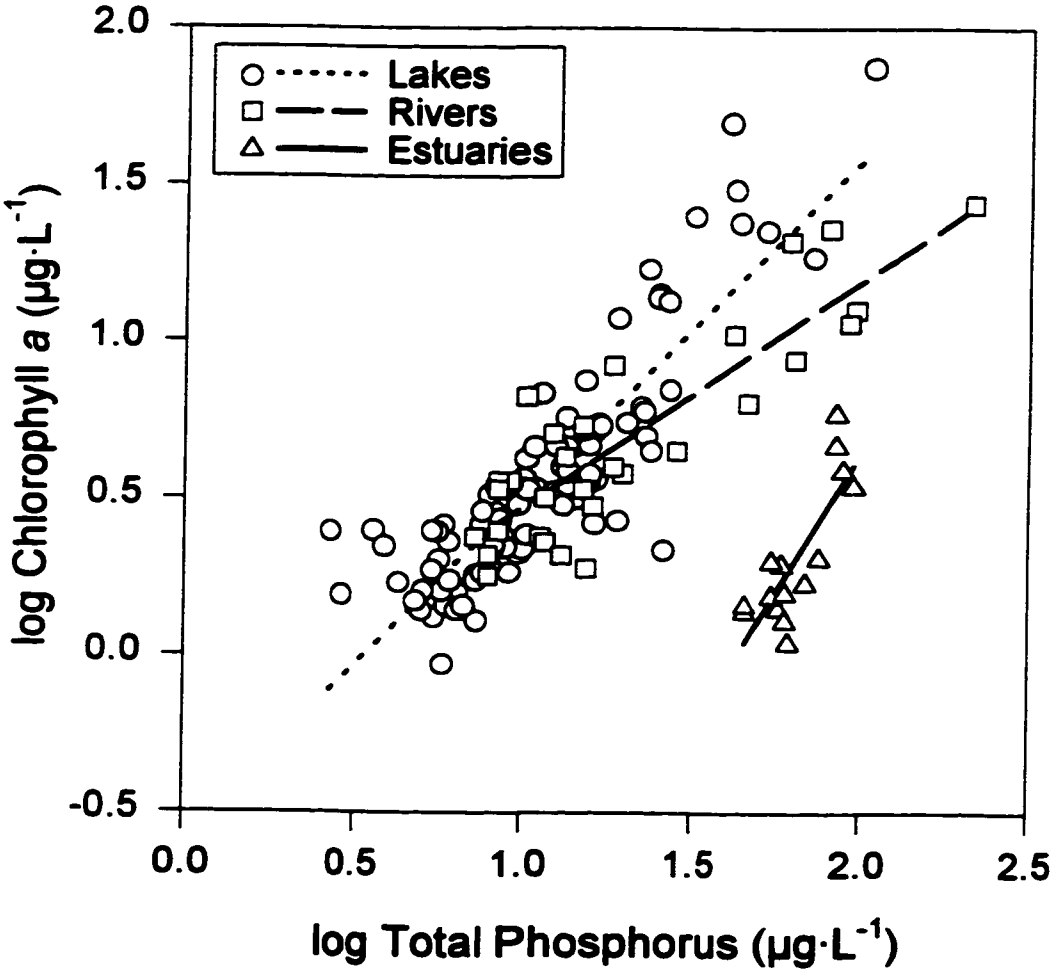
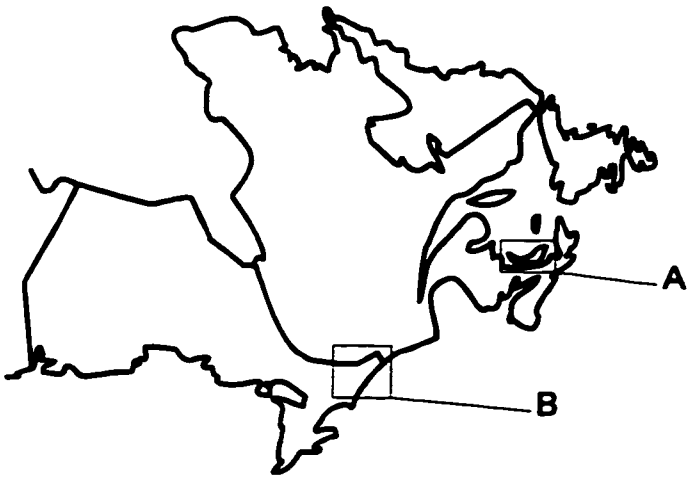
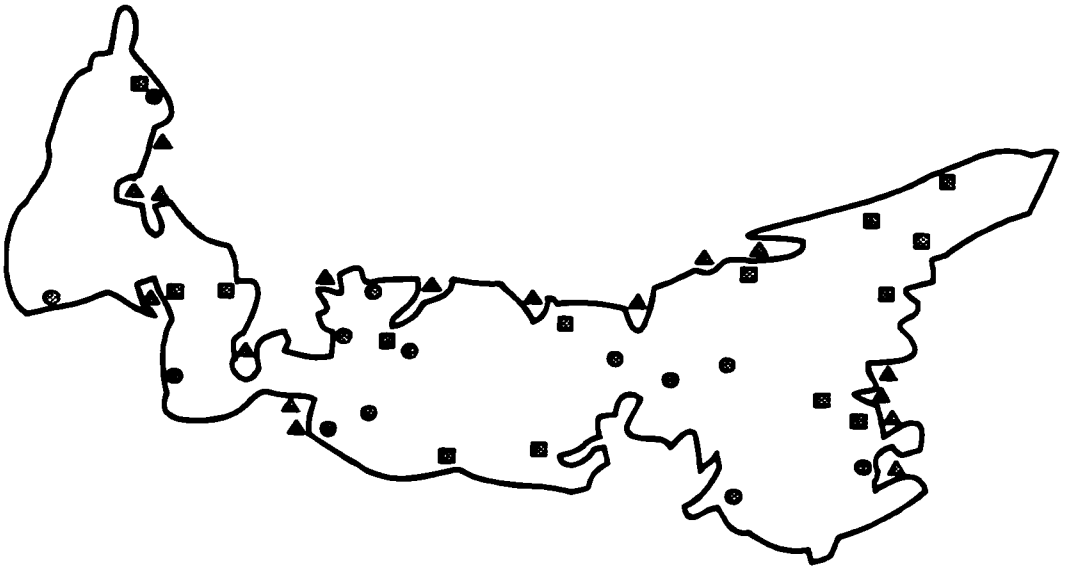

Lakes
Rivers
Estuaries
2.0
1.5
1.0
0.5
0.0
-0.5
0.0
0.5
1.0
1.5
2.0
2.5
log Chlorophyll a (µg·L-1)
log Total Phosphorus (µg·L-1)

Figure 1.2: Location of the 53 sampling sites in this study; a) Prince Edward Island sites, b) "Outaouais" sites in south eastern Ontario and western Québec. Lakes are represented by circles, rivers by squares and estuaries by triangles.


A
B

Ⓐ



Ⓑ

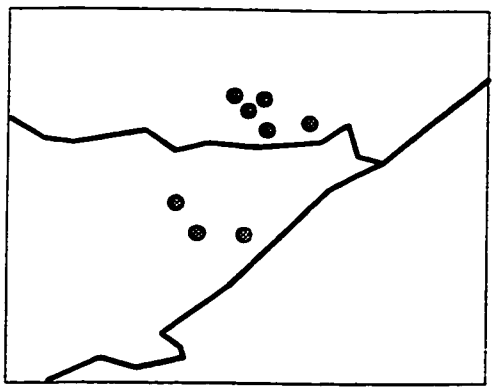


Figure 1.3: Schematic representation of the filtering protocol followed in the study. The procedure was repeated for each of the 4 station replicates. Horizontal lines represent filters, retained for analysis, having the indicated pore size. When two filters are pictured together, one is used for phosphorus (P) analysis and the other for chlorophyll *a* (Chl) analysis. Pre-filtration (not retained) is indicated by the (⊔) symbols. Test tubes represent analyses on direct water samples. TP = total phosphorus; SP = soluble phosphorus; PIP = particulate inorganic phosphorus.

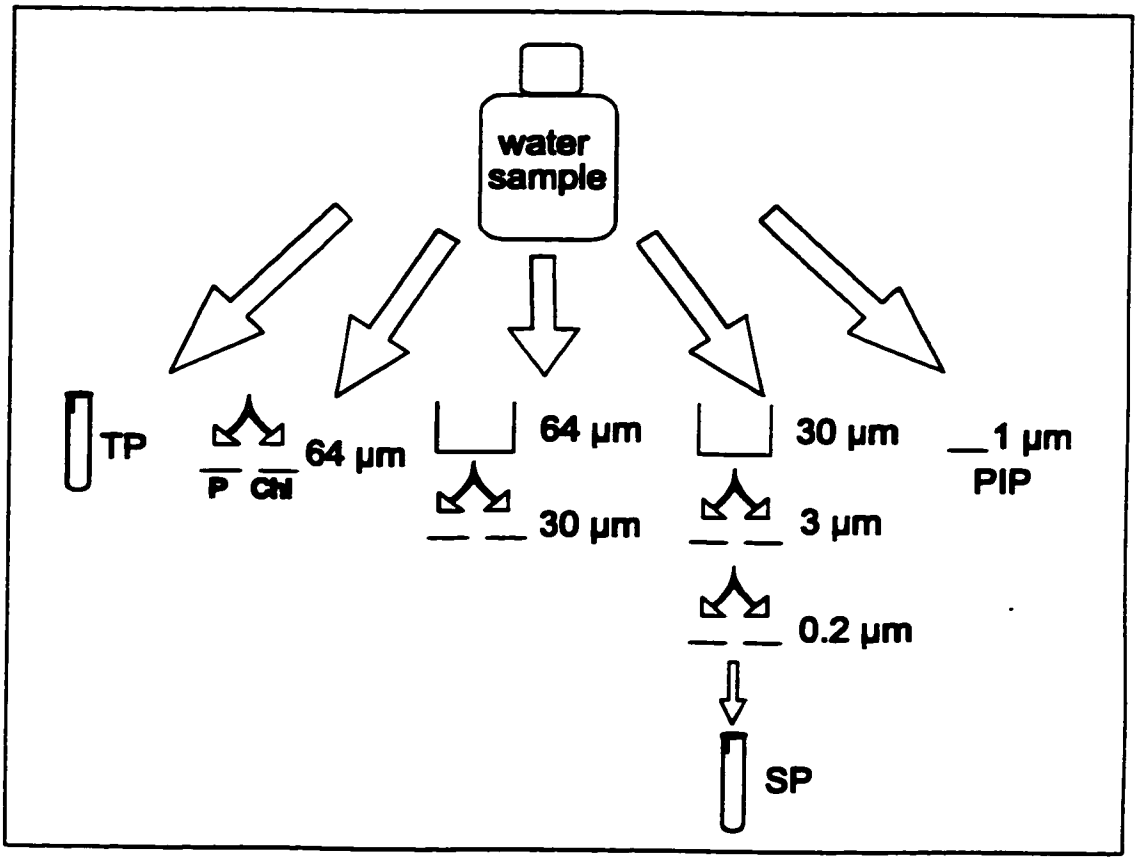

water sample
TP
P
Chl
64 µm
64 µm
30 µm
30 µm
3 µm
0.2 µm
SP
1 µm
PIP

Figure 1.4: The relationship between summer log chlorophyll *a* concentration and log total phosphorus for each aquatic biome studied. The data represent that of Prince Edward Island lakes (n = 13), rivers (n = 15) and estuaries (n = 17) as well as Outaouais lakes (n = 8). The lines represent the regression models fitted to the data of each system.

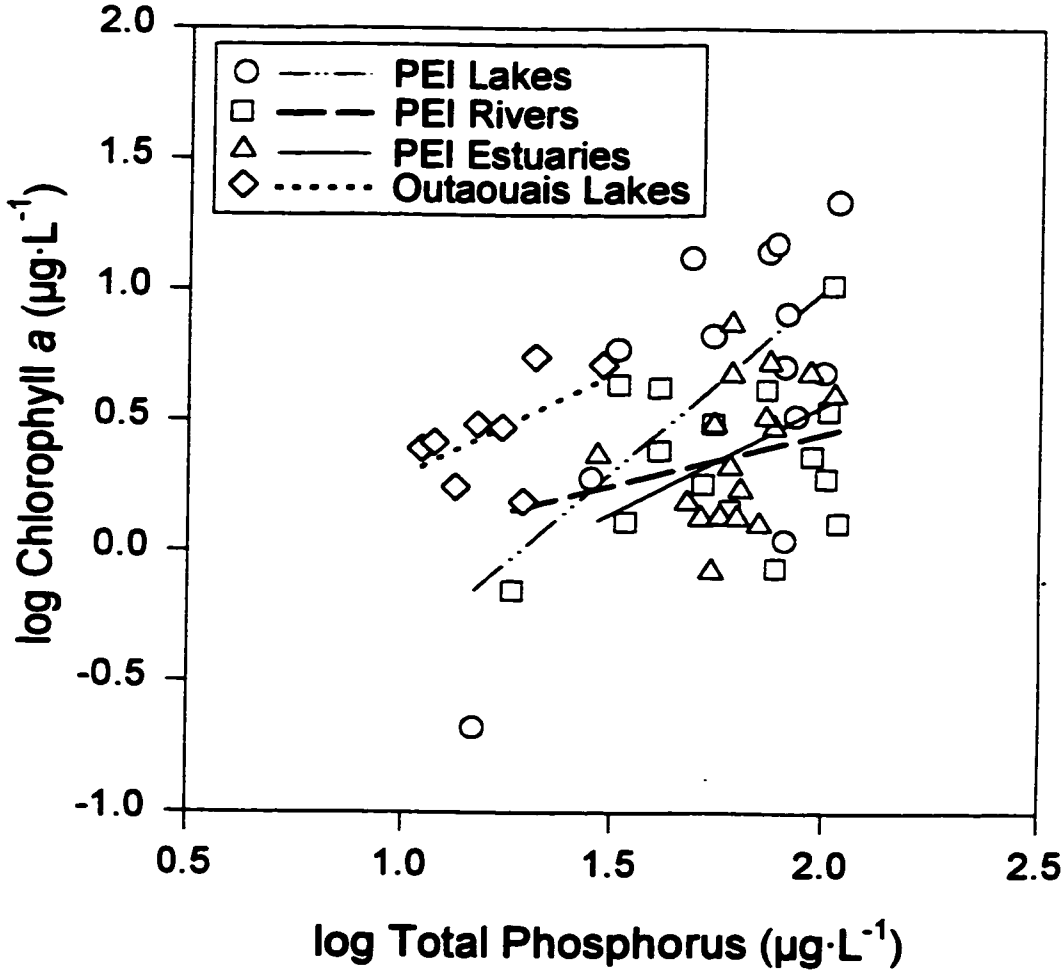

PEI Lakes
PEI Rivers
PEI Estuaries
Outaouais Lakes
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
0.5
1.0
1.5
2.0
2.5
log Chlorophyll a (µg·L-1)
log Total Phosphorus (µg·L-1)

Figure 1.5: The percentage of log chlorophyll *a* concentration ($\mu g \cdot L^{-1}$) predicted by log phosphorus concentration ($\mu g \cdot L^{-1}$) of various size classes and chemical forms. The general linear models developed include data from all four aquatic biomes studied (A-F) unless noted otherwise (I and II). A) total chlorophyll - total phosphorus; B) total chlorophyll - particulate phosphorus; C) total chlorophyll - particulate organic phosphorus; D) chlorophyll - particulate phosphorus (< 64µm); E) chlorophyll - particulate phosphorus (< 30µm); F) chlorophyll - particulate phosphorus (> 3µm). I) chlorophyll - particulate phosphorus (> 3µm), PEI estuaries removed; II) chlorophyll - particulate phosphorus (< 30µm), PEI estuaries only. The percentage of the variability in chlorophyll *a* concentration accounted for by temporal variability (T Error - total chlorophyll only) and spatial variability plus measurement error (S+M Error) is represented at the top of each of the bars.

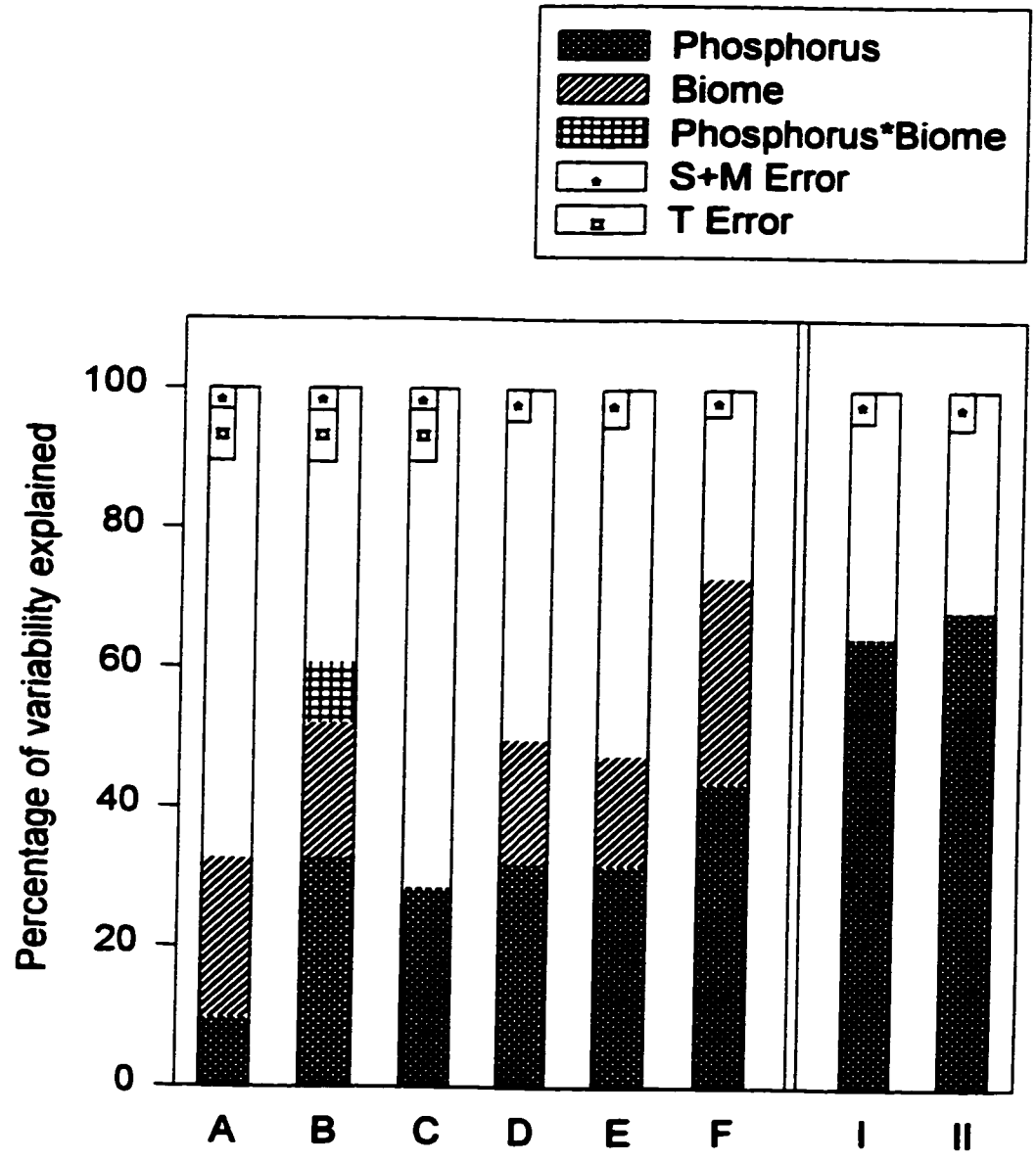

Phosphorus
Biome
Phosphorus*Biome
S+M Error
T Error
Percentage of variability explained
100
80
60
40
20
0
A
B
C
D
E
F
I
II

Figure 1.6: The percentage of log chlorophyll *a* concentration ($\mu g \cdot L^{-1}$) predicted by log phosphorus concentration ($\mu g \cdot L^{-1}$) of various size classes and chemical forms. The general linear models developed include data from the three Prince Edward Island aquatic biomes studied. A) total chlorophyll - total phosphorus; B) total chlorophyll - particulate phosphorus; C) total chlorophyll - particulate organic phosphorus; D) chlorophyll - particulate phosphorus (< 64µm); E) chlorophyll - particulate phosphorus (< 30µm); F) chlorophyll - particulate phosphorus (> 3µm). The percentage of the variability in chlorophyll *a* concentration accounted for by temporal variability (T Error - total chlorophyll only) and spatial variability plus measurement error (S+M Error) is represented at the top of each of the bars.

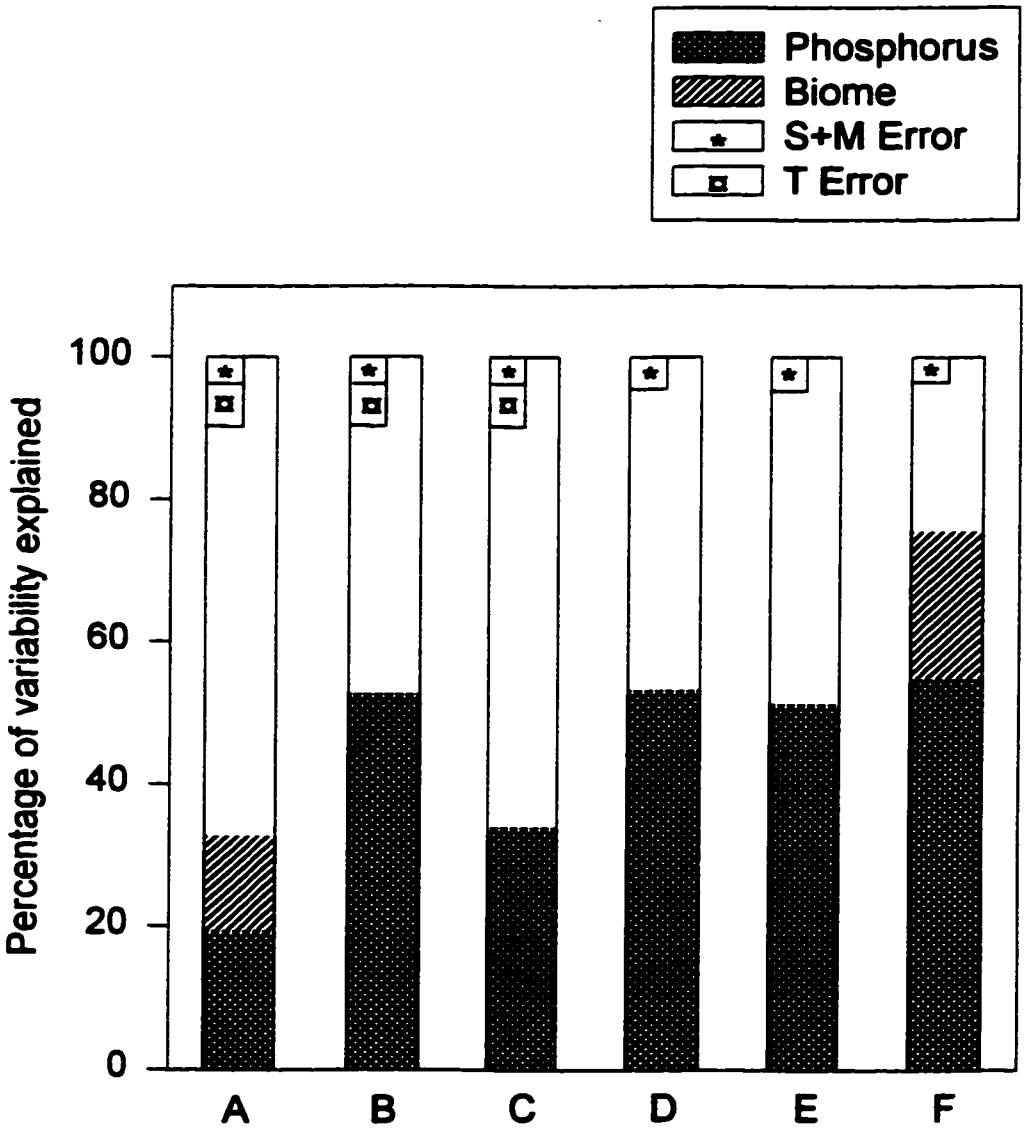

Phosphorus
Biome
S+M Error
T Error
Percentage of variability explained
100
80
60
40
20
0
A
B
C
D
E
F

Figure 1.7: The relationship between log chlorophyll *a* and log phosphorus in the >3µm particulate fraction. Lines represent the regression models fitted to the pooled data of Prince Edward Island (PEI) lakes, rivers and Outaouais lakes (solid line) and PEI estuaries (dashed line).

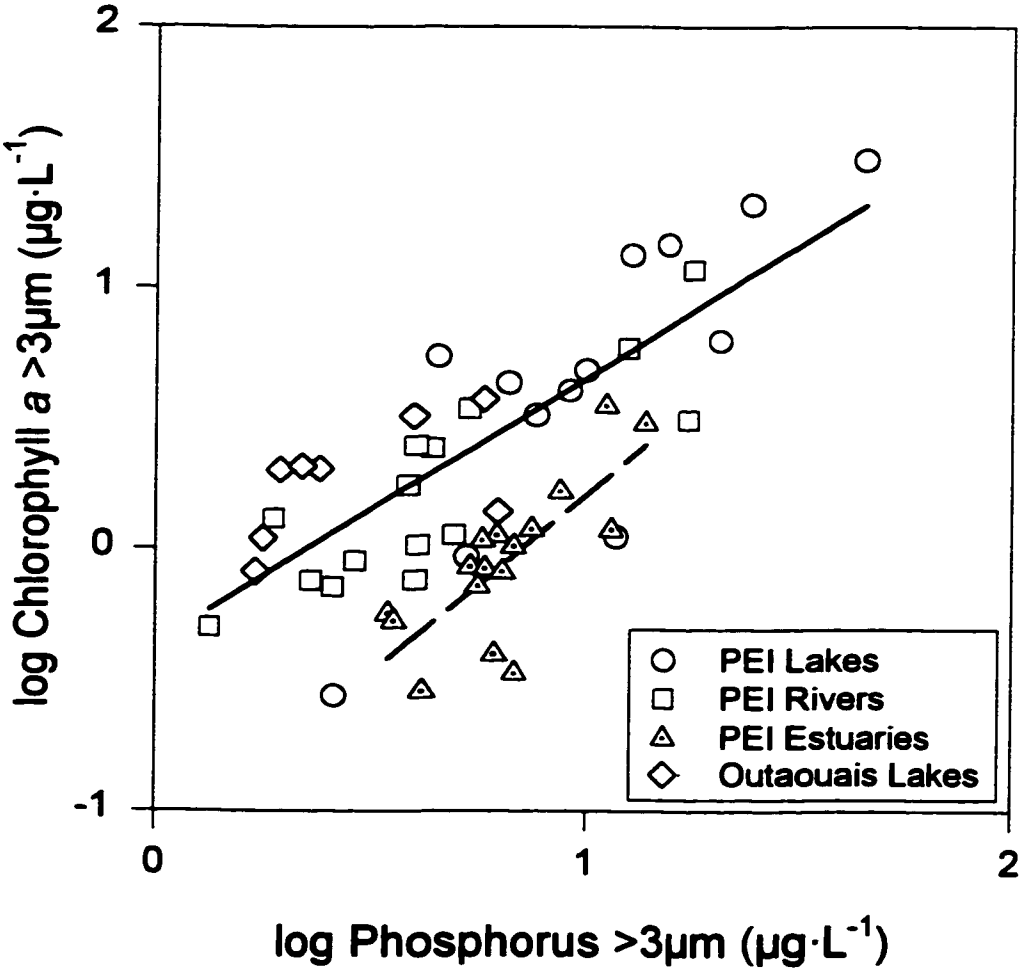

log Chlorophyll a >3µm (µg·L-1)
log Phosphorus >3µm (µg·L-1)
2
1
0
-1
0
1
2
PEI Lakes
PEI Rivers
PEI Estuaries
Outaouais Lakes

Figure 1.8: The relationship between log chlorophyll *a* concentration and log particulate phosphorus concentration of the 0.2 - 3µm size fraction. Data are from Prince Edward Island lakes (circles), rivers (squares), estuaries (triangles) and Outaouais lakes (diamonds). The Pearson correlation coefficient (r), sample number (n) and probability (p) are shown.

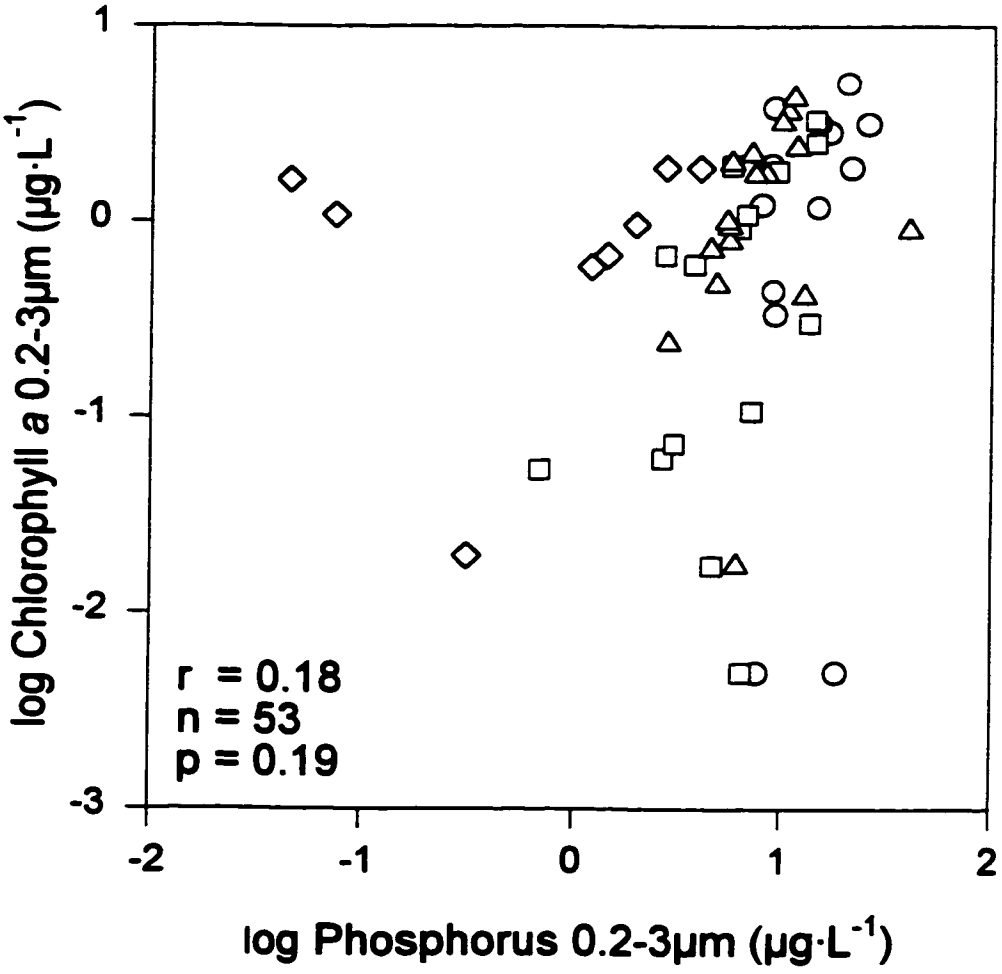
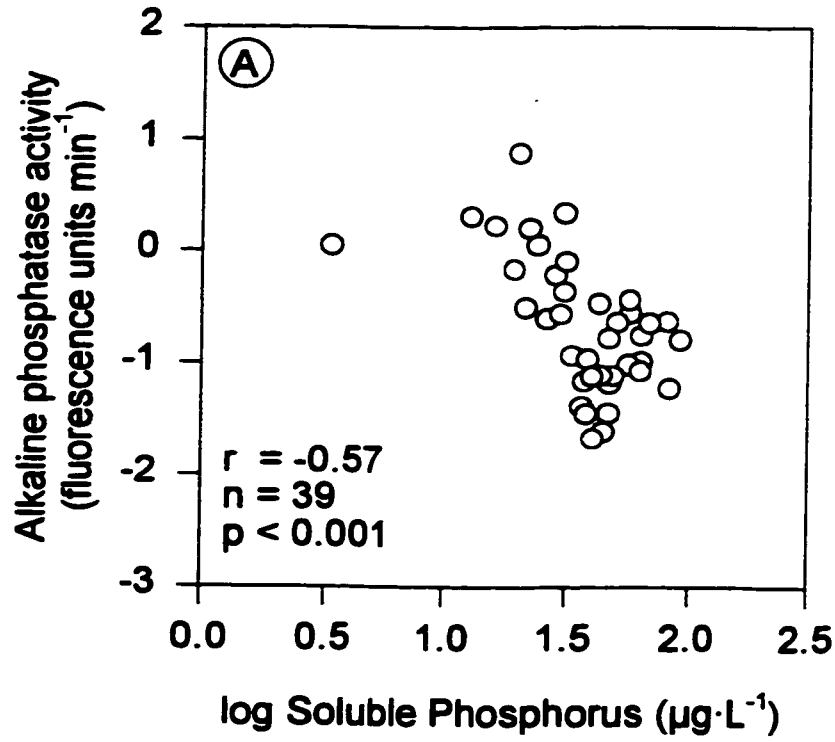
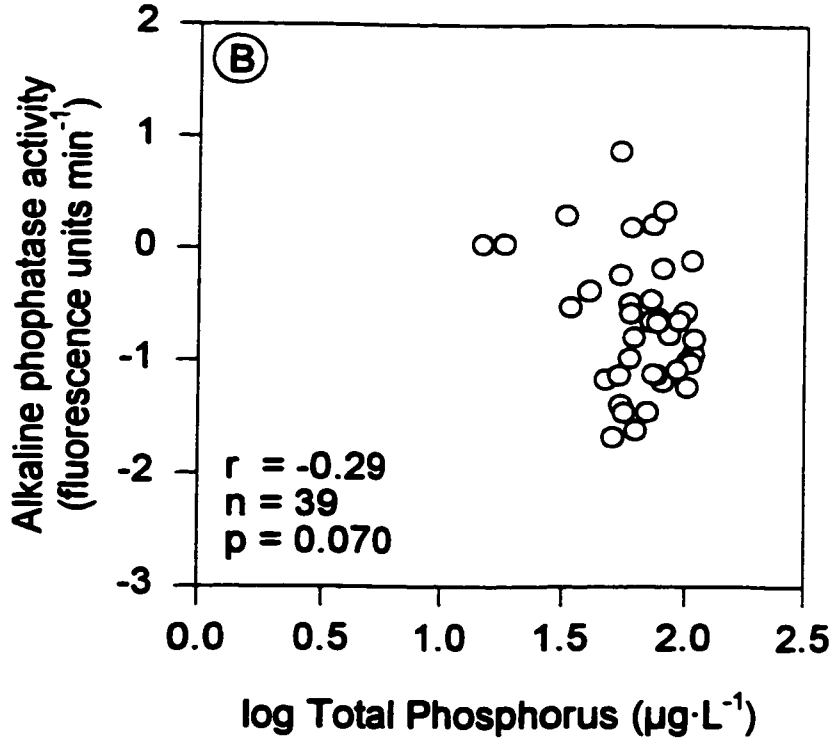

1
0
-1
-2
-3
-2
-1
0
1
2
log Chlorophyll a 0.2-3µm (µg·L-1)
log Phosphorus 0.2-3µm (µg·L-1)
r = 0.18
n = 53
p = 0.19

Figure 1.9: The relationship between log alkaline phosphatase activity (APA) level and log phosphorus in the 39 sites for which APA was measured. A) APA vs soluble phosphorus (<0.2µm). B) APA vs total phosphorus. The Pearson correlation coefficient (r), sample number (n) and probability (p) are shown.


A
Alkaline phosphatase activity (fluorescence units min^{-1})
2
1
0
-1
-2
-3
r = -0.57
n = 39
p < 0.001
0.0
0.5
1.0
1.5
2.0
2.5
log Soluble Phosphorus (µg·L^{-1})


B
Alkaline phophatase activity (fluorescence units min^{-1})
2
1
0
-1
-2
-3
r = -0.29
n = 39
p = 0.070
0.0
0.5
1.0
1.5
2.0
2.5
log Total Phosphorus (µg·L^{-1})

CHAPTER 2

Regional Differences in Chlorophyll - Phosphorus Relationships: The Case of Prince Edward Island Estuaries, Lakes and Rivers

ABSTRACT

Lower than expected chlorophyll *a* to total phosphorus ratios have been reported for Prince Edward Island (PEI) estuaries. Meeuwig et al. (1998) suggest that grazing by mussels and increased turbidity contribute to this low chlorophyll yield. But could the phenomenon be a result of a more general regional effect, perhaps related to the Island's iron rich soils? We tested these hypotheses by comparing the chlorophyll *a* - total phosphorus (Chl-TP) relationships of estuaries, rivers and lakes on PEI to those observed elsewhere. We find that all aquatic systems on PEI have lower chlorophyll *a* yields than predicted by Chl-TP relationships derived for lakes, rivers and estuaries elsewhere in North America. Although lower chlorophyll yields may be related to mussel presence in PEI estuaries as compared to PEI lakes, mussels do not account for differences between the PEI and reference data sets. Turbidity, as estimated by light extinction coefficients, is not higher in PEI systems than in the reference sets, and may be even lower. The results suggest that the lower chlorophyll *a* yields on Prince Edward Island are related to some regional factor such as the geology of the area. Until it is known if regional effects, such as found on PEI and in prairie saline lakes (Prepas and Trew, 1983) are more common than formerly believed, phosphorus loading models based on large scale data should be interpreted with caution.

INTRODUCTION

Phytoplankton abundance can be influenced by nutrient availability, light availability, density, temperature, turbulence, sinking, grazing, bacteria and parasites (Harris 1987, Sze 1998). Of these, nutrient limitation has the largest influence on phytoplankton growth and abundance, and of all nutrients, phosphorus is the nutrient that most commonly limits algal growth in temperate freshwater systems (Elser et al. 1990). Strong relationships between chlorophyll *a* (an estimate of algal biomass) and total phosphorus (an estimate of phosphorus available to algal growth) have been derived for lakes (Prairie et al. 1989), rivers (Basu and Pick 1996) and even for coastal lagoons (Contreras and Kerekes 1993).

Predictive models based on phosphorus loadings have been successfully developed and used to control lake eutrophication (e.g. Dillon 1975). These lake management models have inspired researchers to examine if these may be modified to be of use on other aquatic systems such as estuaries. As it is not clear whether algal growth in estuaries is generally phosphorus or nitrogen limited (Hecky and Kilham 1988), the first step in this process has been to assess the strength of the relationship between nutrients (i.e. phosphorus and nitrogen) and algal production.

Meeuwig et al. (1998) examined this question for a series of estuaries on Prince Edward Island (PEI), on the east coast of Canada. Their results show that

these estuaries do in fact have highly significant chlorophyll *a* - total phosphorus (Chl-TP) relationships. However, the concentration of chlorophyll in the estuaries was more than an order of magnitude lower than predicted from total phosphorus by models derived for lakes (Dillon and Rigler 1974; OECD 1982). Meeuwig et al. (1998) also note that, in contrast to PEI, the relationship between chlorophyll and total phosphorus for a survey of estuaries in North and South Carolina does not differ from that of lakes.

Why is the chlorophyll yield (the ratio of chlorophyll *a* concentration to total phosphorus concentration) in PEI estuaries lower than that in American estuaries or in temperate zone lakes? Meeuwig et al. (1998) suggest the lower chlorophyll *a* yield in PEI estuaries is the result of a combination of the effects of grazing by mussels and/or high turbidity. In support, they offer a phosphorus mass-balance model, calculating the reduction in algal biomass that might result from particular rates of mussel grazing and light limited algal growth. They conclude that grazing and turbidity could account for 68% of the "chlorophyll deficit" (i.e. the lowered chlorophyll yield).

Two predictions of the mussel grazing hypothesis that Meeuwig et al. (1998) did not test are: 1) that estuaries lacking mussel cultures should have higher chlorophyll yields than estuaries with mussels, and 2) that estuaries lacking mussel cultures should have chlorophyll yields comparable to those observed elsewhere. A prediction of the turbidity hypothesis is that the chlorophyll-

phosphorus residuals should be correlated to turbidity.

It is also possible that the low yield in PEI estuaries is a more general regional effect. Prince Edward Island is well known for its red soils that are high in iron-oxides (MacDougall et al. 1988). As iron oxides have a high affinity for phosphates, phosphorus could conceivably be sequestered into inorganic iron complexes and, as a result, be unavailable to algae. If this were responsible for the low chlorophyll yield in estuaries, then one would expect to see the same phenomenon in PEI lakes and rivers.

Finally, it is also possible that some methodological or analytical error was responsible for the discrepancy.

We test these hypotheses, with data from estuaries, lakes and rivers we sampled on Prince Edward Island.

METHODS

Our survey of Prince Edward Island's aquatic systems was conducted in July and August 1997. In total, 45 sites were sampled: 15 rivers, 13 lakes and 17 estuaries, including 13 of the15 sampled by Meeuwig et al. (1998). General limnological characteristics (i.e. total depth, Secchi depth, water temperature, salinity, conductivity and light intensity) were recorded at 4 stations along a given transect at each site. The presence or absence of mussel aquacultures was also noted. Finally, 4 Litres of water were drawn at each station using a length of 2.5

cm diameter Tygon tubing. This produced an integrated sample over the mixed layer or to 0.5m from the bottom in shallower sites. Each site was only visited once, since we were primarily interested in inter-site variation rather than within-site variation.

Within 6 hours of collection, water samples were filtered through a series of various size Nitex screens (pore size; 64µm and 30µm) and Nucleopore filters (pore size; 3µm, 1µm and 0.2µm) in order to measure the amounts of phosphorus and chlorophyll *a* in particles of varying size. The filtering protocol was developed in order to keep the various size fractions as independent of one another as possible. Details are given in Chapter 1. Total and soluble phosphorus samples were preserved sealed in pre-cleaned, phosphorus-free glass tubes and all filters were wrapped in foil and frozen until analysis.

Total phosphorus in each size fraction (excluding those bound for inorganic particulate analysis) was determined spectrophotometrically using the molybdenum blue method after potassium persulfate digestion (Menzel and Corwin 1965). Chlorophyll *a* concentrations were analysed by spectrophotometer after extraction in 95% ethanol for 24 hours (Ostrofsky and Rigler 1987).

RESULTS AND DISCUSSION

The unusually low chlorophyll yield noted by Meeuwig et al (1998) is unlikely to be due to methodological artifact, unless our work suffers from the

same artifact. A comparison of Chl-TP relationships derived from their data and our estuarine data (by analysis of covariance) shows no significant differences (Figure 2.1). However, the variability in chlorophyll *a* explained by total phosphorus in our data is much lower ($r^2 = 0.15$) than that reported by Meeuwig et al. (1998) ($r^2 = 0.65$). This may be because we sampled each site only once in the summer months, as opposed to six times over the spring and summer months as did Meeuwig et al. (1998). Meeuwig et al. (1998), report that their data are lower than the Dillon and Rigler (1974) and OECD (1983) relationships developed for lakes. Our data are consistent with that result (Figure 2.2).

We also find that chlorophyll *a* yields are low in PEI lakes and rivers (Figure 2.2). We compared the Chl-TP relationships for PEI lakes, rivers and estuaries with published relationships for each of the classes of aquatic systems observed elsewhere in North America (Ontario and Québec lakes: Currie 1990; Ontario and Québec rivers: Basu and Pick 1996; North and South Carolina estuaries: US EPA data from Meeuwig et al. 1998). In all cases, the chlorophyll *a* yield of PEI systems are significantly lower (Figure 2.3, Table 2.1). This suggests that there is a common, regional factor, specific to Prince Edward Island that causes low chlorophyll yields in all aquatic systems. What could this factor be?

Meeuwig et al. (1998) suggest that low chlorophyll yields are the result of aquacultures of the blue mussel, *Mytilus edulis*. Grazers have been postulated to reduce planktonic algal abundance (Quirós 1990, Mellina et al. 1995, Nicholls et

al. 1999). However, mussel aquacultures are present only in estuaries and therefore cannot be responsible for the decreased yield in lakes and rivers. Consequently, mussel grazing cannot constitute a common factor decreasing the chlorophyll *a* yield on Prince Edward Island.

If grazing were responsible for the lower chlorophyll yields in PEI estuaries, then one would expect systems without mussels to have higher chlorophyll yields than those with mussels. Mussel farms were present in six of the estuaries sampled by Meeuwig et al. (1998) and in ten of the estuaries we sampled. We find a significant difference in the chlorophyll yield of estuaries with and without mussel farming for our data set. However, this was not the case for the Meeuwig et al. (1998) data (Table 2.2; Figure 2.4).

Mussels are suspension feeders, retrieving food particles of particular sizes out of the water column. Therefore, if grazing by mussels is the cause of the differences in chlorophyll yield, then one would expect chlorophyll *a* size structure to differ between sites with and without mussel farms (e.g. Hansson et al. 1998). To test for effects of mussel grazing on chlorophyll *a* size structure in estuaries, we used the following analysis of covariance model:

CHL_FRACTION = TOTAL PHOSPHORUS
+ SIZE FRACTION
+ MUSSEL
+ FRACTION * MUSSEL

where CHL_FRACTION is the concentration of chlorophyll in one of four SIZE FRACTIONS (>64µm, 30-64µm, 3-30µm and 0.2-3µm) and MUSSEL is a binary variable distinguishing mussel presence or absence.

If mussels influence chlorophyll *a* size structure, then the interaction term of the model above should be significant. We find no significant difference (Table 2.3). Previous studies have discovered that PEI estuaries have more chlorophyll in the 0.2-3µm fraction than the other PEI aquatic systems (Chapter 1). However, mussel grazing is not responsible for this difference.

Does the presence of mussel farms account for the discrepancies between the PEI estuary and Carolina estuary data? We find that significant differences in the chlorophyll *a* yield of the two data sets are still present when only PEI estuaries without mussel farms are used in an analysis of covariance (Table 2.4). In fact, the average difference in chlorophyll yield (as measured by the difference in adjusted least squared means) between PEI estuaries with and without mussels is 0.35 log units. The average difference in chlorophyll yield between PEI and US EPA estuary data is almost double this amount (0.61 log units). Even if mussel presence were responsible for some of the variation in chlorophyll *a* yield among estuaries, a fundamental difference between the data sets remains.

Closer inspection of our PEI data shows that estuaries have a lower average chlorophyll yield than the lakes (Table 2.5a). Mussels may account for the slight difference between these classes of aquatic systems. We find no

significant difference in the chlorophyll yield of estuaries without mussel farms and PEI lakes, which is consistent with the hypothesis that the presence of aquacultures does lower the chlorophyll *a* yield of PEI estuaries compared to PEI lakes (Table 2.5b).

Could the overall differences in chlorophyll yield among all Prince Edward Island systems be influenced by increased turbidity? Meeuwig et al. (1998) suggest turbidity as a possible contributor to the low yield, and show that according to their mass-balance model, turbidity accounts for 35-75 % of the deficit in chlorophyll *a*. However, nowhere in their paper do they show that PEI estuaries have high turbidity levels compared to other systems. On the contrary, they mention that certain PEI estuaries support a large biomass of the benthic alga *Ulva lactuca* (sea lettuce) (a characteristic of estuaries that we also noticed) and that this would not be possible if light were limiting (Meeuwig et al. 1998).

To test if PEI systems have higher turbidity levels than systems from which are derived the more conventional Chl-TP relationships we first compared the light extinction coefficients of our PEI lake, river and estuary data with those given in Currie (1990) and Basu and Pick (1996). The light extinction coefficients for PEI systems were found to be similar to those of Currie's (1990) lakes ($n = 67$, $F = 0.57$, $p = 0.452$) and they were significantly lower than those of Basu and Pick's (1996) riverine systems ($n = 56$, $F = 10.46$, $p = 0.002$). In other words, Prince Edward Island systems are not more turbid than central Canadian sites, and they

may even be clearer. A second test of the turbidity hypothesis was to test whether the chlorophyll a - total phosphorus residuals were related to either the light extinction coefficient or to the ratio of euphotic depth (i.e. depth of 1% surface light intensity) : mixed depth. Neither of these variables was significant when added to the Chl-TP model.

The last of the hypotheses left to explain the lower chlorophyll *a* yield in PEI systems is one that we cannot directly test with the data available to us. Is the geology of Prince Edward Island, its red iron rich soils, the reason why chlorophyll yields are low? It is certainly a possibility. Adsorbtion of phosphorus onto iron oxide rich dust particles has been suggested as a mechanism controlling phosphorus concentrations in the eastern Mediterranean Sea (Krom 1991). Froelich (1988) states that iron oxides containing clays have a strong affinity for absorbing phosphates. These are the types of soils found in Prince Edward Island (MacDougall et al. 1988 cited from Meeuwig et al. 1998). Phosphorus, once sorbed, is not easily desorbed (Barrow 1983) and so any phosphorus sequestered by iron oxides into soil particles would remain there and be unavailable to algae. Meeuwig et al. (1998) discuss this possibility, stating that their turbidity variable could actually be a surrogate for iron control of phosphorus because of the correlation between soil particles and turbidity. The level of phosphorus sequestration by iron-oxides on Prince Edward Island is a question that should be investigated further.

In conclusion, our results show that the depressed chlorophyll *a* yield reported for PEI estuaries is not limited to estuaries but rather is characteristic of aquatic systems in general in the region. This phenomenon is not the result of mussel grazing or increased turbidity but may somehow be related to the high iron-oxide content of the Island's soils. Canadian prairie saline lakes, which also possess decreased chlorophyll yields are another area of North America known to present regionally unique Chl-TP relationships (Prepas and Trew 1983, Bierhuizen and Prepas 1985). Regional effects on chlorophyll *a* - total phosphorus relationships may be a much more common phenomenon than previously thought. If this is the case, regionality might have large implications towards the control of eutrophication through phosphorus loading models. These management models are currently based on data from very large geographical areas. The inclusion of data from regions of differing chlorophyll yields therefore creates additional variation around the overall chlorophyll - total phosphorus relationship, significantly decreasing the accuracy of the model in estimating the algal biomass produced in a given system. Until the extent of regional effects are assessed, current management models should be interpreted with caution.

Table 2.1: Summary of ANCOVA models predicting log chlorophyll *a* concentrations from phosphorus concentrations between our Prince Edward Island (PEI) data and comparable published regression data for each class of aquatic system studied. The interaction between log total phosphorus (TP) and the categorical variable (Study) was not significant. Indep.Var. = independent variable; SS = sum of squares; RMSE = residual mean square error.

A) Lakes: PEI lakes (this study) vs Currie (1990)

Indep.Var.	SS	d.f.	F	P	N	r^2	RMSE
log(TP)	6.94	1	78.0	<0.001	49	0.63	0.09
Study	3.17	1	35.6	<0.001			

B) Rivers: PEI rivers (this study) vs Basu and Pick (1996)

Indep.Var.	SS	d.f.	F	P	N	r^2	RMSE
log(TP)	2.49	1	52.1	<0.001	46	0.63	0.05
Study	2.93	1	61.3	<0.001			

C) Estuaries: PEI estuaries (this study) vs EPA estuaries (in Meeuwig et al. 1998)

Indep.Var.	SS	d.f.	F	P	N	r^2	RMSE
log(TP)	4.22	1	69.3	<0.001	85	0.63	0.06
Study	3.00	1	49.2	<0.001			

Table 2.2: Summary of ANCOVA models predicting log chlorophyll *a* concentrations from phosphorus concentrations between Prince Edward Island (PEI) estuaries with and without mussel aquacultures for our data and that of Meeuwig et al. (1998). The interaction between log total phosphorus (TP) and the categorical variable (Mussel) was not significant. Indep.Var. = independent variable; SS = sum of squares; RMSE = residual mean square error.

A) PEI estuaries with vs without mussel aquacultures (this study)

Indep.Var.	SS	d.f.	F	P	N	r^2	RMSE
log(TP)	0.30	1	8.31	0.012	17	0.57	0.04
Mussel	0.49	1	13.5	0.003			

B) PEI estuaries with vs without mussel aquacultures (Meeuwig et al. 1998)

Indep.Var.	SS	d.f.	F	P	N	r^2	RMSE
log(TP)	0.36	1	18.2	0.001	15	0.66	0.02
Mussel	0.01	1	0.47	0.507			

Table 2.3: Summary of the general linear model predicting top-down effects on chlorophyll *a* size structure (i.e. the concentrations of chlorophyll in four algal size fractions) in Prince Edward Island estuaries. TP = total phosphorus; Fraction = categorical variable distinguishing four size fractions; Mussel = binomial variable distinguishing presence or absence of mussel aquacultures; Indep.Var. = independent variable; SS = sum of squares; RMSE = residual mean square error.

Indep.Var.	SS	d.f.	F	P	N	r^2	RMSE
log(TP)	0.51	1	2.12	0.151	67	0.72	0.24
Fraction	34.1	3	47.6	<0.001			
Mussel	0.04	1	0.15	0.703			
Fraction*Mussel	1.46	3	2.03	0.119			

Table 2.4: Summary of the ANCOVA model predicting log chlorophyll *a* concentrations from phosphorus concentrations between our Prince Edward Island (PEI) estuaries without mussel aquacultures and the EPA estuary data (from Meeuwig et al. 1998). The interaction between log total phosphorus (TP) and the categorical variable (Study) was not significant. Indep.Var. = independent variable; SS = sum of squares; RMSE = residual mean square error.

Indep.Var.	SS	d.f.	F	P	N	r^2	RMSE
log(TP)	4.13	1	70.1	<0.001	75	0.54	0.06
Study	0.46	1	7.89	0.006			

Table 2.5: Summary of ANCOVA models predicting log chlorophyll *a* concentrations from phosphorus concentrations between our Prince Edward Island (PEI) lakes and estuaries (with and without mussel aquacultures). The interaction between log total phosphorus (TP) and the categorical variable (System = aquatic system class) was not significant. Indep.Var. = independent variable; SS = sum of squares; RMSE = residual mean square error.

A) PEI lakes vs PEI estuaries (all data)

Indep.Var.	SS	d.f.	F	P	N	r^2	RMSE
log(TP)	1.61	1	13.7	0.001	30	0.42	0.12
System	0.76	1	6.46	0.017			

B) PEI lakes vs PEI estuaries (without mussel aquacultures)

Indep.Var.	SS	d.f.	F	P	N	r^2	RMSE
log(TP)	1.52	1	10.7	0.005	20	0.40	0.14
System	0.06	1	0.40	0.538			

Figure 2.1: The relationship between log chlorophyll *a* and log total phosphorus for estuaries on Prince Edward Island. Data from this study (n = 17) and from Meeuwig et al. (1998) (n = 15) are shown. The lines represent the regression models fitted to the data of each set.

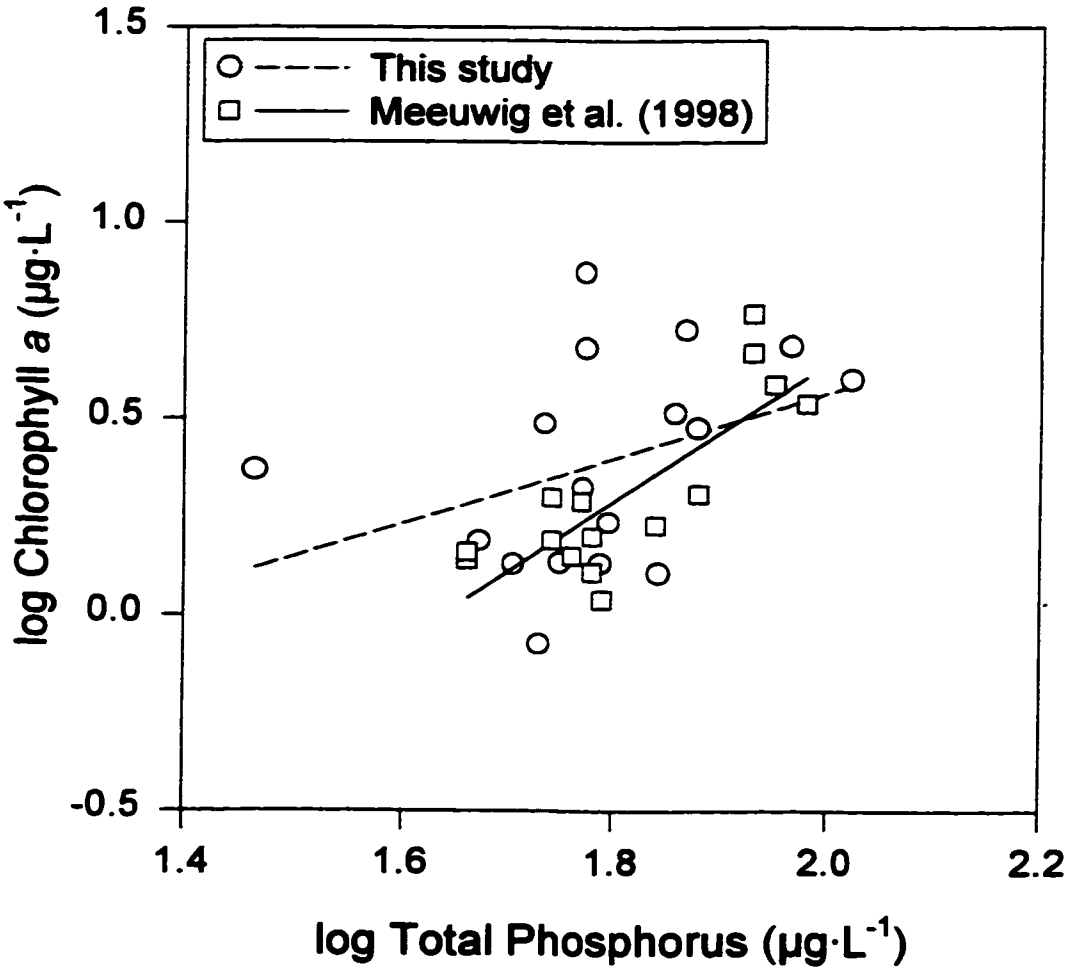

This study
Meeuwig et al. (1998)
log Chlorophyll a (µg·L-1)
log Total Phosphorus (µg·L-1)
1.5
1.0
0.5
0.0
-0.5
1.4
1.6
1.8
2.0
2.2

Figure 2.2: The relationships between log chlorophyll *a* and log total phosphorus for five data sets. Data come from Dillon and Rigler (1974), OECD (1982), and from lakes, rivers and estuaries on Prince Edward Island (PEI) (this study).

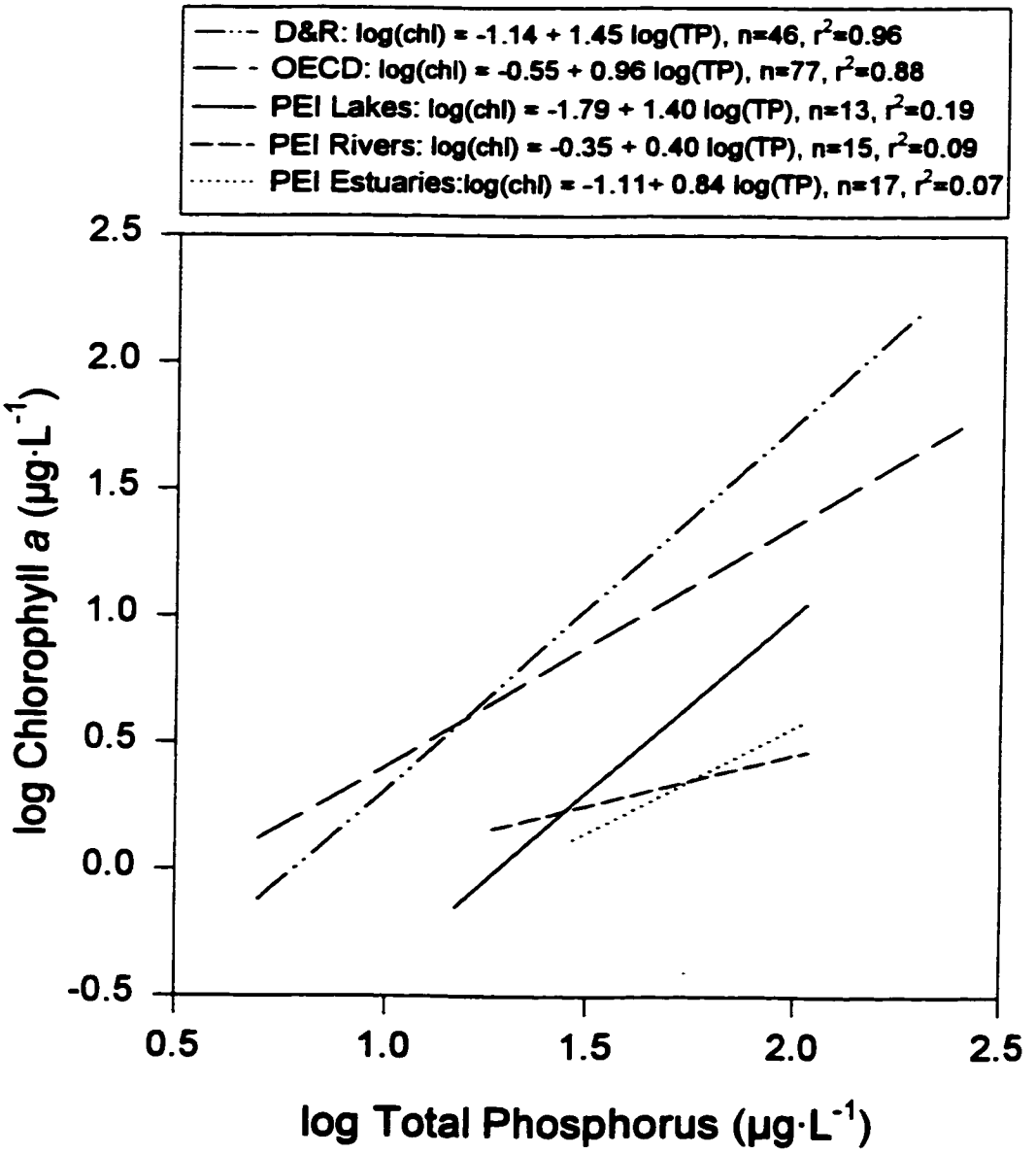
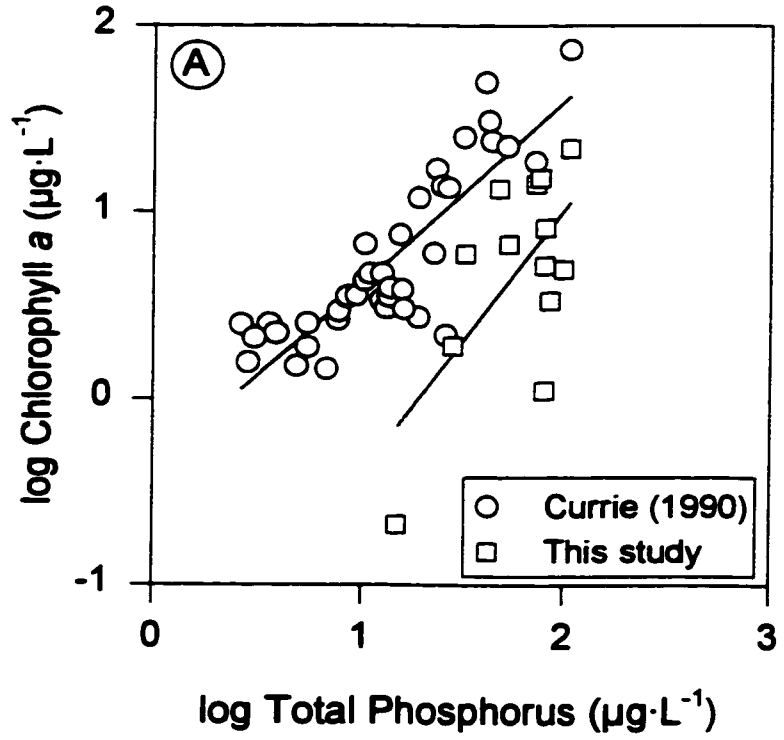
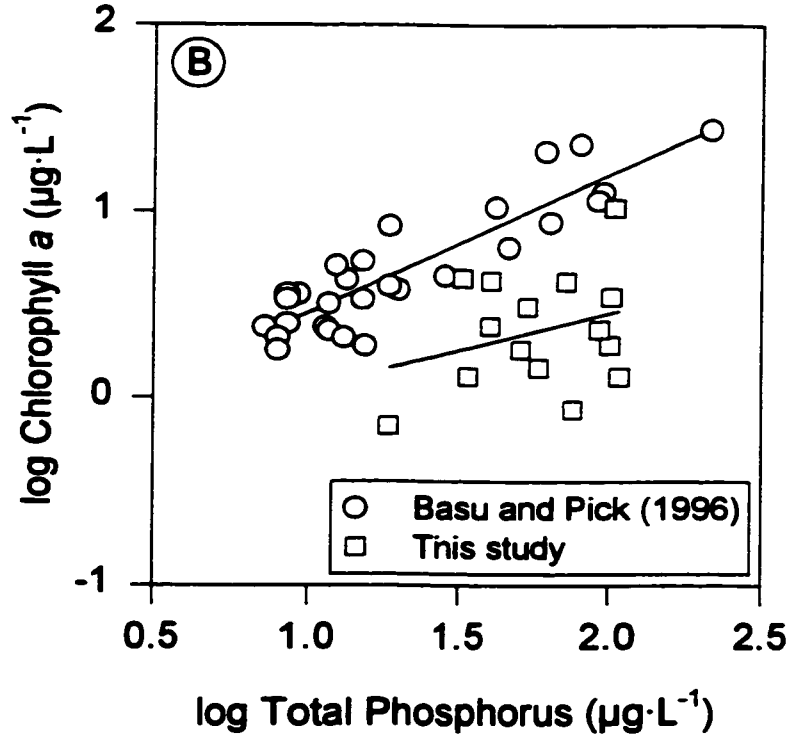

D&R: log(chl) = -1.14 + 1.45 log(TP), n=46, r²=0.96
OECD: log(chl) = -0.55 + 0.96 log(TP), n=77, r²=0.88
PEI Lakes: log(chl) = -1.79 + 1.40 log(TP), n=13, r²=0.19
PEI Rivers: log(chl) = -0.35 + 0.40 log(TP), n=15, r²=0.09
PEI Estuaries:log(chl) = -1.11+ 0.84 log(TP), n=17, r²=0.07
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
0.5
1.0
1.5
2.0
2.5
log Chlorophyll a (µg·L-1)
log Total Phosphorus (µg·L-1)

Figure 2.3: The relationships between log chlorophyll *a* and log total phosphorus for lakes, rivers and estuaries. A) Lake data from this study (n = 13) and Currie (1990) (n = 38). B) River data from this study (n = 15) and Basu and Pick (1996) (n = 28). C) Estuary data from this study (n = 17) and EPA data for Carolina estuaries (n = 68) (from Meeuwig et al. 1998) The lines represent the regression models fitted to the data of each set.


A
log Chlorophyll a (µg·L-1)
log Total Phosphorus (µg·L-1)
Currie (1990)
This study
2
1
0
-1
0
1
2
3


B
log Chlorophyll a (µg·L-1)
log Total Phosphorus (µg·L-1)
Basu and Pick (1996)
This study
2
1
0
-1
0.5
1.0
1.5
2.0
2.5

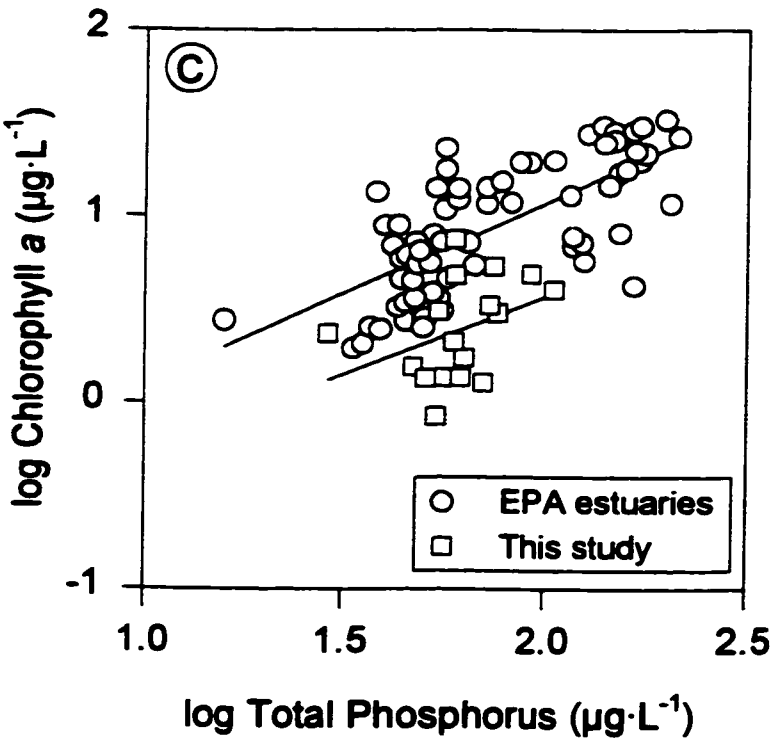
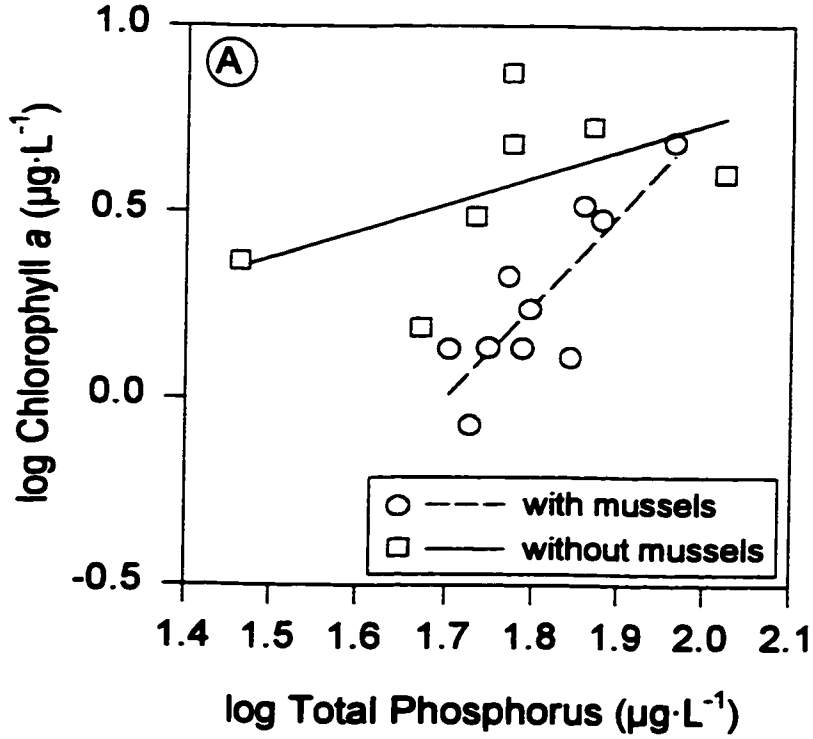
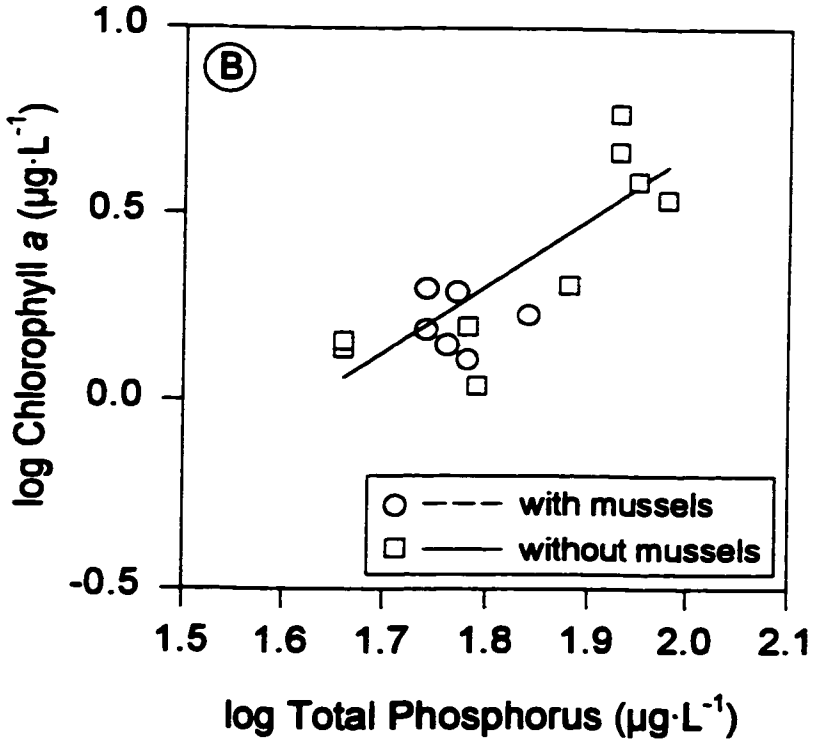

©
2
1
0
-1
1.0
1.5
2.0
2.5
log Chlorophyll a (µg·L-1)
log Total Phosphorus (µg·L-1)
EPA estuaries
This study

Figure 2.4: The relationships between log chlorophyll *a* and log total phosphorus for estuaries with and without mussel aquacultures. A) Data from this study. B) Data from Meeuwig et al. (1998). The lines represent the regression models fitted to the data of each set. The relationship for estuaries with mussels in the data of Meeuwig et al. (1998) is not significant.


A
1.0
0.5
0.0
-0.5
log Chlorophyll a (µg·L-1)
with mussels
without mussels
1.4 1.5 1.6 1.7 1.8 1.9 2.0 2.1
log Total Phosphorus (µg·L-1)


B
1.0
0.5
0.0
-0.5
log Chlorophyll a (µg·L-1)
with mussels
without mussels
1.5 1.6 1.7 1.8 1.9 2.0 2.1
log Total Phosphorus (µg·L-1)

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APPENDICES

Appendix A - Site code and latitude and longitude coordinates for the 53 study sites.

Site	Code	Latitude	Longitude
Wrights Pond	1-01	46° 20' N	63° 43' W
O'Keefe Lake	1-02	46° 15' N	62° 49' W
Campbells Pond	1-03	46° 32' N	63° 33' W
Glenfinnan Lake	1-04	46° 18' N	62° 57' W
Indian River Pond	1-05	46° 28' N	63° 40' W
Officers Pond	1-06	46° 19' N	63° 04' W
Round Pond	1-07	46° 55' N	63° 59' W
Scales Pond	1-08	46° 20' N	63° 36' W
Pinette Pond	1-09	46° 05' N	62° 52' W
Finlaysons Pond	1-10	46° 02' N	62° 34' W
Bell Pond	1-11	46° 23' N	63° 20' W
Arsenault's Pond	1-12	46° 29' N	64° 06' W
Glenwood Pond	1-13	46° 36' N	64° 19' W
West River	2-01	46° 12' N	63° 20' W
Enmore River	2-02	46° 37' N	64° 03' W
Hunter River	2-03	46° 24' N	63° 21' W
Valleyfield River	2-04	46° 08' N	62° 41' W
Morrell River	2-05	46° 24' N	62° 42' W
Tryon River	2-06	46° 16' N	63° 32' W
Little Tignish River	2-07	46° 55' N	64° 03' W
Miminegash River	2-08	46° 51' N	64° 12' W
Black River	2-09	46° 22' N	63° 11' W
Trout River	2-10	46° 33' N	63° 57' W
Souris River	2-11	46° 23' N	62° 18' W
Cross River	2-12	46° 27' N	62° 16' W
Naufrage River	2-13	46° 26' N	62° 25 W
Sturgeon River	2-14	46° 06' N	62° 35' W
Big Brook	2-15	46° 20' N	62° 26' W
Percival Bay	3-01	46° 12' N	64° 05' W
Foxley Bay	3-02	46° 43' N	64° 04' W
Grand River	3-03	46° 30' N	63° 55' W
Darnley Basin	3-04	46° 33' N	63° 40' W
Dunk River	3-05	46° 21' N	63° 45' W

Appendix A ... (cont.)

Site	Code	Latitude	Longitude
Wilmot River	3-06	46° 23' N	63° 45' W
Murray River	3-07	46° 01' N	62° 36' W
Cardigan River	3-08	46° 13' N	62° 33' W
Rustico Bay	3-09	46° 25' N	63° 14' W
New London Bay	3-10	46° 29' N	63° 28' W
Savage Harbor	3-11	46° 25' N	62° 50' W
Tracadie Bay	3-12	46° 23' N	63° 00' W
St.Peters Bay	3-13	46° 26' N	62° 40' W
Kildare River	3-14	46° 51' N	64° 03' W
Mill River	3-15	46° 45' N	64° 09' W
Brudenell River	3-16	46° 12' N	62° 36' W
Montague River	3-17	46° 10' N	62° 38' W
Mulvihill Lake	4-01	45° 29' N	75° 51' W
Carmen Lake	4-02	45° 36' N	75° 55' W
Bellamy lake	4-03	44° 43' N	76° 01' W
Silver Lake	4-04	44° 49' N	76° 35' W
Stump lake	4-05	44° 56' N	76° 38' W
Ramsay Lake	4-06	45° 35' N	76° 06' W
Taylor lake	4-07	45° 36' N	76° 03' W
McGregor Lake	4-08	45° 39' N	75° 40' W

Appendix B - Morphometric and physico-chemical data measured in the 53 study sites. Max Depth = Maximum depth; Temp = Temperature; K = light extinction coefficient; APA = Alkaline phosphatase activity levels (fluorescence units / minute); n/a represents measurements which were not available.

Site	Max Depth (m)	Mean Depth (m)	Secchi Depth (m)	Temp (°C)	Salinity (‰)	Conductivity (μmhos·cm^2)	K (m^{-1})	APA
1-01	2.4	1.6	2.4	18.6	0.0	229.6	2.03	0.81
1-02	2.3	2.3	2.3	24.0	0.0	27.4	0.55	1.11
1-03	3.4	3.1	3.4	21.4	0.0	182.2	1.25	1.77
1-04	3.1	2.5	3.1	22.2	0.0	32.0	1.71	n/a
1-05	1.3	1.1	1.3	22.5	0.0	212.8	1.22	7.58
1-06	2.4	1.3	2.4	19.0	0.0	235.2	1.22	0.30
1-07	1.4	1.1	1.4	19.6	3.5	5812.5	0.82	2.34
1-08	2.9	1.8	2.9	13.9	0.0	200.6	1.18	0.12
1-09	2.5	1.8	2.5	18.4	0.0	136.9	0.67	0.12
1-10	2.7	1.5	2.7	17.0	0.0	215.6	0.79	0.17
1-11	3.5	2.3	3.5	14.2	0.8	7835.4	n/a	0.27
1-12	1.3	0.9	1.3	17.3	1.1	2005.2	n/a	n/a
1-13	1.7	1.2	1.7	13.7	0.0	369.4	n/a	2.35
2-01	3.4	2.5	3.4	15.1	0.2	498.0	1.30	0.10
2-02	1.1	0.8	1.1	21.2	1.0	1644.4	2.17	n/a
2-03	1.7	1.0	1.7	19.7	0.8	1485.9	n/a	0.10
2-04	3.2	2.0	3.2	15.5	0.0	160.9	0.68	0.06
2-05	2.9	2.5	2.9	21.5	9.2	14562.5	1.37	0.23
2-06	0.3	0.2	0.3	16.0	0.5	1133.3	n/a	0.33
2-07	1.0	0.6	1.0	10.8	0.0	257.9	n/a	0.34
2-08	0.8	0.4	0.8	16.5	0.1	452.1	n/a	1.34
2-09	0.4	0.3	0.4	10.2	0.9	1133.3	n/a	0.24
2-10	0.2	0.2	0.2	15.7	0.1	620.0	n/a	0.60
2-11	0.4	0.3	0.4	10.1	0.0	758.3	n/a	0.22
2-12	1.2	0.8	1.2	13.9	0.5	1006.3	n/a	n/a
2-13	2.7	1.9	2.7	21.0	0.3	834.3	n/a	n/a
2-14	0.6	0.4	0.6	10.1	0.0	1316.3	n/a	0.16
2-15	0.7	0.4	0.7	14.6	0.0	349.2	n/a	0.44
3-01	3.0	2.5	3.0	23.6	24.1	36437.5	1.14	n/a
3-02	3.6	2.8	2.7	21.7	20.5	31835.9	1.06	0.07

Appendix B ... (cont.)

Site	Max Depth (m)	Mean Depth (m)	Secchi Depth (m)	Temp (°C)	Salinity (‰)	Conductivity (μmhos·cm^2)	K (m^{-1})	APA
3-03	7.6	5.0	3.3	18.6	26.4	40295.6	0.61	0.05
3-04	5.9	4.8	3.3	19.0	25.3	35354.2	0.57	0.02
3-05	2.6	2.2	1.5	22.0	23.0	35625.0	0.85	1.69
3-06	2.3	1.8	1.7	19.8	21.8	31968.8	0.61	0.31
3-07	7.0	5.7	4.8	20.2	24.0	35970.2	0.42	0.02
3-08	8.0	5.1	4.7	17.7	23.2	33871.5	0.42	0.14
3-09	4.5	4.0	2.3	19.8	21.5	32831.3	1.04	0.09
3-10	6.0	4.6	2.8	19.8	22.4	34302.1	0.52	0.03
3-11	3.2	3.2	2.8	21.1	22.5	34593.8	0.82	0.08
3-12	4.9	4.0	3.1	23.4	28.5	43000.0	0.51	0.20
3-13	6.3	5.5	4.9	21.6	22.7	39760.7	0.40	0.03
3-14	4.3	3.0	1.2	21.0	17.2	27104.2	1.14	1.01
3-15	4.9	4.2	2.2	21.4	19.6	30181.3	0.77	0.08
3-16	7.6	6.6	4.0	20.6	24.1	35237.5	0.12	0.17
3-17	6.6	6.3	4.5	21.2	27.2	39531.3	0.08	0.08
4-01	4.1	2.9	3.1	24.3	0.0	140.6	0.86	n/a
4-02	5.4	4.6	3.1	26.0	0.0	80.0	0.81	n/a
4-03	2.3	1.9	2.3	26.1	0.0	255.2	0.74	n/a
4-04	7.1	6.2	3.5	25.2	0.0	254.5	0.54	n/a
4-05	3.4	2.7	3.4	26.5	0.0	122.5	0.68	n/a
4-06	9.0	6.5	3.3	25.0	0.0	75.0	0.81	n/a
4-07	5.9	4.3	3.8	25.2	0.0	87.4	0.53	n/a
4-08	13.0+	13.0+	7.2	18.0	0.0	100.0	0.58	n/a

Appendix C - Total phosphorus concentration ($\mu g \cdot L^{-1}$) measured in each size fraction. TP = total phosphorus; POP = particulate organic phosphorus (>1µm); PIP = particulate inorganic phosphorus (>1µm); n/a represents measurements which were not available.

Site	TP	>64µm	30-64µm	3-30µm	0.2-3µm	Soluble	POP	PIP
1-01	81.7	n/a	1.3	19.5	15.3	19.8	31.6	45.1
1-02	15.0	n/a	0.6	2.1	7.8	3.3	3.0	5.5
1-03	73.4	7.4	3.2	5.0	25.5	16.5	16.7	16.5
1-04	28.1	2.8	0.2	2.2	9.3	11.3	9.8	4.5
1-05	54.0	0.8	0.8	8.4	16.8	21.4	11.2	1.8
1-06	106.1	1.1	1.1	4.4	22.3	62.4	13.1	3.4
1-07	84.9	3.6	2.4	3.2	21.3	31.6	9.0	12.2
1-08	80.7	0.1	0.3	11.4	18.7	47.6	10.7	7.5
1-09	127.7	0.2	1.2	43.4	9.5	33.6	34.0	13.2
1-10	85.6	0.7	0.2	6.8	9.1	64.1	6.4	2.1
1-11	80.7	13.6	4.4	6.6	9.2	26.6	17.6	16.2
1-12	48.4	2.5	1.0	9.2	15.0	16.1	9.9	10.7
1-13	32.4	1.9	0.7	2.0	8.1	13.2	4.2	3.4
2-01	103.8	n/a	1.3	16.2	14.2	64.0	18.3	21.9
2-02	32.7	0.3	0.1	4.0	9.8	14.7	7.9	8.8
2-03	110.5	5.5	4.6	7.8	14.6	59.8	14.9	33.4
2-04	101.2	0.6	1.0	2.5	6.5	83.0	5.3	5.1
2-05	72.3	0.0	0.2	2.7	14.3	51.0	2.8	4.9
2-06	59.0	0.4	1.0	2.6	3.5	43.3	4.4	4.0
2-07	34.0	1.5	1.0	1.5	2.8	22.9	4.9	4.2
2-08	18.9	0.4	0.7	1.5	6.8	24.8	3.3	2.8
2-09	93.3	0.4	0.6	2.9	3.9	81.4	3.8	2.7
2-10	55.9	5.9	2.2	4.5	7.4	29.7	10.6	6.6
2-11	76.4	0.3	0.6	1.3	0.9	69.1	2.2	2.2
2-12	51.2	0.3	0.4	1.3	3.1	38.6	2.9	0.7
2-13	41.0	2.3	0.6	2.4	6.9	21.8	7.0	2.9
2-14	107.8	1.3	1.9	1.7	5.8	91.9	4.1	3.2
2-15	40.3	0.5	0.2	0.7	5.8	31.3	0.7	1.3
3-01	29.3	n/a	0.3	3.2	8.5	17.5	2.4	12.5
3-02	47.7	2.7	0.1	3.3	12.8	38.7	2.3	9.1
3-03	54.5	1.5	0.3	3.6	7.6	36.7	1.0	9.2
3-04	62.6	1.5	0.5	3.8	5.0	45.0	1.3	12.1

Appendix C ... (cont.)

Site	TP	>64µm	30-64µm	3-30µm	0.2-3µm	Soluble	POP	PIP
3-05	59.6	2.6	0.4	10.7	7.5	22.7	3.2	22.5
3-06	59.4	1.0	0.1	10.1	10.5	31.6	2.2	23.7
3-07	50.7	1.1	0.4	2.1	5.8	40.7	1.0	6.8
3-08	59.3	2.8	1.6	3.1	5.7	38.8	1.8	8.7
3-09	92.5	1.4	0.2	7.1	10.0	63.5	3.8	16.0
3-10	69.9	2.5	0.1	3.7	6.2	47.3	5.1	5.5
3-11	76.2	2.8	0.3	3.7	5.7	49.2	3.8	8.3
3-12	72.3	1.6	0.6	4.1	11.6	57.2	1.8	11.5
3-13	56.1	0.8	1.1	3.7	2.9	38.3	4.0	4.8
3-14	105.4	1.7	0.3	9.3	41.5	31.9	4.6	18.7
3-15	73.9	2.0	0.0	3.7	11.3	43.9	2.3	12.2
3-16	61.6	4.4	0.1	2.3	5.4	47.3	2.0	6.2
3-17	53.4	1.8	0.4	2.1	4.9	40.2	2.8	4.6
4-01	20.7	1.8	1.3	2.6	4.1	9.4	3.8	4.2
4-02	29.9	1.4	0.5	2.1	3.1	14.2	6.1	0.9
4-03	17.3	1.3	0.0	1.1	2.0	16.3	2.9	0.7
4-04	19.7	2.2	0.0	4.0	1.6	12.1	3.9	0.5
4-05	11.6	0.3	0.2	1.1	0.8	8.9	2.4	0.2
4-06	15.1	0.9	0.4	0.7	0.6	6.3	3.3	1.7
4-07	12.1	1.0	0.0	1.2	1.2	8.6	3.2	0.7
4-08	13.5	0.8	0.2	0.9	1.5	10.2	2.4	0.7

Appendix D - Chlorophyll *a* concentration ($\mu g \cdot L^{-1}$) measured in each size fraction; n/a represents measurements which were not available.

Site	Total	>64µm	30-64µm	3-30µm	0.2-3µm
1-01	7.6	n/a	0.4	5.9	1.3
1-02	0.3	n/a	0.1	0.1	0.0
1-03	17.8	12.8	0.5	1.4	3.2
1-04	2.1	0.1	0.1	0.8	1.1
1-05	8.5	1.4	0.2	3.3	3.6
1-06	6.4	3.0	0.3	1.0	2.0
1-07	9.5	1.1	0.7	2.3	5.4
1-08	1.1	0.0	0.0	1.1	0.0
1-09	35.9	0.3	0.5	29.9	5.2
1-10	3.8	1.4	0.0	1.8	0.6
1-11	23.0	13.7	2.8	4.4	2.2
1-12	16.6	8.6	0.4	4.3	3.3
1-13	6.8	3.9	0.7	0.9	1.3
2-01	4.1	n/a	0.1	3.0	0.9
2-02	4.5	0.1	0.1	2.2	2.0
2-03	14.4	7.6	1.6	2.6	2.6
2-04	2.0	0.1	0.1	0.8	0.9
2-05	4.2	0.0	0.0	0.9	3.3
2-06	1.4	0.2	0.1	0.4	0.7
2-07	2.9	1.9	0.3	0.2	0.4
2-08	0.7	0.0	0.2	0.5	0.0
2-09	2.4	0.2	0.1	1.5	0.6
2-10	6.0	4.0	1.0	0.9	0.1
2-11	1.1	0.1	0.2	0.5	0.3
2-12	1.9	0.3	0.3	0.7	0.6
2-13	4.9	2.0	0.0	1.4	1.4
2-14	1.3	0.3	0.2	0.6	0.2
2-15	2.5	0.1	0.1	0.3	2.0
3-01	2.4	n/a	0.1	0.5	1.9
3-02	2.1	0.0	0.0	0.4	1.8
3-03	3.1	0.1	0.0	0.8	2.3
3-04	2.1	0.1	0.1	0.7	1.2
3-05	4.8	0.0	0.0	3.0	1.8

Appendix D ... (cont.)

Site	Total	>64µm	30-64µm	3-30µm	0.2-3µm
3-06	7.6	0.0	0.1	3.4	4.0
3-07	1.5	0.0	0.0	0.4	0.9
3-08	2.2	0.4	0.4	0.3	1.0
3-09	5.0	0.1	0.0	1.5	3.4
3-10	1.3	0.0	0.0	1.1	0.2
3-11	3.1	0.1	0.1	0.9	2.0
3-12	3.5	0.2	0.1	0.5	2.7
3-13	1.5	0.0	0.0	0.7	0.8
3-14	6.6	0.1	0.0	1.1	5.4
3-15	5.4	0.2	0.0	0.9	4.3
3-16	1.5	0.1	0.0	0.2	1.2
3-17	0.9	0.1	0.1	0.1	0.6
4-01	5.7	0.4	0.8	2.6	2.0
4-02	5.2	0.7	0.6	2.0	2.0
4-03	3.0	0.3	0.2	1.5	1.0
4-04	1.7	0.4	0.1	0.8	0.3
4-05	2.5	0.1	0.1	0.6	1.7
4-06	3.1	0.4	0.5	1.2	1.1
4-07	2.7	0.6	0.5	1.0	0.6
4-08	1.8	0.3	0.2	0.6	0.7

Appendix E - Champion, M. and Currie D.J. 1999. Phosphorus-chlorophyll relationships in lakes, rivers and estuaries. Internationale Vereinigung für Theoretische und Angewandte Limnologie, Verhandlungen (in press)

PHOSPHORUS-CHLOROPHYLL RELATIONSHIPS IN LAKES, RIVERS AND ESTUARIES

Introduction

In aquatic systems, phytoplankton abundance is strongly influenced by nutrient availability. For temperate-zone lakes, it has been clearly shown that the variation among lakes in plankton chlorophyll *a* concentrations (Chl *a*) is strongly related to total phosphorus (TP) levels (DILLON & RIGLER 1974, PRAIRIE et al. 1989, MCCAULEY et al. 1989). Further experimental manipulations have shown that phosphorus often limits algal growth (SCHINDLER 1977). This has allowed lake managers to manipulate TP levels to control algal biomass. However, there still remains a higher level of residual variability in the Chl *a*-TP relationship than managers might wish (CARPENTER et al. 1985). Although many studies have attempted to relate this residual variability to other lake characteristics (CURRIE 1990, QUIRÓS 1990), no general explanation has yet been identified.

It has also been shown that Chl *a* levels in rivers and in estuaries are related to TP (BASU & PICK 1996, MEEUWIG et al. 1998). However, these relationships differ from those derived in lakes in that there is typically less Chl *a* per unit TP in rivers than in lakes, and still less in estuaries (Table App.E.1a). By examining these three relationships more closely, we hope to determine the source of some of the residual variability mentioned above.

Why do some systems produce more chlorophyll per unit phosphorus than do others? One hypothesis is that the relationship between chlorophyll *a* and the amount of phosphorus actually in algae (i.e. algal phosphorus) is the same everywhere, but that differing proportions of the total phosphorus are sequestered

Appendix E ... (cont.)

in non-algal components of the water column. The lower TP-Chl *a* ratios in rivers and estuaries would therefore be the result of phosphorus in non-algal components of the plankton. The alternative hypothesis is that the amount of chlorophyll produced per unit algal phosphorus differs among systems.

Methods

In order to test our hypotheses, a series of sites (13 lakes, 15 rivers, 17 estuaries) were each sampled once in the summer of 1997 in the province of Prince Edward Island on the east coast of Canada (Figure App.E.1). At four stations in each site, whole water samples were obtained by tube sampler, integrating over the epilimnion. Physical characteristics of the sites were also recorded (temperature, salinity, Secchi depth, etc.).

In the laboratory, the water was filtered onto a set of various size Nitex and Nucleopore filters in order to measure the amounts of phosphorus and chlorophyll in particles of varying size. Total phosphorus was measured in unfiltered water, as well as in the particles retained on a 64 µm filter (i.e. >64µm particles). To yield a 30-64 µm fraction, a sub-sample of integrated water was first filtered through a 64 µm filter before being passed through a 30µm filter on which TP was measured. The procedure was repeated to produce a 3- 30 µm fraction. The filtrate of this last fraction was passed through a 0.2µm filter to produce a 0.2-3µm fraction and the final filtrate was collected as the soluble fraction (<0.2 µm). Chlorophyll *a* was similarly separated into >64 µm, 30-64 µm, 3-30 µm and 0.2-3 µm fractions. Total chlorophyll *a* was calculated as the sum of the chlorophyll in all fractions.

Total phosphorus in each size fraction was determined spectrophotometrically with the molybdeum blue method following potassium persulfate digestion (MENZEL & CORWIN 1965). Chlorophyll *a* levels were also

Appendix E ... (cont.)

analyzed by spectrophotometer after extraction in 95% ethanol for 24 hours (OSTROFSKY & RIGLER 1987). Chlorophyll *a* concentrations were calculated using the calibration equation of WINTERMANS & DEMOTS (1965). All statistical analyses were performed with the SYSTAT (version 7.0) software package.

Results and Discussion

Relationships between Chl *a* and TP for the three systems studied are shown in Figure App.E.2. Chlorophyll - TP relationships based on a very large collection of lakes are typically non-linear (PRAIRIE et al. 1989, MCCAULEY et al. 1989). However in our data, TP only varies by one order of magnitude, whereas it varies by >3 orders in global studies. Over our limited range of TP, the relationship is not detectably non-linear (i.e. a quadratic $(\log TP)^2$ term added to the model is non-significant).

The differences in the TP-Chl *a* relationships among lakes, rivers and estuaries were smaller than the literature had led us to anticipate. We also find that the variability explained by the individual regressions is lower for our data than for other published equations (Table App.E.1a,b). However, the higher residual variability in our samples may be the result of only visiting each of our sites once. Other studies typically sampled individual sites more often. Analysis of covariance (ANCOVA) shows that mean chlorophyll levels differ significantly among the three kinds of systems we studied, after controlling for TP (Table App.E.2a). *A posteriori* comparisons showed that lakes, on average, have more chlorophyll than rivers or estuaries.

To characterize the "algal fraction" of the water column we have used the phosphorus and chlorophyll values measured in the 3-30 µm size fraction. Earlier work has shown that this size fraction contains mostly algae, with relatively few bacteria or crustacean zooplankton (CURRIE et al. 1986). In this predominantly

Appendix E ... (cont.)

algal size fraction, we find no statistical differences between the P-Chl a relationship among lakes, rivers and estuaries (Figure App.E.3, Table App.E.2b). Moreover, the relationships have less residual variability than the corresponding total phosphorus relationships (Table App.E.1b,c). Also, the slope of the relationship is not significantly different than 1.0 ($t=1.471$, $d.f.=2$, $p=0.279$). These observations are consistent with the hypothesis that, at least in the mesotrophic systems that we studied, one unit of phosphorus incorporated into phytoplankton yields one unit of chlorophyll, regardless of the system in question.

If the surplus phosphorus present in the river and estuarine TP-Chl *a* relationships is not represented in the algal phosphorus relationships, then the phosphorus must be found elsewhere in these systems. But where? We addressed this question by examining the residuals of the model

$P_i = C_0 + C_1 (\log TP) + C_2 (F_i)$

where P_i is the amount of phosphorus in size fraction F_i. We find that, relative to rivers and estuaries, a greater proportion of phosphorus in lakes is associated with particles ranging from 0.2-64μm (Figure App.E.4). This corresponds to size fractions in which 80% of the chlorophyll is found and phosphorus is probably associated mainly with algae. In the case of estuaries, disproportionately large amounts of phosphorus are found in the >64μm fraction, indicating that a large proportion of phosphorus may be associated with flocculated particles, or fragments of macrozooplankton. Rivers have greater amounts of phosphorus in the soluble fraction (<0.2μm).

The goal of this paper was to examine the TP-Chl *a* relationships of three aquatic systems in an attempt to explain some of the remaining residual variability surrounding the individual relationships. We find that irrespective of the overall differences between the TP-Chl *a* relationships of lakes, rivers and estuaries, the Algal P-Chl *a* relationships are indistinguishable. Examination of the size

Appendix E ... (cont.)

distribution of phosphorus suggests that, in rivers and estuaries, a larger portion of the total phosphorus in the water column is found in non-algal fractions. Determining where this "extra" phosphorus is ending up, and why, will be the next steps of this work.

Acknowledgments

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Appendix E ... (cont.)

Table App.E.1: Summary of the regression equations predicting chlorophyll *a* concentrations from phosphorus concentrations in lakes, rivers and estuaries. Equations taken from the literature are presented in section a), equations derived from this study are presented in sections b) and c). The "algal" fraction (section c) corresponds to P and Chl *a* levels measured in the 3-30µm fraction. The residual mean-square error is abbreviated by RMSE.

		Equation	N	r^2	RMSE
a)	Published relationships - Total Phosphorus				
	lakes[1]	log(Chl *a*) = -0.41 + 1.01 log(TP)	36	0.74	0.06
	rivers[2]	log(Chl *a*) = -0.26 + 0.73 log(TP)	31	0.76	0.03
	estuaries[3]	log(Chl *a*) = -2.89 + 1.76 log(TP)	15	0.65	0.02
b)	This study - Total Phosphorus				
	lakes	log(Chl *a*) = -1.79 + 1.40 log(TP)	13	0.42	0.19
	rivers	log(Chl *a*) = -0.35 + 0.40 log(TP)	15	0.1	0.09
	estuaries	log(Chl *a*) = -1.11 + 0.84 log(TP)	17	0.15	0.07
c)	This study - Algal Phosphorus				
	lakes	log(Chl *a*) = -0.78 + 1.25 log(AP)	13	0.55	0.18
	rivers	log(Chl *a*) = -0.49 + 0.94 log(AP)	15	0.64	0.06
	estuaries	log(Chl *a*) = -1.04 + 1.44 log(AP)	17	0.74	0.04

[1] CURRIE 1990

[2] BASU & PICK 1996

[3] MEEUWIG et al. 1998

Appendix E ... (cont.)

Table App.E.2: Summary of ANCOVA models for a) Total P-Chl *a* relationships and b) Algal P-Chl *a* relationships between lakes, rivers and estuaries on Prince Edward Island, Canada. A log(TP)*system term added to model a) is non-significant. The algal fraction corresponds to P and Chl *a* levels measured in the 3-30µm size fraction of the water column. Elimination of one of the terms containing the variable "system" from the model b) does not cause the remaining terms to become significant. The residual mean-square error is abbreviated by RMSE.

	Dependent variable	Independent variables	SS	d.f.	F	P	r^2	RMSE
a)	log(Chl *a*)	log(TP)	1.4	1	12	0	0.3	0.12
		system	1	2	4.09	0.02		
b)	log(Chl *a*)	log(AP)	4.3	1	59.6	< 0.001	0.7	0.09
		system	0.5	2	2.89	0.07		
		log(AP)*system	0.2	2	0.87	0.426		

Appendix E ... (cont.)

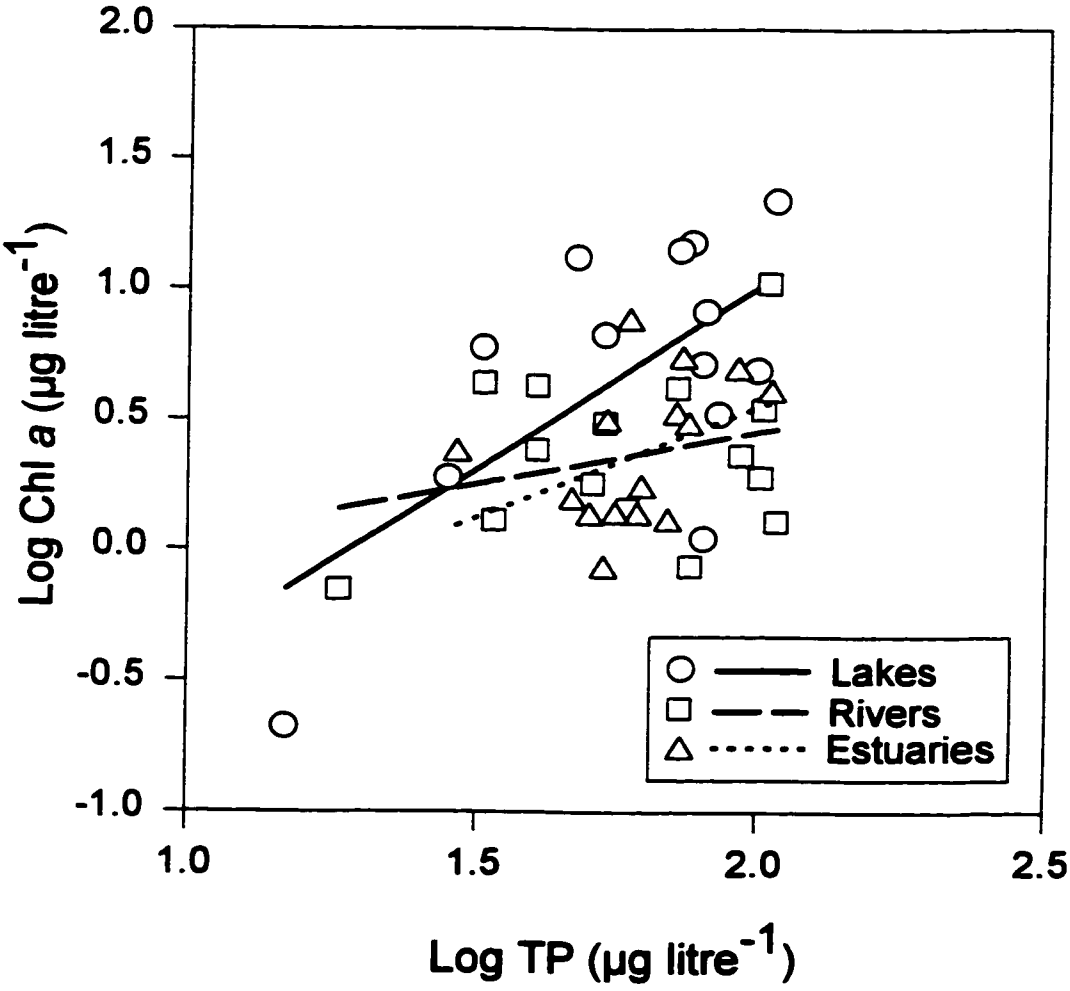
Figure App.E.1. **Location of Prince Edward Island, Canada.**

Appendix E ... (cont.)

Appendix E ... (cont.)

Figure App.E.2. The relationship between TP and Chl *a* for lakes, rivers and estuaries in Prince Edward Island. The regression slopes for each system are presented. The lake relationship differs significantly from the other two.

Appendix E ... (cont.)



Appendix E ... (cont.)

Figure App.E.3. The relationship between P and Chl *a* measured in the 3-30µm size fraction (algal fraction) for lakes, rivers and estuaries in Prince Edward Island. The regression slopes for each system are presented. There are no statistical differences between the three relationships.

Appendix E ... (cont.)

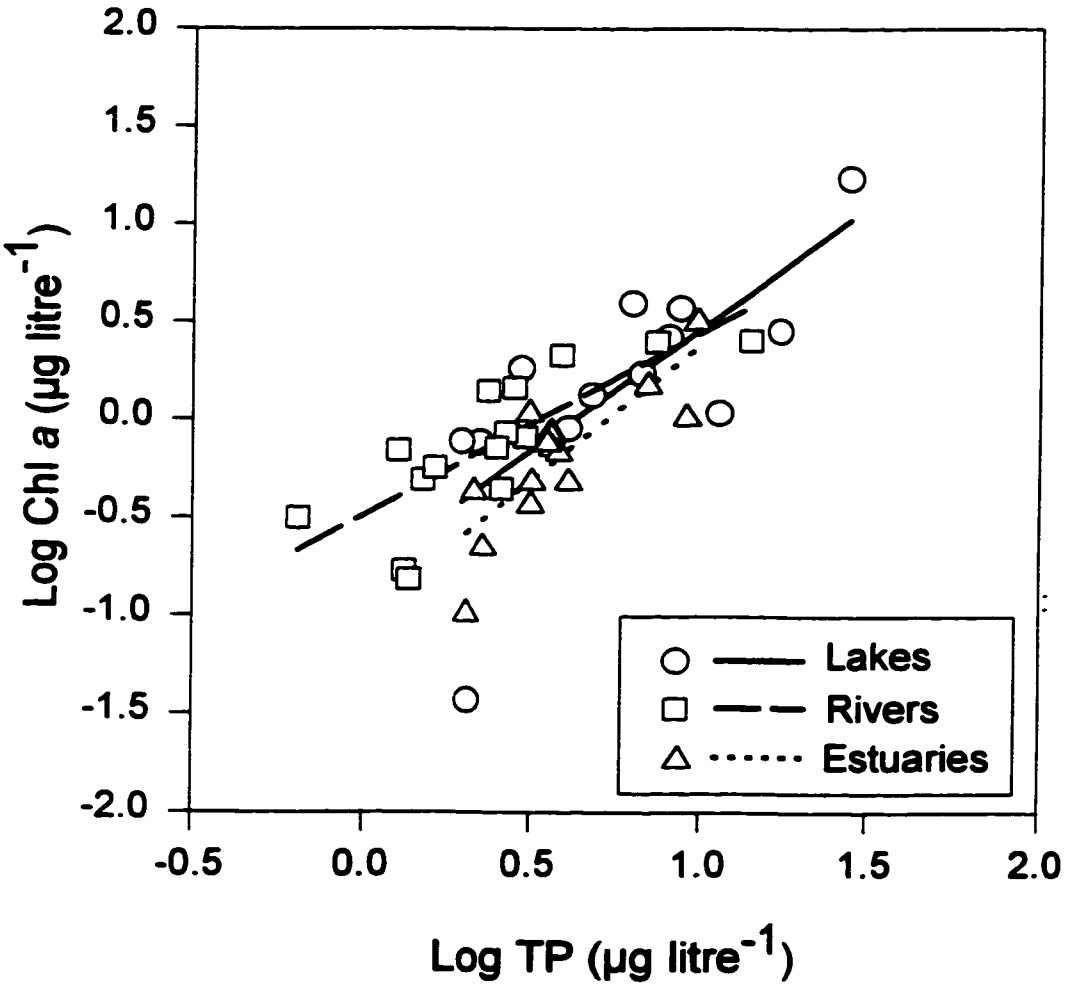


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Figure App.E.4. The mean residual amount of phosphorus (P) in each size fraction in lakes, rivers and estuaries, after statistically controlling for log(TP) and for the mean amount of P in the size fractions. This figure shows that, in lakes, a disproportionate large amount of P is in particles between 0.2 and 64µm. In contrast, rivers have disproportionately large amounts of soluble P (<0.2µm), while estuaries have P concentrated in the largest size fraction. The standard errors are also presented. Asterisks (*) denote means statistically different from zero.

Appendix E ... (cont.)