

Linking Ecology and Management of Water Quality: The Distribution and Growth of  
Phytoplankton in Coastal Lakes of British Columbia

by

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### **Abstract**

Processes regulating the growth and successional pattern of phytoplankton and the production of odour compounds in lakes of coastal and interior British Columbia were examined. An emphasis was placed on the role of nutrients, the role of size in determining nutrient deficiency, and the importance of winter for understanding the functioning of coastal lakes. Although the study lakes were all phosphorus limited (TN:TP molar ratio >22), plankton, especially the greater than 3  $\mu\text{m}$  size fraction, were often nitrogen deficient. This demonstrates the importance of nitrogen as a growth regulating nutrient for larger plankton in these lakes. Seasonal patterns of productivity varied among lakes, and Maxwell Lake was found to reach maximal photosynthetic rates in February. Lakes without a dominant seasonal physical influence (e.g. ice-cover) and those subject to short-scale stochastic events that play dominant roles may not have their "successional clock" set. This can lead to an apparent chaotic seasonal pattern of species distribution. In coastal lakes the lack of strong seasonal patterns is more likely to occur in lakes with lower nutrients (e.g.  $<10 \mu\text{g TP}\cdot\text{L}^{-1}$ ) than in lakes with relatively high nutrients ( $>15 \mu\text{g TP}\cdot\text{L}^{-1}$ ) because of the seasonal cycling of nutrients within eutrophic lakes.

The origin of odours in drinking water was examined from nineteen lakes and reservoirs to determine links between limnological variables and classification and intensity of odour. Total phosphorus (TP) was the best single predictor of odour intensity. Vegetation and grassy odours were more prevalent in lakes with TP less than  $13 \mu\text{g}\cdot\text{L}^{-1}$ , while earthy odours were common at higher TP. Drinking water quality issues were reviewed and the relationship between policy, management and science was examined. This work stresses the importance of sound science to ensure the legality, legitimacy, efficiency and effectiveness of implementing water quality policies and for establishing best management practices.



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## **Chapter 1: Introduction**

### *Introduction*

Understanding phytoplankton species composition and succession is a vital component for the ecological management of drinking water lakes and reservoirs. Phytoplankton represent the base of the classical aquatic food web, so phytoplankton biomass, growth rates, and species composition can constitute a major component of the overall aquatic community. From an applied perspective, it is known that certain algal species produce taste and odour compounds and toxins. Additionally, algae can decrease filtering efficiency, discolour water, decrease the effectiveness of treatment processes, and increase disinfection byproducts (Suffet et al. 1995). Therefore, it is important to know the composition of phytoplankton and to understand their growth and loss processes in order to appreciate the effect of various management decisions on the quality of source water.

The functioning of lakes is influenced by past and present geomorphological, physical, and biological processes. Lake functioning is fundamentally influenced by local geography and climate. There is a general coherence of lakes in areas of similar geography and climate (Hughes and Larsen, 1988; Omernik et al. 1991; Benson et al. 2000). Lakes of coastal British Columbia (BC) are different from most temperate lakes because they are not typically covered with ice during winter and the major period of water inflow (freshet) occurs during winter (November – February/March). The coastal region in which this research was conducted has previously been classified as part of the Coastal and Insular Mountain Region of British Columbia (Northcote and Larkin 1956). Winters are mild and wet, while summer epilimnetic temperatures are typically greater than 20°C. These coastal study lakes are best classified as being warm monomictic

(Hutchinson 1957; Wetzel 2001; Kalff 2002). For management purposes it is necessary to couple general knowledge of lake functioning with an understanding of regional lake processes, and, if possible, specific characteristics of the water body or water bodies of management interest. The distinctiveness of lakes in this region warranted further investigation to understand how they differed from more studied continental dimictic lakes. Choosing several lakes across a gradient of size, depth and trophic status provided a means for simultaneously evaluating the diversity of lake types within this region.

*Measures of Nutrient Deficiency and Limitation (Chapter 2)*

Growth of phytoplankton and the success of individual species depends on both external conditions and individual physiologies (Reynolds 1984a). Nutrients, temperature, light, grazers, and stratification patterns (mixing/sinking) all influence the population dynamics and spatial distribution of phytoplankton in lakes. One of the most studied associations in phytoplankton ecology is the relationship between nutrients and phytoplankton biomass and composition.

An approach to studying the relationship between nutrients and phytoplankton is to measure water column nutrients, however, these concentrations do not necessarily reflect the nutrient status of a cell. For example, nutrient cycling within the food web may provide an adequate source of that nutrient (Lehman 1980) or an algal cell may obtain nutrients mixotrophically (Caron et al. 1993). Nutrient debt assays, such as nitrogen debt (N-debt), phosphorus debt (P-debt), or ammonium enhanced response (AER) directly assess deficiency of nutrients (Healey and Hendzel 1979a; 1979b and 1980; Mitchell and Malthus 1984). Indicators of phytoplankton nutrient status (e.g.

nutrient ratios and nutrient debt assays) are more reliable when two or more are used in conjunction (Guildford et al. 1994).

Nutrient ratios are useful for understanding nutrient-limited conditions (Droop 1974; Healey and Hendzel 1980), species domination (Tilman et al. 1982; Sommer 1985, Watson et al. 1997), and stoichiometric nutrient transfer (Elser and Urabe 1999). Individual species of phytoplankton have different physiological requirements (Tilman and Kilham 1976; Tilman et al. 1986). Species competitiveness will depend upon the ratio of available nitrogen to phosphorus and their ability to obtain those nutrients. For example, it is thought that certain cyanobacteria may have a competitive advantage when the N:P ratios are low because they can fix nitrogen.

Chapter 2, examines nutrient dynamics in coastal and interior lakes of British Columbia. Nitrogen and phosphorus were considered because of the central role of these nutrients in limnological studies. A differentiation was made between deficiency (as reflected by bioassays) and limiting nutrients (total nutrients available). Particulate ratios (PN:PP) represent the stoichiometric composition of the plankton community, so ratios should reflect the limiting nutrient as constrained by the dominant species. Two size fractions were considered in nutrient deficiency assays (<3  $\mu\text{m}$  and >3  $\mu\text{m}$ ). This fractionation was conducted because of the rapid ability of cells smaller than 3  $\mu\text{m}$  to take up dissolved nutrients. This study stresses the importance of defining limitation to an appropriate time and spatial scale. Despite all lakes being classified as P-limited (as determined by TN:TP), plankton communities were often N-deficient emphasizing the potential role of nitrogen in shaping species composition.

### *Phytoplankton Productivity (Chapter 3)*

During the first year of this study, wintertime sampling revealed a wintertime chl *a* maximum in several of the study lakes. Lake successional theories predict winter to represent the period of lowest biomass and growth (Sommer et al. 1986), so the question immediately arose as to how active the plankton communities were during winter. The <sup>14</sup>C-technique was used to estimate productivity on each sampling date during the second year of research (starting June 2001). Although the overall study finished during the winter of 2002, productivity measurements were conducted into the spring of 2002 (May) in order to obtain measurements for a full year.

The third chapter addresses the issue of productivity in the coastal lakes and reservoirs of this study. Productivity reflects phytoplankton growth and provides insight into when communities are active, which is important both from limnological and management perspectives. The results from this study demonstrate the need for evaluating productivity during winter and points to the diversity of lakes within this region. I discuss several explanations for inter-lake variance, including trophic status and zooplankton community composition.

### *Species Composition and Succession (Chapter 4)*

Species composition and abundance are dependent on many variables. There is, however, a general pattern to phytoplankton species succession in temperate lakes (Reynolds 1984a; Sommer et al. 1986). The written model proposed by Sommer et al. (1986) predicts that the increase of light and temperature in spring accompanied by an abundance of nutrients causes a bloom of phytoplankton dominated by small fast-growing species, or r-selected organisms (MacArthur and Wilson 1967; Kilham and

Hecky 1988). Species composition remain similar between years because more inoculum is available from species present during the previous year (Reynolds 1984a). When thermal stratification occurs, further changes in community structure are more strongly related to biotic interactions and there is an increased resilience to physical disturbances (Reynolds 1988). Species present during spring blooms tend to be edible, which gives rise to a corresponding increase in zooplankton abundance. Increased abundance of zooplankton causes a clearing of algae which subsequently causes zooplankton abundance to decrease. At this point models for oligotrophic and eutrophic systems diverge. In eutrophic systems, nutrients are still abundant enough to sustain high algal biomass, however, due to selective grazing, larger more inedible forms of algae are selected for (K-selected species). In oligotrophic systems low nutrients prevent a second algal bloom, and algal biomass remains low during the summer. In autumn, physical control dominates phytoplankton biomass and composition because when mixing occurs nutrients increase concurrently with a decline in temperature and light (Sommer 1996).

Sommer et al. (1986) suggest that phytoplankton seasonal succession is based upon physiological constraints, resource competition and grazing and therefore is predictable and directional. They note, however, that disturbances, like the lack of thermal stratification and high or irregular flushing rates, may cause phytoplankton seasonal succession to become unpredictable.

The fourth chapter addresses the issue of seasonal succession in coastal lakes and probes the relationship between species composition and indicators of nutrient status. Phytoplankton seasonal succession in most temperate lakes begins in spring after a period of low growth and competition during winter. Because the plankton communities are

active throughout winter, the inconsistency of this starting point for coastal lakes is an important consideration for seasonal cycles. Upon analyzing species associations and seasonal trends for each lake, the issue of disturbance, or perturbation, in relation to internal forces is addressed.

*Source Water Odours (Chapter 5)*

Water purveyors are required to deliver safe drinking water, however, consumer perception of safe drinking water is also of great importance. Consumers often judge the safety of tap water by its taste, odour and colour. Odours can originate from many sources, including from the biota of surface source waters, disinfection processes and distribution systems. The fifth chapter uses flavour profile analysis (FPA) to evaluate source-water odours. Common limnological parameters are used to predict both odour intensity and odour quality. During the second year of study, select samples were analyzed for target taste and odour compounds using a gas chromatograph mass spectrometer. Analysis of odours at the terminal end of water mains in the Capital Regional District (CRD; Victoria, BC) distribution system was also undertaken to determine the principal source of odour in the CRD drinking water system.

*Drinking Water Management, Policy and Science (Chapter 6)*

The sixth chapter summarizes water quality issues and reviews the link between management, policy and science. Sound policy must have effective instrumentation to maintain a legal, legitimate, efficient and effective basis for managing water systems. Science plays an integral role because it provides the defensible knowledge upon which policy and management decisions can be made. Gaining an understanding of management issues through scientific investigation, is therefore imperative. The theme

of using scientific studies to better understand issues important for water resource management leads to the study presented in the sixth chapter.

### *Research Objectives*

The principal objectives of this research were two fold. The first was to examine patterns of phytoplankton distribution and abundance, and the second was to examine the relationship between plankton and drinking water odours. The emphasis on nutrient dynamics is due to both the bias of research towards understanding the role of nutrients and because nutrients remain one of the few major factors affecting phytoplankton that can be effectively managed. Ultimately, the goal was to evaluate the predictability of phytoplankton species biomass and distribution in southern west-coast warm monomictic lakes.

**Chapter 2: Temporal Changes in Nitrogen and Phosphorus Co-Deficiency of  
Plankton in Lakes of Coastal and Interior British Columbia**

***Abstract***

Plankton nutrient limitation and deficiency were assessed in six coastal and four interior lakes and reservoirs in British Columbia. Ultimate nutrient limitation was defined as occurring over longer time scales (months to years) and representing the potential attainable biomass or yield. Proximate nutrient limitation reflects the physiological status and therefore represents limitation of instantaneous growth rates. All the lakes and reservoirs were considered to be ultimately P-limited according to TN:TP. Bioassay responses were used to assess the physiological status of plankton in whole water and  $< 3 \mu\text{m}$  size-fractions. Both P and N deficiency were found to occur at the same time, suggesting that when deficiency occurs, co-deficiency is common. The  $< 3 \mu\text{m}$  size-fraction accounted for a large proportion of P-debt, whereas the  $> 3 \mu\text{m}$  fraction accounted for most of the AER. Thus, size is important to understanding nutrient deficiencies in plankton communities. These results stress 1) the importance of measuring proximate deficiencies at greater temporal resolution, 2) that N and P were commonly found to be co-deficient, 3) the need to define nutrient limitation/deficiency in the context of the methods used and, 4) several measures of deficiency are required to assess the nutrient status of a plankton community.

## *Introduction*

A central objective of limnological research has been to understand and predict the abundance and composition of phytoplankton. Studies examining phytoplankton-nutrient interactions have dominated this research because nutrients play a fundamental role in the limitation of algal production and biomass. The role of phosphorus (P) is central to our current understanding of phytoplankton abundance and distribution in lakes (Schindler 1978; Hecky and Kilham 1988). P-limitation in lakes is supported by many studies and is important with respect to both community biomass (Dillon and Rigler 1974) and composition (Watson et al. 1997). However, nitrogen (N) is also important and may have a secondary role in limiting community abundance and affecting species distribution (Smith 1982; Hecky and Kilham 1988; Elser et al. 1990).

Studies assessing limitation have generally compared phytoplankton biomass and species composition in lakes across nutrient gradients or have conducted nutrient addition experiments. Other studies have sought to demonstrate nutrient deficiency in un-manipulated lakes (Healey and Hendzel 1980). There is a fundamental distinction between knowing how lakes respond to a perturbation (e.g., nutrient addition) and knowing how lakes function without manipulation. The concept of ultimate vs. proximate nutrient limitation highlights this difference. Ultimate nutrient limitation of lakes must be considered over longer time scales (months to decades) and is paramount to understanding biomass and production at the temporal scale. Proximate nutrient limitation, on the other hand, concerns the growth or physiological status of algal species (Healey 1979) at a specific time and place within a lake.

During summer stratification in many temperate lakes biological uptake often drives inorganic nutrient concentrations to near or below detection limits. Under these circumstances, availability of dissolved inorganic nutrients should be an important variable controlling phytoplankton species composition and productivity. On the other hand, the total amount of a nutrient present (especially TP and TN) plays a key role in determining the carrying capacity or potential attainable biomass. This relationship is exemplified by the abundance of published TP vs. chlorophyll *a* (Chl *a*) relationships (e.g., Dillon and Rigler 1974; OECD 1982; McCauley et al. 1989). Thus, ultimate limitation is best viewed as the total concentration of a *limiting* nutrient with respect to biomass (i.e., yield sensu Liebig, Odum 1997), whereas nutrient *deficiency* is best viewed in the proximate sense of limiting instantaneous growth rates (Beardall et al. 2001). The distinction between *limitation* and *deficiency* is critical because *limiting* nutrients do not necessarily equate to *deficient* nutrients for all phytoplankton at all times and places (De Baar 1994; Beardall et al. 2001). A deficient nutrient, while available, may be energetically costly to obtain (e.g., N<sub>2</sub> for N-fixing cyanobacteria).

Nutrient ratios have been used as a basis for evaluating nutrient limitation for several decades. Plankton nutrient ratios originally were found to be similar across much of the world's oceans (Redfield 1958). The consistency of nutrient ratios in the ocean was postulated to result from the interaction between elemental requirements of plankton and available nutrient pools resulting from biogeochemical nutrient cycling (Redfield 1958). Studies have since shown that these ratios often deviate across the oceans (Hecky et al. 1993; Falkowski 2000) but the original ratio postulated by Redfield is still used as a benchmark for comparison. Since inorganic carbon is available in excess, deviations

from Redfield's ratio of 106:16:1 (C:N:P molar) result from limitation in either N or P, intracellular nutrient storage, or detritus. Particulate nutrient ratios, especially N:P and C:N, are thought to be one of the simplest measures of phytoplankton nutrient status, although deviations from the Redfield ratio are common in lakes (Hecky et al. 1993).

One distinction between bioassay experiments and total nutrient ratios is that total nutrient ratios represent an integrated measure of nutrient availability over longer time scales (Redfield 1934; Kilham 1990; Falkowski 2000) whereas bioassays, such as N- and P-debt, alkaline phosphatase activity (APA) and  $\text{PO}_4^{3-}$ -turnover time measure deficiency at shorter time scales (Healey 1975, 1979). Total nutrient ratios, therefore, should provide better indices of nutrient *limitation* whereas physiological assessments should be more reflective of nutrient *deficiencies*. Particulate nutrient ratios often reflect total nutrient ratios (Hecky et al. 1993) but since they are a measure of organism stoichiometry they may also reflect nutrient deficiencies. It is important to note that limitation and deficiency are not mutually exclusive with respect to any one nutrient and it is not uncommon for a limiting nutrient to also be deficient (Hecky et al. 1993). Therefore, within both a community and a species more than one nutrient can be deficient at the same time.

Morphological and physiological differences between plankton result in different nutrient competitive abilities (Tilman 1982). Other attributes aside, small plankton and those with large surface area to volume ratios have an advantage in nutrient uptake. Bacteria have been shown to be especially proficient at taking up phosphate, and bacteria-sized particles ( $< 3 \mu\text{m}$ ) account for most of phosphate uptake in the epilimnion of P-limited lakes (Currie and Kalff 1984; Mazumder et al. 1988; Taylor and Lean 1991).

Studies also suggest that in many instances bacteria may be limited by P and not organic carbon (Toolan et al. 1991; Coveney and Wetzel 1992). The proportion of P and N deficiency accounted for by bacterial-sized particles is, therefore, important for understanding the dynamics of both the plankton community as a whole and larger-sized phytoplankton.

The purposes of the study presented are 1) to better understand the role of nutrients (N and P) in plankton communities of British Columbia lakes and reservoirs, 2) to examine the role and magnitude of both nutrient limitation and seasonal nutrient deficiency, 3) to assess the relative importance of bacteria/picoplankton and  $> 3 \mu\text{m}$  sized fractions in nutrient deficiency bioassays, and 4) to examine the correspondence of different methods of assessing nutrient limitation and deficiency. N and P were considered because of their general importance in nutrient studies and specific studies suggesting both N and P limitation in coastal lakes (e.g., Suttle and Harrison 1988).

I examined the above questions in lakes and reservoirs located on Vancouver Island and in the East Kootenay region of British Columbia. P and N deficiency were examined in two plankton size classes ( $< 3 \mu\text{m}$  and  $> 3 \mu\text{m}$ ) at least monthly during the stratified summer season and at least once during winter. Three measures of P limitation (TN:TP, PC:PP, PN:PP), one measure of P deficiency (P-debt), one measure of N-limitation (PC:PN) and two measures of N deficiency (N-debt and ammonium enhanced  $^{14}\text{C}$  uptake response (AER)) were used. P- and N-debt bioassays were developed using chemostat and batch culture experiments by Healey and Hendzel (1979, 1980). They measure dark uptake at saturating concentrations of  $\text{PO}_4^{3-}$  and  $\text{NH}_4^+$ , standardized to Chl *a*. The principal is that nutrient deficiency results in high capacity for uptake in the

dark. AER is based on the enhanced dark fixation of carbon in the presence of ammonium and indicates N deficiency (Yentsch et al. 1977, Mitchell 1989). The principal behind AER is that carbon skeletons are required for the incorporation of N into amino acids, so N deficient cells increase their dark carbon fixation rates compared to N replete cells (Elrifi and Turpin 1987).

### ***Methods***

#### *Study sites, sampling and chemical analysis:*

Six lakes and reservoirs near Victoria BC (coastal) and 4 lakes and reservoirs near Cranbrook BC (interior) were sampled and tested for plankton nutrient deficiency (Table 2.1). The coastal lakes are best classified as temperate warm monomictic and the interior lakes are temperate dimictic lakes. During winter, the coastal lakes reach a minimum water temperature of approx. 4 °C and, if it occurs, ice cover is typically transitory. Summer temperatures reach a maximum of 20 to 25 °C in both the coastal and interior lakes. A summary of the lakes, their locations, and basic morphological and limnological parameters is given in Table 2.1.

Each lake was sampled at its point of maximum depth; Shawnigan Lake and Sooke Lake Reservoir were sampled at the point of maximum depth in two different basins because they each have morphometrically and hydrodynamically distinct basins. Coastal lakes were sampled monthly from May to September in 2000 and May to November in 2001, and during the winter (January – February) of 2001 and 2002. Shawnigan Lake and Sooke Lake Reservoir were sampled every two weeks during the summer of 2001. Interior lakes were sampled three times during 2000 (May, July, and September). Temperature profiles (YSI Model 58) were used to define epilimnetic and

metalimnetic layers. An integrated epilimnetic sample was taken using a 5 cm diameter 6 m length of Tygon tubing with a weight attached at one end. Epilimnetic samples were taken over the whole epilimnion if it was less than 5.5 m deep or otherwise the top 5.5 m. Metalimnetic samples were taken near the centre of the metalimnion using a Niskin bottle. Triplicate water samples were taken from both the epilimnion and metalimnion. Secchi depth measurements were taken with a 20 cm black and white Secchi disk (Table 2.1).

Light extinction coefficients for photosynthetically available radiation (PAR) were determined using a  $2\pi$  quantum sensor (Li-Cor LI-192SA). PAR measurements were made at least every metre through the mixed layer. Measurements of PAR in air were made prior to and after underwater measurements to ensure the incident light remained similar. Extinction coefficients ( $k$ ) were calculated from the slope of the linear regression of the logarithm of light vs. depth. Mean mixing layer irradiance ( $\bar{I}$ ) was calculated using the same formula as Guildford et al. (2000):

$$\bar{I} = I_s ((1 - e^{-kZ_m})(kZ_m)^{-1}).$$

where:  $I_s$  is the sum of daily incident solar PAR and  $Z_m$  is the mixing depth.  $I_s$  was only measured on Vancouver Island. The sensor (Li-Cor LI-90SZ) was at the top of the drinking water intake tower on Sooke Lake Reservoir. In order to integrate variation due to cloud cover,  $I_s$  was averaged one week prior to, and one week after  $k$  was measured and adjusted for 6% surface reflectance (Wetzel and Likens 2000). During winter, the coastal lakes do not freeze so mixing depth ( $Z_m$ ) for determining mean epilimnetic PAR

was defined as the shallower of: the surface to (but not including) the depth where the temperature changed by 0.5°C over 0.5 m, or the mean depth of the lake. An average daily (24 hr) limitation threshold for  $\bar{I}$  of 3.5 mmol quanta·m<sup>-2</sup>·min<sup>-1</sup> was used; below this value algae in the mixed layer were considered to be light limited (Hecky and Guildford 1984).

Chl *a* was analyzed by filtering samples through GF/F filters (Whatman), then extracting the filters with 95% ethanol at 4 °C overnight, and analyzing on a spectrophotometer (Ultrospec® 2000, Amersham) using a 10 cm quartz cell. Chl *a* was calculated according to Wintermans and De Mots (1965). A VWR pH probe (Model 2000) was used to determine pH. Total P (TP), total dissolved phosphorus (TDP), total N (TN) and NO<sub>3</sub><sup>-</sup>/NO<sub>2</sub><sup>-</sup> were analyzed on a Lachat automated ion analyzer (Zellweger Analytics, QuickChem® 8000). NH<sub>4</sub><sup>+</sup>/NH<sub>3</sub> was analyzed manually on a spectrophotometer using the phenol blue method (Stainton et al. 1977). TP was measured by digesting unfiltered samples in an autoclave with potassium persulfate. The digested phosphate was analyzed using the ascorbic acid method (APHA 1998). TDP was determined by filtration through 0.45 µm membrane filters and then analyzing the filtrate the same way as TP. TN was measured by autoclaving unfiltered samples with an alkaline potassium persulfate solution (APHA). After digestion, samples were measured as nitrite using the cadmium column reduction method (APHA 1998). NO<sub>3</sub><sup>-</sup>/NO<sub>2</sub><sup>-</sup> was analyzed using water filtered through 0.45 µm membrane filters and measured using the cadmium reduction method. Particulate nutrients were only measured from the epilimnion. PC and PN were collected on pre-combusted GF/F's and analyzed on a CHN analyser (Costech) with a Delta<sup>plus</sup> Advantage mass spectrometer as the detector.

Standard curves using acetanilide were generated for each run. Particulate phosphorus (PP) was collected on acid-rinsed membrane filters (0.2 $\mu$ m pore size) and analyzed in a similar manner to TP. Average dissolved and total nutrients, Chl *a*, and pH, were determined from triplicate water samples, while particulate analysis was estimated from one sample. The test of discordancy (Barnett and Lewis 1984) was used to identify and remove outliers from samples conducted in triplicate.

*Nutrient deficiency bioassays:*

Nutrient status of plankton was evaluated using six different methods (Table 2.2). PC:PN was used as a measure of N-limitation, PC:PP and N:P (TN:TP and PN:PP) were used as measures of P limitation. P and N debt assays were used as indicators of nutrient deficiency of P and N respectively (Healey 1975) and AER is a measure of N deficiency (Yentsch et al. 1977; Mitchell 1989).

P- and N-debt bioassays were conducted according to Healey (1975) and Healey and Hendzel (1979, 1980). Glassware for all nutrient bioassays were acid washed in 10% HCl, rinsed with deionized water six times, and placed in a drying oven. P- and N-debt were conducted on all samples collected in 2000 until the winter of 2002. Water was pooled from the triplicate water samples and bioassays were conducted on this combined sample. Bioassays were conducted both on unfiltered water and water filtered gently through 3  $\mu$ m nuclepore membrane filters. Filtered water was divided into separate flasks for P- and N-debt. Na<sub>2</sub>HPO<sub>4</sub> and NH<sub>4</sub>Cl were added to the samples to a final concentration of approx. 5  $\mu$ mol·L<sup>-1</sup>. Nutrient analyses for P- and N-debt were conducted manually with a spectrophotometer using the ascorbic acid and Berthelot reaction methods, respectively (Stainton et al. 1977). Triplicate (P-debt) and quadruplicate (N-

debt) measurements were taken immediately after spiking the samples and again after incubating in the dark at room temperature for 24 hours. Deficiency values used for nutrient debt assays are from Healey (1975) and Healey and Hendzel (1980).

AER bioassays were conducted on epilimnetic samples from coastal lakes and reservoirs from July 2001 to the winter of 2002. Dark inorganic- $^{14}\text{C}$  incubations were conducted in a similar manner to that outlined by Shearer et al. (1985). The water source used for these bioassays was the same as that used in nutrient debt bioassays. Incubations were conducted in 65 mL glass bottles without headspace. 400 mL of water was spiked with 0.8 mL of  $\text{NaH}^{14}\text{CO}_3^-$  to a final activity of 590 kBq. Samples were gently mixed using a magnetic stirrer and three subsamples were removed and incubated. Immediately after these samples were removed, 1 mL of 1mM  $\text{NH}_4\text{Cl}$  was added to the remaining lake sample (final concentration of ammonium was ca.  $5\mu\text{mol}\cdot\text{L}^{-1}$ ) and this was stirred before being transferred to three separate incubation bottles. Incubations were conducted for 4.5 – 5 hours (Elser et al. 1988) at approximately in situ lake temperatures. Immediately following incubation, 3 mL was transferred from incubation bottles to scintillation vials containing a  $\text{CO}_2$  trapping agent (3-methoxypropylamine, Fluka). The 3 mL sample was used to determine the total available  $^{14}\text{C}$  in each sample. The remaining water in the incubation bottles was filtered through  $0.45\mu\text{m}$  Gelman membrane filters. Filters were fumed in a desiccator with concentrated HCl before being transferred to scintillation vials and counted (Beckman LS6000IC).

Alkalinity of each sample was determined using Gran titration and dissolved inorganic carbon (DIC) was calculated from pH and alkalinity (Park 1969). Carbon

uptake rates were determined by solving the following equation (Wetzel and Likens 2000):

$$C - fixation = \frac{{}^{14}C - assimilated \times {}^{12}C - available}{{}^{14}C - available} \times 1.06$$

where  ${}^{14}C - assimilated$  is the  $dpm \cdot mL^{-1}$  of the particulates,  ${}^{14}C - available$  is the total  $dpm \cdot mL^{-1}$ ,  ${}^{12}C - available$  is  $DIC \cdot L^{-1}$  and “1.06” is an isotope correction factor. AER ratios (Yentsch et al. 1977) were calculated by dividing the average rate of carbon fixation ( $\mu g C \cdot \mu g Chl a^{-1} \cdot hr^{-1}$ ) of samples incubated with added  $NH_4^+$  ( $+NH_4^+$ ) by the average of those incubated without  $NH_4^+$  ( $-NH_4^+$ ).

The threshold ratio of 2, defining N limitation using AER, was somewhat arbitrarily assigned by Yentsch et al. (1977). Mitchell and Malthus (1984) found ratios less than 1.5 were significantly different and may represent N deficiency. Mitchell (1989) suggested that carbon fixation rate measurements represent a more robust means of evaluating N-limitation and proposed the threshold enhancement rate of  $0.02 \mu g C \cdot \mu g Chl a^{-1} \cdot h^{-1}$ .

Whole water and  $< 3 \mu m$  size fractions were incubated for all of the physiological nutrient assays. The  $< 3 \mu m$  fraction was subtracted from the whole water lake samples to calculate the nutrient status of the  $> 3 \mu m$  size fraction. On occasion, the  $< 3 \mu m$  size fraction had a slightly greater response than the whole water. When this occurred the  $> 3 \mu m$  fraction was assumed to have zero deficiency. Chlorophyll was not fractionated during the study, so only the relative proportion that these two size fractions contributed to the total deficiency of each bioassay could be assessed because the fractionated Chl *a*

biomass corrected rates were unavailable for comparison to the nutrient thresholds (Table 2.2). Pearson correlation was used to describe the relationship between AER ratios and AER rate measurements.

### ***Results***

AER measurements are usually analyzed as either an enhancement ratio or an enhancement rate. Comparison of whole water AER, as expected, showed that these two analyses were well correlated,  $r^2 = 0.84$  (Figure 2.1a). An enhancement ratio of 2 (Yentsch et al. 1977) corresponded to an enhanced fixation rate of  $0.05 \mu\text{g C} \cdot \mu\text{g Chl } a \cdot \text{h}^{-1}$  while an enhanced fixation rate of  $0.02 \text{ C} \cdot \mu\text{g Chl } a \cdot \text{h}^{-1}$  (Mitchell 1989) corresponded to an enhancement ratio of 1.42. The  $< 3 \mu\text{m}$  fraction was enhanced by a ratio of more than 1.42 on only two dates (2.16 on 16 July 2001 and 1.42 on 16 Oct. 2001, both in Shawnigan North). However, the  $< 3 \mu\text{m}$  fraction did account for a large proportion ( $\bar{x} = 62\%$  range from 28 to 90%) of the total dark C-fixation (Appendix 2.1). Thus, enhancement ratios for the  $> 3 \mu\text{m}$  fraction are higher because the  $< 3 \mu\text{m}$  fraction component was subtracted from whole water (Figure 2.1b).

A comparison of AER and N-debt in the  $> 3 \mu\text{m}$  fraction showed a weak correlation ( $r^2 = 0.35$ ,  $p < 0.01$ ). This comparison was conducted using volumetric rates (Figure 2.2) because I don't have measurements of the  $> 3 \mu\text{m}$  chlorophyll fraction. N-deficient samples are marked with either an "x" (N-debt) or a "+" (AER). Overall, AER appeared to be a more sensitive measure. Of the 54 samples with results from both nutrient assays, 38 had zero net N-debt uptake in the  $> 3 \mu\text{m}$  fraction, while only 7 had no  $> 3 \mu\text{m}$  AER enhancement.

Seasonal water column mean irradiances for Council Lake, Shawnigan Lake and

Sooke Lake Reservoir suggest that light was not limiting photosynthesis in the mixed layer from May to October (Figure 2.3). The same trend was found for the other coastal lakes (data not shown).

TN:TP of the epilimnion and metalimnion suggest that if/when my study lakes were nutrient limited, P was most likely limiting (Figure 2.4). Average particulate ratios are greater than Redfield (Table 2.1) and PC:PN was high in interior lakes (Jimsmith, Mark Creek, New and Phillips). Other ratios fall within the typical range of north temperate lakes (Hecky et al. 1993; Guildford and Hecky 2000). JSL and NEL (interior lakes) had the greatest average TN:TP and high PC:PP. Both JSL and NEL have extensive macrophyte communities, which may partly explain the relatively high particulate carbon concentrations. Overall, PN:PP ratios were lower than TN:TP, with a few points falling below the Redfield ratio (Figure 2.4a). Data for PC:PP fell on both sides of threshold line (Figure 2.4c). PC:PN, a measure of nitrogen limitation, is suggestive that the plankton communities were also nitrogen limited on some dates although most points fall below the threshold line (Figure 2.4d). Measures of N- and P-debt and AER, corroborate that plankton communities were both phosphorus and nitrogen deficient (Figure 2.5). P-debt was associated with limiting TN:TP ratios, which was not surprising because on most dates TN:TP was considered to be phosphorus limiting (Figure 2.5a). Many samples that showed N deficiency (N-debt and AER) were also P-limited according to TN:TP (Figures 2.5a, 2.5b). There was not a clear trend observed between increasing deficiency as indicated by the bioassays and the particulate ratio limiting thresholds (Figures 2.5d, 2.5e, 2.5f). However, severe deficiency determined using bioassays was observed to

occur at elemental ratios nearer to Redfield (Fig. 5d, 5e,5f) than is commonly assumed (Table 2).

As expected, nutrient debt was greater when dissolved nutrients were low (Figure 2.6). Note that total dissolved phosphorus was used in this comparison because dissolved inorganic phosphorus (DIP) measurements were routinely near zero (below our analytical detection limit). When DIP is low the ascorbic acid method is known to give overestimates (Hudson et al. 2000). Deficiency, especially of phosphorus was more pronounced in the epilimnion (Figures 2.6a vs. 2.6b). The concentrations of DIN and TDP above which nutrient deficiency was not observed, is similar to the limiting-resource thresholds of growth for nitrate (approx.  $7.1 \mu\text{mol}\cdot\text{L}^{-1}$ ) and phosphate (approx.  $0.27 \mu\text{mol}\cdot\text{L}^{-1}$ ) reported by Interlandi and Kilham (2001). On average, most N- bioassays were deficient at lower values of DIN than the threshold reported by Interlandi and Kilham (2001), but my P-debt assay is in close agreement.

Both size fractions usually contributed to the total deficiency for N- and P-debt of those epilimnetic samples that were considered to be nutrient deficient (i.e. greater than deficiency threshold). The  $< 3 \mu\text{m}$  fraction most frequently contributed more than half of the total P debt (Figure 2.7). On the other hand, AER was principally a result of the  $> 3 \mu\text{m}$  fraction. When AER enhancement occurred in the  $< 3 \mu\text{m}$  fraction it was usually small and was not considered deficient. Over a third of samples had less than 25% of N-debt accounted for by  $< 3 \mu\text{m}$  fraction, while the  $< 3 \mu\text{m}$  fraction accounted for more than 75% of the total N-debt in 41% of the samples. Like AER, these latter deficiencies were usually close to threshold values.

Seasonal patterns in nutrient deficiency were examined in North Shawnigan Lake and Sooke Lake Reservoir (deepest stations) because these sites were sampled every two weeks during the summer (2001). These lakes are in close proximity (within 4 km) and have many similar limnological parameters (Table 2.1). Despite this, there were noticeable differences in nutrient deficiency measures. Both showed P-debt during the summer months, but in Shawnigan the  $> 3 \mu\text{m}$  size fraction was consistently deficient from early July until late September, while the  $< 3 \mu\text{m}$  size fraction constituted a consistent proportion of the total debt (Figure 2.8a). Sooke Lake Reservoir was extremely P deficient from late May until mid-July (Figure 2.8b). However, unlike Shawnigan, the  $< 3 \mu\text{m}$  size fraction accounted for most of the P-debt during this time.

One of the lakes that showed a consistent annual pattern of N-debt over the two years of this study was Council Lake. Council Lake has high TN:TP for most of the year ( $\bar{x} = 64.3 \text{ molar} \pm 5.8 \text{ SE}$ ) but was strongly N deficient (N-debt assay) during the spring with the deficiency decreasing over the course of the summer (Figure 2.9). Despite relatively high TN:TP, DIN was less than  $1 \mu\text{mol}\cdot\text{L}^{-1}$  for all dates except February 2001. TDP concentrations were also low, less than  $0.1 \mu\text{mol}\cdot\text{L}^{-1}$ , for all dates except May 2001. P-debt indicated that Council Lake plankton were frequently P-deficient. Breaking the nutrient assays into  $> 3 \mu\text{m}$  and  $< 3 \mu\text{m}$  fractions suggests that despite high TN:TP, the  $< 3 \mu\text{m}$  fraction often contributed a large proportion of the P-debt (Figure 2.9). N deficiency for the  $> 3 \mu\text{m}$  fraction remained severe during the spring and early summer of both years. AER suggested strong N-deficiency for the  $> 3 \mu\text{m}$  fraction in July, August, and September 2001.

In general, plankton communities of the coastal lakes had higher nutrient debt during the summer and lower debt in the winter, although nutrient deficiency was noted to occur during the winter. None of the lakes exhibited enhanced AER during January and February. Three of my lakes did, however, have severe P-debt during the winter (Council, Sooke North, Shawnigan South). Apart from Council in the winter of 2001, it was the  $> 3 \mu\text{m}$  sized fraction that contributed most of this P-debt.

### *Discussion*

TN:TP suggests that my study lakes are P-limited, that is the biomass of phytoplankton is ultimately controlled by P. Analysis of particulate ratios demonstrated that the plankton community was actually closer to threshold limits than the TN:TP ratio suggested. Physiological indicators suggested both severe P- and N-deficiency occurred simultaneously. As such, TP may be ultimately limiting in these lakes, whereas particulate data may be more reflective of the plankton species composition that is most competitive under the particular resource supply ratios (Kilham 1990). Particulate ratios should more closely reflect deficiency bioassays, however, they do not necessarily reflect proximate nutrient deficiencies since they are measures of biomass and not rate processes. Nutrient assays should reflect the proximate nutrient deficiencies of those plankton species and may in turn affect species competition. Hecky and Kilham (1988) suggest that the paradigm of N-limited oceans and P-limited lakes may be due in part to differences of technique. Because different techniques define limitation at different scales (e.g. ultimate vs. proximate) it is necessary that limitation studies consider the methods used and the implied time-scales associated with the methods.

More than one nutrient seems to be deficient at a given time in a plankton community, which is illustrated temporally for three lakes. This suggests that in my coastal oligotrophic study lakes during periods of nutrient stress, co-deficiency may be a common occurrence rather than an exception. P-deficiency was more often dominated by the  $< 3 \mu\text{m}$  sized fraction, which corresponds with previous findings that small plankton have high P-requirements (Elser et al 1996). N-deficiency was not as clearly dominated by the  $< 3 \mu\text{m}$  fraction, and the AER assay suggests that, apart from a couple of days the bacterial/picoplankton-sized fraction was not N-deficient.

Larger phytoplankton are at a disadvantage regarding dissolved nutrient uptake because they have greater volume to surface area ratios. Because dissolved nutrients ( $\text{PO}_4^{3-}$ ,  $\text{NH}_4^+$ ,  $\text{NO}_3^-$ ) were usually at low concentrations in the surface and metalimnetic waters of my study lakes, the larger phytoplankton must either grow more slowly, rely on luxury uptake and internal nutrient stores, or rely on phagotrophy. For example, many of the chrysophytes that are dominant in oligotrophic lakes are phagotrophic (Raven 1988; Jones 2000). Bacterial P has been found to serve as a substitute source of P for mixotrophic algae (Rothhaupt 1996). Jansson et al. (1996) suggest that N deficiency in larger plankton may be induced by mixotrophic consumption of P-rich bacteria. This offers one explanation for why phytoplankton show N deficiency despite high TN:TP. P-limitation itself may also offer an explanation for higher PC:PN in some of my samples since extreme P-limitation induces limiting PC:PN ratios in some phytoplankton species (Healey 1975).

While no two structural nutrients can limit (*sensu stricto* Liebig) the yield of a phytoplankton species at the same time (Odum 1997), more than one non-structural

nutrient can limit the growth rate (Tilman 1982; Odum 1997; Beardall et al. 2001).

Deficiency of non-structural nutrients (e.g., nutrients needed for enzymes, co-factors) will not directly affect the carrying capacity (i.e., potential yield) but will affect the rate at which phytoplankton will grow towards that yield. While I am not aware of clear empirical demonstration of *co-limitation* by two nutrients for a single species, Healey (1985) demonstrated co-limitation by nutrients and light, implying energetic- or rate-limiting steps lower growth rate, regardless of the availability of structural nutrients. For example, iron deficiency in certain diatoms has been found to cause flavodoxin to be used in place of ferridoxin (La Roche et al. 1996). Thus, in theory, if iron is present in low concentrations diatom growth rate would be reduced because of the lower energy efficiency of transfer of the photosystems while simultaneously being limited in yield by another nutrient. Lower growth rates resulting from iron deficiency may also affect the competitive ability of certain diatoms. While plankton diversity provides the most probable explanation for the co-deficiency observed in this study, it is interesting to postulate about the idea of co-deficiency within a single species since nutrients required in the greatest amount usually have both structural and non-structural roles. This study provides no direct evidence for this idea. However, it is interesting to note that severe P-debt, N-debt and AER were observed to occur at particulate nutrient ratios closer to Redfield than the literature values used to define deficiency in this study (Table 2).

An alternative explanation for N-deficiency in lakes with high TN:TP is that dissolved organic N is less available than dissolved organic P. Allochthonous organic matter often has high N:P ratios (Wetzel 2001) so phytoplankton may still be N-deficient despite high TN:TP. Previous investigators (Healey and Hendzel 1980) found nutrient

ratios of plankton and detritus were not distinguishable, but there is a good possibility of interference in my study lakes because TN:TP and PN:PP were poorly correlated ( $r^2 = 0.001$ ). N- and P-turnover times both remained rapid despite high N:P loading (50:1 molar) in coastal BC lakes (Suttle et al. 1991) corroborating my finding of N-deficiency.

Bacteria are important with respect to nutrient uptake and affect bioassays both directly (e.g., representing a large percentage of P-debt) and indirectly (e.g., masking enhancement effect in AER). Accounting for the bacterial component is critical if Chl *a* is used as the measure of biomass or when deficiency thresholds are derived from studies using axenic algal cultures. Correction for bacterial influence on larger plankton is most easily made by size fractionating chlorophyll and conducting bioassays on the bacterial-sized community in parallel with normal bioassays. Size fractionation of plankton has the unfortunate consequence of removing possible nutrient linkages, for example, micrograzer recycling of phosphorus that is utilized by bacteria. The magnitude of this effect was not measured in the current study, however, I believe that the contribution of bacterial P-deficiency should be recognized when applying modelled nutrient thresholds and will allow for a more direct evaluation of deficiency severity in larger plankton. If a relatively large proportion of chlorophyll is in the  $< 3 \mu\text{m}$  fraction, but a larger proportion of nutrient deficiency is in the larger fraction ( $> 3 \mu\text{m}$ ) then the larger fraction will be more severely deficient than deficiency thresholds based on whole water would indicate.

Size fractionation is a convenient way of looking at different plankton functional groups (Sheldon et al. 1972). Although I only looked at two size fractions, the difference in P-debt for Shawnigan Lake and Sooke Lake Reservoir highlights the value of looking at these two sizes. During stratification the  $> 3 \mu\text{m}$  fraction in Shawnigan Lake

accounted for a large proportion of the P-debt whereas, in Sooke Lake Reservoir, the  $>3\mu\text{m}$  size fraction accounted on average for much less of the total P-debt. Overall, however, Sooke Lake Reservoir showed greater P-deficiency in June and July because of the large phosphorus uptake by the  $< 3 \mu\text{m}$  size fraction. Since bacteria can dominate P uptake (Curry and Kalff 1984; Taylor and Lean 1991) it is important to clarify exactly what limitation bioassays represent

The most striking differences in nutrient deficiency between the two size classes occurred in the AER assays. The threshold value for enhancement rates of  $0.02 \mu\text{g C} \cdot \mu\text{g Chl } a^{-1} \cdot \text{h}^{-1}$  suggested by Mitchell (1989) corresponds to an enhancement ratio of 1.42 in this study, close to the significant ratio of 1.45 reported by Mitchell and Malthus (1984). AER deficiency threshold rates for just the  $> 3 \mu\text{m}$  fraction will typically be lower, whereas the threshold ratios will be higher than those obtained using whole water.

The most probable explanation for the lack of enhanced uptake by the AER  $< 3 \mu\text{m}$  fraction is that the fraction was not N deficient on most occasions. However, several potential explanations may account for these results. Dark inorganic carbon fixation results from anaplerotic carboxylation (Raven 1997). Under N limitation these reactions provide carbon skeletons necessary for amino acids (Elrifi and Turpin 1987). Since the AER  $< 3 \mu\text{m}$  fraction was generally not enhanced it suggests, 1) it was N limited, but used stored organic carbon as the carbon source, 2) it had a means of storing N that does not require C-uptake, 3) it was N limited but utilized a different N source (i.e., organic), or 4) that this size fraction was not N limited. Since the  $< 3 \mu\text{m}$  size fraction comprised a significant portion of the non-enhanced dark carbon fixation ( $\bar{x} = 62\%$  range from 28% to 90%), it is unlikely that cellular organic carbon would be exclusively used as the

carbon skeleton source under  $\text{NH}_4^+$  enhanced conditions. I am unaware of any studies demonstrating luxury dark N-fixation that doesn't involve C. While this explanation cannot be ruled out, it is considered unlikely. However, N-storage independent of C would offer an explanation for samples with high N-debt and corresponding low AER. The  $< 3 \mu\text{m}$  size fraction may have used organic N sources, but it is unlikely that they would not utilize  $\text{NH}_4^+$  and C anaerobically if they were deficient in N.

Every nutrient deficiency analysis is unique in how it measures and defines deficiency. This means that in order to better understand the nutrient status of a community it is necessary to use several measures of deficiency/limitation. There was a weak relationship between severity of deficiency of the  $> 3 \mu\text{m}$  fraction for AER and N-debt. The AER bioassay suggested greater N-deficiency for most samples, either because the bioassay is more sensitive or because the thresholds for N-debt and AER are not comparable. Guildford and Hecky (2000) found that N-deficiency measures (N-debt, C:N) did not follow a consistent pattern with either TN or TN:TP, whereas samples were consistently P-deficient when TN:TP was more than 50 (molar). N-debt, therefore, may be less predictable with respect to TN:TP than P-debt. This could be explained by the many different forms of nitrogen available, and the demonstrated ability of plankton to store P.

The context of defining and measuring nutrient limitation is of importance for understanding plankton ecology. The lakes and reservoirs in my study are P-limited in an ultimate sense (as indicated by TN:TP ratios) although the actual plankton community itself reflected a lower ratio. Many of the study lakes and reservoirs demonstrated proximate deficiency of both N and P. When deficiency was exhibited it was common

for both N and P to be deficient at the same time. Suttle et al. (1991) found relative uptake rates in the  $< 3 \mu\text{m}$  fraction more P-limited, whereas the  $> 3 \mu\text{m}$  fraction was more N-limited. Using nutrient deficiencies I found the  $< 3 \mu\text{m}$  sized fraction constituted a large proportion of P-debt, whereas the  $> 3 \mu\text{m}$  sized fraction constituted a large proportion of AER and N-debt, which agrees with Suttle et al.'s (1991) results. These findings underscore the importance of both plankton size and time scales in defining the impact of nutrient deficiency and limitation in plankton communities. Previous studies have stressed the importance of N-limitation under high TP (Smith 1982; McCauley et al. 1989), however, N-deficiency may also be important for understanding phytoplankton distribution in lakes with low TP. The most fundamental observations from this study are the distinction between nutrient limitation and deficiency, that plankton communities can be co-deficient in both N and P at a given time within a single lake, and that no single measure of deficiency reflects the complete nutrient status of the plankton community.

Table 2.1: Morphological and limnological characteristics of lakes and reservoirs in this study.

Area, maximum depth, and mean depth are for full stage conditions. Secchi, pH, DOC (dissolved organic carbon), chlorophyll *a* (Chl *a*), TP (total phosphorus), TN (total nitrogen), PC:PN (particulate carbon to particulate nitrogen), PC:PP (particulate carbon to particulate phosphorus) and PN:PP (particulate nitrogen to particulate phosphorus) are summer epilimnetic averages (May – September). For comparison, Redfield Ratios are: PC:PN (6.6:1), PC:PP (106:1) and PN:PP (16:1). Shawnigan Lake and Sooke Lake Reservoir each have three major basins; the two sampling stations were in the north and south basins. Morphometric data from Spafard et al. (2002).

\* Reservoirs and lakes that may experience seasonal drawdown. † Sooke Lake Reservoir physical parameters are valid from 1989 when the dam was last raised until winter 2003 when the dam was again raised.

Lake	Lat °N	Long °W	Surface area (ha)	Max depth (m)	Mean depth (m)	Secchi (m)	pH	DOC (mg·L <sup>-1</sup> )	Chl <i>a</i> (µg·L <sup>-1</sup> )	TP (µg·L <sup>-1</sup> )	TN (µg·L <sup>-1</sup> )	PC:PN (molar)	PC:PP (molar)	PN:PP (molar)
Council Lake	48°31'													
	123°40'		16	17.0	5.2	8.3	7.5	2.1	0.9	3.6	96.0	12.4	312	26.0
Cusheon Lake	48°48'													
	123°28'		31	9.5	4.4	3.8	7.6	5.2	5.9	16.1	417.6	8.8	202	24.2

Table 2.1 continued.

Lake	Lat °N	Surface area (ha)	Max depth (m)	Mean depth (m)	Secchi (m)	pH	DOC (mg·L <sup>-1</sup> )	Chl <i>a</i> (µg·L <sup>-1</sup> )	TP (µg·L <sup>-1</sup> )	TN (µg·L <sup>-1</sup> )	PC:PN (molar)	PC:PP (molar)	PN:PP (molar)
Elk Lake	48°31'												
	123°23'	246	20	7.7	5.0	8.2	5.4	2.9	17.6	401.5	9.0	200	21.8
-----													
Jimsmith Lake	49°28'												
	115°50'	21	7.5	4.9	6.0	8.4	7.7	1.4	8.9	368.2	15.7	489	28.9
-----													
Mark Creek	49°41'												
Reservoir*	116°01'	5	17.5	5.8	7.2	7.4	2.2	0.8	4.6	59.8	17.7	457	26.9
-----													
Maxwell Lake*	48°49'												
	123°31'	27	17.0	6.5	4.6	7.2	4.6	2.4	9.6	257.9	9.8	244	24.5
-----													
New Lake	49°30'												
	115°50'	27	10.5	3.0	5.2	8.4	18.0	2.3	10.3	1077.7	17.5	484	27.3
-----													
Phillips Reservoir*	49°27'	30	17.5	7.7	6.2	8.5	2.0	0.8	7.2	112.9	16.6	354	21.6

Table 2.1 continued.

Lake	Lat °N	Surface area (ha)	Long °W	Max depth (m)	Mean depth (m)	Secchi (m)	pH	DOC (mg·L <sup>-1</sup> )	Chl <i>a</i> (µg·L <sup>-1</sup> )	TP (µg·L <sup>-1</sup> )	TN (µg·L <sup>-1</sup> )	PC:PN (molar)	PC:PP (molar)	PN:PP (molar)
Shawnigan	48°37'													
Lake	123°38'	552		53.0	13.0									
Shawnigan														
North Basin		359		53.0	14.2	5.9	7.5	3.1	1.7	4.9	152.4	11.7	357	30.2
Shawnigan														
South Basin		57		27.0	11.9	5.9	7.5	3.1	1.6	4.4	153.4	11.7	406	35.4
Sooke Lake	48°33'													
Reservoir*†	123°41'	605		70.0	19.5									
Sooke North Basin*†		434		70	23.2	8.5	7.5	2.1	0.7	3.1	80.9	12.2	274	23.7
Sooke South Basin*†		45		22	8.6	7.4	7.4	2.0	0.9	3.3	85.6	12.2	319	27.0

Table 2.2: Values for phytoplankton nutrient limitation and deficiency thresholds.

Adapted from Guildford and Hecky (2000). \* Healey (1975) and Healey and Hendzel (1980). † Particulate ratios. § Mitchell (1989) and Yentsch et al. (1977).

Indicator	Nutrient	Deficiency Threshold
C:N*†	N	> 14.6 (molar ratio)
C:P*†	P	> 258 (molar ratio)
N:P*†	P	> 22 (molar ratio)
P-debt†	P	> 0.075 $\mu\text{mol P} \cdot \mu\text{g chl}^{-1} \cdot \text{day}^{-1}$
N-debt*	N	> 0.15 $\mu\text{mol N} \cdot \mu\text{g chl}^{-1} \cdot \text{day}^{-1}$
Dark $\text{NH}_4^+$ Enhanced $^{14}\text{C}$ -Uptake§	N	> 0.02 $\mu\text{g C} \cdot \mu\text{g chl}^{-1} \cdot \text{h}^{-1}$ > 2 ( $+\text{NH}_4^+/-\text{NH}_4^+$ )

Figure 2.1: (a) Chlorophyll specific – ammonium enhanced  $^{14}\text{C}$  uptake response (AER) enhancement rates vs. AER enhancement ratios for whole water. (b) Comparison of absolute enhancement rates for different size fractions.

(a) The horizontal dotted line represents an uptake deficiency threshold ratio of 2 (Yentsch et al. 1977) and the vertical dotted line represents an enhancement deficiency threshold rate of  $0.02 \mu\text{g C} \cdot \mu\text{g Chl } a \cdot \text{h}^{-1}$  (Mitchell 1989).

Ratio = Rate  $\times 18.13 + 1.05$ ,  $r^2 = 0.84$ . (b)  $< 3 \mu\text{m}$  (—□—),  $> 3 \mu\text{m}$  (—◆—) and whole water (—●—). Lines of best fit determined using bivariate principle axes analysis.

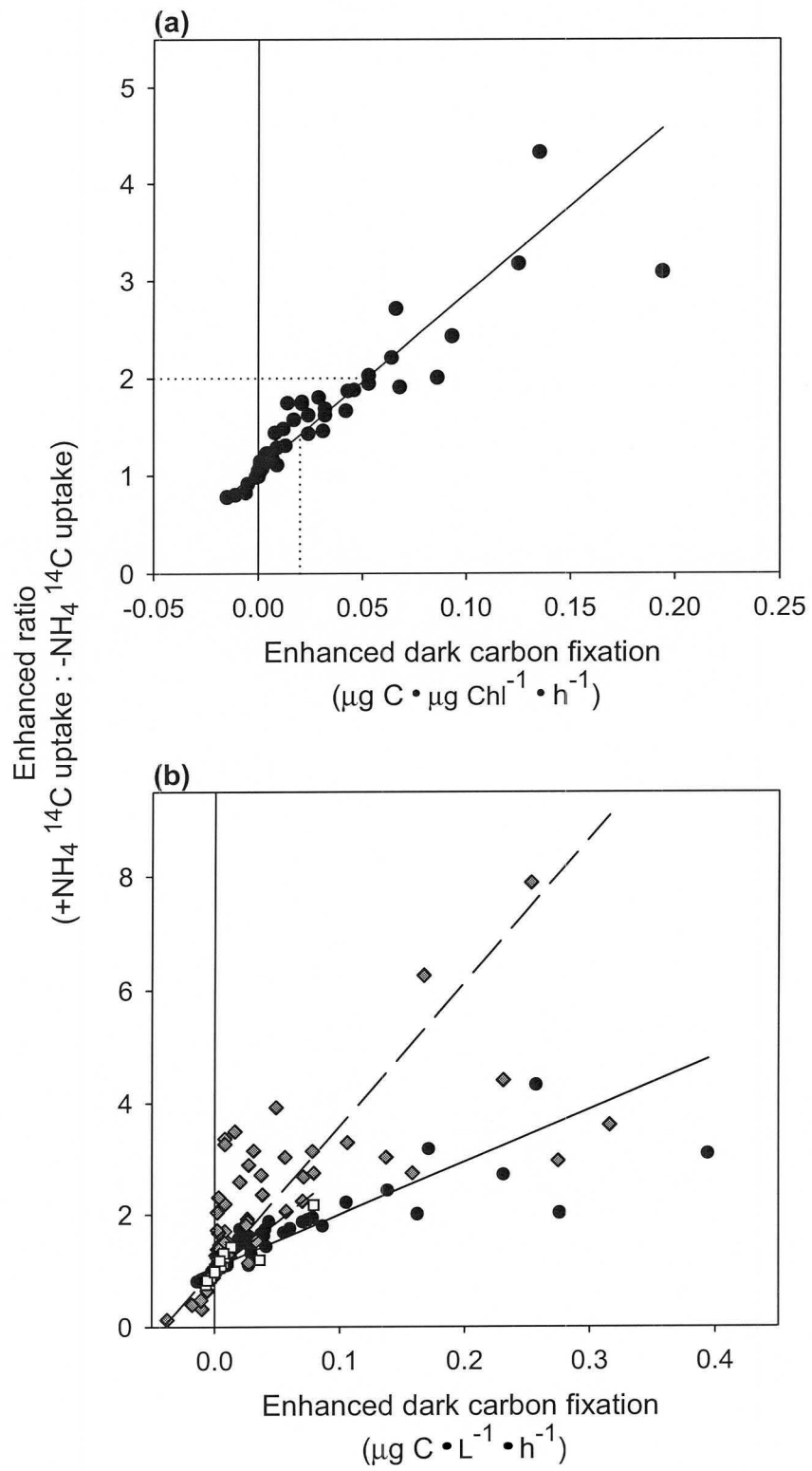


Figure 2.2: N-debt vs. ammonium enhanced  $^{14}\text{C}$  uptake response (AER) for all dates when both N-debt and AER assay were conducted.

All points are represented by a circle ( $\circ$ ). When either whole water N-debt or AER were considered to be deficient the contribution of the  $> 3 \mu\text{m}$  fraction is noted either with a  $\oplus$  (AER) or an  $\otimes$  (N-debt). If both the AER and N-debt indicated deficiency then the symbol is solid ( $\bullet$ ). ( $r^2 = 0.35$ ,  $p < 0.01$ ).



Figure 2.3: Daily mean water column photosynthetically available radiation (PAR),  $\bar{I}$ , of the mixed layer.

Council (—○—), Shawnigan (—●—), and Sooke (—●—). Solar incident PAR was measured and corrected for 6% surface reflectance. The dashed line represents the threshold below which Hecky and Guildford (1984) found light limitation.

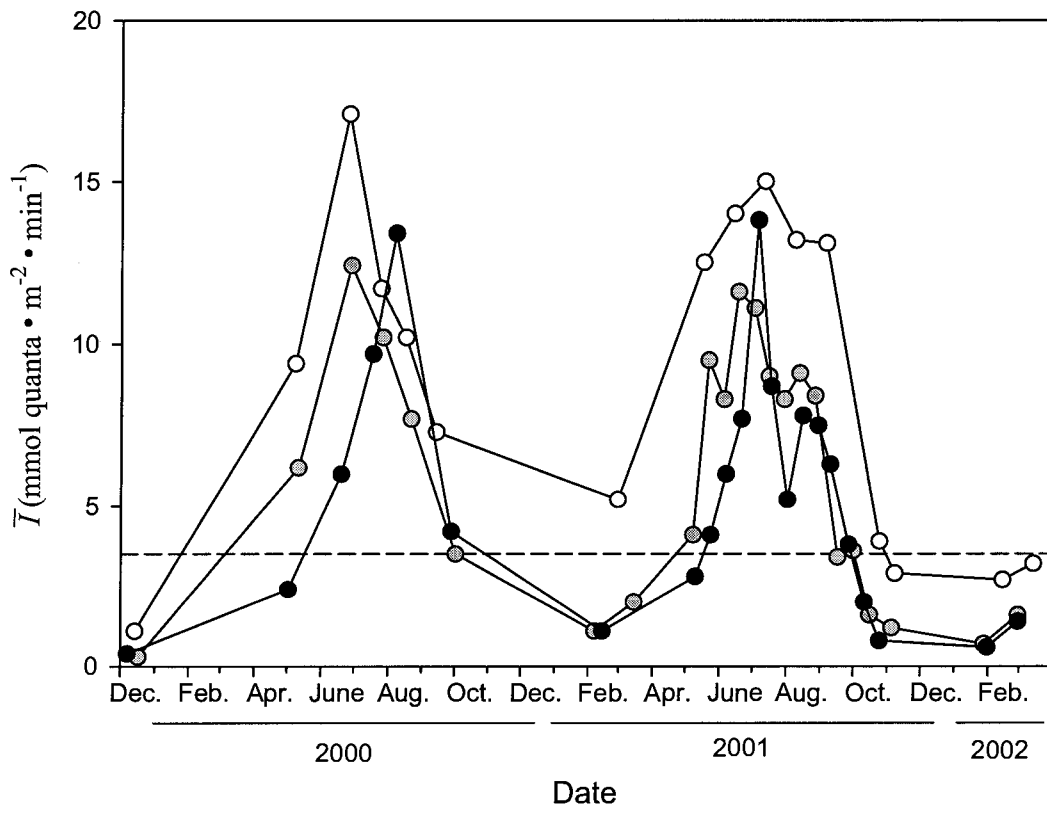


Figure 2.4: Nitrogen vs. phosphorus for (a) the epilimnion and (b) the metalimnion of all study lakes during stratified periods. Total (♣) and particulate (♠) data are presented for the epilimnion. Comparison of (c) particulate carbon vs. particulate phosphorus (PC vs. PP) and (d) particulate nitrogen vs. PP (PN vs. PP) are given for epilimnetic samples.

Inset figures show the non-log transformed data. Inset figure in (a) is for total nutrients only. Dashed lines are the 22:1 and 16:1 N:P ratios (molar) for N vs. P (a) and (b), 258:1 for PC:PP ratio (c) and 14.6:1 for PC vs. PN (d).

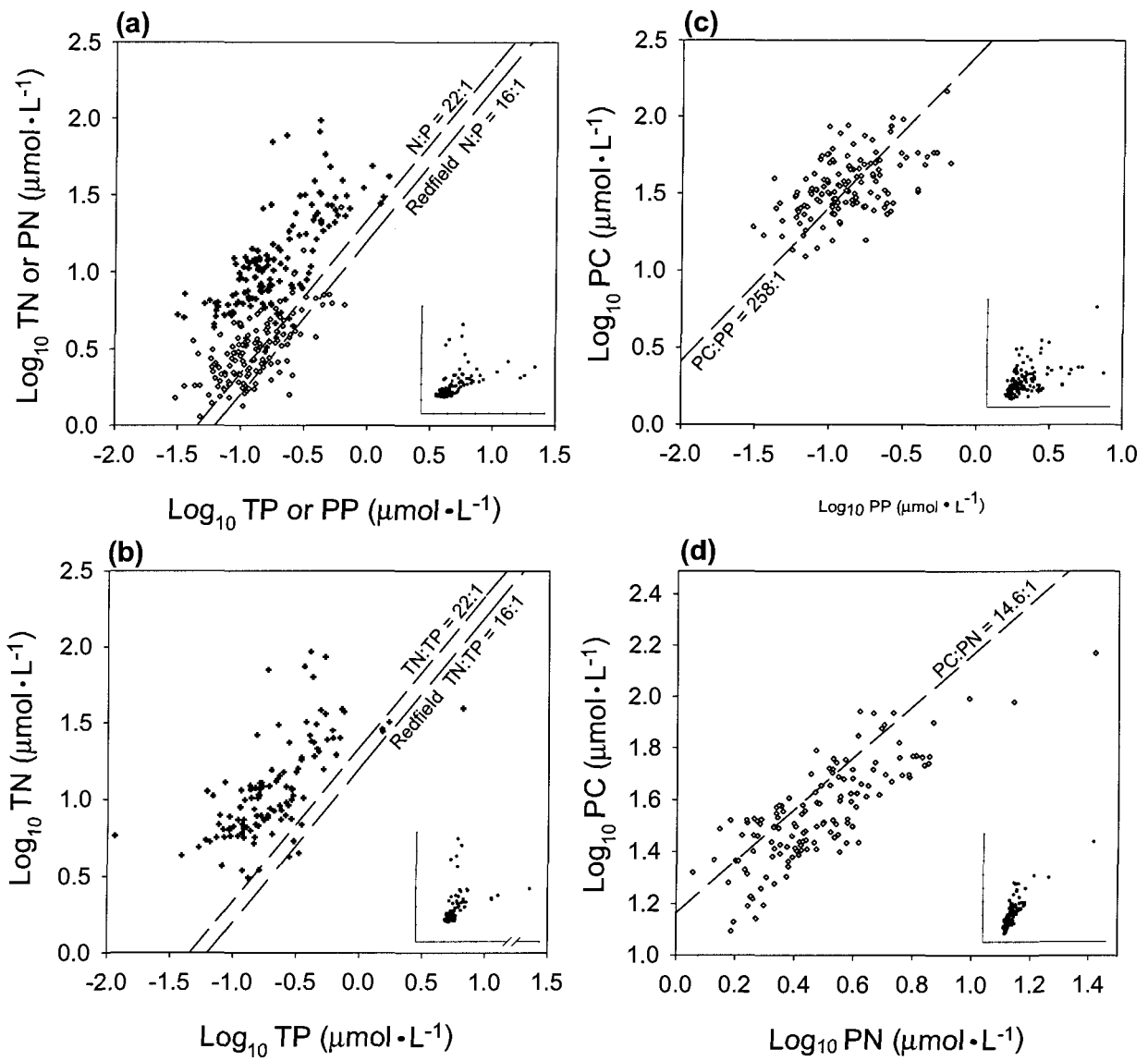


Figure 2.5: Whole water nutrient debt assays plotted against total nitrogen to total phosphorus ratios (TN:TP molar) and particulate carbon (PC) to particulate phosphorus (PP) and particulate nitrogen (PN) ratios (PC:PP and PC:PN molar). (a) P-debt vs. TN:TP; (b) N-debt vs. TN:TP; (c) ammonium enhanced  $^{14}\text{C}$  uptake response (AER) vs. TN:TP; (d) P-debt vs. PN:PP; (e) N-debt vs. PC:PN; (f) AER vs. PC:PN. Dashed lines represent nutrient thresholds.

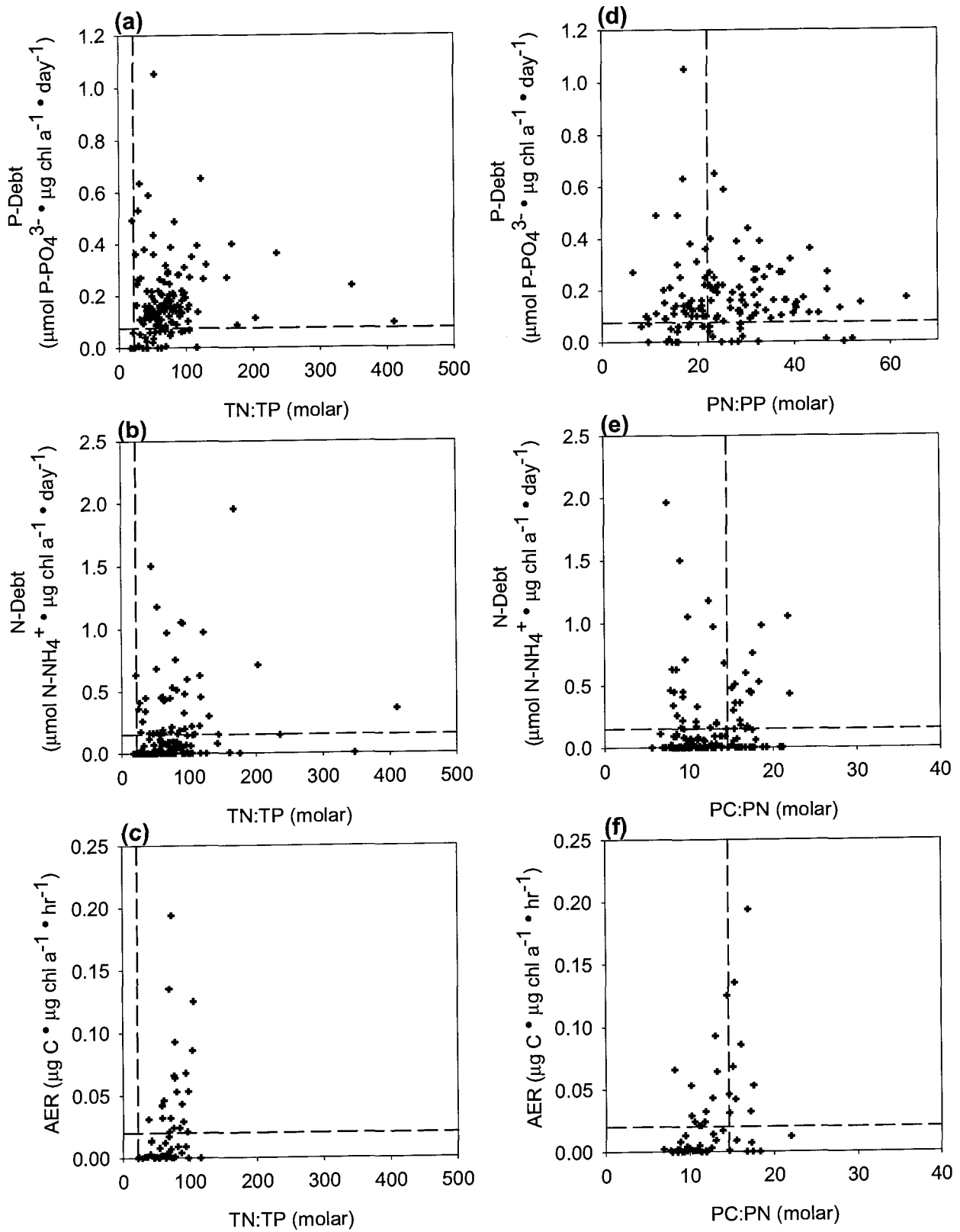


Figure 2.6: Epilimnetic and metalimnetic P-debt (a,b) and N-debt (c,d) and epilimnetic ammonium enhanced  $^{14}\text{C}$  uptake response (AER) (e) vs. total dissolved phosphorus (TDP) and total inorganic nitrogen (DIN) respectively.

Dashed lines represent nutrient thresholds.

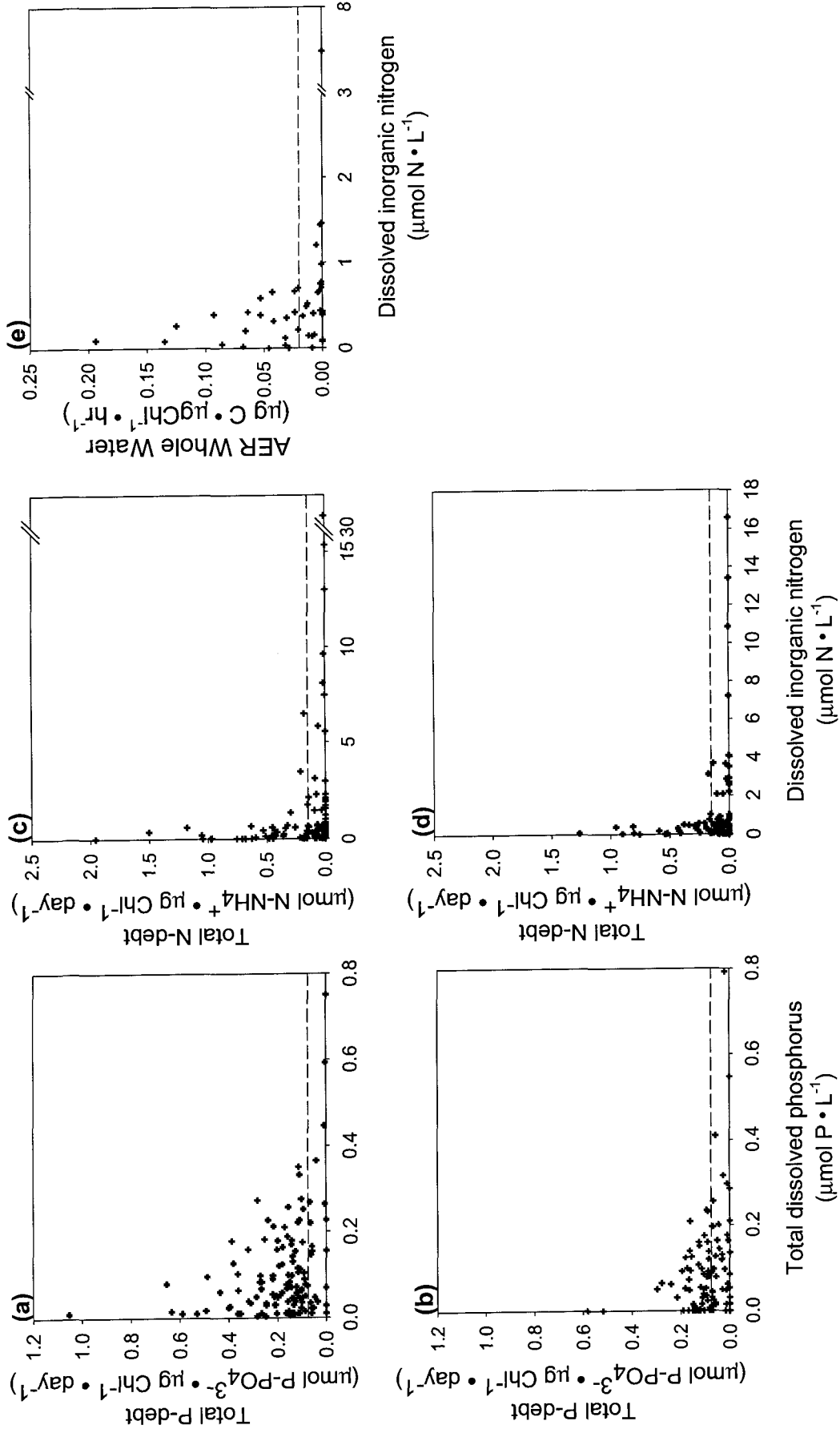


Figure 2.7: Proportion of deficiency accounted for by the  $< 3 \mu\text{m}$  fraction for samples that were considered to be deficient.

P-debt (■), AER (▨) and N-debt (□).

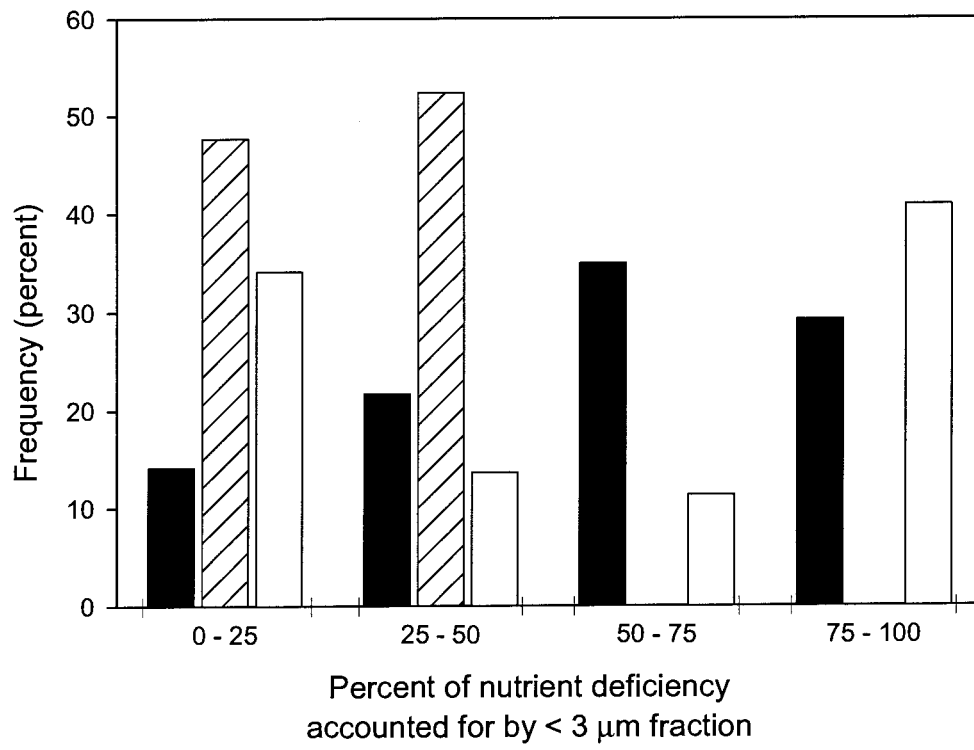


Figure 2.8: Temporal size fractionated epilimnetic P-debt for (a) Shawnigan north basin, and (b) Sooke north basin during 2001.

The  $> 3 \mu\text{m}$  sized fraction is solid (■) and the  $< 3 \mu\text{m}$  fraction is crosshatched (▨). The sum of both is equal to the whole water deficiency. The  $< 3 \mu\text{m}$  fraction was measured independently and is presented as a proportion of the whole water deficiency with the  $> 3 \mu\text{m}$  fraction constituting the remaining amount. Ammonium enhanced  $^{14}\text{C}$  uptake response (AER) for  $> 3 \mu\text{m}$  fraction is given on right axis (—●—). The horizontal dashed line represents the nutrient and the vertical dashed lines represent the period of stratification.

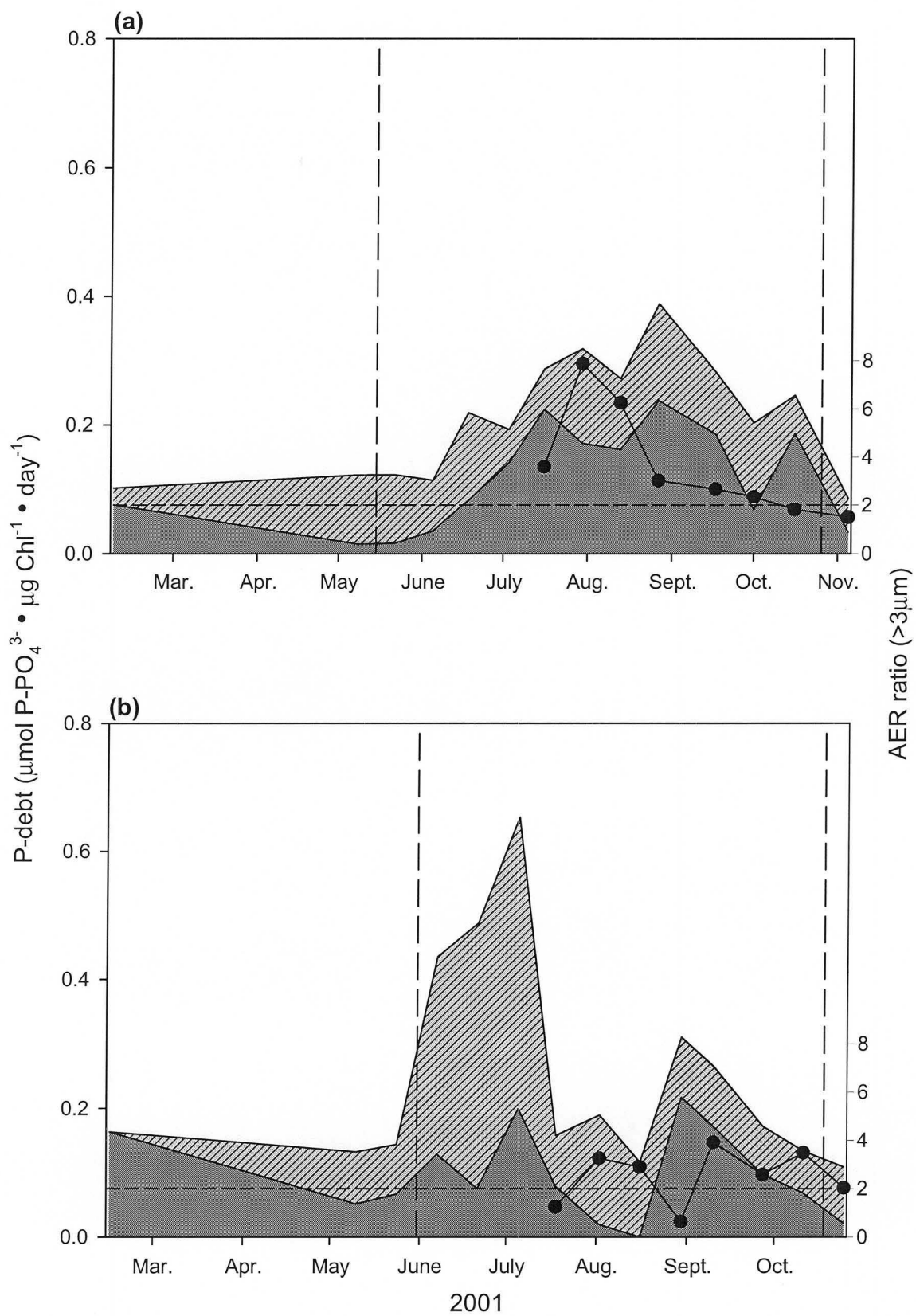
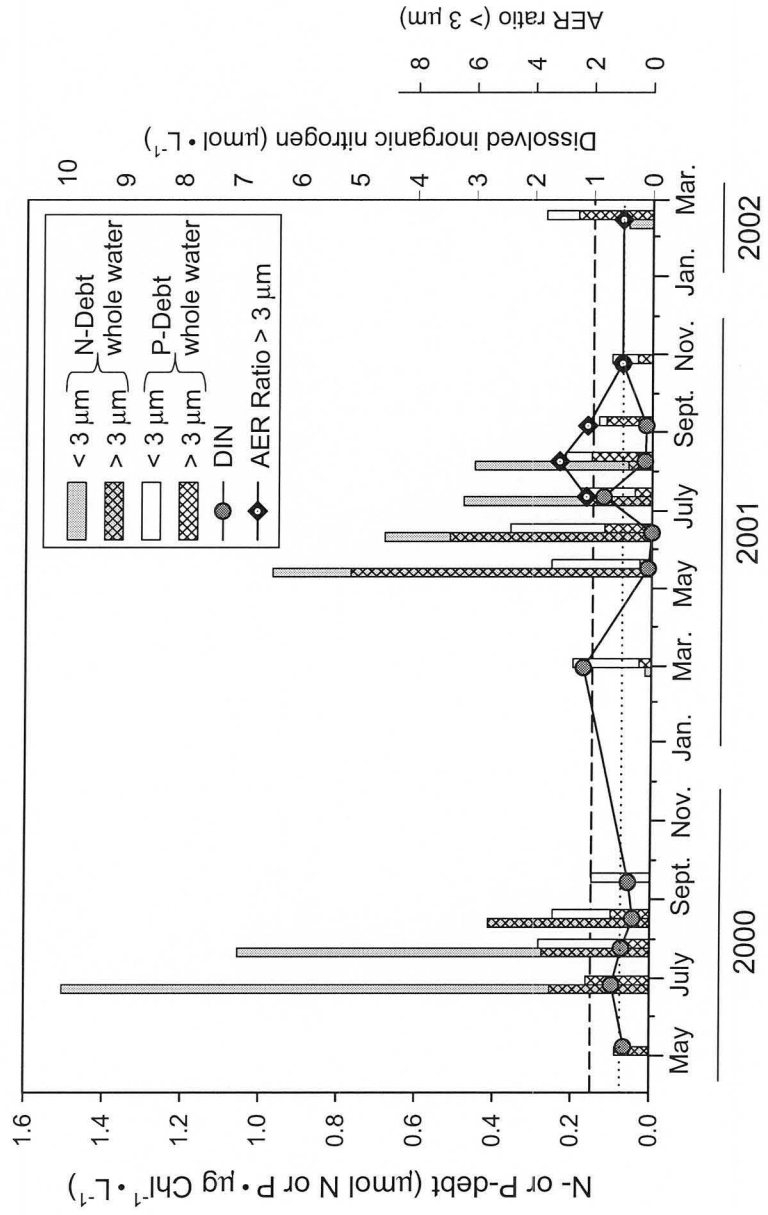


Figure 2.9: Seasonal trends in N-debt, P-debt, dissolved inorganic nitrogen (DIN), and ammonium enhanced  $^{14}\text{C}$  uptake response (AER) for council lake.

Size fractions calculated in the same manner as figure 2.8. Dashed horizontal line represents both the N-debt and AER deficiency thresholds and the dotted line represents the P-debt deficiency threshold.



**Appendix 2.1:** Table D1: Dark carbon fixation rates (mean  $\pm$  standard deviation (St.Dev.)) for whole water and the  $< 3 \mu\text{m}$  sized fraction.  $-\text{NH}_4^+ / +\text{NH}_4^+$  represents samples without and with ammonium added respectively. Standard deviations for  $n = 3$ ; N/A= cases where 2 replicates were lost.

Lake	Sampling Date	Whole water carbon fixation ( $\mu\text{g C}\cdot\text{L}^{-1}\cdot\text{h}^{-1}$ )		$<3\mu\text{m}$ carbon fixation ( $\mu\text{g C}\cdot\text{L}^{-1}\cdot\text{h}^{-1}$ )	
		$-\text{NH}_4^+ \pm \text{St.Dev.}$	$+\text{NH}_4^+ \pm \text{St.Dev.}$	$-\text{NH}_4^+ \pm \text{St.Dev.}$	$+\text{NH}_4^+ \pm \text{St.Dev.}$
Council	12-Jul-2001	$0.081 \pm 0.0243$	$0.156 \pm 0.0052$	$0.025 \pm 0.0074$	$0.029 \pm 0.0011$
Council	09-Aug-2001	$0.044 \pm 0.0128$	$0.072 \pm 0.0039$	$0.030 \pm 0.0083$	$0.026 \pm 0.0007$
Council	06-Sep-2001	$0.030 \pm 0.0019$	$0.039 \pm 0.0007$	$0.023 \pm 0.0005$	$0.024 \pm 0.001$
Council	25-Oct-2001	$0.037 \pm 0.0003$	$0.040 \pm 0.0012$	$0.029 \pm 0.0013$	$0.032 \pm 0.0008$
Council	14-Feb-2002	$0.032 \pm 0.0019$	$0.033 \pm 0.002$	$0.019 \pm 0.0001$	$0.020 \pm 0.0032$
Cusheon	07-Aug-2001	$0.096 \pm 0.0129$	$0.125 \pm 0.011$	$0.068 \pm 0.0023$	$0.071 \pm 0.0026$
Cusheon	04-Sep-2001	$0.274 \pm 0.0013$	$0.301 \pm 0.0152$	$0.077 \pm 0.0009$	$0.078 \pm 0.0015$
Cusheon	29-Oct-2001	$0.055 \pm 0.0005$	$0.055 \pm 0.0019$	$0.038 \pm 0.0011$	$0.037 \pm 0.0015$
Cusheon	07-Feb-2002	$0.048 \pm 0.0053$	$0.040 \pm 0.0038$	$0.034 \pm 0.0011$	$0.035 \pm 0.0053$
Elk	12-Jul-2001	$0.231 \pm 0.0233$	$0.229 \pm 0.0084$	$0.187 \pm 0.031$	$0.223 \pm 0.023$
Elk	09-Aug-2001	$0.269 \pm 0.0103$	$0.544 \pm 0.0311$	$0.128 \pm 0.0131$	$0.129 \pm 0.0067$
Elk	06-Sep-2001	$0.135 \pm 0.0102$	$0.367 \pm 0.0411$	$0.067 \pm 0.0028$	$0.068 \pm 0.0018$
Elk	22-Oct-2001	$0.101 \pm 0.0013$	$0.110 \pm \text{N/A}$	$0.082 \pm 0.0021$	$0.082 \pm 0.0031$
Elk	14-Feb-2002	$0.089 \pm 0.0026$	$0.094 \pm 0.0004$	$0.050 \pm 0.0022$	$0.047 \pm 0.0005$
Maxwell	07-Aug-2001	$0.079 \pm 0.0016$	$0.138 \pm 0.0097$	$0.051 \pm 0.002$	$0.055 \pm 0.0013$
Maxwell	04-Sep-2001	$0.108 \pm 0.0065$	$0.194 \pm 0.0053$	$0.062 \pm 0.0031$	$0.069 \pm 0.0045$
Maxwell	29-Oct-2001	$0.063 \pm 0.0019$	$0.091 \pm 0.0017$	$0.034 \pm 0.0004$	$0.036 \pm 0.001$
Maxwell	07-Feb-2002	$0.071 \pm 0.0044$	$0.081 \pm 0.0018$	$0.023 \pm 0.0023$	$0.022 \pm 0.0011$

## Appendix 2.1 continued.

Shawnigan-S	16-Jul-2001	0.161 ± 0.0161	0.322 ± 0.0083	0.070 ± 0.0029	0.073 ± 0.0076
Shawnigan-S	30-Jul-2001	0.080 ± 0.006	0.135 ± 0.0094	0.026 ± 0.0042	0.025 ± 0.0007
Shawnigan-S	13-Aug-2001	0.083 ± 0.0074	0.161 ± 0.0153	0.046 ± 0.0013	0.047 ± 0.0024
Shawnigan-S	27-Aug-2001	0.087 ± 0.0013	0.192 ± 0.0052	0.041 ± 0.0027	0.040 ± 0.001
Shawnigan-S	17-Sep-2001	0.095 ± 0.0207	0.136 ± 0.0133	0.033 ± 0.0006	0.041 ± 0.0039
Shawnigan-S	01-Oct-2001	0.037 ± 0.0014	0.046 ± 0.0033	0.025 ± 0.0012	0.025 ± 0.0002
Shawnigan-S	16-Oct-2001	0.026 ± N/A	0.030 ± 0.0033	0.022 ± N/A	0.023 ± 0.0031
Shawnigan-S	05-Nov-2001	0.029 ± 0.0006	0.033±4.55x10 <sup>-5</sup>	0.020 ± 0.0002	0.019 ± 0.0008
Shawnigan-S	28-Jan-2002	0.032 ± 0.0019	0.031 ± 0.0023	0.023 ± 0.0007	0.024 ± 0.0006
-----					
Shawnigan-N	16-Jul-2001	0.189 ± 0.0391	0.583 ± 0.046	0.068 ± 0.0103	0.146 ± 0.0032
Shawnigan-N	30-Jul-2001	0.077 ± 0.0123	0.334 ± 0.0149	0.040 ± 0.0021	0.044 ± 0.003
Shawnigan-N	13-Aug-2001	0.078 ± 0.0048	0.249 ± 0.0074	0.047 ± 0.0024	0.051 ± 0.0052
Shawnigan-N	27-Aug-2001	0.097 ± 0.0143	0.235 ± 0.0165	0.029 ± 0.0002	0.031 ± 0.0008
Shawnigan-N	17-Sep-2001	0.080 ± 0.0008	0.150 ± 0.0034	0.038 ± 0.0059	0.036 ± 0.0007
Shawnigan-N	01-Oct-2001	0.054 ± 0.0013	0.094 ± 0.0003	0.026 ± 0.0004	0.028 ± 0.0002
Shawnigan-N	16-Oct-2001	0.062 ± 0.0081	0.101 ± 0.0059	0.031 ± 0.0006	0.045 ± 0.0082
Shawnigan-N	05-Nov-2001	0.040 ± 0.0004	0.049 ± 0.0018	0.022 ± 0.0008	0.023 ± 0.0007
Shawnigan-N	28-Jan-2002	0.026 ± 0.0004	0.027 ± 0.0012	0.023 ± 0.0002	0.023 ± 0.0007
-----					
Sooke-S	18-Jul-2001	0.085 ± 0.0056	0.084 ± 0.0019	0.060 ± 0.0048	0.059 ± 0.0028
Sooke-S	02-Aug-2001	0.025 ± 0.0002	0.037 ± 0.0009	0.022 ± 0.0009	0.026 ± 0.0063
Sooke-S	16-Aug-2001	0.055 ± 0.0157	0.091 ± 0.0159	0.033 ± 0.001	0.033 ± 0.0023
Sooke-S	30-Aug-2001	0.044 ± 0.0003	0.041 ± 0.0046	0.023 ± 0.0076	0.031 ± 0.0021
Sooke-S	10-Sep-2001	0.071 ± 0.0328	0.057 ± 0.0079	0.041 ± 0.0161	0.045 ± 0.003
Sooke-S	27-Sep-2001	0.025 ± 0.0013	0.026 ± 0.0004	0.020 ± 0.0001	0.019 ± 0.0002
Sooke-S	11-Oct-2001	0.027 ± 0.0002	0.029 ± 0.0008	0.024 ± 0.0004	0.024 ± 0.0005
Sooke-S	25-Oct-2001	0.028 ± 0.0005	0.031 ± 0.0006	0.021 ± 0.0005	0.022 ± 0.0004
Sooke-S	31-Jan-2002	0.022 ± 0.0007	0.025 ± 0.001	0.015 ± 0.0006	0.016 ± 0.0005

## Appendix 2.1 continued.

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Sooke-N	18-Jul-2001	$0.082 \pm 0.0058$	$0.091 \pm 0.0125$	$0.067 \pm 0.0022$	$0.072 \pm 0.0021$
Sooke-N	02-Aug-2001	$0.035 \pm 0.0017$	$0.041 \pm 0.0039$	$0.032 \pm 0.0007$	$0.029 \pm 0.0006$
Sooke-N	16-Aug-2001	$0.042 \pm 0.0023$	$0.062 \pm 0.0043$	$0.028 \pm 0.0128$	$0.021 \pm 0.0039$
Sooke-N	30-Aug-2001	$0.041 \pm 0.0008$	$0.032 \pm 0.003$	$0.025 \pm 0.0049$	$0.022 \pm 0.0003$
Sooke-N	10-Sep-2001	$0.049 \pm 0.004$	$0.093 \pm 0.0162$	$0.033 \pm 0.0058$	$0.027 \pm 0.0002$
Sooke-N	27-Sep-2001	$0.034 \pm 0.0006$	$0.053 \pm 0.0033$	$0.021 \pm 0.0017$	$0.021 \pm 0.0003$
Sooke-N	11-Oct-2001	$0.027 \pm 0.0009$	$0.046 \pm 0.0004$	$0.020 \pm 0.0002$	$0.024 \pm 0.0028$
Sooke-N	25-Oct-2001	$0.019 \pm 0.0002$	$0.022 \pm 0.0004$	$0.017 \pm 0.0004$	$0.017 \pm 0.0003$
Sooke-N	31-Jan-2002	$0.018 \pm 0.0009$	$0.020 \pm 0.0013$	$0.016 \pm 0.0006$	$0.015 \pm 0.0006$

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**Chapter 3: Variation in temporal  $^{14}\text{C}$ -plankton photosynthesis among warm-monomictic lakes of coastal British Columbia**

***Abstract***

Seasonal patterns of  $^{14}\text{C}$ -phytoplankton photosynthesis (PPhot) were examined in six warm monomictic lakes of coastal British Columbia. Four of my study lakes followed typical lake patterns with maximum PPhot occurring in the spring and minimal rates occurring during the winter. However, the spring maximum occurred several weeks earlier than lakes in other climatic regions. In addition, maximum rates of daily photosynthesis were observed to occur during the winter months in Maxwell Lake, rather than during the standard growing season. All study lakes except Maxwell Lake had large *Daphnia* in the plankton community. Maxwell was dominated by small crustacean zooplankton implying the importance of trophic-structure in mediated seasonal patterns of productivity. The four oligotrophic lakes in my study also exhibited P-deficiency during winter, as indicated by P-debt bioassays and rapid  $^{32}\text{PO}_4^{3-}$  turnover rates. This data suggest that these coastal oligotrophic lakes were co-limited by nutrients and light during winter. The importance of winter (November-March) photosynthetic production to the total annual carbon budget in the six lakes studied here is greater than that typically reported for other temperate zone lakes. If plankton community respiration decreases more than photosynthetic production with wintertime temperatures, than more than 50% of annual net pelagic carbon fixation could occur in winter in some coastal lakes.

### *Introduction*

Factors that limit phytoplankton photosynthesis (P<sub>Phot</sub>) commonly include light (photosynthetically available radiation or PAR), temperature (Kirk 1994), and nutrients (Schindler 1977). Zooplankton grazing can also influence photosynthetic production (Pace 1984; Perin et al. 1996). Seasonally these factors vary greatly in north temperate lakes and typically P<sub>Phot</sub> is low during winter, – increases in the spring, is high in the summer, and declines in autumn (Alvarez-Cobelas and Rojo 1994; Wetzel 2001). In ice-covered lakes roughly 10% of annual areal P<sub>Phot</sub> occurs under ice (range: 2% to > 30%; Alvarez Cobelas and Rojo 1994). Nutrients and grazing are thought to be the principal factors limiting production during the summer, while PAR and temperature are considered most important during winter months. Quantifying and interpreting seasonal variations of P<sub>Phot</sub> rates (Fee et al. 1992) and photosynthetic parameters (Wetzel 2001; Knoll et al. 2003) is critical for evaluating lake energetics.

There is growing appreciation of the photosynthetic contribution made by phytoplankton growing under low light conditions, especially at the interface between nutrient-replete and nutrient-depleted water (Goldman and McGillicuddy 2003). Planktonic photosynthesis resulting from imported nutrients has received special attention because it is considered to represent “new” production (Dugdale and Goering 1967; Eppley and Peterson 1979). New production occurs in zones of upwelling or mixing and the ratio of “new” versus “old” (recycled nutrient driven) production varies seasonally. For example, Brooks and Edgington (1994) found that P<sub>Phot</sub> peaked during spring isothermal conditions in Lake Michigan and that this was also an important time of new production.

Coastal lakes of British Columbia (BC) often exhibit elevated chlorophyll *a* (chl *a*) concentrations during winter (see results). Despite occurring in several countries (e.g. Canada, Chile, Norway) temperate coastal lakes have been the subject of fewer seasonal PPhot studies than have temperate dimictic lakes. The lack of permanent ice cover results from relatively mild air temperatures that typically do not remain below zero during the diurnal cycle. Thus, these lakes are best classified as being warm monomictic lakes (Stockner and Shortreed 1985). The lack of permanent ice cover means that PAR is not attenuated to the same extent as in ice-covered lakes at a similar latitude. However, similar seasonal changes in both PAR and temperature offers an opportunity to study their relative importance on plankton photosynthesis among lakes, and to examine which factors control photosynthesis during winter months in warm monomictic lakes.

Two physiologically important parameters define the relationship between photosynthesis and light:  $\alpha^B$  and  $P_m^B$ ;  $\alpha$  is the initial slope of the photosynthesis-irradiance (P-E) curve,  $P_m$  is the maximum rate of photosynthesis, and the superscript B indicates that each parameter is biomass-corrected for chl *a* (Fee 1990; Kirk 1994). At saturation ( $P_m^B$ ) the rate of photosynthesis is limited by enzymatic activity (carboxylation enzymes) so, at a physiological level, the same saturating PAR will have a correspondingly smaller  $P_m^B$  at lower temperatures (Steemann Nielsen and Jorgensen 1968).  $\alpha^B$  represents the PAR-limited portion of photosynthesis, and in this region of the P-E curve cells have sufficient carboxylation enzymes. Therefore, temperature has much less affect on  $\alpha^B$  than it does on  $P_m^B$ . Thus, during winter months, lower temperatures (ca. 4°C) should depress  $P_m^B$  and limit areal PPhot. Kirk (1994) hypothesized that low

PAR during the winter is less important because it affects a smaller portion of the P-E curve. The ratio of  $P_m^B$  to  $\alpha^B$  is known as  $E_k$  and is a useful parameter for examining seasonal changes of photosynthesis parameters. During winter,  $P_m^B$  should decrease, while  $\alpha^B$  should increase due to the decrease in available PAR (Kirk 1994). Under such conditions  $E_k$  will be smallest when temperature and light are low, which can make it difficult to differentiate which factor is of greater importance.

Algal physiological studies (i.e. culture studies) are vital to our understanding of organism response to factors that can limit production (e.g.  $\alpha^B$ ,  $P_m^B$ ). However, ecological studies (i.e. field studies) offer the opportunity to examine how communities respond to changes in growth conditions and offer insight into how communities compensate for these changes. In temperate lakes, temperature and PAR co-vary and nutrient availability and zooplankton grazing rates change seasonally. The seasonal temperature cycle of coastal BC lakes examined by this study is similar to that of lakes with permanent winter ice cover and therefore offered the opportunity to examine the interaction of variables controlling plankton photosynthesis.

The overall goal of this study was to improve our understanding of seasonal carbon dynamics in coastal BC lakes. Specifically, I examined the relative importance of light and temperature during winter months to test the hypothesis that temperature plays a direct role in limiting ecosystem pelagic photosynthesis (Kirk 1994). I compare the results to published patterns of PPhot to determine both how PPhot seasonal trends in BC coastal lakes compare to other temperate lakes and how PPhot rates match total phosphorus (TP) models. The objectives were 1) determine the relative importance of annual  $^{14}\text{C}$ -fixation during winter, 2) examine the roles of temperature and light on PPhot

during winter, 3) examine the seasonal variability of  $^{14}\text{C}$ -PPhot and photosynthetic parameters, and 4) examine other lake processes, including nutrient deficiency and grazer community structure to determine if these influence  $^{14}\text{C}$ -PPhot among lakes.

### ***Methods***

#### *Study Lakes, Field Sampling:*

$^{14}\text{C}$ -PPhot was measured on epilimnetic and metalimnetic water samples from six coastal British Columbia lakes. Council Lake (COL), Elk Lake (ELL), Shawnigan Lake (SHL) and Sooke Lake Reservoir (SOL) are located near the southern end of Vancouver Island, and Cusheon Lake (CUL) and Maxwell Lake (MXL) are on Saltspring Island (Figure 3.1). Surface area ranged from 16 ha to 605 ha and maximum depth from 9.5 m to 70 m (Table 3.1, Spafard et al. 2002). All the lakes are soft-water, with near-neutral pH and average annual dissolved inorganic carbon (DIC) ranging from 313 to 1057  $\mu\text{mol}\cdot\text{L}^{-1}$  and average dissolved organic carbon (DOC) less than 5.6  $\text{mg}\cdot\text{L}^{-1}$  (Table 3.1). Flushing rates vary from less than one year, to approximately 4.5 years (Table 3.1). Based on Chl *a*, TP and  $^{14}\text{C}$ -PPhot the lakes were classified as being oligotrophic to meso-eutrophic (Table 3.1). COL, MXL, SHL and SOL are considered oligotrophic, although MXL has greater temporal change in classification than do the other lakes and might be considered oligo-mesotrophic during winter and spring - up to the onset of stratification. CUL and ELL are best described as being meso- to mesoeutrophic.

Lake water samples were collected at the point of maximum depth in each lake with either a 6 m integrated tube (epilimnion) or a vertically oriented Niskin bottle (metalimnion). Epilimnetic samples were taken from the surface to the depth where temperature changed by  $>1^{\circ}\text{C}\cdot\text{m}^{-1}$ , or if the epilimnion was greater than 5.5 m, then

integrated samples were taken to a depth of 5.5 m. Metalimnetic samples were taken near the middle of the metalimnion at the point of maximum temperature change with depth. Water samples were taken in triplicate and immediately placed in opaque Nalgene containers and stored in coolers until laboratory processing later the same day. A Li-Cor  $2\pi$  quantum sensor (Li-Cor LI-192SA) was used to measure light extinction ( $k_d$ ) of PAR ( $\mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) on each sampling date. PAR measurements were made at least every metre to a depth of ca. 7–9 m. To minimize changes in incident light affecting  $k_d$ , surface PAR was measured immediately prior to, and after profile measurements to ensure that incident light remained similar. Mean daily epilimnetic PAR values were calculated using the same formula as Guildford et al. (2000) and were based on a 24 hour time period to remove seasonal bias. The epilimnion for calculating mean PAR was defined as the shallower of: the depth at which temperature changed by  $1^\circ\text{C}\cdot\text{m}^{-1}$  or the mean depth of the lake. Temperature profiles were obtained using a YSI model 58 temperature/oxygen meter.

*Laboratory analyses:*

Chl *a* was determined in triplicate by filtration through GF/F filters (Whatman) and each sample was frozen until analysis. Chl *a* was extracted in 95% ethanol overnight at  $4^\circ\text{C}$  and read on a spectrophotometer using a 10 cm quartz cuvette. Chl *a* calculations were based on Winternans and Mots (1965). TP was measured as phosphate on a Lachat autoanalyzer (Zellweger Analytics, QuickChem 8000) after digesting unfiltered water with potassium persulfate in an autoclave. Alkalinity was determined in at least duplicate for each sampling date using Gran Titrations. DIC was calculated from alkalinity and pH. DOC was determined from water filtrate passed through ashed GF/F filters

(Whatman), and measured on a Shimadzu analyser (TOC 5000) using the combustion/infrared technique.

*Plankton photosynthesis determination:*

Methods used for determining  $^{14}\text{C}$ -PPhot follow Fee (Shearer et al. 1985; Fee et al. 1989; Fee 1990) with slight modifications. In a darkened room water from triplicate samples was pooled.  $\text{Na}^{14}\text{CO}_3$  was added to 1.25L of the pooled sample to a final activity of approximately 0.75MBq per 1.25L. The sample was gently mixed with a stirring bar and siphoned into nine (seven light and two dark bottles) 125mL Pyrex glass bottles with ground glass stoppers. Dark bottles had aluminium foil sandwiched between 4-5 layers of black vinyl (Plasti Dip). Bottles were incubated across a light gradient in a flatbed incubator (Shearer et al. 1985) at in situ water temperatures (crushed ice was added, when needed, to maintain constant incubator temperature). The light source was a 150-watt high-pressure sodium vapor lamp. PAR at each bottle location for all incubations was measured with a  $4\pi$  sensor (Li-Cor LI-193SA) approximately halfway through the incubation period. On dates with metalimnetic samples, two incubators were used, one each for epilimnetic and metalimnetic samples. Incubations lasted 3 hours. Immediately after incubations, 3 mL of sample was removed from three random bottles and placed in a scintillation vial with 150  $\mu\text{l}$  of 3-methoxypropylamine (Fluka) as a  $\text{CO}_2$  trapping agent. The average of these three samples was used to determine the available  $^{14}\text{C}$ . From each bottle 30 mL was filtered through 0.45  $\mu\text{m}$  membrane filters (Gelman). Samples were counted for at least two minutes with a precision of 95% in 10 000 disintegrations or for 50 minutes, whichever came first. Samples were counted on

Beckman LS6000IC scintillation counter. CPMs were converted to DPMs based on a standard curve. Light bottle DPM was corrected for average dark bottle fixation.

Photosynthesis rates were calculated using DIC,  $^{14}\text{C}$  available and  $^{14}\text{C}$  fixed with an isotopic correction factor of 1.06 (Shearer et al. 1985). P-E parameters ( $\alpha^B$ ,  $P_m^B$ ) were calculated using the computer program PSPARMS (Fee 1990) and P-E curves were fit using the following equation (Fee 1990):

$$\text{If: } E < \frac{E_k}{20} \quad \text{then } P_{\text{Phot}} = 0$$

$$\text{If: } E \geq 2E_k \quad \text{then } P_{\text{Phot}} = BP_m^B$$

$$\text{Otherwise: } P_{\text{Phot}} = B\alpha^B E' \left[ 1 - \frac{E'}{4E_k} \right]$$

where:  $E$  = irradiance (PAR) at a given depth,  $E_k = P_m^B / \alpha^B$ ,  $E' = E - E_k/20$  and  $B$  = biomass measured as chl  $a$ .

Ambient surface PAR at the top of the SOL drinking water intake tower (within 40 km of all study lakes) was logged for the duration of this study (Li-Cor LI-90SZ sensor). Surface PAR for photosynthesis versus depth profiles were calculated using averages of surface PAR for the sampling date, the three days prior to and the three days after the sampling date. Lake PAR at 0.1 m intervals was calculated based on  $k_d$  and these 7-day PAR averages corrected for 6% surface reflectance. Areal  $^{14}\text{C}$ -PPhot was calculated at 0.1 m intervals. Lake-wide annual estimates of areal PPhot were based on daily PAR measurements. Photosynthetic parameters ( $\alpha^B$ ,  $P_m^B$ ), chlorophyll and epilimnetic/metalimnetic depths were linearly interpolated between sampling dates for these calculations. Lake-wide productivity was corrected for lake bathymetry (Spafard et

al. 2002). Literature data were digitized using the computer program Grafula 3 (ver. 2.10), and cross correlation analysis conducted using SPSS 10.

*Nutrient deficiency:*

P-debt was used to assess the physiological status of phosphorus deficiency in the plankton community (Chapter 2). The P-debt bioassay is based on chemostat and lake studies that have demonstrated P-deficient plankton have a high capacity for phosphate uptake in the dark (Healey and Hendzel 1979, 1980). Briefly, 100 mL of lake water was placed in an Erlenmeyer flask,  $\text{Na}_2\text{HPO}_4$  was added to bring the concentration to approximately  $5 \mu\text{mol}\cdot\text{L}^{-1}$ , and was incubated for 24 hours in the dark. Immediately after adding phosphate, samples were gently stirred and three subsamples were taken. Using the ascorbic acid/molybdate method, subsamples were analyzed for  $\text{PO}_4^{3-}$  on a spectrophotometer (Stainton et al. 1977). After the 24-hour incubation  $\text{PO}_4^{3-}$  was again determined on three subsamples. Uptake rates were biomass corrected for chl *a*. P-deficiency thresholds are assumed to be those given by Healey (1975), Healey and Hendzel (1979, 1980) and Guildford and Hecky (2000).

$^{32}\text{PO}_4^{3-}$ -turnover time was measured in a similar manner to Lean and White (1983) and Mazumder et al. (1988). To 100 mL of lake water, carrier-free  $^{32}\text{PO}_4^{3-}$  (Sigma-Aldrich) was added to a final activity  $0.9\text{--}3.2 \text{ kBq}\cdot\text{mL}^{-1}$ ; 2 mL subsamples were withdrawn and passed through  $0.2\mu\text{m}$  Nuclepore filter at 0.5, 1, 2, 4, and 10 minutes. These filtrates were placed in scintillation vials and counted on a Beckman LS6000IC liquid scintillation counter. The uptake constant was calculated as the slope of the least-squares regression of the natural log of percent  $^{32}\text{PO}_4^{3-}$  left in the water over time. The reciprocal of the uptake constant is  $^{32}\text{PO}_4^{3-}$  turnover time (Lean 1973).

*Zooplankton:*

Zooplankton were collected in triplicate by vertical tows through the entire water column to a maximum depth of 30 m using a 64  $\mu\text{m}$ -mesh 30 cm-diameter Wisconsin net. Crustacean zooplankton length measurements were made from at least 150 individuals or all individuals in 10% of the sample. Biomass was calculated from length measurements using published formulas for length-mass relationships (Culver et al. 1985; Yan and Mackie 1987). The dominant zooplankters numerically and by biomass were nauplii, cyclopoid and calanoid copepods, *Bosmina* spp., and *Daphnia* spp. The more common non-dominant zooplankters included *Diaphanosoma brachyurum*, *Holopedium gibberum*, *Ceriodaphnia* sp., and Chydoridae.

**Results**

During winter months a thin, temporary layer of ice formed on some of the smaller lakes; however, parts of all the lakes remained open and any ice present was thin (<10 mm). Seasonal surface water temperatures fluctuated in an expected manner for lakes in a north temperate climate, ranging from <3°C during winter to >23°C in summer (Figure 3.2). Likewise, ambient surface PAR changed in a typical seasonal pattern.

*Plankton photosynthesis:*

Seasonal physiological adaptations reflected by photosynthetic parameters followed expected trends (Table 3.2).  $E_k$  was lowest during winter months (Jan. to mid March) in COL (Council Lake), CUL (Cusheon Lake), and MXL (Maxwell Lake), while SHL (Shawnigan Lake) and SOL (Sooke Lake Reservoir) had low  $E_k$  values from autumn until spring (Oct – April). ELL (Elk Lake) was similar to SHL and SOL, except for October when  $E_k$  was unexpectedly large. During spring,  $E_k$  increased rapidly in

COL, CUL and MXL because  $\alpha^B$  decreased at the same time  $P_m^B$  increased. This rate of increase was smaller in SHL and SOL because  $\alpha^B$  did not increase until later in the season. The equation between  $P_m^B$  and  $\alpha^B$  for all lakes when  $E_k$  was less than 200 was:  $P_m^B = 0.31(\alpha^B) + 0.61$  (Type II regression;  $r^2=0.50$ ;  $n=41$ ). The slope of this relationship is less than 1 indicating that  $P_m^B$  varied to a lesser extent than did  $\alpha^B$ .

COL and MXL had winter near-surface  $^{14}\text{C}$ -PPhot rates similar to, or higher than summertime values (Figure 3.3). The greatest  $^{14}\text{C}$ -PPhot rates in COL occurred in May and June, while rates from February and March were similar to near-surface rates measured in July. MXL had the highest rates during the winter months. Seasonal trends in COL and MXL are in contrast to those observed in the other four lakes. CUL and ELL had highest rates during bloom events in late August to early September (CUL) and April and to a lesser August in ELL. Seasonal trends in SHL and SOL were similar to one another with highest near-surface rates occurring in spring, while the lowest rates occurred in late autumn and winter. MXL had the highest rates, after those measured in February and early March, in April, and May. SHL and SOL had relatively deep metalimnia, and although these may be important for other reasons, they did not have a large impact on integral photosynthetic rates. In the oligotrophic to mesotrophic lakes 1% PAR extended into the hypolimnion. Phytoplankton growth in the hypolimnion may be of interest, but is of minor importance for estimating integral photosynthetic rates. MXL had the most pronounced metalimnetic  $^{14}\text{C}$ -PPhot peak of all the lakes (Figure 3.3).

There was no apparent seasonal trend in water column transparency as determined by Secchi depth; however, average seasonal compensation depths were lower during winter than in the stratified period (Table 3.1). The average wintertime compensation

depths for all lakes except COL (54%) were > 74% of the average compensation depth during the stratified period. While decreased transparency during winter may have affected the available PAR to a small extent, the decrease in ambient solar PAR intensity and duration during winter was of greater importance. Seasonal PPhot depth profiles are strongly influenced by ambient light. Although COL and MXL had high near-surface  $^{14}\text{C}$ -PPhot in February and March, as expected there was a rapid decrease of this rate with depth compared to summer. This trend can be observed in the other lakes; however, because wintertime rates are lower in these lakes it may be less important to their annual carbon-fixation budgets.

Daily  $^{14}\text{C}$ -PPhot estimates for the six lakes were summed by month over the year to estimate annual carbon-fixation budgets using daily PAR measurements (Table 3.3). Every month accounted for between 2% and 23% of the annual production. The integral rates follow similar seasonal trends with  $^{14}\text{C}$ -PPhot versus depth. However, despite similar light saturated near-surface photosynthetic rates in COL during winter and summer, estimates of annual C-fixation demonstrate that COL is similar to SHL and SOL and different from MXL. The last autumn sampling date in MXL occurred immediately prior to autumn overturn (29 Oct. 2001). If  $^{14}\text{C}$ -PPhot rates increased after autumn mixing then carbon budget estimates presented here for November to January are conservative.

Daily estimates were used to calculate average weekly  $^{14}\text{C}$ -PPhot rates and these were compared to seasonal trends from Alvarez-Cobelas and Rojo (1994). The percent difference of weekly  $^{14}\text{C}$ -PPhot was plotted against the annual average, starting from summer solstice (Figure 3.4). COL, ELL, SHL, and SOL closely follow the typical

seasonal trend in PPhot for deep lakes ( $Z_{\text{mean}} > 5$  m). A cross correlation plot for COL versus a typical deep lake is shown in the bottom left hand corner of Figure 3.4a. The other lakes in Figure 3.4a have similar cross correlation plots (data not shown). Note that there is approximately a three week lag in the correlation. MXL did not follow the typical pattern, and the zero lag correlation is approximately 0 (Figure 3.4b). With a  $Z_{\text{mean}}$  of 4.4 m CUL is classified as a shallow lake by Alvarez-Cobelas and Rojo (1994); however, the pattern of PPhot in CUL is not similar to either their typical deep or shallow lake.

The relationship between  $^{14}\text{C}$ -PPhot and TP for daily measurements was compared to the seasonal predictive model of Smith (1979) for 58 north temperate lakes and the model of Stockner and Shortreed (1985) from BC coastal lakes (Figure 3.5). All data points fell within the 95% confidence limits of Smith (1979) except for CUL and ELL winter measurements and the ELL October measurement. On average, Stockner and Shortreed (1985) found greater photosynthetic rates at a given TP than I did; however, their rates over  $2\mu\text{g}\cdot\text{L}^{-1}$  TP are from fertilized lakes and may therefore not apply to unmanipulated coastal lakes.

*Nutrient and light deficiency:*

P-debt was used as a physiological assessment of community phosphorus deficiency and compared to culture-modelled thresholds. Greater P-debt values indicate greater P-deficiency (Healey and Hendzel 1980). COL and MXL were strongly P-deficient during summer and relative to late summer and autumn, COL had greater P-debt during winter (Figure 3.6). CUL and ELL only showed deficiency during summer

months. SHL and SOL were strongly deficient in summer and this level of deficiency decreased during winter months.

$^{32}\text{PO}_4^{3-}$  turnover time (minutes) was also used as a measure of P-deficiency because it is thought to represent the  $\text{PO}_4^{3-}$  uptake efficiency of the plankton community and has been found to correspond well with the phosphorus deficiency index (Millard et al. 1996). Turnover times less than 10 minutes are considered to represent extremely P-deficient systems (Millard et al. 1996; Hudson et al. 2000). In all lakes the majority of summertime  $\text{PO}_4^{3-}$  turnover times were less than 10 minutes (Nowlin 2003). During February, P-turnover in COL was ca. 11 minutes, which was higher than rates in the spring and autumn, but lower than those during July and August. P-turnover in MXL was always less than 10 minutes, including the winter (Figure 3.7). CUL and ELL had the longest wintertime  $^{32}\text{PO}_4^{3-}$  turnover times (>35 minutes), while SHL and SOL had winter turnover times between 18 and 23 minutes.

Light deficiency was evaluated both with a physical assessment of the light environment measured as mean epilimnetic PAR ( $\bar{I}_E$ ) and a physiological assessment of epilimnetic phytoplankton. The ratio of  $\bar{I}_E$  to the light saturation parameter  $E_k$ , or light limiting ratio (LLR), was also used to assess the available light compared to that required for maximal photosynthesis (Millard et al. 1996). In theory the threshold for determining light deficiency occurs when the average epilimnetic PAR is less than  $E_k$ , which corresponds to a ratio of 1. However, Millard et al. (1996) found LLR values of 0.8 or greater to still represent light sufficiency.  $\bar{I}_E$  values during the summer were typically greater than limitation thresholds reported elsewhere (Hecky and Guildford 1984; Millard et al. 1996). The epilimnion of SHL and SOL are deeper (July and August average, 7.0

m and 9.8 m respectively) than those of COL (5.75 m) CUL, ELL and MXL (all 4.0 m). The greater epilimnetic depth in SHL and SOL is a function of greater fetch and results in a lower  $\bar{I}_E$ . Since summertime  $E_k$  does not differ vastly between the lakes, LLR was lowest in SHL and SOL during summer.

#### *Zooplankton:*

Large crustacean zooplankton dominated all the study lakes except MXL. Biomass-weighted mean length was typically greater than 0.8 mm for COL, CUL, ELL, SHL, and SOL (Figure 3.8). *Daphnia* spp. were present in all of these lakes and together with calanoid or cyclopoid copepods constituted the majority of crustacean zooplankton biomass. MXL was distinct from the other lakes in this study with a biomass-weighted mean length of ca. 0.4 mm. Zooplankton in MXL was dominated by *Bosmina* spp. and cyclopoid copepods.

#### ***Discussion***

There were distinct differences in the temporal patterns of  $^{14}\text{C}$ -PPhot among lakes. Seasonal patterns in COL, ELL, SHL and SOL were typical for deep lakes ( $Z_{\text{mean}} > 5\text{m}$ ), with the greatest near-surface PPhot rates in the spring and summer and the lowest rates in autumn and winter. In contrast, MXL had highest rates during winter and early spring. COL had highest near surface rates in the spring, similar near-surface rates during winter and summer and lowest rates in the autumn. CUL had greatest PPhot rates in late summer (Figure 3.3, Table 3.3). The cross-correlation of the four lakes fitting the typical PPhot seasonal trends of Alvarez-Cobelas and Rojo (1994) was offset by several weeks suggesting that these coastal lakes reach maximal photosynthesis at an earlier time than other lakes.

High near surface winter  $^{14}\text{C}$ -PPhot rates in COL and MXL indicate that neither temperature nor PAR was limiting surface photosynthetic production to a greater extent than that observed during summer. Available light was considered to be limiting during the winter (using both  $\bar{I}_E$  and LLR measurements) and is reflected in the physiological parameters. The relatively high wintertime rates are, therefore, primarily a result of increased chlorophyll biomass and light adaptation by the phytoplankton (Table 3.2).

Temperature changes elicit algal physiological responses (Raven and Geider 1988; Falkowski and Raven 1997) and can affect species composition. Kirk (1994) suggested that low temperature is an important limiting factor of photosynthesis in lakes. However, other research suggests that temperature may not be as important at the ecosystem level (Goldman 1977; Goldman and McGillicuddy 2003) except under conditions of light and nutrient sufficiency (cf. Lomas et al. 2002). The concomitant seasonal decrease in ambient PAR and the decrease of maximum depth where C-fixation occurred implicates PAR as a principal factor limiting integral  $^{14}\text{C}$ -PPhot during winter. The larger range of  $\alpha^B$  compared to  $P_m^B$  suggests that PAR ( $\alpha^B$ ) was more important than temperature ( $P_m^B$ ) and had more influence on  $E_k$  (Jones 1978). In addition, most C-fixation occurred within the light-limited portion of the P-E curve (Figure 3.3) where temperature should have a minimal effect on PPhot.

Besides affecting  $P_m^B$ , temperature can also regulate other processes, such as bacteria growth (Pomeroy and Wiebe 1988; Shiah and Ducklow 1994) and zooplankton growth and grazing rates (Peters and Downing 1984; Gillooly 2000). Low temperatures may therefore affect phosphorus dynamics by changing competitive interactions between algae and bacteria and/or reduced grazing pressure from zooplankton. The extent to

which algae increase their competitiveness for nutrient acquisition as a function of temperature was not; however, directly addressed by this study.

The interaction of light, internal loading of phosphorus from sediments, and the extent of mixing influences the size and duration of spring blooms in Lake Michigan (Brooks and Edgington 1994). In the lakes there was an increase of TP in the near-surface water during winter (Table 3.2), so the lakes might be considered comparable to such a situation; however, light levels are still relatively low in the winter (February). The hydrologic cycle of southern BC-coastal lakes is different when compared to north temperate lakes that experience winter ice cover. Ice-covered lakes can receive the bulk of runoff and associated allochthonous nutrients during spring runoff, whereas BC-coastal lakes receive the greatest inputs during the winter rainy season (Stockner and Shortreed 1985; Nowlin et al. 2004). Nutrients from runoff, therefore, enter these lakes in late autumn and early winter. However, the slight increase in nutrients alone cannot explain observed temporal variations in  $^{14}\text{C}$ -PPhot because all lakes but MXL had lower integral  $^{14}\text{C}$ -PPhot during winter compared to spring or summer. There was also no clear relationship between  $^{14}\text{C}$ -PPhot and TP within individual lakes (Figure 3.5). In MXL, the combination of increased TP together with other variables may help explain the greater wintertime  $^{14}\text{C}$ -PPhot rates, and importantly, from an ecosystem perspective, this fixed carbon may represent new production.

P-deficiency indices (P-debt and  $^{32}\text{PO}_4^{3-}$  turnover time) suggest that microbial communities in COL and MXL were phosphorus deficient for most of the year, including winter. This contrasts with other studies that have found severe P-deficiency in summer (when  $\bar{I}_E$  is greatest) that declines when ambient PAR is lower, to the point where

phytoplankton are light and not nutrient limited (Millard et al. 1996). Despite having lower wintertime PPhot rates, SHL and SOL were moderately P-deficient during winter ( $^{32}\text{PO}_4^{3-}$  turnover time, some P-debt). Even CUL and ELL, which indicated no P-deficiency with P-debt, had  $^{32}\text{PO}_4^{3-}$  turnover times lower than the 100 – 1000 minute range measured by Millard et al. (1996) in Lake Ontario during spring and autumn. The coastal lakes in my study therefore maintained a moderate P-deficiency throughout winter, so the data suggests light and nutrients may co-limit (cf. Healey 1985) coastal oligotrophic lakes in winter.

Lake trophic structure has long been known to affect plankton size (Brooks and Dodson 1965; Pace 1984) and productivity (Hrbáček et al. 1961). Studies examining the interactions between trophic structure, nutrients, and plankton photosynthesis suggest that small zooplankton-dominated systems will have greater rates of photosynthesis under conditions of nutrient enrichment (Schindler et al. 1997). MXL is populated with threespine sticklebacks (*Gasterosteus aculeatus*), which consequently leads to a zooplankton community characteristic of lakes that experience intense zooplanktivorous fish predation (i.e., dominated by smaller zooplankton taxa such as *Bosmina* sp. and cyclopoid copepods; Brooks and Dodson 1965). In contrast to the zooplankton in MXL, large *Daphnia* were present in all the other study lakes. The presence of large *Daphnia* has been demonstrated to reduce or depress algal biomass with increasing total phosphorus (Mazumder 1994). In contrast, odd-linked lakes (*sensu* Mazumder 1994) demonstrate greater responsiveness to changes in potential productivity. Under similar nutrient conditions, or the slightly enriched nutrient conditions during lake mixing, MXL may therefore be expected to show greater increases in production. However, the role of

temperature on zooplankton may mitigate direct (i.e. grazing effects) wintertime differences between even- and odd-linked lakes.

Since I did not directly test the respective importance of temperature, light, nutrient dynamics, and zooplankton on  $^{14}\text{C}$ -PPhot I cannot conclusively identify which factor was most influential at different times of the year within individual lakes. However, I can assess the relative importance of each by comparing seasonal trends within and among lakes. Seasonal changes of light and temperature typically have a strong control of temporal variability in  $^{14}\text{C}$ -PPhot (Alvarez Cobelas and Rojo 1994) but there is also a tight coupling of plankton-structure with these physical variables. Bacterial respiration is more strongly influenced by temperature than is primary productivity (Lefèvre et al. 1994; Lomas et al. 2002) and decreases in bacterial activity may release phytoplankton from nutrient competition. The interaction between light and nutrients (Fahnenstiel et al. 2000) and large zooplankton (Litaker et al. 2002) can also play an important role in the formation of cold-water blooms. Since within-lake TP and productivity were uncorrelated and seasonal temperature and light changes were similar between lakes, I suggest that a major difference in seasonal PPhot among lakes is based on the composition of the plankton community.

Considering primary production seasonally is important because of demonstrated temporal uncoupling of periods of net photosynthesis and respiration (Blight et al. 1995; Smith and Kemp 1995, 2001; Serret et al. 1999). Productivity under low light conditions can be seasonally important (Goldman and McGillicuddy 2003), represent new carbon (Dugdale and Goering 1967; Eppley and Peterson 1979) and is necessary for calculating annual photosynthetic budgets (Brooks and Edgington 1994). Measuring community

respiration rates is also vital, especially if respiration of the non-algal component (esp. bacteria) are influenced more by temperature than are phytoplankton. Based on the interpolated seasonal data, I estimated that  $^{14}\text{C}$ -fixation from November to March constituted between 22% and 47% of the annual budget for the oligotrophic-oligomesotrophic lakes (i.e., not including CUL and ELL). I have no estimate of the net carbon-fixation over a season since respiration was not measured. However, if community respiration decreases more with decreasing temperature than C-fixation, then the net contribution of wintertime C-fixation would be greater than the estimates presented here.

This study also challenges definitions of ideal growth conditions for species and/or communities, since “ideal” or “optimal” laboratory growth is not necessarily transferable to the field. Whilst low temperature (3-4°C) and low light are not typically considered ideal growth conditions for phytoplankton communities, the interplay of both physical and biological processes (e.g., competition and grazing) presented a niche in one of my study lakes that was quite different from that which would be expected from culture studies. This complicates our ability to develop predictive models, even those that incorporate light, nutrients, temperature, and photosynthetic parameters (Geider et al. 1998) and stresses the importance of studying rate processes and community composition under a spectrum of environmental conditions.

Table 3.1: Lake characteristics and trophic classification categories based on chl *a*, TP (total phosphorus) and PPhot (<sup>14</sup>C-primary productivity).

1% PAR compensation depths (m) are averages from May to October and January to March respectively. Average DIC (dissolved inorganic carbon) is in units of  $\mu\text{mol}\cdot\text{L}^{-1}$ , and DOC (dissolved organic carbon) is in units of  $\text{mg}\cdot\text{L}^{-1}$ . Average flushing rates are from Nordin et al. (1982), McKean (1992), Holms (1999), and Nowlin et al. (2004). Flushing rate for COL was calculated from precipitation and watershed area. For trophic categories: U-O=ultraoligotrophic, O=M=meso-oligotrophic, M=mesotrophic, M-E=meso-eutrophic and E=eutrophic. Noticeable temporal deviations in trophic classification are noted below. Trophic classification follow Wetzel (2001).

Lake	Z <sub>max</sub> (m)	Z <sub>mean</sub> (m)	1% Depth May -Oct. (m)	1% Depth Jan. - Mar. (m)	Depth Jan. - Mar. (m)	DIC ( $\mu\text{mol}\cdot\text{L}^{-1}$ )	DOC ( $\text{mg}\cdot\text{L}^{-1}$ )	Flushing Rate (years)	Chl <i>a</i>	TP	PPhot
Council (COL)	17.0	5.2	16.6	9.0	9.0	346	2.3	<1 year	O	U-O	O
Cusheon (CUL)	9.5	4.4	6.5	4.8	4.8	591	5.4	1.5	O <sup>a</sup>	E	M
Elk (ELL)	19.4	7.7	9.1	7.7	7.7	1075	5.6	4.5	M	M-E	M-E <sup>b</sup>
Maxwell (MXL)	17.0	6.5	8.6	7.2	7.2	313	4.4	1.5	O <sup>c</sup>	O-M	O <sup>c</sup>
Shawnigan (SHL)	53.0	13.0	11.2	8.4	8.4	369	3.1	<1 year	O	O	O
Sooke (SOL) <sup>d</sup>	70.0	19.5	13.9	11.4	11.4	354	2.1	<1 year	O	U-O	O

a: M in September

b: E in April

c: (Chl *a*) M-E in October-April; (PPhot) M in February

d: due to changes in dam height, SOL depth values are valid from 1989 until winter 2003.

Table 3.2: Limnological variables; chl  $a$  ( $\mu\text{g}\cdot\text{L}^{-1}$ ), total phosphorus (TP,  $\mu\text{g}\cdot\text{L}^{-1}$ ) and epilimnetic photosynthesis parameters for COL, CUL, ELL, MXL, SHL, and SOL:  $\alpha^B$  ( $\mu\text{gC}\cdot\mu\text{gChl}^{-1}\cdot\text{mol quanta}^{-1}\cdot\text{m}^{-2}$ ),  $P_m^B$  ( $\mu\text{gC}\cdot\mu\text{gchl}^{-1}\cdot\text{hr}^{-1}$ ),  $E_k$  ( $P_m^B / \alpha^B$ ,  $\mu\text{mol quanta}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) and integrated PPhot using 7-day average PAR ( $^{14}\text{C}$ -PPhot,  $\text{mgC}\cdot\text{m}^{-2}\cdot\text{day}^{-1}$ ).  $^{14}\text{C}$ -PPhot values represent average pelagic fixation for the entire lake (i.e. calculations account for bathymetry). Morphometric data from Spafard et al. (2002).

COL							MXL						
Date	Chl <i>a</i>	TP	$\alpha^B$	$P_M^B$	$E_k$	PPhot	Date	Chl <i>a</i>	TP	$\alpha^B$	$P_M^B$	$E_k$	PPhot
14-Jun-01	1.0	4.0	3.7	3.8	285	159	12-Jun-01	1.7	13.1	5.8	3.4	164	247
12-Jul-01	1.1	3.0	2.4	2.8	327	127	9-Jul-01	2.4	7.3	2.3	1.7	204	174
9-Aug-01	0.9	3.6	3.1	2.5	218	96	7-Aug-01	2.9	7.4	2.4	1.9	219	219
6-Sep-01	1.0	3.5	3.4	2.7	221	113	4-Sep-01	3.0	8.3	3.1	2.3	205	236
25-Oct-01	1.3	4.4	4.5	2.0	125	63	29-Oct-01	3.7	10.0	3.1	1.9	169	87
14-Feb-02	1.9	4.8	8.0	2.1	71	104	7-Feb-02	15.5	11.0	7.4	1.2	46	416
14-Mar-02	2.5	NS	5.7	1.3	65	107	7-Mar-02	10.4	NS	5.8	1.0	48	331
19-Apr-02	1.2	3.6	5.6	2.7	133	122	30-Apr-02	2.1	11.9	5.9	3.2	145	223
21-May-02	1.3	4.8 <sup>c</sup>	3.6	3.2	245	179	16-May-02	1.5	11.3 <sup>c</sup>	6.1	4.4	201	180
CUL							ELL						
Date	Chl <i>a</i>	TP	$\alpha^B$	$P_M^B$	$E_k$	PPhot	Date	Chl <i>a</i>	TP	$\alpha^B$	$P_M^B$	$E_k$	PPhot
12-Jun-01	2.7	16.9	3.9	2.32	164	216	14-Jun-01	1.9	15.9	3.9	3.3	238	300
9-Jul-01	2.1	11.8	5.2	4.34	232	288	12-Jul-01	2.2	20.8	9.3	5.5	166	664
7-Aug-01	2.3	13.6	4.7	4.1	239	281	9-Aug-01	5.2	12.8	6.8	4.4	179	936
4-Sep-01	12.8	19.3	3.5	2.3	180	642	6-Sep-01	3.5	13.0	9.6	3.3	96	648
29-Oct-01	2.4	38.7	8.4	1.34	44	73	22-Oct-01	13.0	40.6	1.82	2.8	430	258
7-Feb-02	1.5	14.4	7.8	2.8	99	54	14-Feb-02	10.8	46.0	4.67	1.5	90	388
7-Mar-02	2.6	NS	6.0	2.7	124	95	14-Mar-02	7.5	NS	5.4	1.7	86	426
30-Apr-02	1.1	10.7	6.0	5.40	252	119	19-Apr-02	11.0	20.5	8.5	2.7	90	1549
16-May-02	1.2	9.6 <sup>c</sup>	7.9	4.7	166	151	21-May-02	2.1	22.5 <sup>c</sup>	9.0	5.9	182	474
SHL							SOL <sup>a,b</sup>						
Date	Chl <i>a</i>	TP	$\alpha^B$	$P_M^B$	$E_k$	PPhot	Date	Chl <i>a</i>	TP	$\alpha^B$	$P_M^B$	$E_k$	PPhot
18-Jun-01	1.6	3.9	2.1	3.1	419	244	21-Jun-01	0.4	2.6	3.9	6.7	472	121
3-Jul-01	1.6	3.6	4.1	2.8	192	335	5-Jul-01	0.3	1.6	6.8	4.9	202	114
16-Jul-01	2.0	3.9	2.8	3.3	335	238	18-Jul-01	1.0	1.9	2.9	1.6	155	104
30-Jul-01	1.9	4.5	3.0	2.7	247	263	2-Aug-01	0.8	2.3	3.8	1.7	127	72
13-Aug-01	1.4	3.4	2.5	2.4	269	171	16-Aug-01	0.6	4.8	2.7	2.5	257	75
27-Aug-01	1.5	4.2	5.0	2.8	155	348	30-Aug-01	0.6	2.0	3.9	2.8	198	90
17-Sep-01	1.6	4.2	3.8	2.8	201	131	10-Sep-01	1.0	3.2	2.5	1.8	203	75
1-Oct-01	1.9	3.9	6.1	2.4	111	212	27-Sep-01	1.2	2.8	3.0	1.9	181	63
16-Oct-01	1.6	4.9	6.1	2.1	95	117	11-Oct-01	1.4	4.6	4.8	1.9	110	71
5-Nov-01	1.9	5.0	4.6	1.0	60	82	25-Oct-01	1.5	5.3	4.7	1.6	94	75
28-Jan-02	1.4	6.9	10.4	2.8	76	102	31-Jan-02	0.7	5.5	8.6	2.4	78	52
28-Feb-02	1.1	NS	10.7	2.5	66	112	28-Feb-02	0.6	NS	7.6	1.9	71	63
18-Apr-02	1.0	6.7	23.6	7.3	86	314	16-Apr-02	0.7	5.7	14.8	4.3	81	189
22-May-02	0.9	4.9 <sup>c</sup>	12.4	6.8	153	302	23-May-02	0.8	3.9 <sup>c</sup>	10.7	5.3	138	232

a: This is a drinking water reservoir and it experiences large seasonal changes in water level.

b: Due to changes in dam height, SOL depth values are valid from 1989 until winter 2003.

c: May TP values from samples approximately 1 week prior to other measurements.

NS = no sample was analyzed on these dates.

Table 3.3: Monthly estimates of  $^{14}\text{C}$ -fixation for the six study lakes. Rates are based on lake size, so are not comparable between lakes.

See text for details on calculations.

Month	Lake $^{14}\text{C}$ -PPhot ( $\text{kg C}\cdot\text{lake}^{-1}\cdot\text{month}^{-1}$ )					
	COL	CUL	ELL	MXL	SHL	SOL
June (2001)	692	2093	20 463	1899	36 582	18 928
July	586	2552	43 159	1590	48 938	21 957
August	445	4352	46 675	1783	36 883	14 411
September	471	4736	43 076	1623	28 051	13 128
October	360	1840	29 570	1055	21 076	13 081
November	250	570	13 352	1011	11 150	8720
December	225	430	11 206	1443	11 051	7427
January (2002)	277	414	13 325	2278	13 456	8177
February	398	559	18 508	2964	14 553	8346
March	550	903	32 453	2978	25 744	15 970
April	600	1003	75 687	2575	47 104	32 663
May	779	1346	48 320	1610	52 106	40 563
Total	5633	20 798	395 794	22 809	346 694	203 371

Figure 3.1: Location of study lakes. Council Lake (COL), Cusheon Lake (CUL), Elk Lake (ELL), Maxwell Lake (MXL), Shawnigan Lake (SHL) and Sooke Lake Reservoir (SOL).

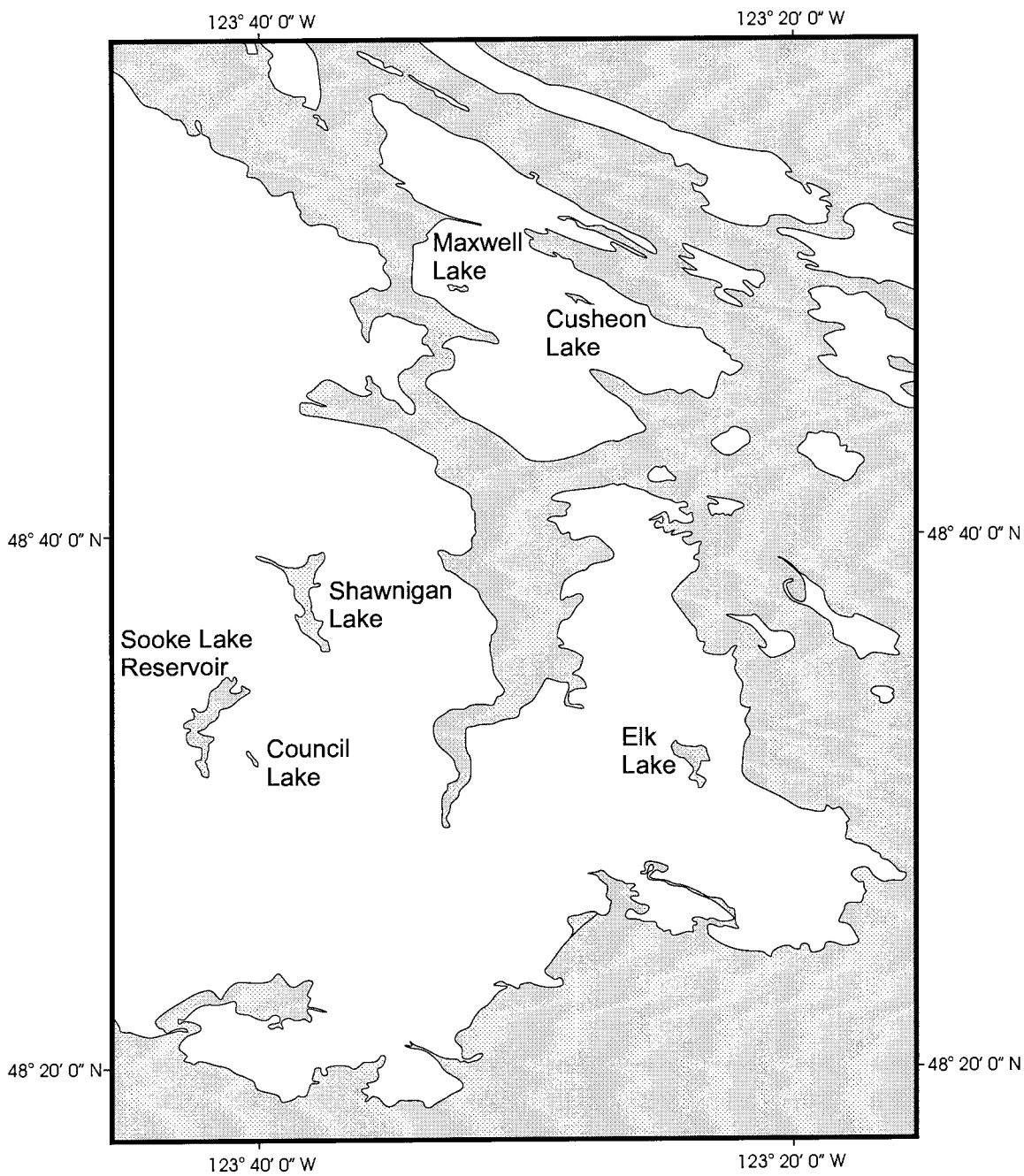


Figure 3.2: Seasonal changes in average surface temperature (0 – 2.0m) for Council (COL), Cusheon (CUL), Elk (ELL), Maxwell (MXL), Shawnigan (SHL) and Sooke (SOL) lakes.

Solid line represents ambient surface PAR measured at SOL drinking water intake tower, dashed lines encompass the PAR range.

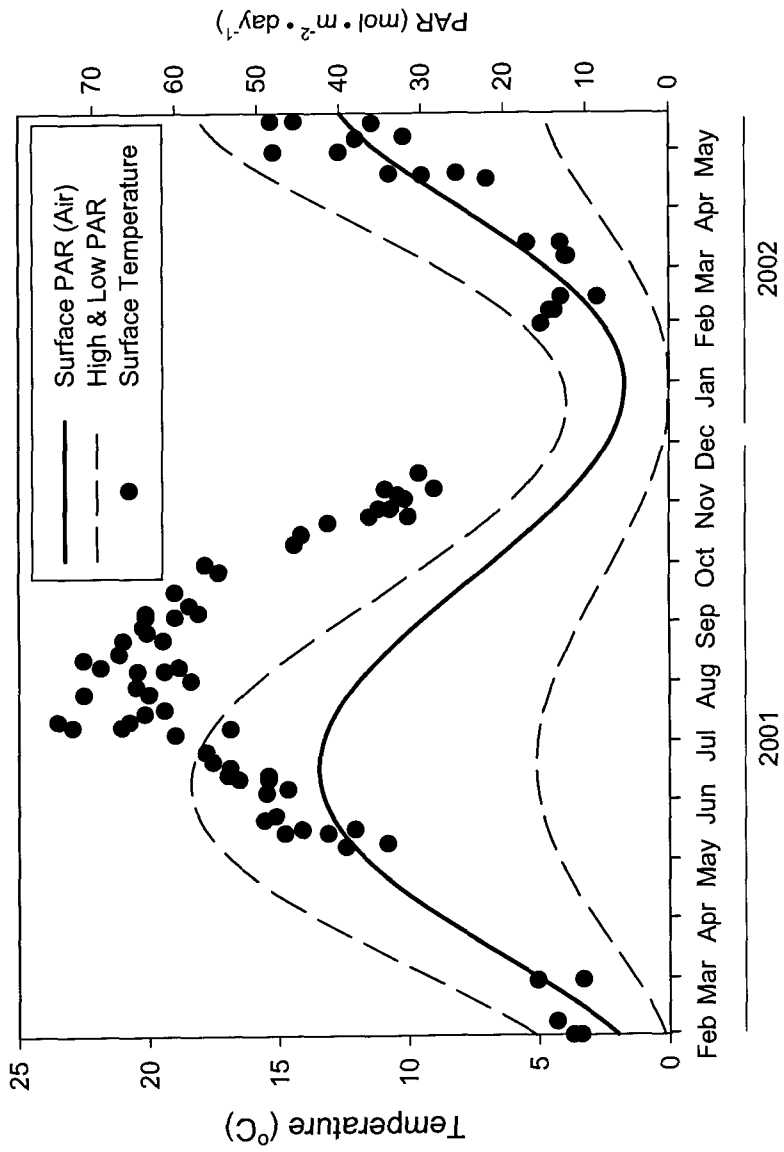


Figure 3.3:  $^{14}\text{C}$ -PPhot vs. depth for each lake.

The corresponding month for each profile is given immediately above the line at depth = 0 m. For clarity, not all months are shown for each lake. Data for months not shown are similar to that of the preceding month.

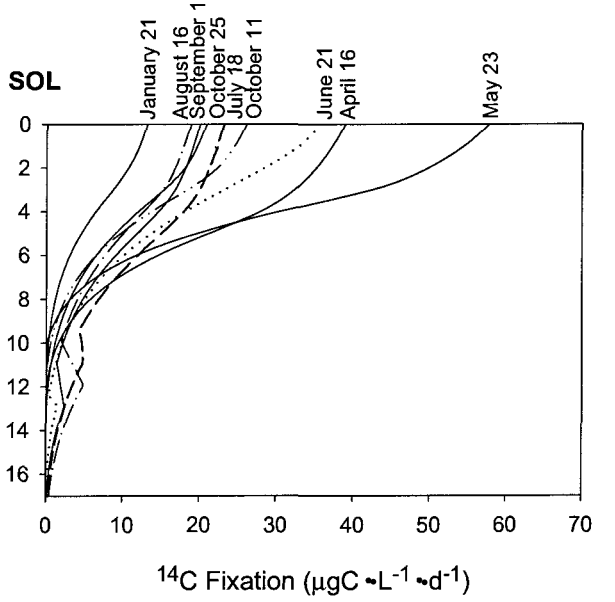
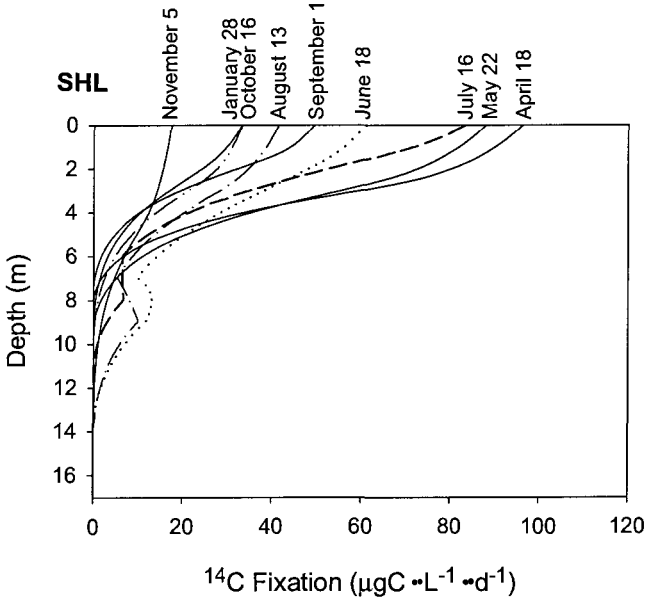
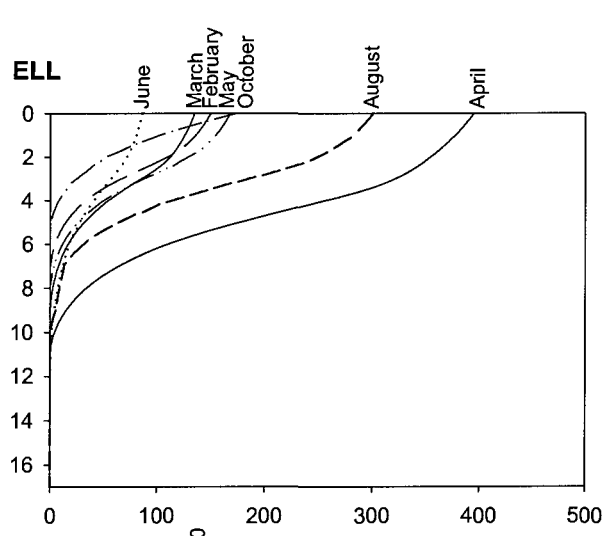
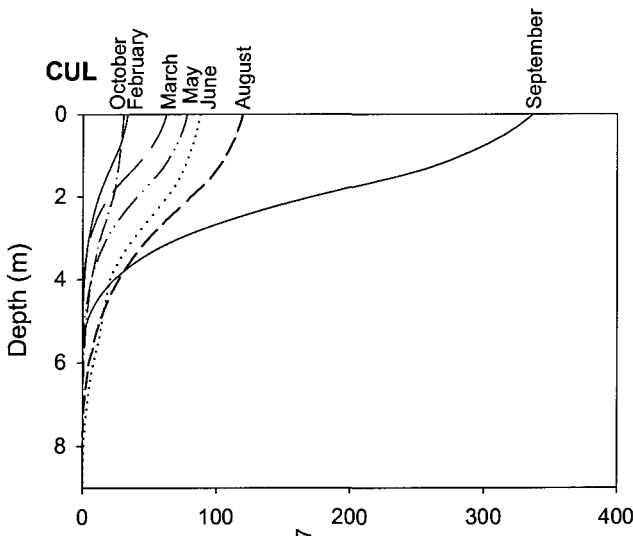
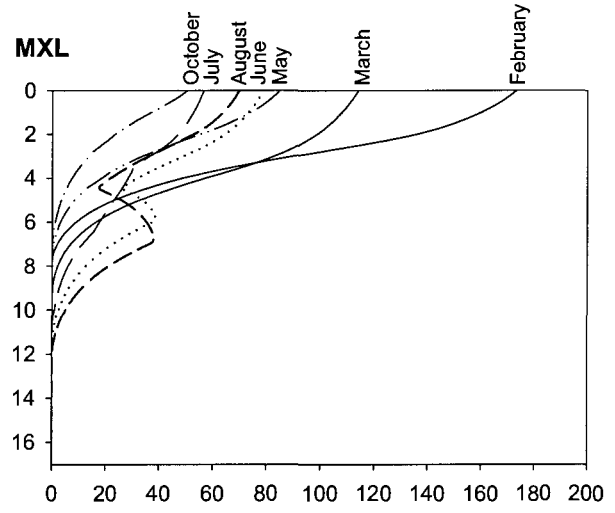
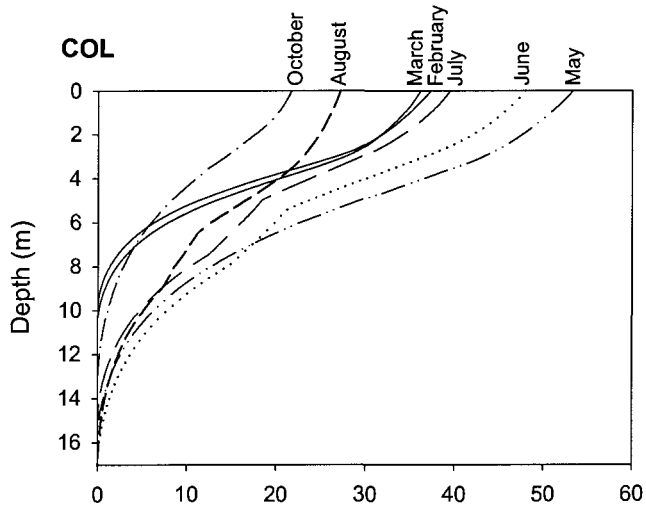


Figure 3.4: Weekly production as percent difference from annual mean compared to the typical seasonal productivity trends of deep lakes

Typical seasonal trends from Alvarez-Cobelas and Rojo (1994). COL, ELL, SHL and SOL follow the seasonal trend **(a)**, whereas CUL and MXL do not **(b)**. Inset figures are cross-correlations between the productivity trend of Alvarez-Cobelas and Rojo and COL **(a)** and MXL **(b)**. Lines on inset figures represent significance level ( $\alpha=0.05$ ). Cross correlations for ELL, SHL and SOL were similar to COL and the cross correlation for CUL, like MXL, suggested significantly different seasonal patterns than that of Alvarez-Cobelas and Rojo (1994).

\* Deep lakes considered to be those with  $Z_{\text{mean}} > 5\text{m}$ .

\*\* ELL and CUL values should be read off the right-hand axis, all other lakes are read from the left hand axis. The units are the same for both axes.

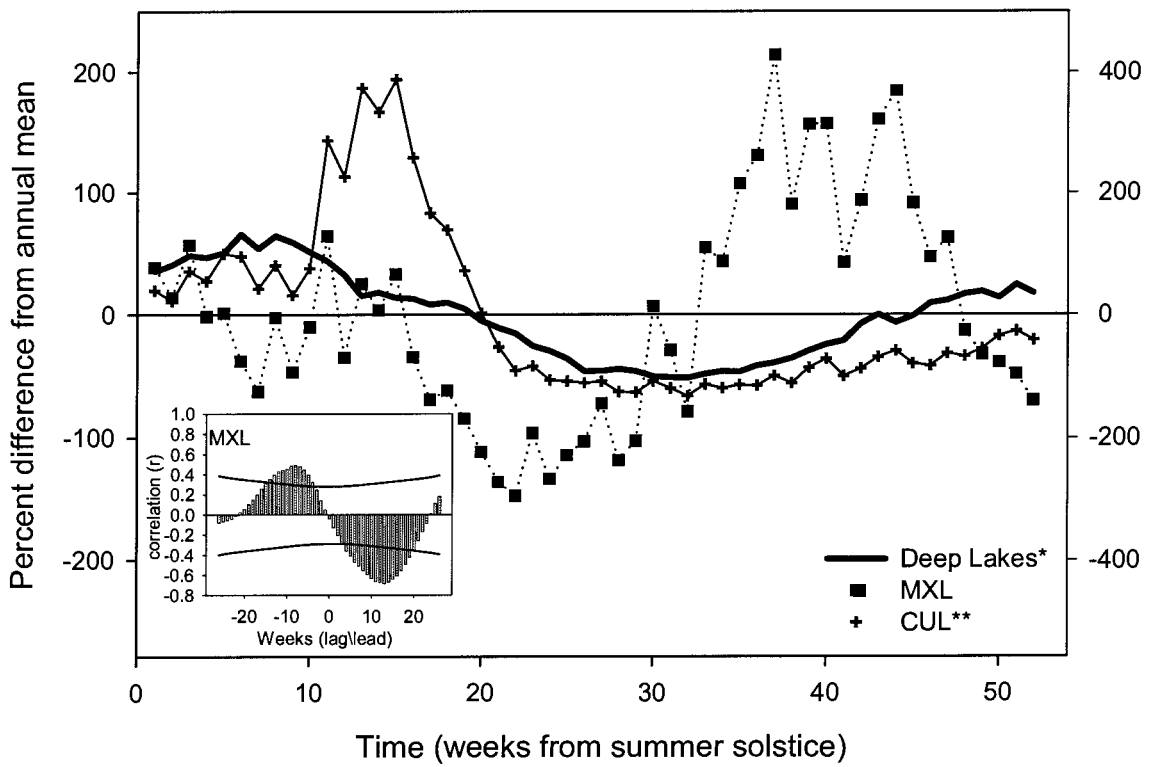
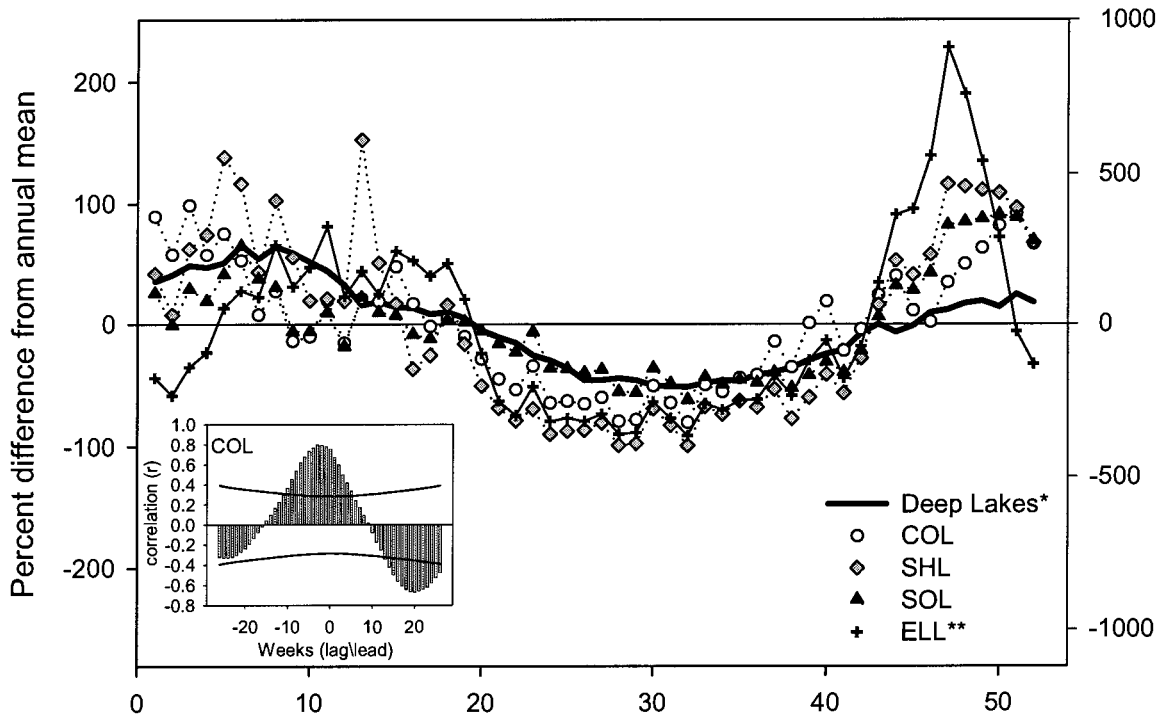


Figure 3.5: BC-coastal lake P<sub>Photo</sub> determined in this study compared to the linear regressions of Smith (1979) and Stockner and Shortreed (1985). Dashed lines represent 95% confidence interval from Smith (1979). Smith's study predicted seasonal averages and encompassed a larger range in total phosphorus, only that portion of the line corresponding to total phosphorus concentrations found in the current study lakes is shown. For more direct comparison, seasonal summertime averages (May-Sept.) have also been plotted for the lakes. Data of Stockner and Shortreed over  $2 \mu\text{g}\cdot\text{L}^{-1}$  TP are from fertilized lakes.

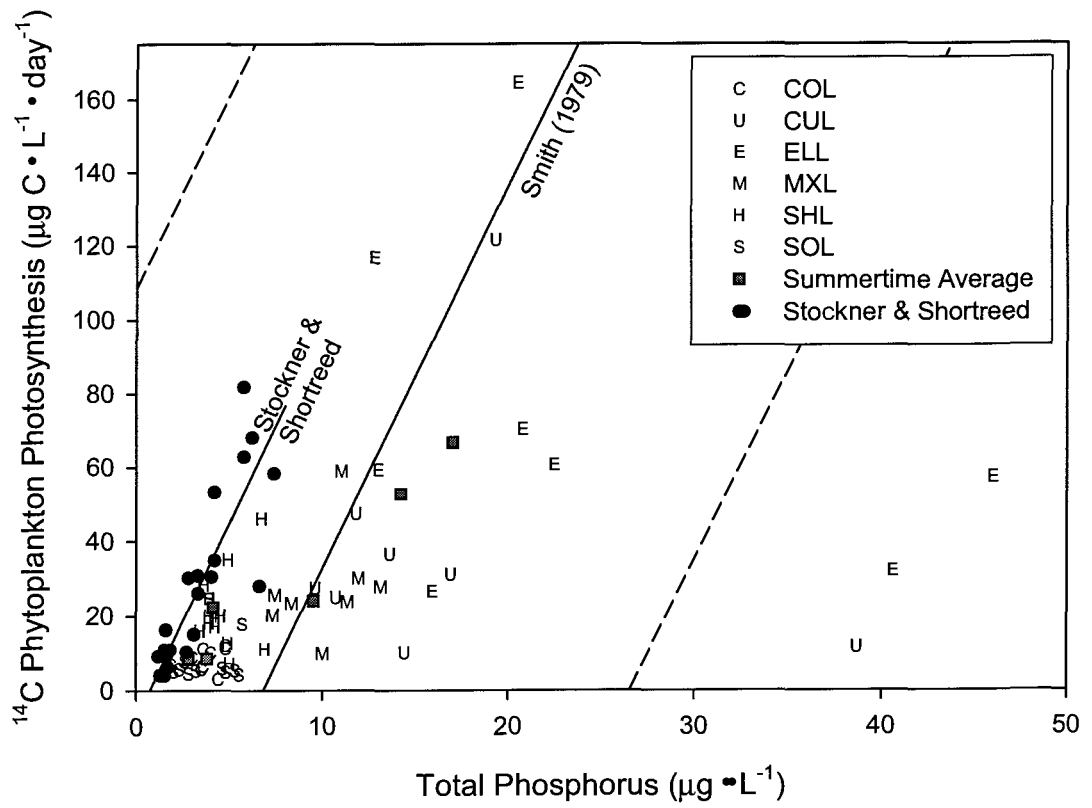
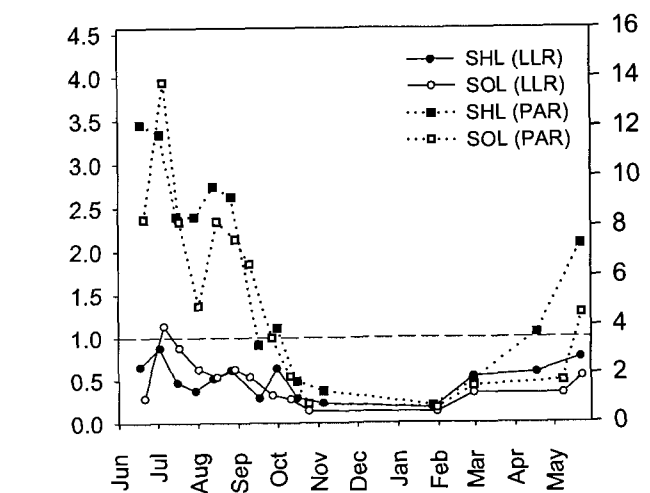
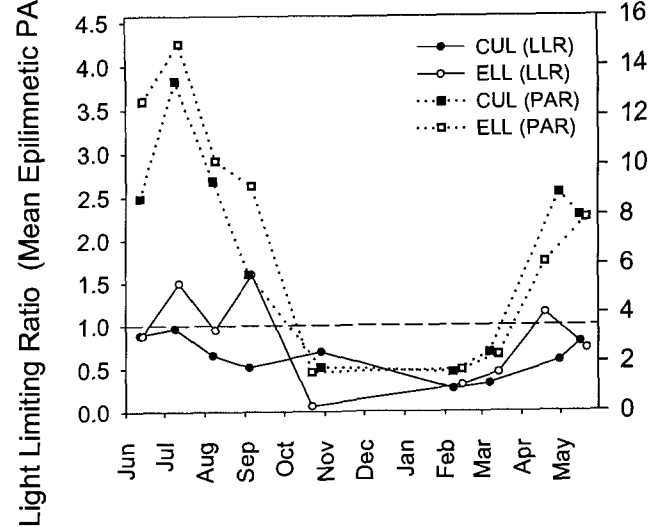
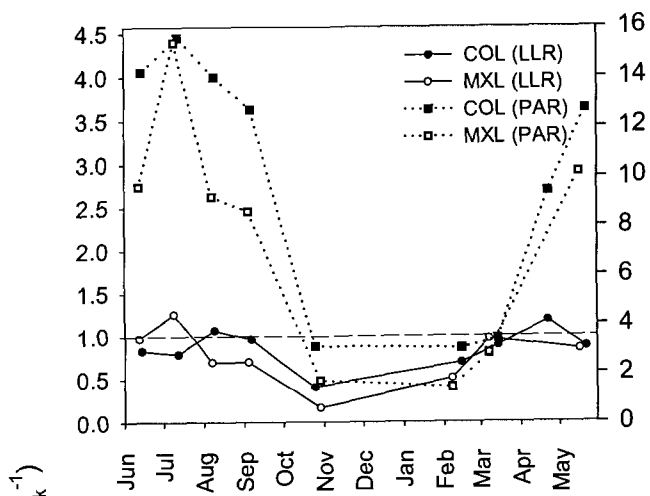
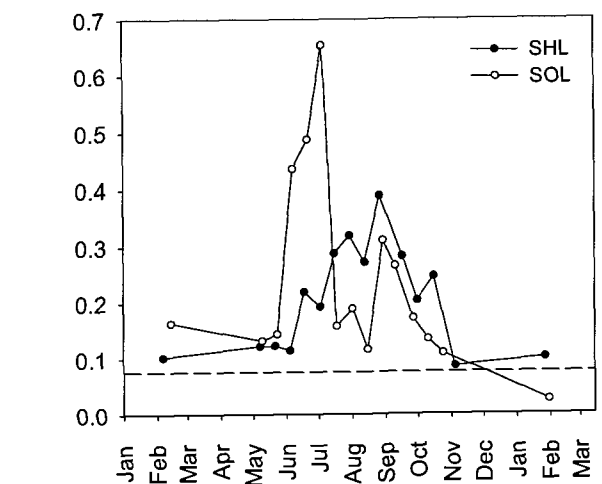
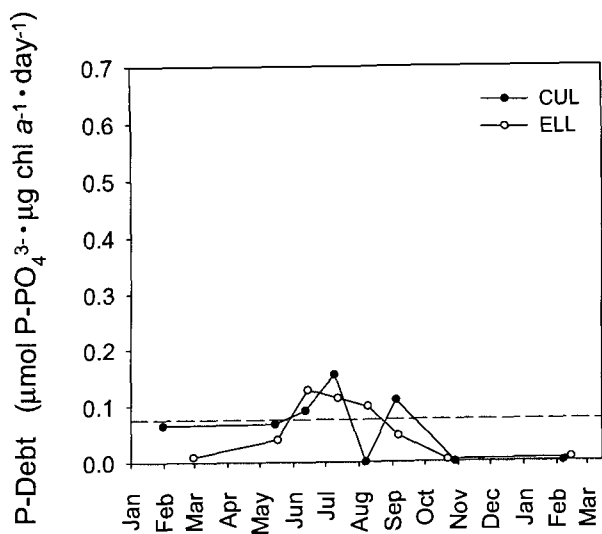
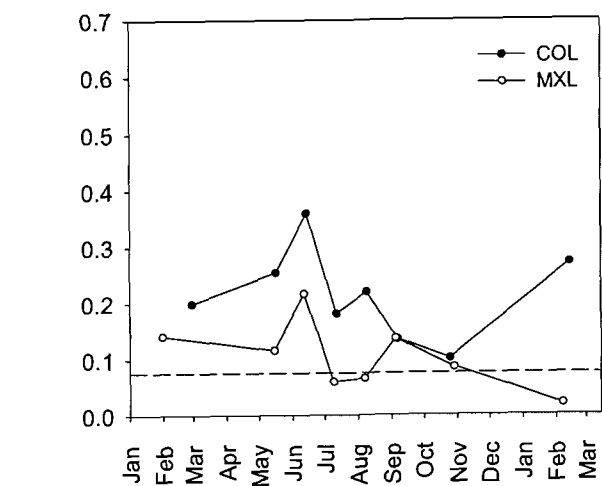


Figure 3.6: Left Panel: whole plankton P-debt from epilimnetic water during the length of this study. Right Panel:  $\bar{I}_E$  and the light limiting ratio (LLR).

Left Panel: greater P-debt is a measure of greater phosphorus deficiency. Dashed lines represent limitation thresholds (Guildford et al. 2000). Right panel: threshold for  $\bar{I}_E$  from Hecky and Guildford (1984). The plankton community is not considered to be light deficient when  $\bar{I}_E$  is greater than  $E_k$ , so the LLR threshold is defined as 1 (Millard et al. 1996). Note that date sequences are different for the left and right panels.



Mean Daily Epilimnetic PAR ( $\text{mmol quanta} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$ )

Figure 3.7: Winter  $^{32}\text{PO}_4^{3-}$  turnover time (minutes).

Shorter turnover times represent greater deficiency, the dashed line (10minutes) is an approximate threshold for defining severe phosphorus deficiency (Millard et al. 1996; Hudson et al. 2000). Thus,  $^{32}\text{PO}_4^{3-}$  turnover times less than 10 minutes (below the threshold line) are considered to be severely deficient

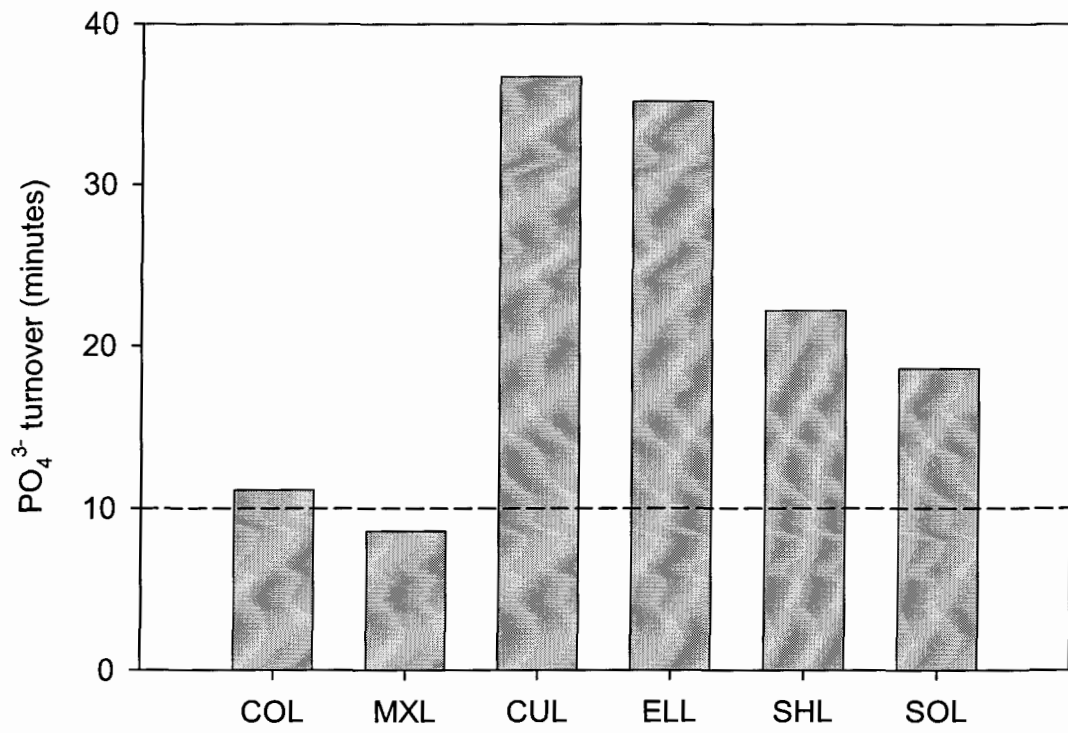
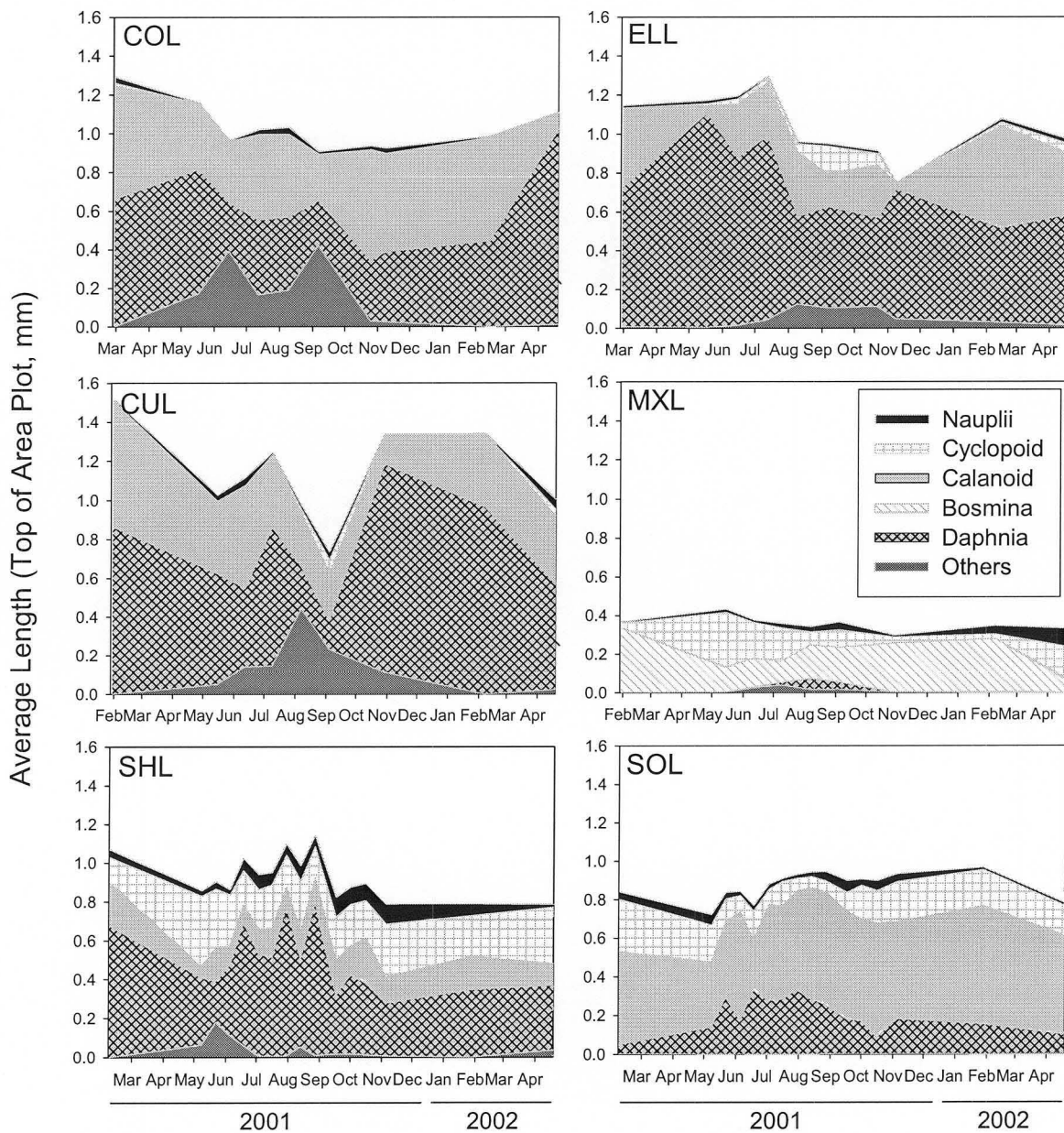


Figure 3.8: Average size (length) of the crustacean zooplankton community weighted by zooplankton biomass.

The top of all area curves represents the average community length. Taxa-specific areas represent the proportion of biomass that each respective group contributes to the total biomass. For example, in COL on 14-June-2001, the average community length was 0.96mm and the proportion of biomass contributed by each group was, other=40.8%, Daphnia= 26.1%, Bosmina=0%, Calanoid=32.5%, Cyclopoid=0.3% and Nauplii=0.3%



**Chapter 4: Phytoplankton succession in warm monomictic lakes of coastal British  
Columbia: is the clock ever set?**

### *Abstract*

Seasonal patterns of biomass and species composition were examined in lakes of southern coastal British Columbia. Lakes ranged from oligotrophic to meso-eutrophic. Among lakes there was a weak association of species with several nutrient indicators. Species known to be found in oligotrophic lakes were most closely associated with indicators of nutrient deficiency and nutrient limitation (chrysophytes and some diatoms). *Cyclotella* sp. was most strongly associated with conditions of nitrogen deficiency. Data indicated that several cyanobacteria were associated with high particulate nitrogen to particulate phosphorus ratios (PN:PP). While nutrient ratios are an important factor in determining species composition, particulate ratios were not necessarily reflected by physiological assessments of nutrient deficiency.

Seasonal succession varied among the lakes, the pattern in Cusheon Lake was dominated by an autumn bloom of *Aphanizomenon flos-aquae* and the period of greatest biomass in Maxwell Lake occurred during winter. The two more productive lakes (Cusheon and Elk) demonstrated a similar plankton physiological pattern between years. The results suggests that, while there is an underlying pattern due to seasonal changes, the species composition of oligotrophic lakes is influenced by weak stochastic events. This explains some of the observed variance between years. In oligotrophic lakes soluble reactive phosphorus ( $\text{PO}_4^{3-}$ ) remained low throughout the year. Another study demonstrated significant wintertime productivity in these lakes (Chapter 3). The wintertime activity of plankton in these oligotrophic lakes points to the lack of an obvious seasonal successional “starting point” and offers an explanation for the interannual variability of species in these lakes.

### ***Introduction***

A goal in ecology is to understand natural systems so that it is possible to predict the abundance and distribution of organisms and the cycling and transformation of energy and matter (Likens 1992). The basis for predictions vary from the recognition of repeatable patterns (Lund 1954) to the understanding of mechanisms that allow species to become abundant, such as growth rates and competitive ability, under various environmental conditions (Tilman 1982). Observed repeatable patterns provide a sound starting point for identifying mechanistic factors that ultimately drive the temporal pattern of species abundance and eventually lead to the development of models to describe those patterns.

Succession describes changes in species composition over time. Changes in the environment that are internally generated by the living component (autogenic forces) and those that are generated from outside the community (allogenic forces) are the two causative mechanisms of succession. Examples of autogenic forces include depletion of wintertime nutrients and zooplankton cropping of the spring bloom, whereas allogenic forces include seasonal changes in mixing regimes. Autogenic succession is typically orderly, directional and thus somewhat predictable (Odum 1975; Amblard 1992). It has recently been postulated that purely autogenic succession can result in several outcomes depending on small changes present at the onset of succession (Huisman and Weissing 1999). Allogenic forces can also lead to predictable changes in community composition, if the forces themselves are predictable (e.g. annual cycles) and of sufficient magnitude to allow specific species or groups to become abundant. In lakes, the interplay of autogenic

and allogenic forces produce the typical pattern of plankton succession (Sommer et al. 1986, Sommer 1989; Talling 1993).

Phytoplankton seasonal succession in lakes is based upon the inherent morphological, physiological and life history strategies of species in relation to environmental changes (Reynolds 1988). Models of plankton succession in temperate lakes assume annual biomass minima during winter (Sommer 1985; Talling 1993; Kalff 2002). Low light levels and temperature in the winter result in low incident energy and slow metabolic rates, resulting in low biomass despite high concentrations of available nutrients. Spring blooms following the initiation of stratification are typically considered to represent the first stage in seasonal succession and the beginning of the phytoplankton growth season (Sommer 1985). The increase in light and temperature in conjunction with available nutrients favours rapidly growing diatoms and small flagellates (i.e. r-selected species). Blooms decline due to a combination of depleted nutrients and high grazing pressure, which leads to the clear water phase. As grazing pressure declines and available nutrients increase, the summertime phytoplankton community develops. During the summertime stage of succession, oligotrophic lakes exhibit smaller changes in algal biomass compared to eutrophic lakes (e.g. Sommer et al. 1986). In fall, autogenic succession ends as species selection is dominated by changes in the physical environment. Though there is considerable variability in seasonal patterns among lakes, within lakes the seasonal transition from allogenic to autogenic processes occurs at a similar time, which results in predictable successional patterns between years (Anneville et al. 2002).

The similarity of inter-annual successional patterns ultimately relies upon the nature and strength of allogenic forces. Dramatic changes in the physical environment can select species with different growth and life history strategies than were present prior to the change. Thus, the initiation of a dramatic allogenic force results in the selection of specific species adapted to grow under those conditions. Changes in strength or importance of allogenic factors has been used to explain interannual variability of phytoplankton (Harris and Baxter 1996) and to examine long-term environmental changes (Anneville et al. 2002). In turn, the plankton community as a whole changes and adapts (e.g. grazers increase during spring phytoplankton bloom).

Earlier studies of seasonal succession focused more on describing seasonal patterns in lakes (e.g. Sommer et al. 1986), while recent studies use multivariate techniques to describe seasonality (Salmaso 1996) and the strength of association between algal species and various parameters (e.g. Wolfinbarger 1999; Fabbro and Duivenvoorden 2000). Both methods rely upon the strength of associations between phytoplankton groups and/or species and the parameters considered. Compositional changes that are consistently associated with dramatic changes in a set of parameters increase the ability to predict the timing of bloom events.

Accurate predictions of phytoplankton blooms are important for economic, social and health reasons. This is especially true of marine coastal regions susceptible to harmful algal blooms (HABs), drinking water supplies that experience cyanobacterial blooms and environmental impact assessments of both inland and coastal waters. Our ability to predict relies upon our capacity to correlate input variables (e.g. time of year, pH, nutrients, light, temperature, zooplankton community etc.) with the growth

characteristics of algal species. The initiation of autogenic species succession is more predictable if initially punctuated by dramatic changes in the physical environment.

Phytoplankton-nutrient relations are a vital component of plankton succession. Allogenic and autogenic forces are both driving forces of seasonal nutrient dynamics. The number of studies examining plankton-nutrient relationships and the central role of nutrients in seasonal successional models is testament to the importance of understanding nutrient dynamics for ecological prediction and management purposes. The associations between common phytoplankton species and ambient levels of dissolved nutrients, physiological measures of nutrient deficiency and particulate ratios, therefore was considered to be an integral component to understanding successional patterns in the lakes of this study.

The purpose of the present study was to examine seasonal successional trends in phytoplankton communities in lakes of coastal British Columbia. The immediate goal was to describe and understand these patterns and the long-term objective was to provide information to local managers of drinking water lakes and reservoirs. The design of the study was based upon the supposition of conventional wisdom regarding seasonal patterns in lakes. This included the presumption that deep monomictic lakes would be light limited in winter, and therefore have an abundance of available nutrients and the lowest algal biomass of the year (Sommer et al. 1986; Kalff 2002). During spring when nutrients are typically readily available and light intensity increases, it was expected that a dramatic increase in biomass would be observed. This biomass was expected to be cropped by zooplankton to produce a clear-water phase and a summer community would develop. I chose lakes and reservoirs (NB: all reservoirs were lake-reservoirs) that

were oligotrophic to meso-eutrophic (*Chapter 3*). It was recognized that seasonal patterns in oligotrophic lakes, while following the general trend described above, would likely not have biomass shifts as dramatic as the mesotrophic lakes. The overall objective of this study was to gain an understanding of the underlying pattern of seasonal succession and gain an insight into whether it is possible to predict annual bloom events or at least to identify periods that were susceptible to algal blooms.

### ***Methods***

#### *Study Lakes, Field Sampling:*

Six coastal British Columbia lakes on Vancouver Island and Salt Spring Island were studied for seasonal succession patterns of phytoplankton. Council Lake (COL), Shawnigan Lake (SHL) and Sooke Lake Reservoir (SOL) were considered oligotrophic, the trophic status of Maxwell Lake (MXL) varied seasonally from oligotrophic to meso-eutrophic and Cusheon (CUL) and Elk (ELL) lakes were considered to be mesotrophic to meso-eutrophic (*Chapter 3*). These lakes typically become stratified in April-May and reach summertime epilimnetic temperatures around 20-22°C. Fall overturn typically occurs in mid to late October or early November. Wintertime surface temperatures are around 4°C, and if ice cover occurs the ice is typically thin and not permanent. Unlike inland temperate lakes, these coastal lakes receive the bulk of stream inflow during the winter rainy season (November – March). Average summertime total phosphorus (TP) ranged from 3.1 to 17.6 µg·L<sup>-1</sup> among all lakes (*Chapter 2*). Shawnigan and Sooke lakes have morphometrically and hydrodynamically distinct basins (Nowlin et al. 2004) so separate samples were taken from the southern (SHL-S and SOL-S) and northern (SHL-N

and SOL-N) most basins in these two lakes. Physical and biological parameters for all six lakes can be found in Spafard et al. (2002), and in *Chapters 2* and *3*.

Field sampling protocols and laboratory methods can be found elsewhere (*Chapter 2; Chapter 3*). Briefly, triplicate integrated epilimnetic samples were collected with a 5 cm diameter, 6 metre-long Tygon tubing. The sampling station for each lake basin was at the point of maximum depth. Standard limnological parameters were measured at each station on each date, including nutrient analyses, temperature and oxygen profiles, and whole-water column zooplankton samples. Upon collection, water samples were placed in opaque Nalgene containers and immediately placed in coolers. Phytoplankton samples were preserved with Lugol's solution (1%). Approximately 500 mL of sample was preserved, with each replicate water sample contributing a third of this volume. Quantitative phytoplankton counts and measurements of phytoplankton biomass were conducted using the preserved sample. The remainder of the water in the 2 L Nalgene containers was used for chlorophyll a (chl *a*) determination, plankton physiological bioassays and measurements of productivity (see *Chapter 2, Chapter 3*). Additional sample water was used for determination of particulate and dissolved nutrients,  $^{32}\text{PO}_4^{3-}$ -turnover time, and grazing rates (Nowlin 2003). In addition to the quantitative phytoplankton samples, approximately 10 L of water was filtered through a 10  $\mu\text{m}$  phytoplankton net in the field. This concentrated sample was not preserved and was used for a qualitative assessment of the phytoplankton community prior to conducting quantitative counts on the preserved sample.

*Phytoplankton Enumeration:*

Phytoplankton counts were conducted according to Utermöhl (1958). All counts for the two-year study were conducted on an Olympus inverted microscope (IMT-2) by the same individual. Hydro-bios Utermöhl chambers were used (diameter=26 mm, volume=3.00 mL). Depending on the concentration of algae, either a 10, 25 or 50 mL settling tower was used. Samples were settled on a settling table (Tangen 1976) for between 24 and 48 hours, depending on the height of the settling tower. A tiered counting method was employed so that larger cells and colonies were counted at low power (100x – 200x) and smaller cells were counted at high power (400x – 630x). Unidentified ultraplankton (2-10 µm) were classified as a group, the dominant phytoplankton were identified to genus or species. Unknown species were grouped together. In the context of classical definitions of species, smaller phytoplankton can be difficult or impossible to definitively identify (e.g. Otsuka et al. 1996) and in these instances species were grouped at the genera level (e.g. *Microcystis*). Due to the morphological ambiguity of some species in the context of classical definitions of species, a minimum of 100 ultraplankton cells and 400 other cells/colonies were counted. If a particular species was deemed to dominate cell numbers (e.g. *Microcystis* sp. or *Anabaena* sp.) then counts were continued until at least 300 cells of the non-dominating species were counted.

Cellular biomass (wet biomass) was estimated from cell measurements assuming a volume to biomass conversion factor of  $1 \text{ mm}^3 = 1 \text{ mg}$ . A video camera was mounted on the inverted microscope and connected to a computer. Live images were viewed with Northern Eclipse software (ver. 6) so that computer-mouse driven cell measurements

could be made concurrently with counts. Shapes used for each species are after Hamilton (1990, pers. com.). Every cell of species that had high variance in size was measured until a minimum of ten individuals were counted. Species with low variance in cellular dimensions between individuals (e.g. some species of *Microcystis*) had at least three measurements made.

Biomass data for twenty-one genera and species that were common to my study lakes were further analyzed to determine algal, nutrient and zooplankton species associations; these algae were: *Microcystis* spp., *Chroococcus* spp., *Anabaena* spp., *Aphanizomenon flos-aquae*, *Chryso-sphaerella longispina*, *Dinobryon bavaricum*, *D. cylindricum*, *D. divergens*, *D. elegantissimum*, *Mallomonas* spp., *Asterionella formosa*, *Aulacoseira granulata*, *Aulacoseira* sp., *Cyclotella* spp., *Fragilaria crottenensis*, *Tabellaria fenestrata*, *Urosolenia eriensis*, *Ceratium hirundinella*, *Chroomonas* sp. *Cryptomonas erosa*, and *Cryptomonas* spp. Several taxonomic keys were used for identification, including Smith (1950), Prescott (1962), Bourrelly (1966, 1968, 1970), Huber-Pestalozzi (1941), Whitford and Schumacher (1984), Cumming et al. (1995), Barber and Haworth (1981) and Patrick and Reimer (1966).

*Statistical analysis:*

Ordination of phytoplankton taxonomic groups, select species and representation of temporal change within each lake was conducted using nonmetric multidimensional scaling, NMDS (Kruskal 1964a, 1964b). One of the advantages of NMDS is that it requires no assumptions about data, other than its ordinal relationship, so it is particularly well suited for analysis of nonlinear data and has been found to represent data in fewer dimensions than other ordination techniques (Gower 1966). Similarities are assumed to

relate to Euclidean distances and tests with ordinal relationships have demonstrated accurate reconstruction of metric space structure (e.g. Shepard 1980). NMDS algorithms were applied to Pearson dissimilarity matrices calculated from either  $\ln(1+B)$  data (where B equals biomass) if only algae were included in the analysis (Sprules 1980), or standardized data (Z-scores) if non-algal data was analyzed at the same time (Legendre, P. Université de Montréal pers. comm.). The closeness of points is the measure of similarity between those points relative to all the other points. Points can be rotated without loss of information, since it is the distance between points, not the position on the figure that is critical.

NMDS ordines data with the objective of minimizing stress, where stress is the measure of the increasing monotonic relationship between the variable dissimilarities and the forecast Euclidean distances in ordinal space (i.e. how representative the relationship between the variables are in the given number of dimensions). Stress (S) is a measure of the goodness-of-fit that can be calculated as the square root of a normalized residual sum of squares between the fitted dissimilarities of objects i and j ( $d_{ij}$ ) and the true dissimilarities of the same two objects ( $\hat{d}_{ij}$ ) with  $d_{ij}$  as a normalizing factor in the denominator (Kruskal and Wish 1978; Legendre and Legendre 1998).

$$S = \sqrt{\frac{\sum (d_{ij} - \hat{d}_{ij})^2}{\sum d_{ij}^2}} \quad (1)$$

Stress values fall between 0 and 1 with lower stress values being indicative of configurations that explain a greater proportion of the least-squares variance in that ordinal space. Stress depends upon both the number of dimensions considered and the

number of objects, so no strict significance tests exist. Kruskal (1964a) suggested the categories for goodness-of-fit summarized in Table 4.1a.

XLStat (version 6.1.9) was used to generate ordination data. XLStat employs the Scaling by MAjorizing a COnvex Function (SMACOF) algorithm to minimize stress. NMDS results for both taxonomic group and selected genera obtained from XLStat were compared to those from SPSS (version 10, ALSCAL algorithm) and both programs arrived at comparable solutions.

## ***Results***

### *General Trends:*

Models and descriptions of seasonal succession typically begin with winter as the baseline starting point (e.g PEG model, Sommer et al. 1986). Contrary to these models, which predict low biomass during winter, the wintertime phytoplankton biomass in some of my study lakes was comparable to, or greater than, that during summer (Figure 4.1). In CUL and ELL the blooms during late summer/early-fall substantially increased both chl *a* and biomass, however, even in these lakes the wintertime biomass was comparable to that observed during late spring and early summer (Figure 4.1b, 4.1d). Cyanobacteria were less abundant in ELL during the fall bloom of 2000, than they were in 2001. SHL and SOL exhibited a more typical pattern of spring biomass increase followed by a decline in early summer (2001). In SOL, the spring biomass was approximately twice that during the winter and summer periods (Figure 4.1g, 4.1h). In SHL, the fall and wintertime biomass was greater than other times of the year, except for a mid-summer bloom that was observed in both basins during 2001.

The ultraplankton biomass (which was defined in this study as phytoplankton in the 2-10  $\mu\text{m}$  size range) constituted a large proportion of the biomass in all lakes. With respect to larger algae, diatoms and chrysophytes dominated COL and MXL (Fig 1a, 1c). The fall cyanobacteria bloom dominated the seasonal cycle in CUL. Although cyanobacteria were observed in ELL, diatoms consistently constituted a greater portion of the biomass. Algal groups were more evenly represented in SHL than in SOL where diatoms constituted the greatest proportion of the non-ultraplankton biomass.

The abundance of available nutrients in late winter is an integral component of successional models that predict spring phytoplankton blooms. Readily available nutrients typically include soluble reactive phosphorus ( $\text{SRP} = \text{PO}_4^{3-}$ ), dissolved inorganic nitrogen ( $\text{DIN} = \text{NO}_3^- + \text{NH}_4^+$ ) and, for diatoms, soluble reactive silica (SRSi). DIN showed a consistent pattern among most of my study lakes, reaching low levels during the summer and increasing in concentration during winter (Figure 4.2). This trend was not observed in either COL or MXL where DIN concentrations were always low (Figure 4.2a, 4.2e). Unlike DIN, SRP was typically low during winter. SRP is known to overestimate  $\text{PO}_4^{3-}$ , and Nowlin (2003) estimated  $\text{PO}_4^{3-}$  using a steady state bioassay to be at picomolar concentrations in these lakes. Therefore, SRP measurements are only useful for identifying periods of time when concentrations are either at detection (i.e. low) or greater than detection, such as during the winter of 2002 in CUL and ELL. SRSi was only measured during the summer of 2001. ELL and MXL had the lowest concentration of SRSi (range ca. 500 – 1000  $\mu\text{g}\cdot\text{L}^{-1}$ ), whereas the concentration in all other lakes was greater than ca. 3000  $\mu\text{g}\cdot\text{L}^{-1}$ .

*Phytoplankton Associations:*

Chrysophytes, dinoflagellates and cryptophytes were in close proximity on the NMDS association plot for algal groups. Residual stress variance was low for this analysis, increasing confidence in the strength of the observed relationship (Table 4.1b). Diametrically opposed from the chrysophyte/dinoflagellate/cryptophyte group were cyanobacteria (Figure 4.3). The unknown group appears near the middle of the plot.

An NMDS plot for the select species summarizes the associations among the 21 common species in the study lakes, although the explained variance is poor (Figure 4.4, Table 4.1b). *Microcystis* and *Chroococcus* are closely associated with each other, while being distant from the other species. Chrysophyte algae and some diatoms are grouped together in the lower left quadrant (Figure 4.4a). *Anabaena* and *Aphanizomenon flos-aquae* are closely grouped (in all three dimensions, see Figure 4.4b) at the opposite end of the figure from this grouping of chrysophytes. *Fragilaria* and *Tabellaria* are the two diatoms most closely associated with *Anabaena* and *Aphanizomenon flos-aquae*. However, not all of these genera/species were found together in all lakes so a close association does not necessarily imply that the two genera/species always co-occur. For example, the close association between *Microcystis* and *Chroococcus* is primarily driven by their co-occurrence in SHL (Figure 4.5a). Examples of another pairing of closely associated species (*D. cylindricum*-*Cyclotella*), and two pairings of diametrically opposed species (*Microcystis*-*C. erosa* and *Anabaena*-*Cyclotella*) are also given to demonstrate the data underlying these association.

*Select Species Associations:*

Ten indicators of total nutrient status, including: total phosphorus (TP), total nitrogen (TN), particulate C:N (PC:PN) and particulate N:P (PN:PP) ratios, DIN, SRP, total dissolved phosphorus (TDP), N-debt, P-debt and  $^{32}\text{PO}_4^{3-}$  turnover times were analyzed together with the 21 select species (Figure 4.6). As with the species plot, the stress value is a bit higher (stress=0.179) so the strength of the relationships is weak. Indicators of nutrient deficiency (N-debt, P-debt) and nutrient limitation (PC:PN and PN:PP ratios) are grouped together on the left-hand side of the ordination plot, whereas indicators of nutrient sufficiency (DIN, SRP, TDP, long  $^{32}\text{PO}_4^{3-}$  turnover times) are grouped in the upper right quadrant. In the third dimension, indicators of nutrient sufficiency remain closely associated, whereas N-debt and P-debt are more distant (Figure 4.6b). This ordination kept the general species associations from Figure 4.4. The chrysophyte/*Cyclotella* grouping is most closely associated with nutrient poor conditions, whereas *Anabaena* and *Aphanizomenon flos-aquae* are more closely associated with conditions of both available nutrients and high total nutrients. Chroomonads and cryptomonads were also associated with higher available nutrients, as has been previously reviewed (Klaveness 1988).

*Microcystis* was closely associated with high PN:PP ratios (Figure 4.6a, 4.6b) whereas *Asterionella* and *Urosolenia* were diametrically opposed on the ordinal plot. Further examination of data revealed that on dates when *Asterionella* or *Urosolenia* were abundant, PN:PP ratios were low (<22 molar), often around 15, whereas on dates when *Microcystis* was abundant, PN:PP ratios were higher (>22 molar).

The zooplankton – phytoplankton association plot obtained a stress value greater than 0.2 (Table 4.1), indicating the relationships in this model are very weak (Figure 4.7). However, the zooplankton associations obtained were expected based upon knowledge of the zooplankton communities in the lakes. Only one lake (MXL) had abundant *Bosmina*. Cyclopoid copepods were abundant in MXL at certain times of the year, but also occurred at other sites, e.g. in SOL-S (Chapter 3). The remainder of the lakes had a mix of *Daphnia*, *Holopedium*, calanoid copepods, *Ceriodaphnia*, *Diaphanosoma*, and nauplii. Abundance of *Chroococcus*, *Chrysophaerella*, and to an extent *Microcystis* (Figure 4.7b), were associated with low abundances of zooplankton, especially the *Daphnia* group, whereas higher biomass of some of the *Dinobryon* species and *Aphanizomenon* were associated with higher biomass of *Daphnia*.

#### *Seasonal Trends:*

Consistent seasonal trends among phytoplankton groups were either not obvious or not dramatic in the oligotrophic lakes in my study (Figure 4.1). Two-dimensional stress values were poor, except in the case of SOL, where both basins had a stress greater than 0.20 (Table 4.1b). Thus, although seasonal patterns were noted among species in each lake, the relationship was weak. The two more productive lakes, CUL and ELL, did exhibit both consistent and obvious late summer–early fall blooms of cyanobacteria and diatoms, respectively. The temporal change in biomass of the dominant species in each lake is plotted to further examine seasonal trends among the lakes (Figure 4.8).

COL: *Cyclotella bodanica* occurred at a high abundance in the spring of both study years, however, *C. bodanica* persisted for a longer period of time during the second year. *Microcystis* was more abundant in the first year, whereas *Asterionella formosa* was

abundant in the summer of the second year only. A bloom of *Chryso-sphaerella longispina* was only observed during the second summer (Figure 4.8a).

MXL: Maxwell is unique among the study lakes because 1) it has a biomass and chl *a* maximum during the winter (Figure 4.1c), 2) it has its greatest seasonal productivity during winter (*Chapter 3*) and 3) MXL's zooplankton community is dominated by *Bosmina* and cyclopoid copepods, rather than *Daphnia* and calanoid copepods (*Chapter 3*). Different algae were dominant during the two winter periods of my study (*Asterionella*-2000 and *D. divergens*-2001). *Ceratium* was abundant during the first summer, but not the second, whereas *D. bavaricum* and *Microcystis* were more abundant in the second (Figure 4.8b).

CUL: As noted previously, the *Aphanizomenon* bloom in September dominates the phytoplankton seasonal pattern in Cusheon Lake. *Fragilaria* appeared with similar abundance at the same time in both years. *Ceratium*, *Anabaena* and *D. divergens* were abundant in the first, but not the second year, whereas *Tabellaria* was abundant the second year but not the first (Figure 4.8c).

ELL: *Fragilaria* and *Ceratium* both exhibited similar seasonal patterns for 2000 and 2001. *Microcystis* was more abundant during the first year, while filamentous cyanobacterial representatives (*Anabaena*, *Aphanizomenon*) were more abundant during the second year (Figure 4.8d).

SHL: There were notable similarities between the two sites examined in Shawnigan Lake. Unlike COL, *Microcystis* was generally more abundant in SHL during 2001 compared to 2000. Both *Chroococcus* and *Aulacoseira* were also more abundant in 2001. *Cyclotella* appeared in both years, but the timing of maximal abundance was

different between years. *D. divergens* biomass followed the same pattern in both SHL-S and SHL-N in 2000 and was not abundant in either of these sites toward the end of summer 2001 until the end of the study. *D. bavaricum* appeared during this latter part of my study in both basins (Figure 4.8e, 4.8f).

SOL: The species examined in Sooke Lake Reservoir also displayed a convergence of patterns between sites. *Urosolenia* constituted a large portion of the biomass, especially during the spring of 2001, and in SOL-S during the winter of 2002. The time of maximum abundance for each species differed between years and basins, for example *Microcystis* was abundant in SOL-N but not SOL-S during the winter of 2001. During summer *Microcystis* biomass peaked in July 2001 in SOL-01, but in SOL-04 it peaked in early October (Figure 4.8g, 4.8h).

Species composition (i.e. common 21 species) on each date was used to test for consistency of seasonal patterns within each lake (i.e. each lake was analyzed separately). Successive dates on NMDS plots were connected to demonstrate the seasonal pattern (*sensu* Sprules 1980). Since each point is considered separately in the analysis, the proximity of each point, not the actual date, represents the similarity of species composition. The lake with the most consistent inter-annual pattern was COL (Figure 4.9a). Spring samples (May) from both years lie near the middle right-hand portion of the plot, the trend from both years is to move left and up, extending to the upper left quadrant before moving to the central lower portion of the plot and ending in the bottom left-hand quadrant. For the other lakes there is less correspondence between the two years (Figure 4.9). Few successive dates on any of the plots vacillate from one end of the plot and back again (especially for SHL and SOL during 2001 where sampling frequency

was increased). This demonstrates the progression of species change and validates NMDS as a means for examining this data. For example, May, June and the first July (all in 2001) samples from SOL-04 (Figure 4.9h) are in close proximity, however, the data suggests that between the first and second sampling date in July (i.e. #1Jul - #2Jul) the phytoplankton community change dramatically near the end of the clear-water phase (Figure 4.1h).

*Nutrient Deficiency Trends:*

Prior to CUL and ELL fall blooms, the phytoplankton community in both lakes demonstrated an increase in nitrogen deficiency as measured by N-debt (*Chapter 2*). This peak in nitrogen deficiency occurred in July (CUL) and August (ELL) but was not accompanied by a concomitant shift of the C:N ratio that would suggest nitrogen limitation (Figure 4.10a, 4.10b). During August of both years the phosphorus deficiency of the plankton community (as measured by P-debt) became negligible in CUL (Figure 4.10c, 4.10d). During the biomass peak (September), both the phosphorus deficiency and the PN:PP ratio increased to deficient and limiting values. The ELL plankton community was P-deficient during the entire time in both years, however, during 2000 the PN:PP ratio was not indicative of P-limitation (<22 molar), whereas it was in 2001 (>22 molar).

PN:PP ratios reflect the species that respond to relative loading of available nutrients (Kilham 1990). When the carbon biomass (particulate carbon) is plotted as a function of particulate phosphorus there appears to be a dichotomy above a particulate phosphorus concentration of about  $10 \mu\text{g}\cdot\text{L}^{-1}$  (Figure 4.11). Plankton communities with PN:PP ratios below 22 (molar) have lower carbon biomass than those with PN:PP ratios above 22. The lines in Figure 4.11 are included to represent upper limits of particulate

carbon for plankton communities with PN:PP ratios either above or below 22. Data points with particulate phosphorus greater than  $10 \mu\text{g}\cdot\text{L}^{-1}$  have been labelled so that each point can be associated with a lake and date. Points with a PN:PP >22 are from CUL in September (dominated by *Aphanizomenon*), ELL in August 2001 (dominated by *Anabaena flos-aquae* and *Gloeotrichia echinulata* but *Microcystis* and *Lyngbya* were also present), ELL in February 2001 (*Synura uvella* and *Stephanodiscus* were dominant, no dominating cyanobacterial present) and MXL February 2002 (dominant alga was *D. divergens*, no dominating cyanobacterial present). Samples with PN:PP lower than 22 were dominated by diatoms, chrysophytes, or dinoflagellates (e.g. ELL August 2000 was dominated by *Fragilaria crotenensis* and *Ceratium hirundinella*).

### ***Discussion***

The six lakes examined in this study exhibited differences in their respective patterns of seasonal succession and their compliance with classical models of phytoplankton succession (Sommer et al. 1986). These deviations include a lack of a spring bloom in CUL, highest seasonal chl *a* during winter in ELL and MXL, no obvious clear-water phase in COL and SHL-N, and a lack of a winter-time phosphate pool in all the lakes. One of the most striking deviations from existing models is that plankton communities are abundant and active (*Chapter 3*) during winter. Successional models for temperate lakes consider winter to represent the “pre-successional state”. The implication, therefore, is that the “starting” point of succession either does not exist in these lakes, or if one identifies winter as the starting point, the predictability of species abundance should be muted since the successional clock has not been “reset”. A progression of species succession was observed in my study lakes, COL demonstrated a

similar *Cyclotella*-dominated pattern over the two years and SOL exhibited classical seasonal biomass changes for a deep oligotrophic lake. NMDS plots revealed a directional pattern of compositional changes, although these were noted to be weak and to be different in most lakes between years.

Seasonal ranges in temperature, light and mixing patterns in coastal BC are similar to other temperate lakes, although coastal lakes are unique because they lack permanent ice cover and have a different seasonal hydraulic/flushing pattern. Lakes of higher trophic status demonstrate greater seasonal response to external (Sommer et al. 1986, Marshall and Peters 1989) and internal (Benndorf et al. 2002) changes. The predictability of seasonal phytoplankton succession can therefore be characterized by the strength of predictable disturbances, which are usually attributed to seasonal forcing (Scheffer et al. 2003). The two meso-eutrophic lakes (CUL & ELL) displayed consistent seasonal phytoplankton events as indicated by algal biomass measurements, physiological assays and zooplankton clearance rates. The consistent patterns of increased production and biomass in these lakes makes them well suited for developing management protocols to ameliorate the associated poor water quality at these times.

A multitude of factors affect species composition, including available nutrients, nutrient cycling efficiency, total nutrient loading, mixing patterns, light environment (both stochastic weather events and lake attenuation factors), temperature, composition and intensity of grazing and patterns of pathogens (e.g. viruses). The role of nutrients has received disproportionate research effort, in part because of their intrinsically important role in shaping community composition and biomass and because it is the one factor that is most easily recognized by policy makers and managers. The general patterns that

emerged from the NMDS species-nutrient parameter plot (Figure 4.6) conform with general phytoplankton theory. From the left-hand side to the upper right portion of the plot (Figure 4.6) there is a progression from nutrient deficient/nutrient poor conditions to nutrient sufficient and nutrient rich conditions. It is interesting to note that there is greater coherence among the indicators of nutrient sufficiency than of nutrient deficiency. The relationship between nutrient indicators and species composition can be seen by examining the species that occur between the indicators of nutrient sufficiency and deficiency. In a separate analysis, this was also apparent in lakes with particulate phosphorus concentrations above  $10 \mu\text{g}\cdot\text{L}^{-1}$  where cyanobacteria were present at PN:PP greater than 22, while diatoms predominated at PN:PP ratios less than 22.

*Asterionella formosa* and *Urosolenia eriensis* were found at an intermediate distance between nutrient deficient conditions and situations of nutrient abundance, however, they were diametrically opposed to high PN:PP ratios (Figure 4.6).

Competition theory has demonstrated that *Asterionella* is a successful competitor under low conditions of low P compared to Si (Tilman et al. 1982), however, my results suggest that *Asterionella* may be more successful in oligotrophic systems when phosphorus is at a non-limiting supply ratio compared to nitrogen. *Cyclotella* was most commonly found under N-deficient (N-debt) and N-limiting (C:N ratio) conditions. Conversely, *Microcystis* and *Chroococcus* were most abundant under higher PN:PP ratios in contrast with previous predictions (Smith and Bennett 1999).

The species composition of the common species highlighted by this study varied between seasons in the oligotrophic lakes (Figure 4.8). A ten-year study of MXL and five other west-coast lakes (Parks 1995) found dramatic interannual differences in both

chlorophyll and the abundance of individual phytoplankton species. Most long-term studies of seasonal succession have found such differences between years (Round 1971; Lund 1964; Maberly et al. 1994). Why did MXL experience an *Asterionella* bloom during the winter of 2000-2001 while in 2001-2002 the dominant phytoplankton was *D. divergens*? Likewise, why did COL experience a prolonged *Cyclotella* bloom in 2001 and why, during 2001 does *Asterionella* appear when it was nearly absent in 2000?

There are many possible explanations, none of which can be rigorously tested from this data. Maberly et al. (1994) found that fall numbers of *Asterionella* were the best single predictor of the number of individuals in the spring bloom of the following year. During the winter of 2000-2001 the region experienced a 100-year drought, so the flushing rates of lakes was lower than it was in the winter of 1999-2000. *Asterionella* does not form resting spores, but rather relies on persistent individuals to initiate its population. Thus, with reduced flushing, sufficient numbers of *Asterionella* may have been able to persist in these small lakes and become abundant. Lund (1979) found a similar trend, with wet winters yielding lower counts of *Asterionella*.

Interannual fluctuations of factors important to community dynamics will affect phytoplankton composition. The similarity of phytoplankton succession between years depends upon the relative difference in magnitude of these factors (allogenic) the susceptibility of the system to organize itself (autogenic forces), and the predictability of the allogenic factors. Under this assumption communities in oligotrophic systems are hypothesized to be less predictable because allogenic forces, (e.g. stochasticity intrinsic of weather) can shape community composition in several directions. For example, the oligotrophic systems in this study were often nutrient co-deficient and many

phytoplankton are known to be mixotrophic. Any small changes in nutrient loading ratios due to mixing events will therefore affect species composition from year to year. Eutrophic systems with strong allogenic forces (e.g. CUL and ELL there was a large increase of nutrients entrained from the hypolimnion during late summer/early fall) represent stronger selection criteria in the plankton community. Contrary to this hypothesis, PerezFuentetaja et al. (1996) considered oligotrophic lakes to be more resistant to change than eutrophic lakes. It is important to consider the resulting magnitude of a disturbance relative to the intrinsic forces present in the system prior to the disturbance. Thus, if a disturbance fundamentally changes the autogenic force (e.g. change in zooplankton species composition) this should have a noticeable effect on species composition.

Recent chaos models have emphasized the unpredictability of competition outcomes under real world scenarios of multispecies competition (Huisman and Weissing 2001). However, competition exists in the medium of a changing environment, and probability functions of species or group success under certain conditions should still be possible, despite the inability to predict the exact nature of interannual variability. For example, unseasonably warm weather near the period of fall overturn can result in fall cyanobacterial blooms.

Predictability is a goal of both ecology and management, so that we have the knowledge to apply appropriate management techniques. Fortunately the water quality of oligotrophic lakes is higher and the need for predicting typical seasonal patterns of abundance is less urgent (Reynolds 1999). However, this is a fundamental issue for meso

to eutrophic lakes. In CUL, which is a community drinking water source, there is a significant seasonal human health risk associated with the fall cyanobacterial bloom.

Oligotrophic lakes in this study exhibited weak seasonal patterns, however, the interannual predictability was low. The observed patterns were not compliant with general models of seasonal succession in all lakes. This highlights the importance of regional variability and provides an impetus to examine the underlying cause of this difference. It was hypothesized that oligotrophic lakes in general are more susceptible to changes in allogenic forces, for example weather patterns, than are eutrophic lakes. In my study lakes the hypothesized influence of winter freshet and lack of ice results in active plankton communities throughout the year. The phytoplankton community of these lakes is therefore susceptible to selection processes throughout the year so that in spring different species combinations should exist each year. Conditions leading to the initiation of seasonal succession in Sommer et al.'s (1986) model in spring are suggestive of minimal competition with most variability being associated with the species composition of the previous fall (Lund 1979; Maberly et al. 1994). The lack of such conditions in the lakes examined during this study suggest, that in addition to species composition being associated with the factors considered in seasonal successional models, competition and allogenic factors during winter must also be considered. This condition, therefore poses the question as to whether successional processes ever truly have a starting point in these lakes. It could be argued that the starting point of Sommer et al.'s (1986) model coincides with the most predictable event (i.e. the spring bloom) and the patterns that follow are a consequence of this event. Using the same rationale in my study lakes, the starting point in CUL would occur in August at the initiation of the

fall cyanobacteria bloom and at the initiation of the winter bloom MXL. However, there appears to be no advantage to altering the current convention since there is no apparent improvement in the ability to predict species composition between years. While changing seasons constitute an integral component of succession in these lakes, there is no strong indication that successional patterns are reset to a similar starting point between years.

Table 4.1: Nonmetric multidimensional scaling stress values.

Kruskal's evaluative stress values (a). Stress values of analysis conducted in this study (b). Plots for algal groups and selected species are graphed using 3-dimensional data. Seasonal plots were graphed in 2-dimensions.

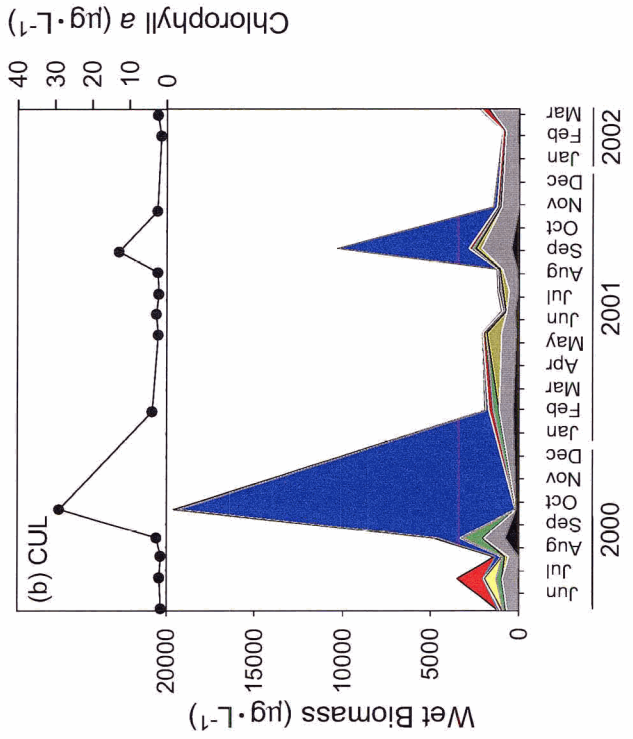
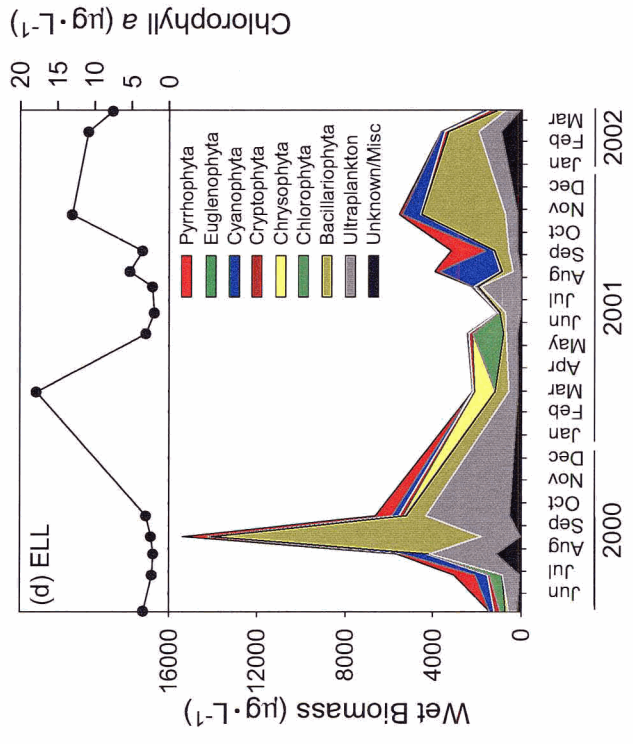
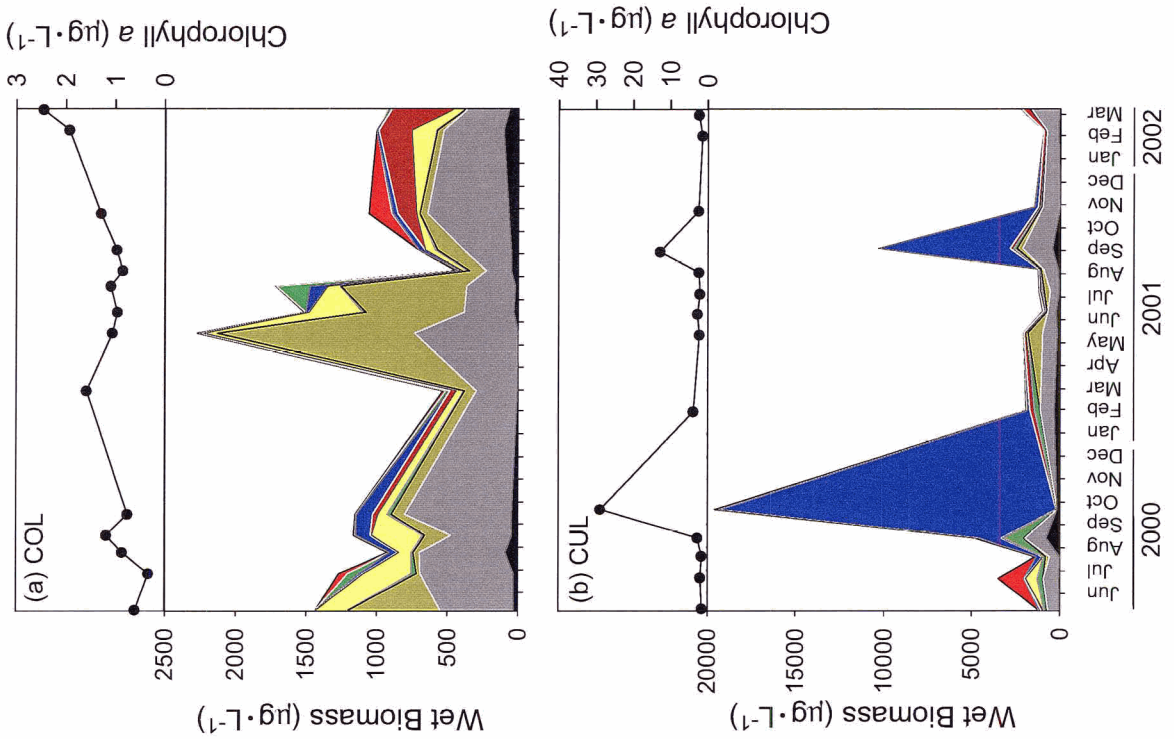
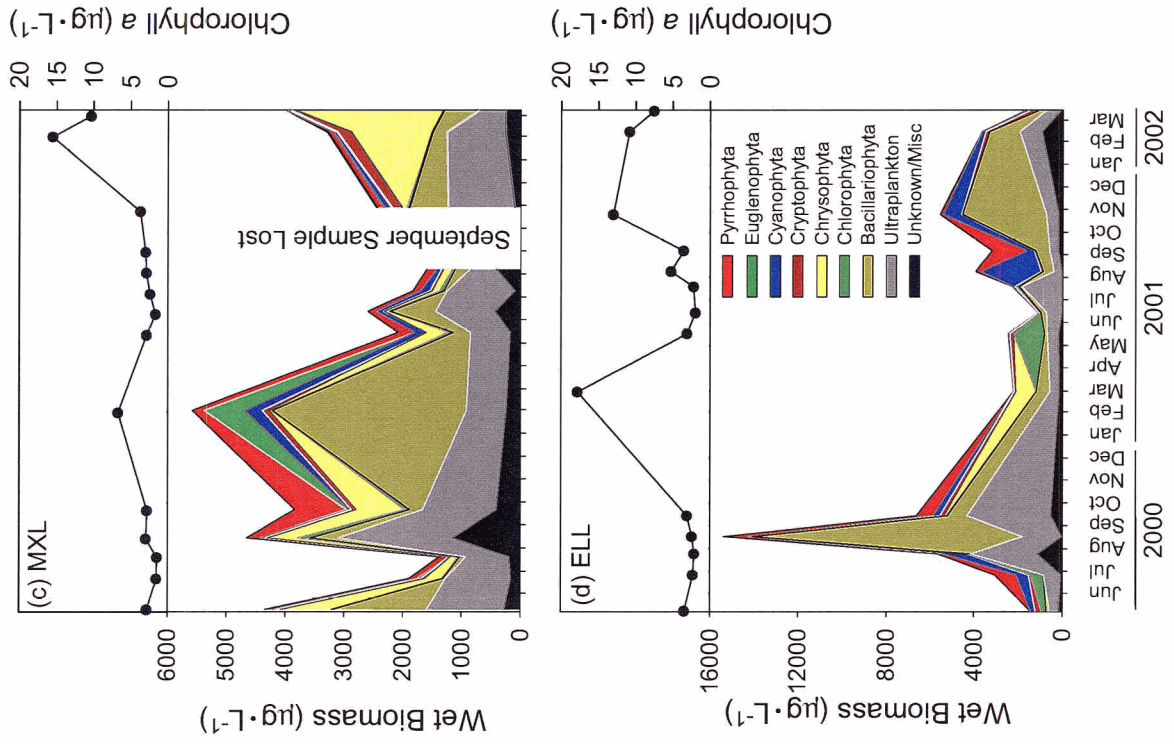
<b>(a)</b>			
Stress Values	Goodness-of-fit		
0.101 to 0.200	poor		
0.051 to 0.100	fair		
0.026 to 0.050	good		
0.001 to 0.025	excellent		
0	perfect		

<b>(b)</b>			
Analysis	2-Dimensions	3-Dimensions	4-Dimensions
Algal groups (Fig. 3)	0.104	<b>0.035</b>	0.003
Select species (Fig. 4)	0.190	<b>0.121</b>	0.087
Select species + nutrients (Fig. 5)	0.231	<b>0.179</b>	0.144
Select species + zooplankton (Fig. 6)	0.295	<b>0.245</b>	0.201
COL – seasonal (Fig. 8a)	<b>0.136</b>	0.088	0.046
CUL – seasonal (Fig. 8b)	<b>0.154</b>	0.064	0.036
ELL – seasonal (Fig. 8d)	<b>0.107</b>	0.059	0.022
MXL – seasonal (Fig. 8c)	<b>0.138</b>	0.074	0.043
SHL-S – seasonal (Fig. 8e)	<b>0.172</b>	0.101	0.065
SHL-N – seasonal (Fig. 8f)	<b>0.153</b>	0.095	0.053
SOL-S – seasonal (Fig. 8g)	<b>0.216</b>	0.148	0.106
SOL-N – seasonal (Fig. 8h)	<b>0.201</b>	0.118	0.067

Figure 4.1: Temporal trends in chlorophyll  $a$  (upper portion of each graph) and phytoplankton group biomass for each lake.

Council Lake, (a) COL; Cusheon Lake, (b) CUL; Maxwell Lake, (c) MXL; Elk Lake, (d) ELL, Shawnigan Lake south basin, (e) SHL-S; Shawnigan Lake north basin, (f) SHL-N; Sooke Lake Reservoir south basin, (g) SHL-S; and Sooke Lake Reservoir north basin, (h) SHL-N.



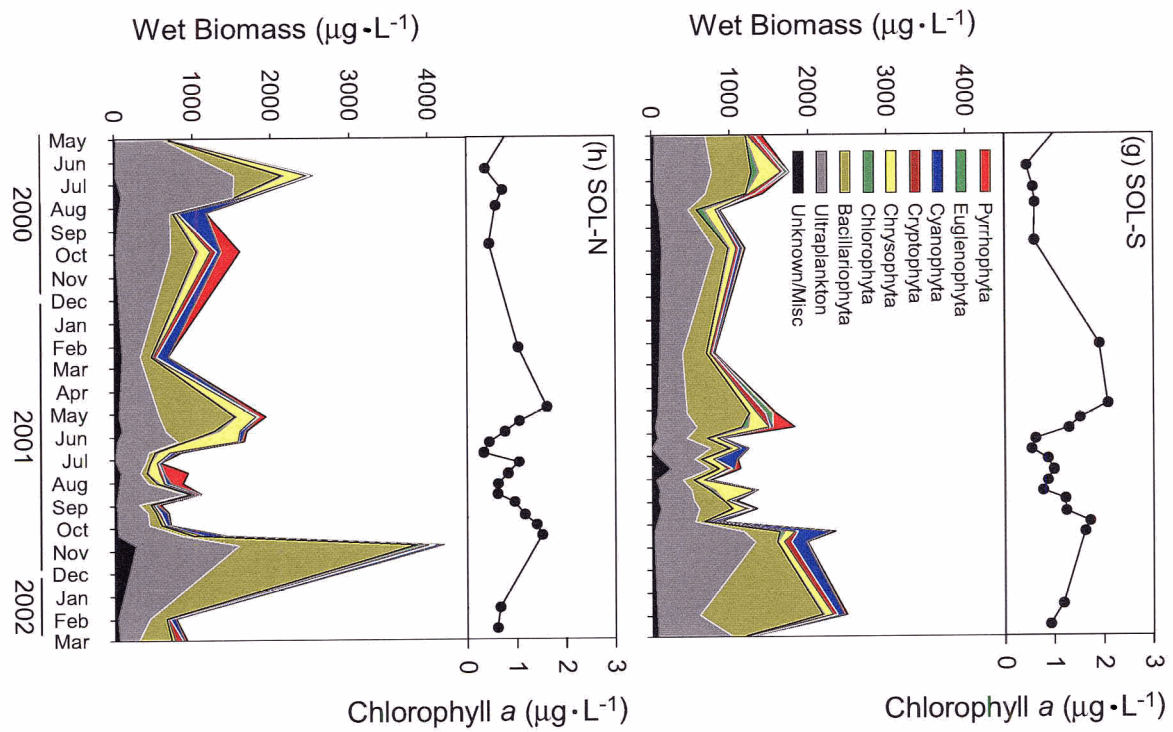
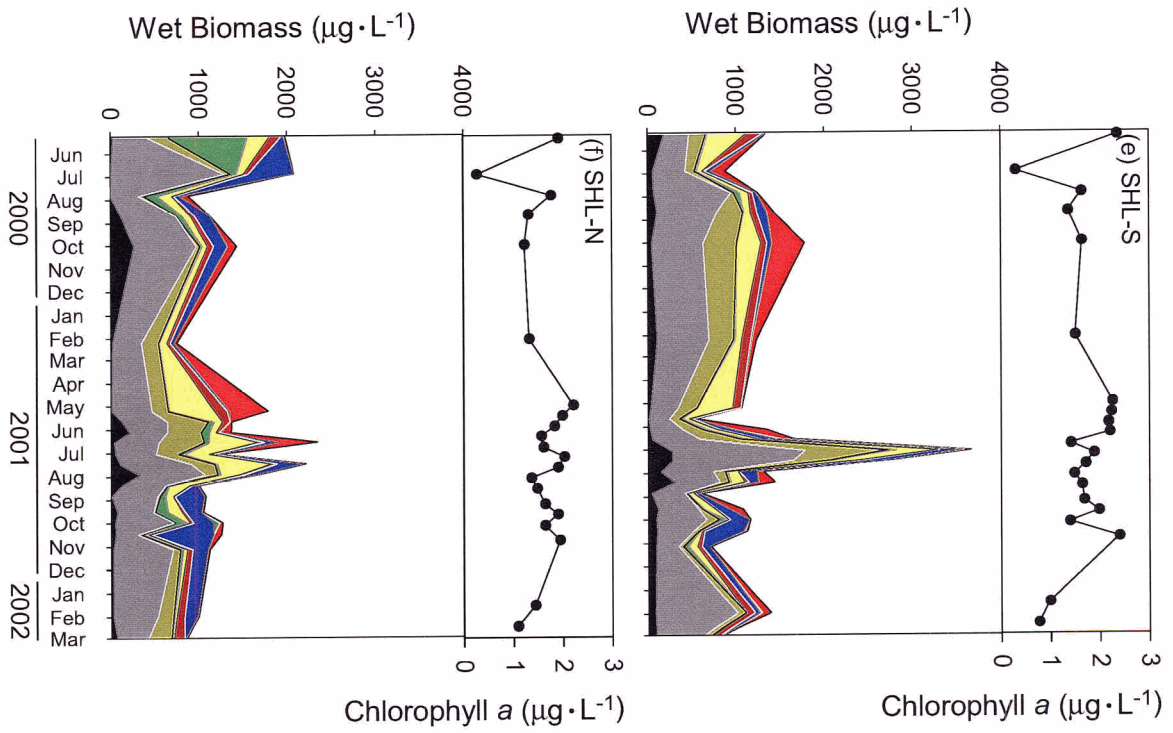


Figure 4.2: Temporal trends in dissolved nutrients; soluble reactive phosphorus (SRP), dissolved inorganic nitrogen (DIN) and soluble reactive silica (SRSI). SRP and SRSI are associated with the left-hand vertical axis and DIN is associated with the right-hand vertical axis. Note different scales. See Figure 1 legend for a summary of lake codes.

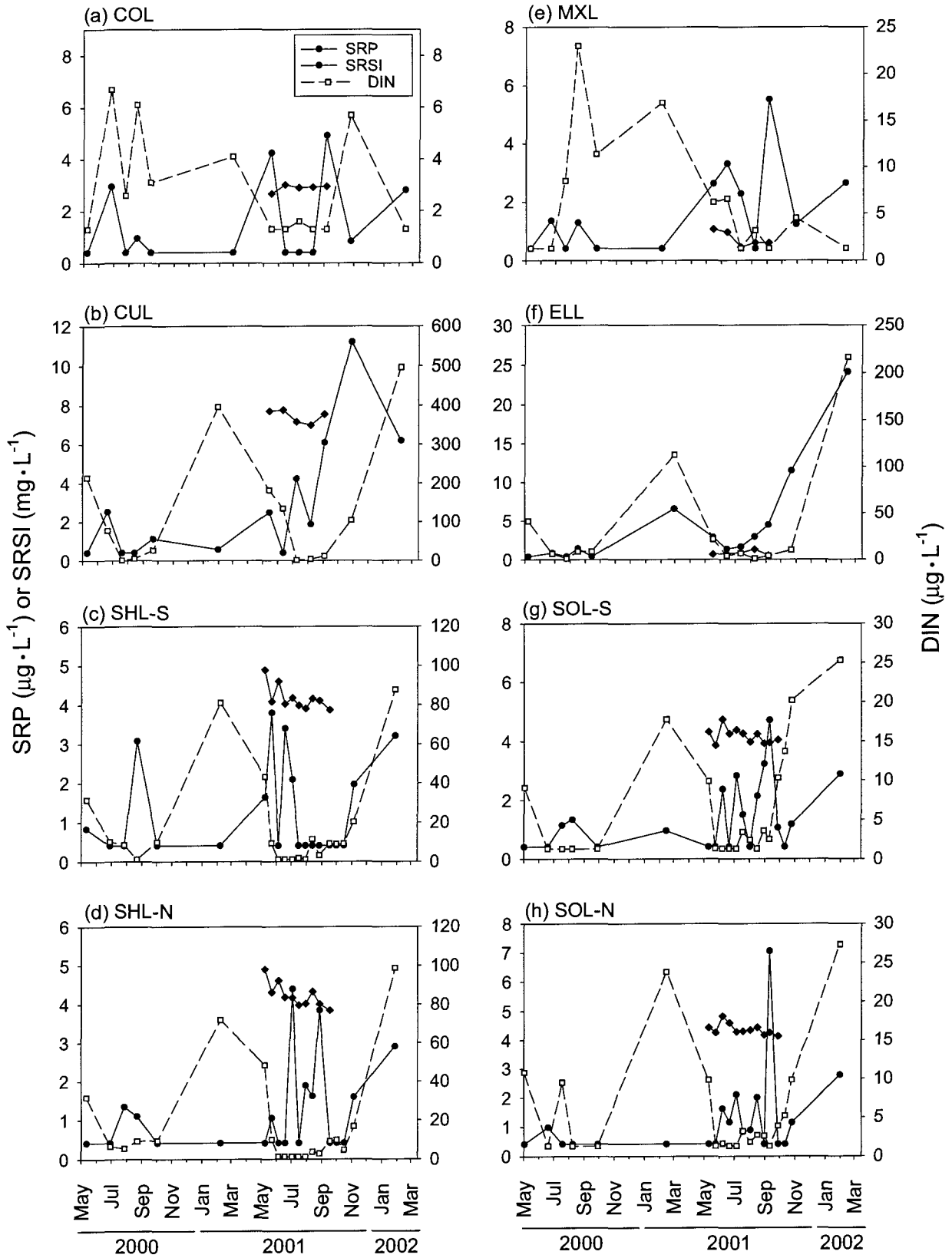


Figure 4.3: Nonmetric multidimensional scaling ordination for biomass abundance of algal groups.

Dimension 1 vs. Dimension 2 (a) and Dimension 1 vs. Dimension 3 (b). The proximity of each point to each other point in 3-dimensional space indicates the strength of their association.

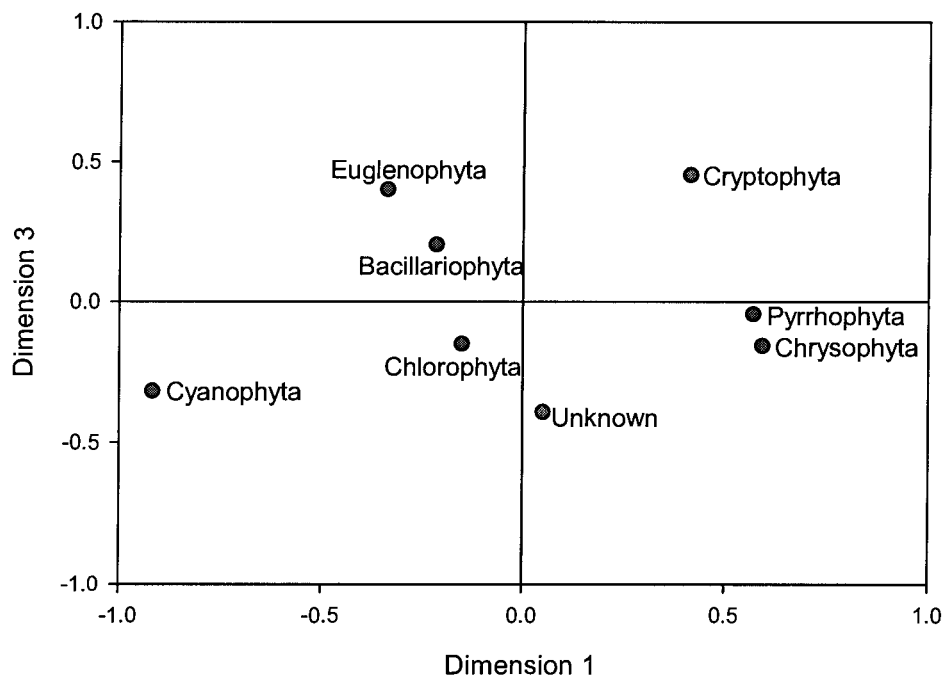
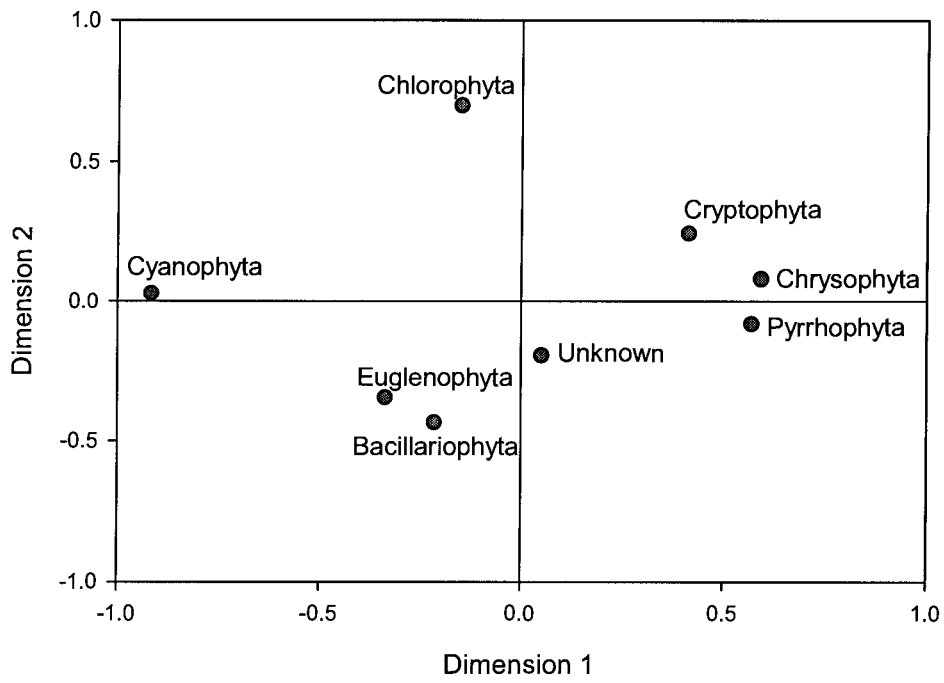


Figure 4.4: Nonmetric multidimensional scaling ordination for the biomass abundance of specific species.

See text for list of species. Dimension 1 vs. Dimension 2 (a) and Dimension 1 vs. Dimension 3 (b). The proximity of each point to each other point in 3-dimensional space indicates the strength of their association.

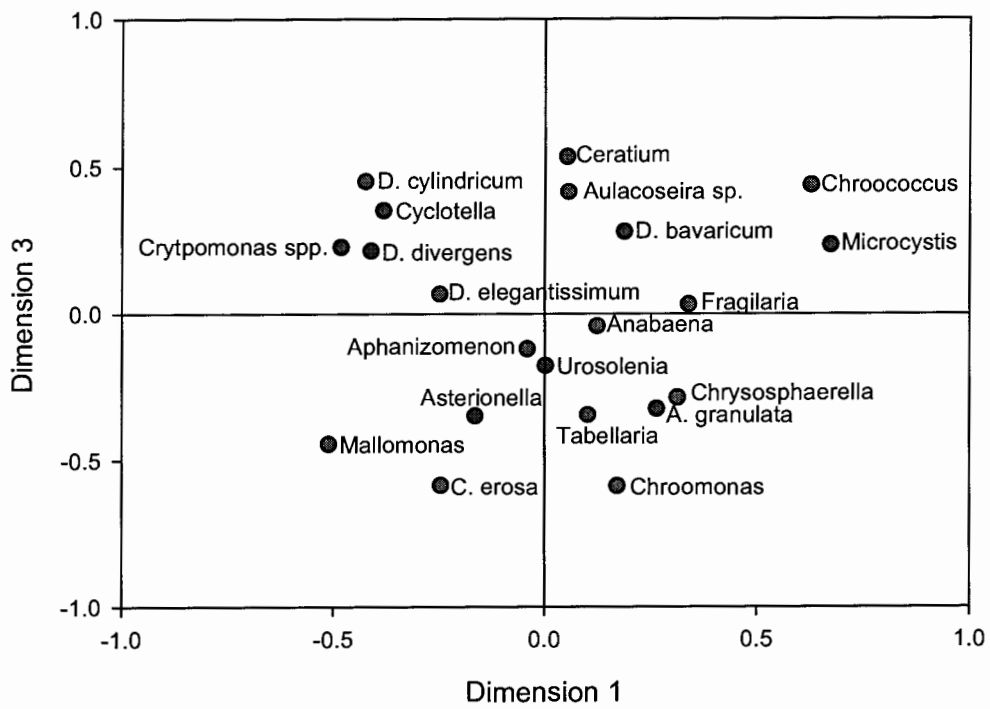
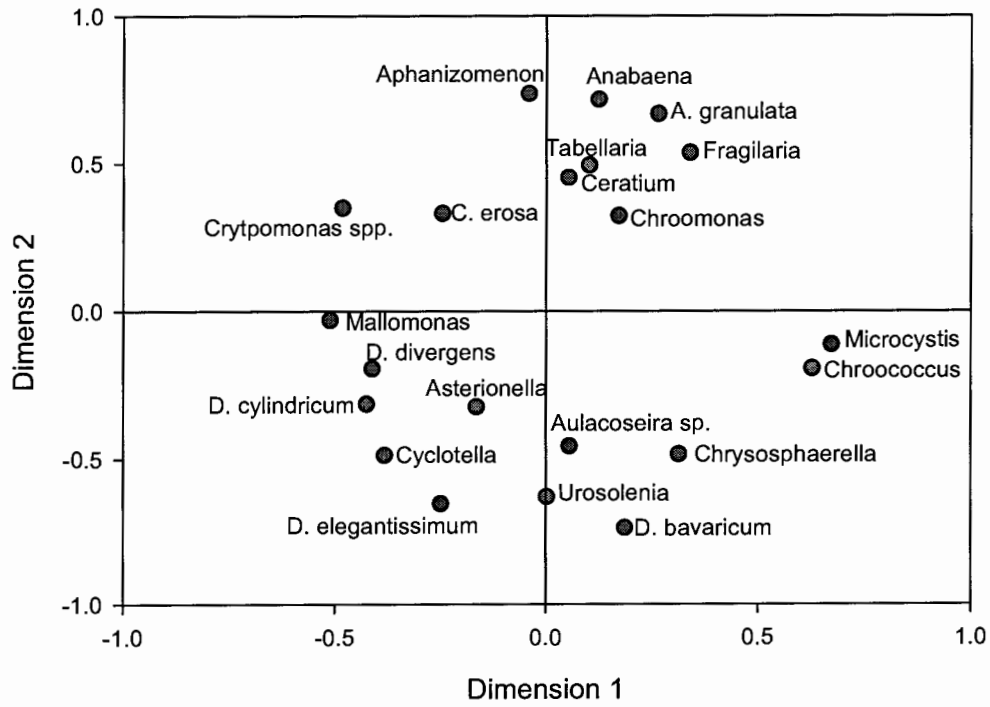


Figure 4.5: An example of two pairs of species found to be closely associated with each other from the NMDS analysis in Figure 4.4.

Closely associated pairs: *Microcystis* spp.–*Chroococcus* spp. (a) and *D. cylindricum*–*Cyclotella* spp. (b). Distally associated pairs: *Microcystis* spp.–*C. erosa* (c) and *Anabaena* spp. –*Cyclotella* spp. (d). Note that the strength of the *Microcystis*–*Chroococcus* relationship is primarily driven by the species composition in SHL (a). The *Microcystis*–*C. erosa* (c) relationship is an example of two species co-occurring in the same lake, but at different times, whereas the *Anabaena*–*Cyclotella* (d) relationship is an example of two species primarily occurring in different lakes. The data are ordered by date, however, the distance between each sampling date is equal for all dates (i.e. this is a line plot, not a scatter plot).

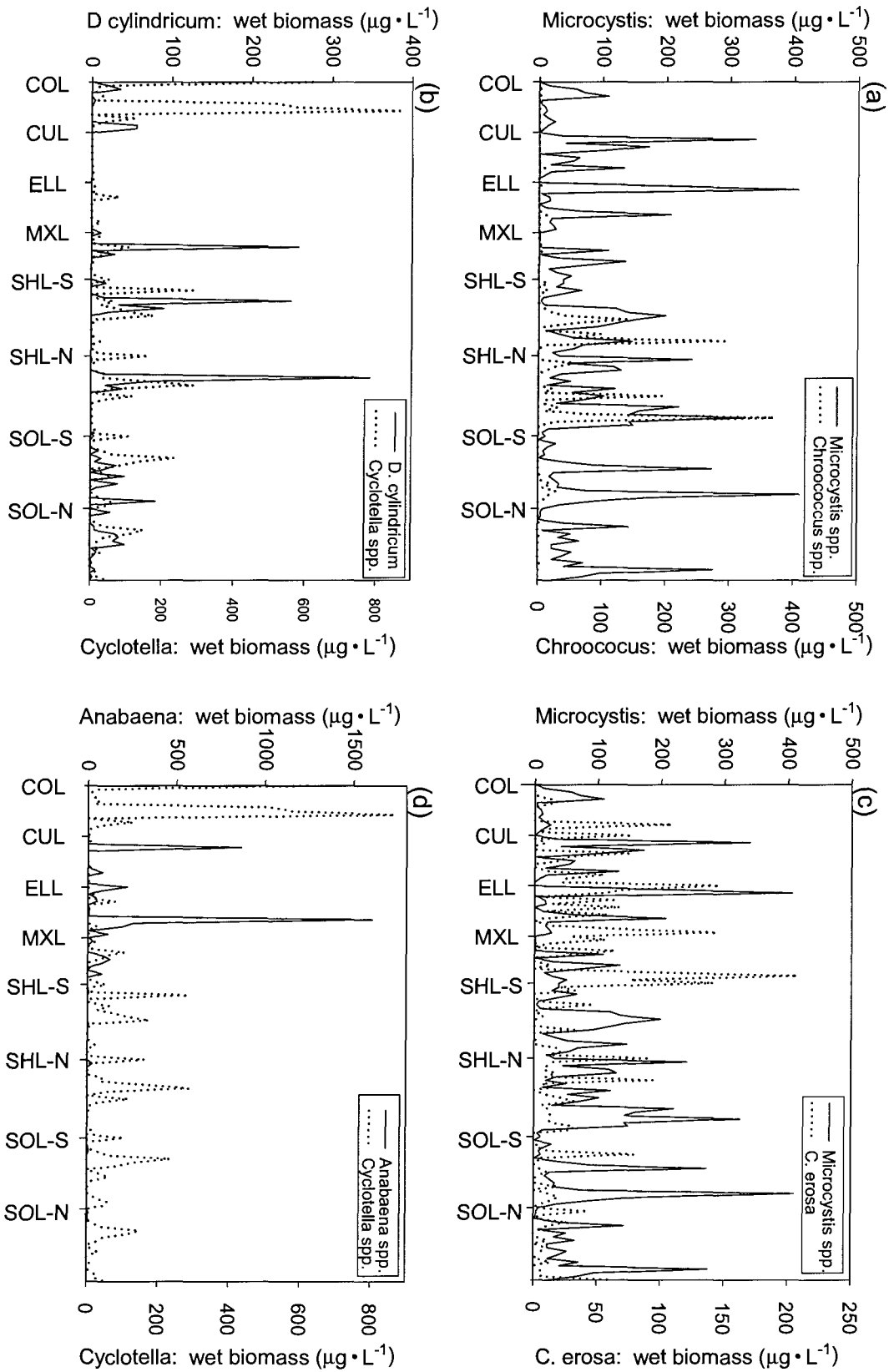


Figure 4.6: Nonmetric multidimensional scaling ordination for the select species and nutrients and nutrient deficiency indicators.

Dimension 1 vs. Dimension 2 (a) and Dimension 1 vs. Dimension 3 (b). The proximity of each point to each other point in 3-dimensional space indicates the strength of their association. See text for details.

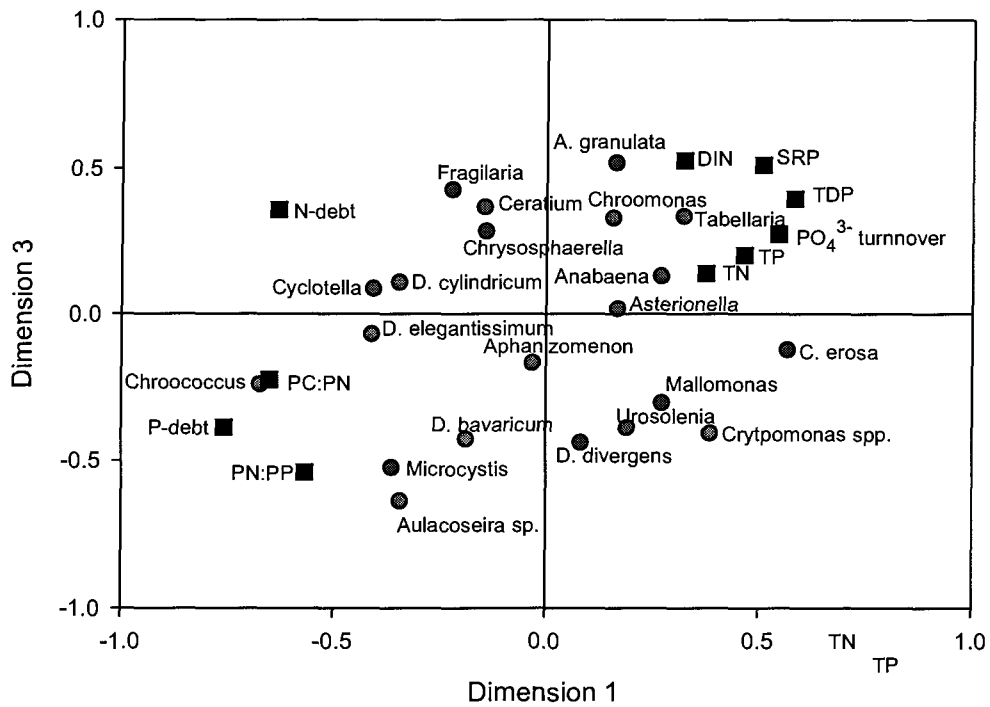
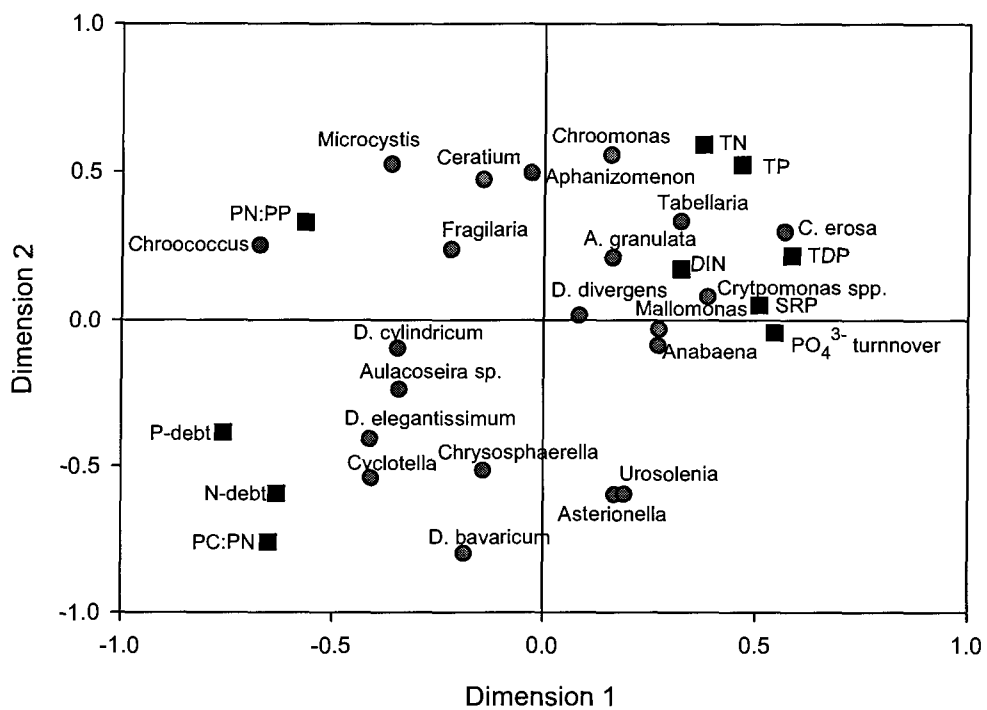


Figure 4.7: Nonmetric multidimensional scaling ordination for the select phytoplankton species and common crustacean zooplankton.

Dimension 1 vs. Dimension 2 (a) and Dimension 1 vs. Dimension 3 (b). The proximity of each point to each other point in 3-dimensional space indicates the strength of their association. See text for details.

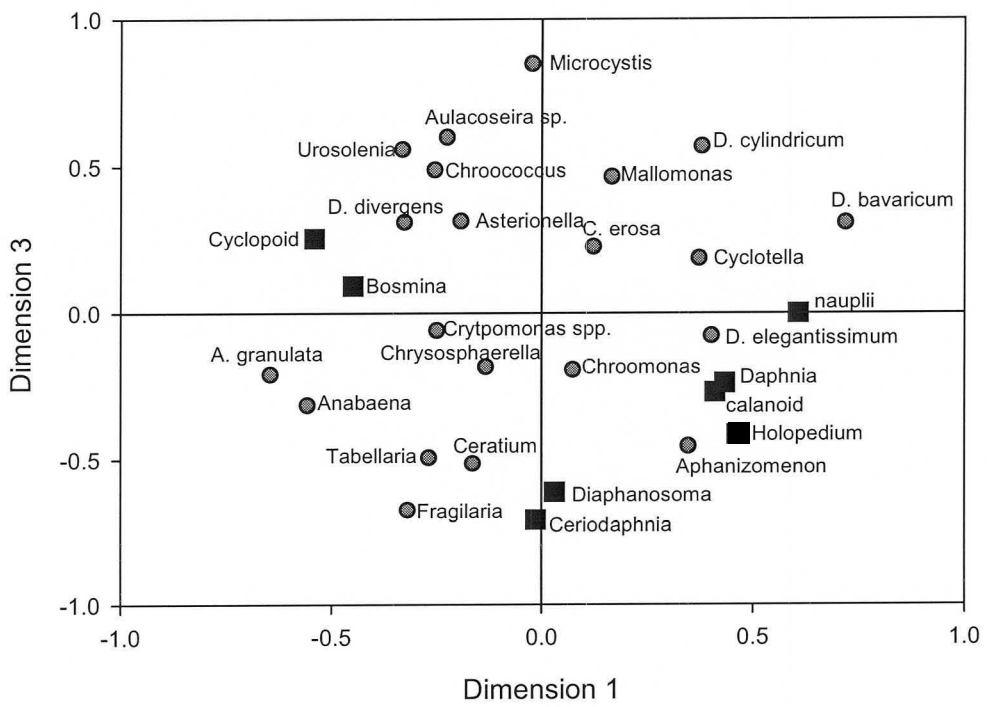
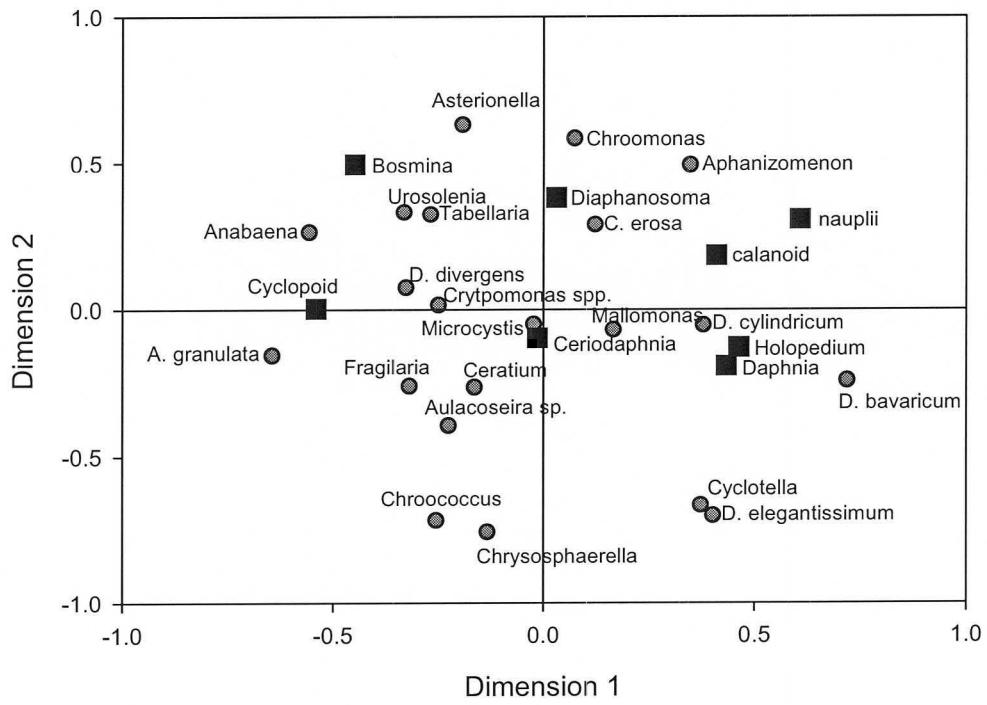
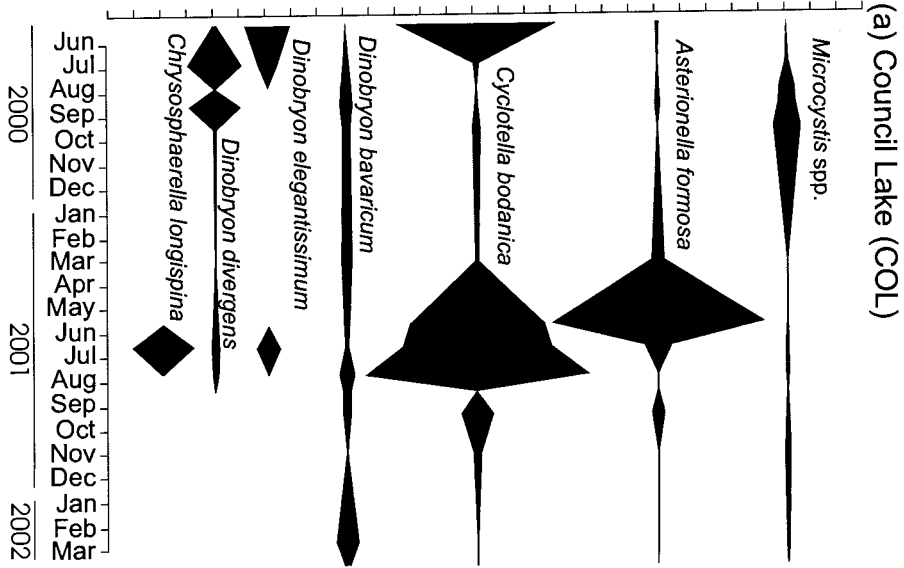


Figure 4.8: Temporal biomass trends for selected species in each lake.

Biomass units on the vertical axis, each scale unit represents the biomass indicated by the adjacent axis label. The width of the area plot for each species represents the biomass measured at that time. Note that for some species the scale unit remains the same, but the size of the scale units change. See Figure 1 legend for a summary of lake codes.

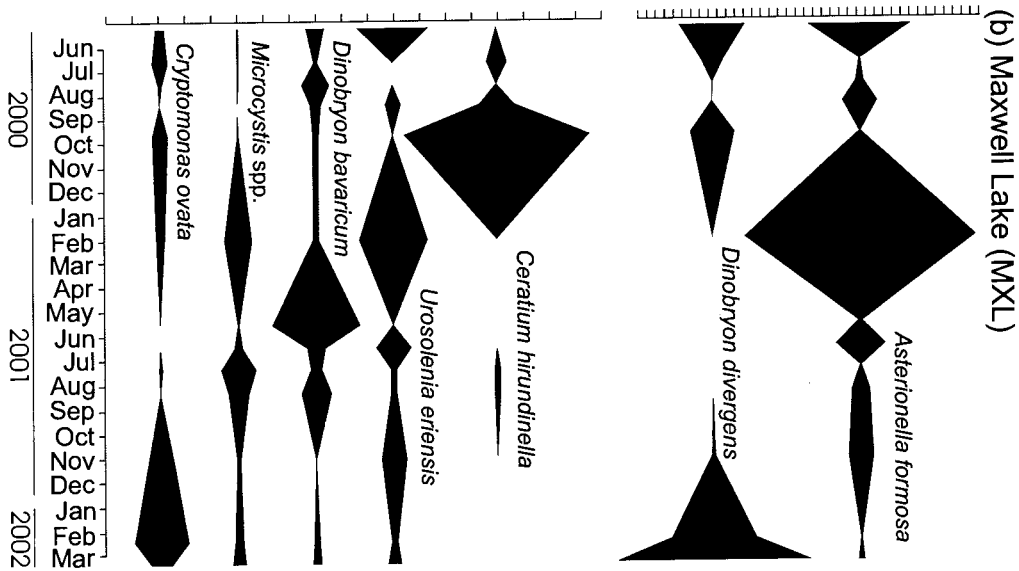
Each Scale Unit Represents Wet Biomass:

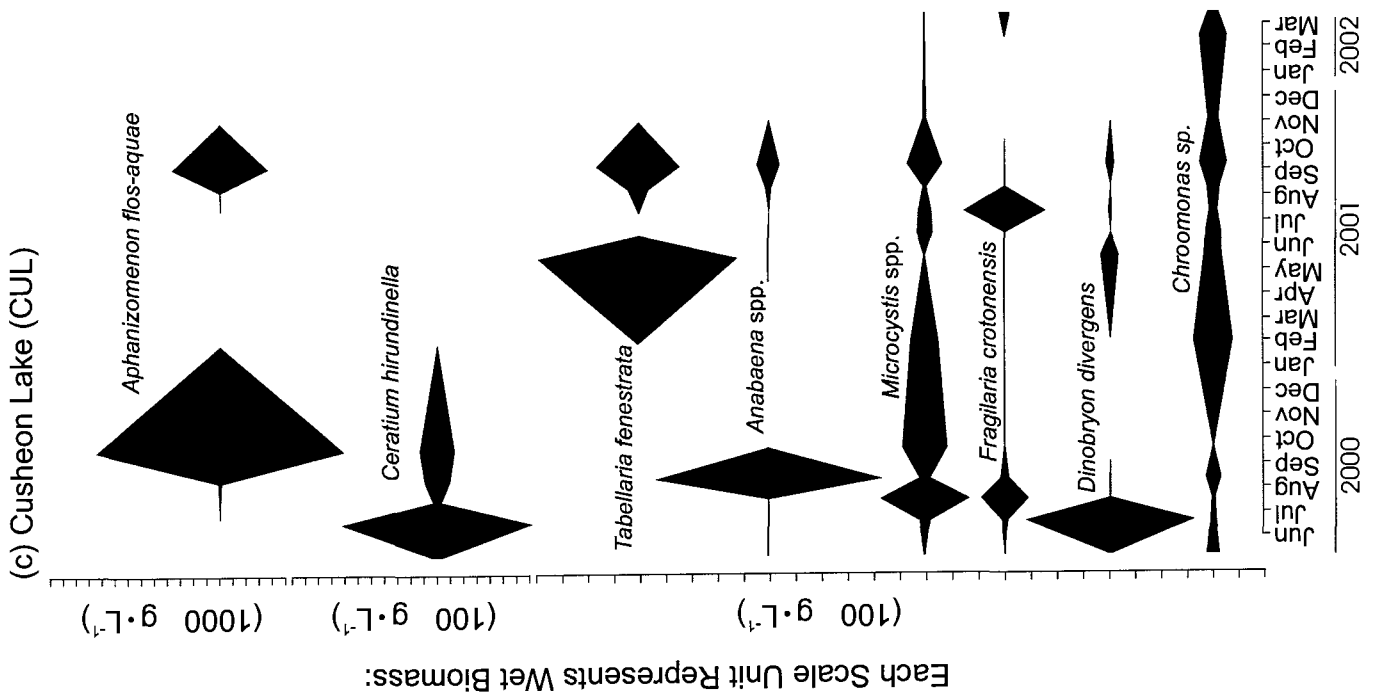
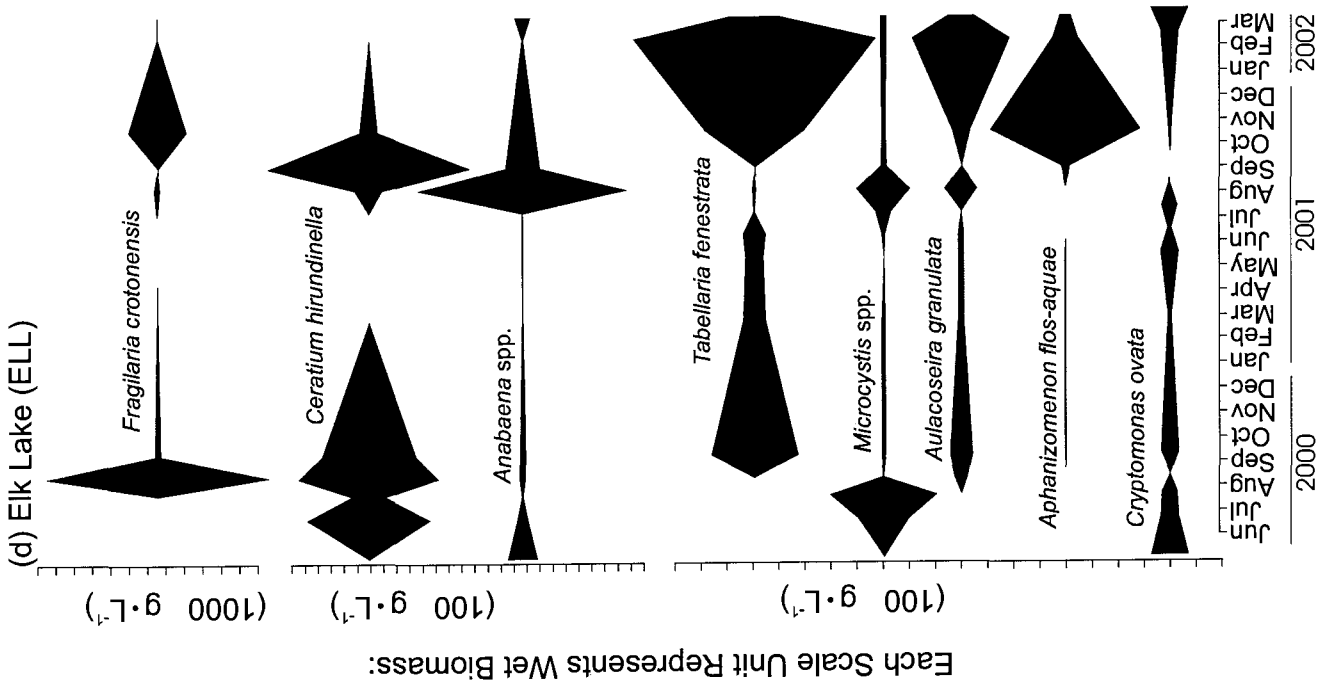
(100 g·L<sup>-1</sup>)

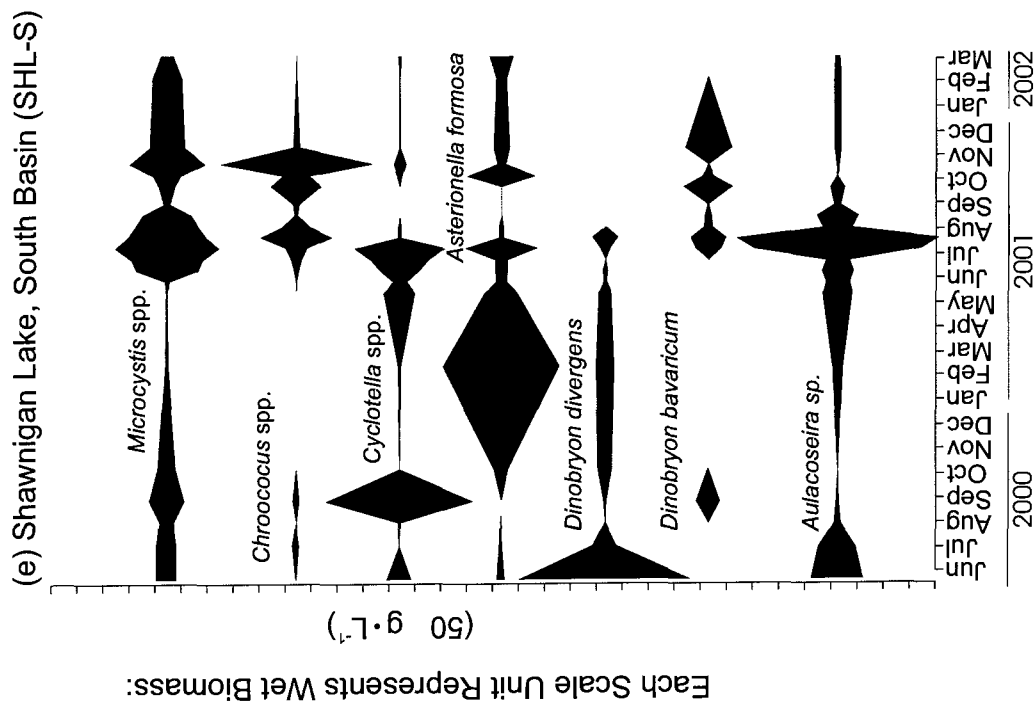
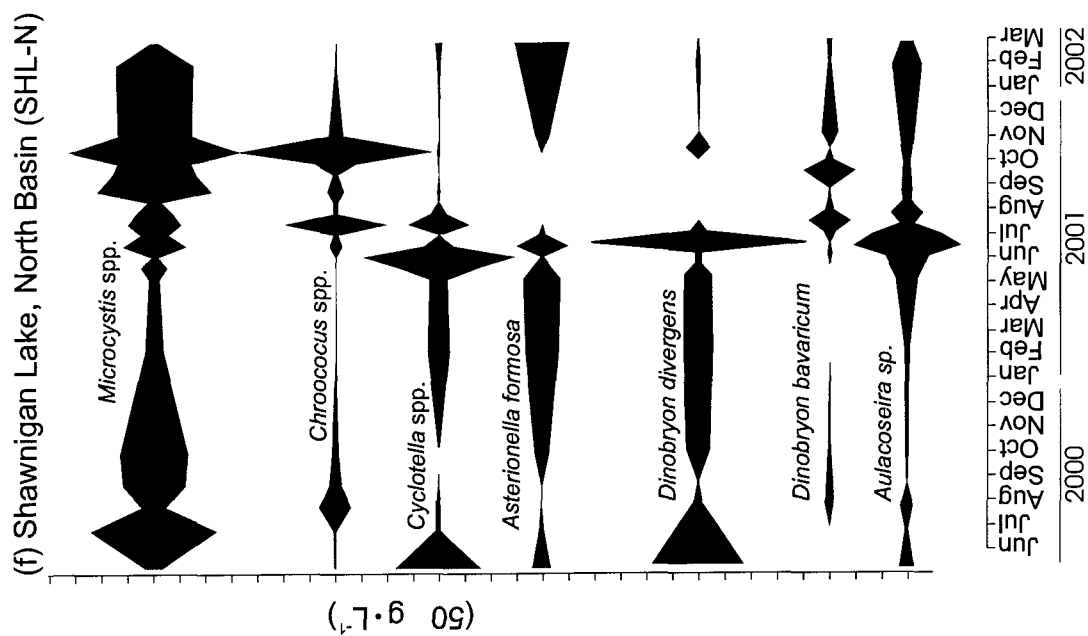


Each Scale Unit Represents Wet Biomass:

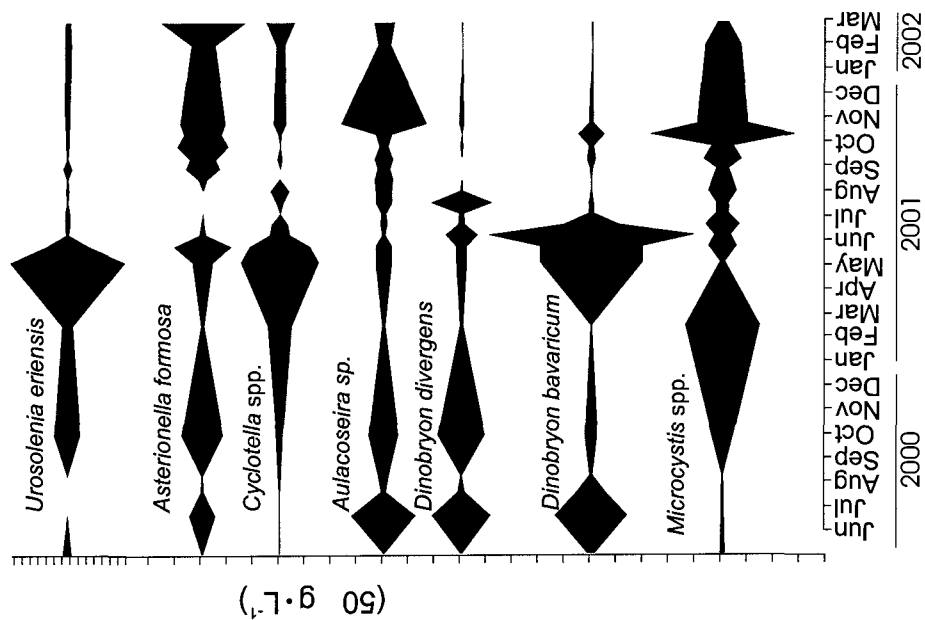
(100 g·L<sup>-1</sup>)





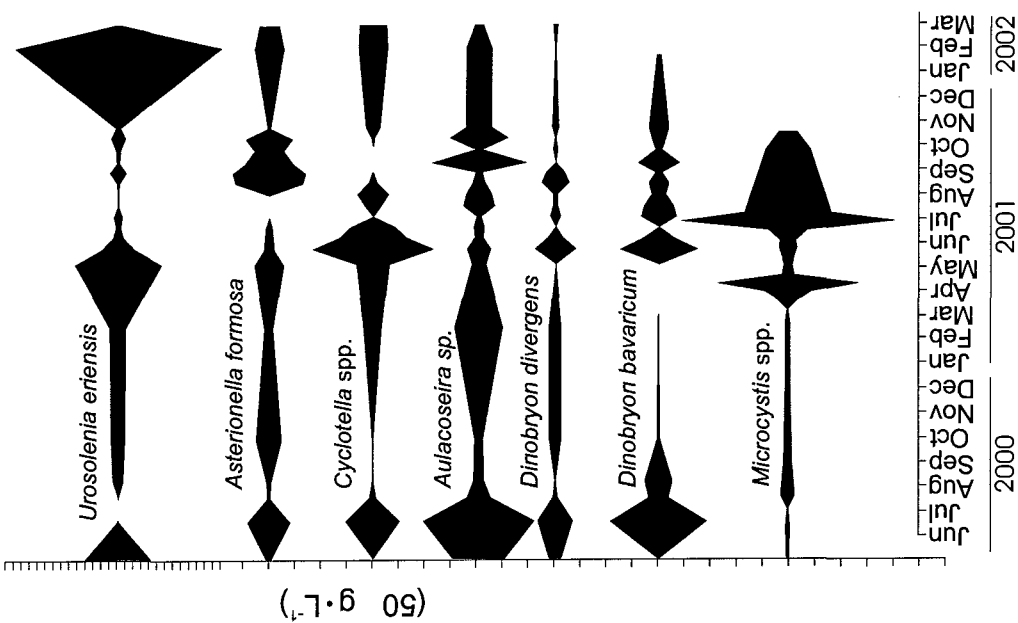


(h) Sooke Lake Reservoir, North Basin (SOL-N)



Each Scale Unit Represents Wet Biomass:

(g) Sooke Lake Reservoir, South Basin (SOL-S)



Each Scale Unit Represents Wet Biomass:

Figure 4.9: Seasonal nonmetric multidimensional scaling ordination for the biomass abundance of specific species for individual lakes.

Solid lines represent data from 2000 to winter 2001 (————) and dashed lines represent data from spring 2001 to winter 2002 (- - - - -). See Figure 1 legend for a summary of lake codes.

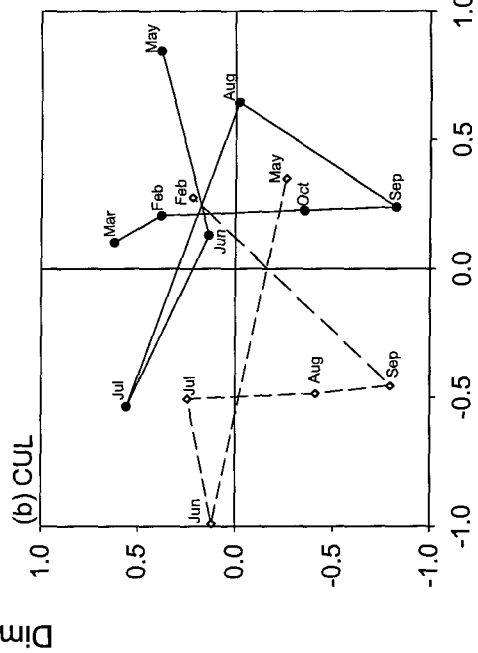
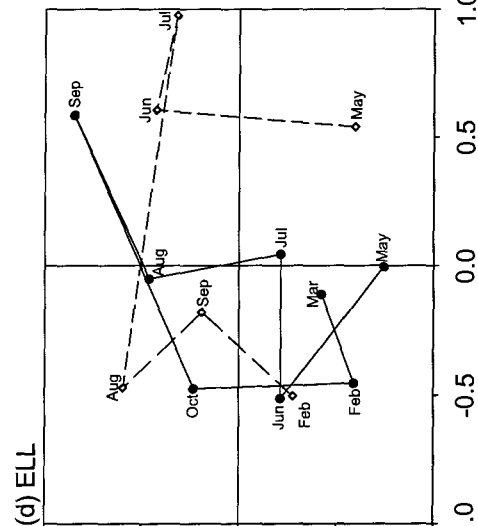
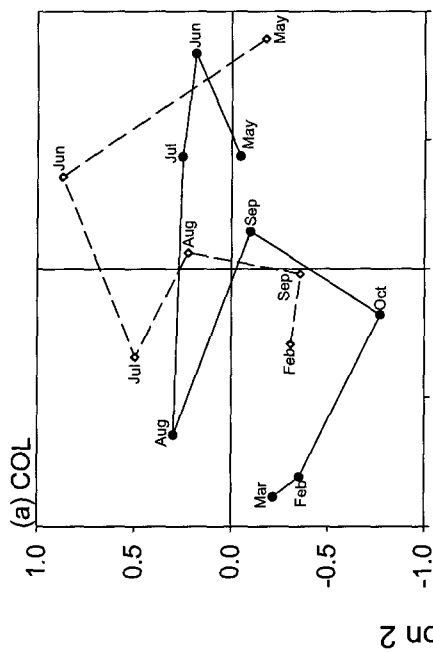
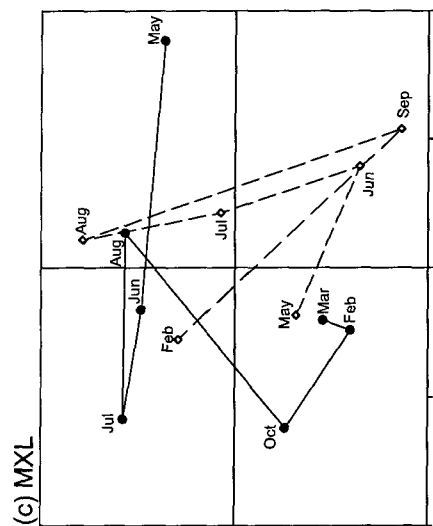




Figure 4.10: Temporal trends in N-debt, PC:PN ratios (C:N molar), P-debt and PN:PP ratios (PN:PP molar) for Cusheon (a, c) and Elk (b, d).

The dashed horizontal lines represents a nutrient deficiency threshold of  $0.15 \mu\text{mol N} \cdot \mu\text{g chl a}^{-1} \cdot \text{day}^{-1}$  (N-debt) and  $0.075 \mu\text{mol P} \cdot \mu\text{g chl a}^{-1} \cdot \text{day}^{-1}$  (P-debt). Dashed horizontal lines also indicate nutrient limitation thresholds for C:N ratios (14.6) and PN:PP ratios (22). Plankton communities with values above these lines are considered to be either nutrient deficient or nutrient limited. Threshold values are from Chapter 2.

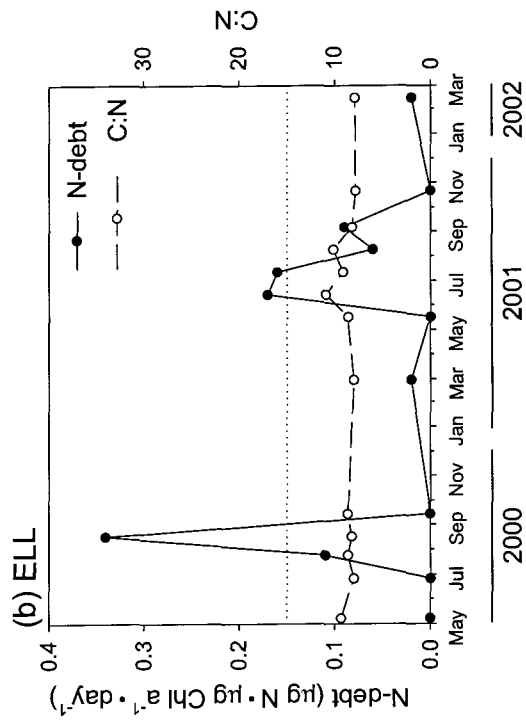
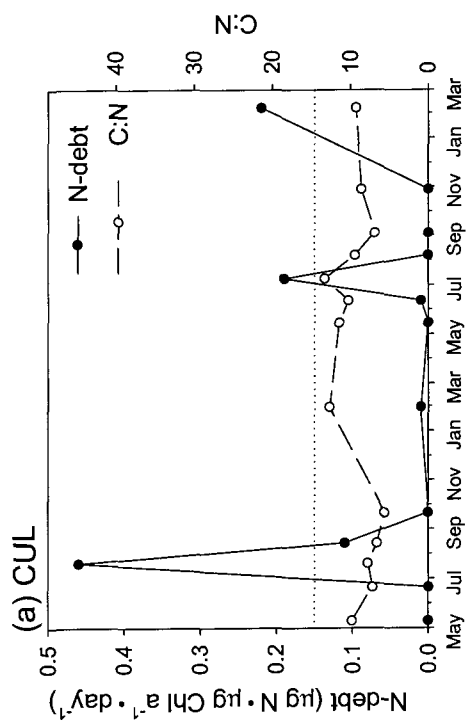
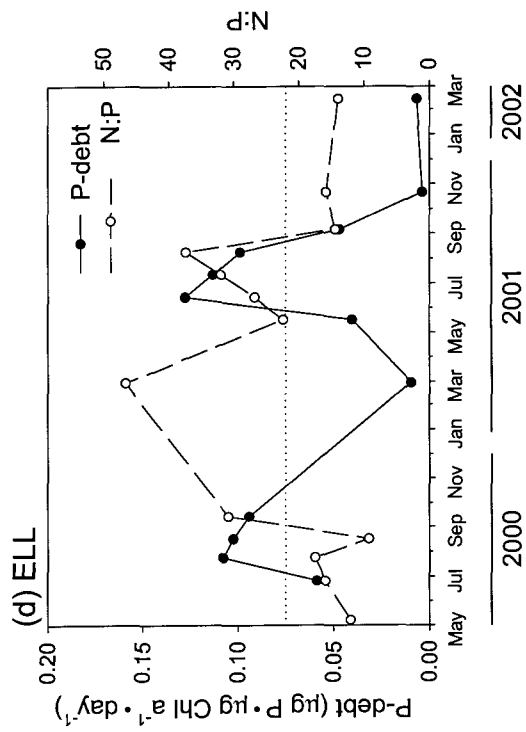
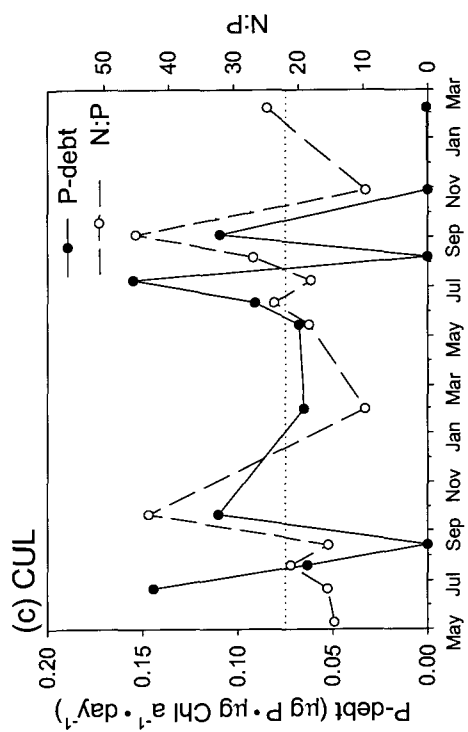
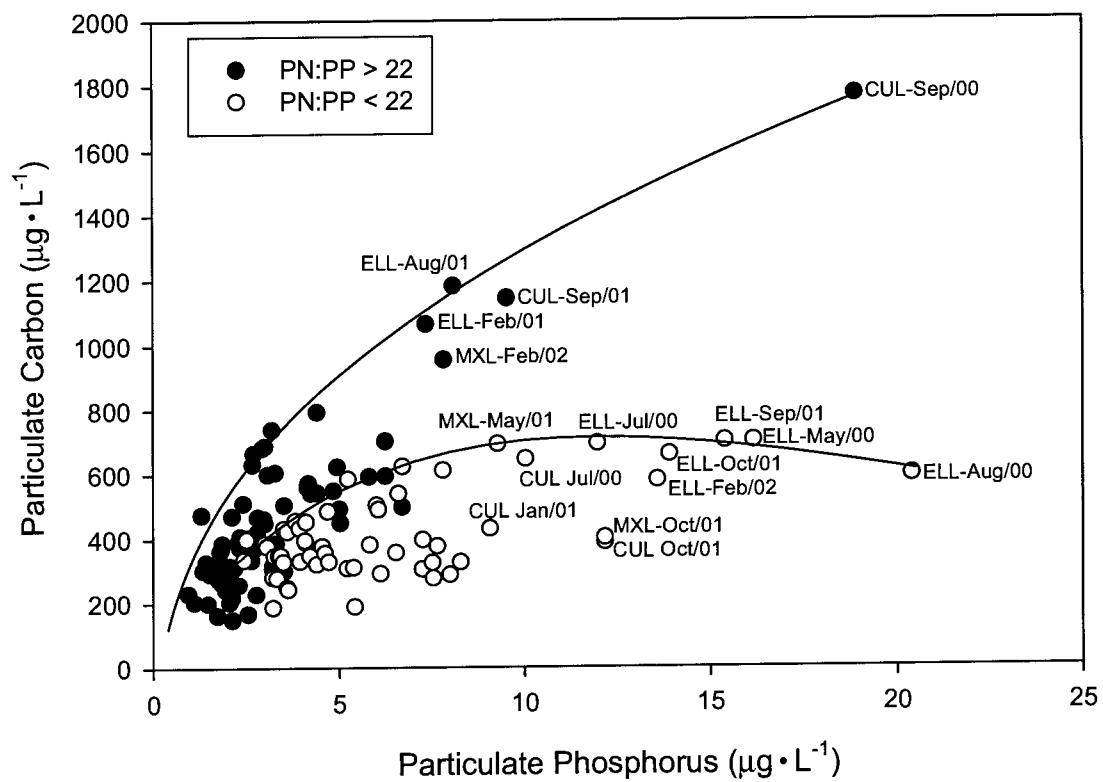


Figure 4.11: Particulate carbon as a function of particulate phosphorus.

Dark points represent samples with PN:PP ratios greater than 22 (molar) and clear points represent samples with PN:PP less than 22 (molar). Points greater than approximately  $10 \mu\text{g P}\cdot\text{L}^{-1}$  are labelled. See text for further discussion regarding dominant species present at these times.



**Chapter 5: Origins and implications of drinking water odours in lakes and  
reservoirs of British Columbia, Canada**

***Abstract***

The relationship between commonly measured limnological parameters and odours was examined in sixteen reservoirs and lakes used as sources for drinking water and three reference lakes. Odour analysis was conducted using flavour profile analysis (FPA) and, on select lakes, gas chromatography ion-trap mass spectrometry (GC-ITMS) for target compounds. Total phosphorus (TP) was the best single predictor of FPA intensity and multiple regression models accounted for 37-39% of intensity variance in the epilimnion and metalimnion respectively. Earthy odours were more prevalent in reservoirs and lakes with higher TP, whereas decomposing vegetation and green vegetation+grassy odours almost exclusively occurred when TP was lower ( $<13\mu\text{g P}\cdot\text{L}^{-1}$ ). Only geosmin was identified with GC-ITMS, and it was found to occur in lakes and reservoirs of higher trophic status (e.g. more algal biomass). Infrequent episodic events in the Greater Victoria's principal reservoir (e.g. algal blooms) have previously been linked with taste and odour problems in their tap water. However, analysis of odours under the conditions of this study (i.e. no strong odour episodes in the source reservoir) suggest that typical odours prevalent in tap water originating from Sooke Lake Reservoir are derived from treatment processes or the distribution system, not directly from the reservoir. This study demonstrates the utility of employing relatively simple and established methods to better understand management issues of a drinking water system.

### ***Introduction***

Controlling taste and odour forming compounds is an important management objective of drinking water suppliers. Consumer perception regarding the safety of drinking water is often based on odour, taste, and appearance (McGuire 1995; Levallois et al. 1999). Perceptions of health risks based on aesthetic properties may result in public demand for greater water treatment, even if actual risks are low (McGuire 1995). Tastes and odours can originate from algae in the source water, result from water treatment processes or develop in distribution systems (Suffet et al. 1995). If the principal taste and odour problems occur at the source, then prevention at the source is preferable to removal during treatment since successful removal of organic taste and odour compounds usually requires increased treatment. Treatment effectiveness for removing such taste and odour compounds varies depending both on the type and concentration of organic molecules present (Suffet et al. 1995).

Taste and odour analysis can be conducted analytically (e.g. gas chromatograph/mass spectrometer - GC-ITMS) or perceptually using trained human panelists (flavour profile analysis - FPA). FPA is based upon Weber-Fechner Law, which quantifies human perception using the method of "just noticeable differences" (Wright 1982). FPA assesses both the intensity (quantity) and description (e.g. earthy, grassy etc.) of tastes and odours. Researchers have advocated the use of both analytical and perception-based analysis for quantifying and understanding taste and odour origins (Rashash et al. 1996; Suffet et al. 1999).

In order to better understand source water taste and odour production in coastal and interior British Columbia (BC) I analyzed odour compounds in raw epilimnetic and

metalimnetic surface waters using FPA and GC-ITMS. Concurrently I measured several limnological and water quality parameters including total nitrogen (TN), total phosphorus (TP), dissolved (DOC) and total (TOC) organic carbon and chlorophyll *a* (chl *a*). I also examined the relationship between odours in source water and those in a distribution system for one of the study sites, the Capital Regional District Water Department, Victoria BC (hereafter referred to as “CRD”). The overall objective was to evaluate if water quality parameters, which are direct and inexpensive to measure, can be used to quantify, characterize, and manage taste and odour problems by identifying critical control points in water systems (Aust. NHMRC. 2002; *Chapter 6*). Critical control points are a component of the HACCP (Hazard Analysis Critical Control Point) quality framework established for the identification, assessment of risk, and control of food hazards. In particular, critical control point analysis allows for the system identification of the greatest potential sources of a specific risk.

### ***Methods***

This study was conducted during 2000 and 2001. Investigated lakes and reservoirs were located on the west coast and in the Kootenay region of BC. Drinking water reservoirs servicing cities and towns were Capilano, Coquitlam, and Seymour (Greater Vancouver), Sooke Lake Reservoir, Goldstream, Lubbe, Butchart, Japan Gulch, Council and Deception (Greater Victoria), Maxwell and Cusheon (Salt Spring Island), Jump Lake and South Forks (Nanaimo) and Phillips and Mark Creek (Cranbrook and Kimberley, respectively). In order to expand the range of lake types I also included Elk lake (Victoria), and Jimsmith and New Lakes (Cranbrook). FPA and limnological parameters were analyzed for all these lakes. Water from a subset of these water bodies

(Cusheon, Council, Elk, Maxwell, Sooke and Shawnigan) was used to test for the presence of specific target taste and odour compounds using headspace solid phase microextraction (SPME) method on a GC-ITMS in 2001.

Samples were collected from the epilimnion (integrated samples collected with a sampling tube, 2000 and 2001) and metalimnion (discrete samples collected with a Niskin bottle, 2001) at the point of maximum depth in each water body (two basins were studied in Shawnigan and Sooke) and placed in odour-free 500 mL glass bottles. Unfiltered samples were refrigerated (4°C) and usually analyzed within 24 hours of collection. *On a few occasions samples arrived at the CRD lab late Friday and were not analyzed until Monday morning.* The FPA panel consisted of three individuals who were all trained in the early 1990s and have been panel members since 1992 at the CRD water department. Analytical protocol followed the methods established at the Philadelphia Taste and Odour Workshop (Philadelphia 1989). Prior to analysis, samples were heated to 45°C in a water bath. Each panel member independently evaluated the odour intensity and descriptor for each sample. *Individual results were discussed and a consensus reached for each sample (Philadelphia 1989).* Odour intensity was ranked on a scale from 0 to 4, where 0 = no odour detectable, #1 = trace, #2 = weak, #3 = moderate, and #4 = strong. In order to quantify the FPA scale used by this panel, five standards at various concentrations were analyzed on four different occasions. The standards were: geosmin (earthy/beets = EA), 2-methylisoborneol (MIB, musty = MU), cis-3-hexen-1-ol (green vegetation+grassy = GV+G), dimethyl sulphide (decomposing vegetation = DV) and diphenyl ether (geranium = GM).

The trophic status of the reservoirs and lakes ranged from ultra-oligotrophic to meso-eutrophic, as reflected by summertime average TP (3.1 to 17.6  $\mu\text{g}\cdot\text{L}^{-1}$ ), chl *a* (0.7 to 6.0  $\mu\text{g}\cdot\text{L}^{-1}$ ) and Secchi depths (8.3 to 3.8 m). I included compounds produced by algal groups that tend to dominate both oligotrophic and eutrophic lakes (Table 1). Based on the range in trophic status in this study, several known algal-produced organoleptic compounds were chosen for GC-ITMS analysis. GC-ITMS analysis was conducted on a subset of lakes and reservoirs (Council, Cusheon, Elk, Maxwell, Sooke and Shawnigan). Immediately upon collection, samples were placed in 40 mL amber vials with Teflon-fitted caps and stored at 4°C. Method development and analysis was conducted by the Pacific Environment Science Centre, Environment Canada. Briefly; headspace SPME and a Varian Saturn 2000 high resolution gas chromatograph/low resolution ion-trap mass spectrometer (GC-ITMS) equipped with a DB-5 column (Supelco, 30m x 0.25mm, 0.25 $\mu\text{m}$  film thickness) were used to quantify taste and odour compounds. SPME fibres were 65  $\mu\text{m}$  polydimethylsiloxane-divinylbenzene (PDMS-DVB) (Supelco) and GC-ITMS analysis was conducted in full scan mode. Commercially available standards used are listed in Table 1 by their name and CAS numbers (except for 2t,4c,7c-decatrienal because no standard was available). Levels of quantification (LOQ) for each compound were determined based on spikes of standards in both distilled and lake water (lake water was first analyzed to ensure absence of these compounds). Method detection limits were lower (c.f. Furtula et al. 2004 for detection limits of this study); however, the greater matrix complexity of lake samples meant that quantification levels were higher.

Lake water collected at the same location and time as FPA samples was also analyzed for TN, TP, chl *a*, TOC and DOC (TOC and DOC from epilimnion only).

Temperature profiles (YSI Model 58) were used to define the epilimnion and metalimnion of the study lakes. TN and TP were analyzed using a Lachat automated ion analyzer (Zellweger Analytics, QuickChem 8000). TN was measured as nitrite after unfiltered samples had been autoclaved in an alkaline potassium persulfate solution and reduced using a cadmium column. Unfiltered samples for TP determination were autoclaved with potassium persulfate and analyzed as orthophosphate using the ascorbic acid method (APHA 1998). Chl *a* was determined by gently filtering samples through GF/F filters (Whatman), extracting chl *a* from the filters overnight at 4°C using 95% ethanol, centrifuging the extracted sample and then determining absorption using a spectrophotometer (*sensu* Wintermans and Mots 1965). TOC and DOC were both measured using an automated Shimadzu analyzer (TOC 5000) with a non-dispersive infrared detector. Analysis for TOC was conducted on whole water, whereas DOC was analyzed on the filtrate from water filtered through ashed GF/F filters (Pace and Cole 2002). Particulate organic carbon (POC) was calculated as the difference between TOC and DOC. True colour was determined spectrophotometrically on filtered samples at 455nm using a platinum-cobalt standard to generate the calibration curve.

### **Results**

I found a strong positive relationship between average TOC and average FPA intensity in the source water of reservoirs and lakes (Figure 5.1a). Typical of lakes, the greatest proportion of organic carbon was in dissolved form with the particulate fraction contributing, on average, less than 10% of the total. There was also a strong relationship between average TP and average FPA intensity and average chl *a* and average FPA

intensity (Figures 5.1b, 5.1c). FPA intensities from 0 – 3 were higher in the epilimnion for a given TP and chl *a* concentration than were those of the metalimnion.

To determine which of the measured limnological variables explained the most FPA variance I conducted a multiple regression with TP, TN, chl *a*, and Secchi depth (DOC was not included for reasons discussed below). Using backwards regression ( $\alpha=0.95$ , removal criterion  $p>0.10$ ) only chl *a* was dropped from the epilimnetic model ( $r^2=0.37$ ), whereas TN and chl *a* were dropped from the metalimnetic model ( $r^2=0.39$ ). TP and TN were the greatest standardized coefficients in the epilimnetic equation and TP was the greatest in the metalimnetic equation. Thus, of variables used, TP was the most parsimonious predictor (strongest single predictor) of FPA intensity.

In order to further understand the relationship between FPA and TP, I chose the most common odour intensities (EA, DV, and GV+G) and used standards to quantify the FPA values (Figure 2). Changes in human perception to odour intensity is logarithmic in nature because as odour intensity increases it requires a greater change in odour to sense the change (Wright 1982). The relationship between FPA standardized intensity and TP in these lakes and reservoirs was different for each odour (Figure 3). When EA odour was detected it was usually present at higher odour intensities (FPA #3 and #4), while the converse is true of GV+G and DV (FPA #1-#3). Thus, there were few cases recorded with odour intensities of #1 for EA and #4 for GV+G and DV. Average TP was similar for EA FPA #1 and #2, and slightly higher for #3. With the exception of one high value that occurred during winter in a more eutrophic lake, DV was relatively constant for a given TP. GV odour increased with TP from FPA #1 to #3, however, the average TP was

lower at high odour intensity (these two samples were recorded in winter and early spring).

The only taste and odour compound identified in the samples using GC-ITMS was geosmin. Not surprisingly geosmin was found in lakes and reservoirs with higher nutrients and higher chl *a* and most often with FPA designations of earthy, at a scale of 4 (Figure 4). GC-ITMS geosmin concentrations are represented as round points with corresponding values on the left hand axis and the FPA scores are shown as square points. Geosmin was also identified once in Council Lake (not shown). In metalimnetic samples, geosmin was identified twice in Cusheon, three times in Elk and once in Maxwell. No geosmin was detected in either Shawnigan or Sooke.

*Source water vs. distribution taste and odour:* The study system for comparing source (reservoir) and tap water odours was Sooke Lake Reservoir. Sooke is a unique drinking water source system because the city of Victoria owns 98% of the watershed and, therefore, controls all access and activities that occur in the watershed. (It is off-limits to the public and there is no fishing, mining, logging, farming, or ranching. No fertilizers, pesticides or herbicides are used, and it is off limits for float planes). Previous taste and odour events have been linked to source water quality (MacKay 1988), so the city is interested in understanding source water odour production as part of its multibarrier drinking water protection plan. The reservoir has a relatively rapid flushing rate (ca. 1 year), typically filling in the fall and winter months during the rainy season. The drinking water intake in Sooke Lake Reservoir is at the south end in a relatively small shallow basin (45 ha, 22m) compared to the north end (434 ha, 70 m)<sup>1</sup>. An old dam

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<sup>1</sup> Morphometric values for Sooke Lake Reservoir are valid from 1989 (when the last small volume increase was made prior to this study) to 2003 when the dam was raised between five and six meters.

immediately in front of the current dam means that drinking water withdrawal is effectively derived from the epilimnion and metalimnion of the south basin (Nowlin et al. 2004).

The predominant south basin odours of the epilimnion and metalimnion in 2001 were Green Vegetation (31%), Decomposing Vegetation (25%), and Hay and Grassy (each 16%). For most of the year the FPA intensity was #2. However, for the five samples taken during the summer (July & August) FPA intensity was #1 for all dates except one (Green Vegetation = #2, early August). Samples during the same period from various sites in the distribution system (Figure 5) were dominated by chlorine (39%), metallic (28%), odour free (13%), musty (10%) and chalky (5%) odours.

### ***Discussion***

I show that basic water quality parameters like TP, TN, and Secchi disk measurements are robust indicators of the odour types and intensities found in the drinking water sources of this study. This observation reinforces the critical need to understand the processes and factors regulating basic water quality parameters of source water. DOC/TOC, like TP, were similarly related to FPA. However, natural organic matter is a complex pool of organic molecules originating from different sources in the watershed and requires further studies.

Previous researchers have demonstrated strong relationships between species of algae and odour intensity, both in culture and in situ (e.g. Rashash et al. 1996; Watson et al. 1999). Smith et al. (2002) found a strong correlation between algal biomass (measured as chl *a*) and geosmin production in a hypereutrophic reservoir in Kansas and identified phosphorus reduction as the principal means of reducing taste and odour

events. This study further extends these results by finding a strong relationship between TP and both odour type and intensity at a lower range of TP (3.1 to 17.6  $\mu\text{g}\cdot\text{L}^{-1}$ ) than is typically studied (e.g. Smith et al. 2002). I found a shift in odour predominance; earthy odours were most commonly associated with high FPA's, whereas green vegetation + grassy odours and decomposing vegetation odours were most common at low FPA's (Figure 3). Different lakes exhibited intermediate scores for both earthy and "vegetation"-based odours, presumably due to the plankton species composition. These results implicate algal biomass as the critical predictor explaining odour intensity. The multiple regressions only explained between 37-39% of the variance so, although it was significant, much of the variance was unexplained. This unexplained variance is presumably largely explained by both algal species composition and algal growth conditions.

The relationships between TP and chl *a* vs. odour intensity were distinctly different in the epilimnion and metalimnion. Light is usually low in the metalimnion so algae will be expected to be low-light adapted, thus having a greater concentration of chl *a* per unit biomass than algae in the epilimnion. Odour production may also be different because different algal species typically predominate in the epilimnion and metalimnion.

There were greater occurrences and higher intensities of earthy odours detected using FPA than could be solely attributed to measured geosmin concentrations. In surface waters there are many odiferous compounds that may act synergistically increasing human perception. Geosmin and MIB are also not the only earthy/musty compounds (Young and Suffet 1999). Both earthy and musty odours are associated with

cyanobacteria, however, geosmin is typically associated with pelagic environments whereas MIB is linked to littoral production (Ridal et al. 1999), which may explain why only geosmin was found in this study. Other target compounds may have been present, however, they were not identified because they were below the method detection limit.

The trophic status of many coastal BC lakes and reservoirs makes them desirable drinking water sources. However, consumers are still sensitive to drinking water quality issues and drinking water suppliers are keenly aware of possible issues arising from concerns derived from consumer perceptions (e.g. odour, taste, colour). Odours detected at the end of the distribution system were not found to occur in the source-water reservoir. Chlorine and metallic odours are typically attributed to chlorination byproducts. Musty odours can also originate from the small distribution reservoirs built to allow for daily fluctuations in water use. The study of Sooke Lake Reservoir suggests that odours in the distribution system were principally derived from either treatment or the distribution system (i.e. chlorine, metallic, and musty odours associated with supply system reservoirs). However, lower threshold odours and phytoplankton blooms from the source reservoir may also contribute to tap water odours.

Source water conditions such as DOC and POC concentration, temperature and pH all influence potential odour production during treatment and distribution. In Sooke at the time of this study, the principal odours in the distribution system were not directly linked to the source water. Current expansion of the reservoir (raising of the dam by ca. 5-6m) may have short-term implications as more nutrients become available and littoral decomposition rates increase. Future water management considerations, including water diversion from an adjacent watershed may also affect odour production both directly in

Sooke Lake Reservoir, and, due to potentially greater concentrations of DOC that can form odour compounds precursors during treatment and distribution.

Most organic carbon in lakes is dissolved (e.g. Figure 1) with DOC typically constituting between 83 – 91% of total carbon (Wetzel 2001), so it is critical to understand the sources of DOC and the relationship with odour compounds. Watersheds are usually the source for the majority of organic carbon in lakes and reservoirs. The timing of allochthonous carbon input is often strongly related to climatic variables (Pace and Cole 2002). One means of determining the timing of terrestrial DOC inputs is to measure colour, since many allochthonous carbon compounds have an associated colour (e.g. humic and fulvic acids). A significant cross-correlation was found between precipitation and true colour in Sooke from 1992 to 2001 (Figure 6). Although colour was only measured in Sooke, coastal lakes in this region should receive the majority of allochthonous carbon input during the first half of the year, since lakes in the same geoclimatic zone generally display synchrony in timing of DOC inputs (Pace and Cole 2002).

The input of new allochthonous carbon during winter and early spring (Jan. – May) may contribute to some odour compounds (Thurman 1985), however, it likely plays a negligible role for the remainder of the year. When allochthonous carbon first enters aquatic systems any labile components are quickly degraded. The DOC that remains in the water column for relatively long periods of time is typically recalcitrant (Wetzel 2001; Leenheer and Croué 2003). The reduction of coloured DOC is usually attributed to bacterial degradation or photodecay (Molot and Dillon 1997). Therefore, a large proportion of DOC is composed of larger molecules that have long turn-over times and

low vapour pressure. In comparison autochthonous DOC has lower molecular weight and higher turnover rates. Because of the different rates of production and degradation of allochthonous and autochthonous DOC, direct comparisons of abundance do not reflect the importance of each to the production of odour compounds or to the energetics of the microbial community. Due to the production rate of autochthonous DOC and higher molecular weight of allochthonous DOC I argue that the relationship between FPA intensity and TP more accurately reflects the principal mechanism of odour production in my study lakes and reservoirs.

TP was most strongly associated with increasing source water odours in this study. Algal biomass increased with increasing nutrients (especially TP) and odour types shifted from grassy-type odours to earthy-type odours. This represents a shift in the spokes of Suffet et al.'s FPA wheel from "grassy/hay/straw/woody" to "earthy/musty/moldy" (Suffet et al. 1999). As nutrients, in particular phosphorus, increase algal biomass generally increases with a concomitant shift in algal species to cyanobacteria (Watson et al. 1997). This study underlies the importance of nutrient reduction to reduce odour production in source water, and highlights the usefulness of using simple and established limnological methods for identifying critical control points in understanding and managing water quality issues.

Table 5.1: Target odour compounds for GC-ITMS analysis.

Compound name and Chemical Abstract Service (CAS) number, level of quantification analysis and human threshold detections for the compounds as reported in the literature.

See following page for table annotations

Compound [CAS number]	Level of Quantification*	Human Odour Threshold	References
Geosmin [19700-21-1]	50 <sup>†</sup> ng·L <sup>-1</sup>	4 - 20 ng·L <sup>-1</sup>	Suffet & Mallevalle 1993, Rashash et al 1997, Rashash et al 1996, Ridal et al 1999, Young & Suffet 1999
MIB [2371-42-8]	50 <sup>†</sup> ng·L <sup>-1</sup>	6-42 ng·L <sup>-1</sup>	Suffet & Mallevalle 1993, Rashash et al 1997, Rashash et al 1996, Ridal et al 1999, Young & Suffet 1999
2t,6c-Nonadienal [557-48-2]	200 ng·L <sup>-1</sup>	4 - 13 ng·L <sup>-1</sup> FI <sup>§</sup> 100 ng·kg <sup>-1</sup>	Kochhar 1993, Rashash et al 1996, Rashash et al 1997, Young & Suffet 1999
2t,6t-Nonadienal [17587-33-6]	200 ng·L <sup>-1</sup>	FI <sup>§</sup> 1000 ng·L <sup>-1</sup>	Kochhar 1993
2,6-Nonadienal		80 ng·L <sup>-1</sup>	Cotsaris et al 1995
2t,4t-Nonadienal [5910-87-2]	400 ng·L <sup>-1</sup>	0.0017nL·kg <sup>-1</sup> 104 nL·kg <sup>-1</sup>	Jensen et al 1999
2t,4t-decadienal [25152-84-5]	400 ng·L <sup>-1</sup>	0.045 nL·kg <sup>-1</sup> 0.08 nL·kg <sup>-1</sup> FI <sup>§</sup> 500 ng·L <sup>-1</sup>	Kochhar 1993, Jensen et al 1999
2t,4c,7c-decatrienal		no standard <sup>‡</sup>	
2t,4t-Heptadienal [4313-03-5]	400 ng·L <sup>-1</sup>	2.5 - 5 µg·L <sup>-1</sup>	Young & Suffet 1999
2t,4t-Octadienal [5577-44-6]	400 ng·L <sup>-1</sup>		Kochhar 1993
Hexanal [1527-97-5]	400 ng·L <sup>-1</sup>	4.5 µg·L <sup>-1</sup> FI <sup>§</sup> 30 µg·kg <sup>-1</sup>	Cotsaris et al 1995, Young & Suffet 1999
Heptanal [111-71-7]	400 ng·L <sup>-1</sup>	3.0 - 7.0 µg·L <sup>-1</sup>	Cotsaris 1995, Rashash et al 1997, Young & Suffet 1999
β-Cyclocitral [432-25-7]	100 ng·L <sup>-1</sup>	3.0 - 19 µg·L <sup>-1</sup>	Cotsaris et al 1995, Rashash et al 1996, Rashash et al 1997, Young & Suffet 1999
β-Ionone [14901-07-6]	100 ng·L <sup>-1</sup>	7 ng·L <sup>-1</sup>	Cotsaris et al 1995
n-Pentanal [110-62-3]	400 ng·L <sup>-1</sup>	60 µg·L <sup>-1</sup>	Cotsaris et al 1995

Table 5.1 annotations:

\* GS/MS level of quantification (LOQ) is ca. 5 to 10 times method detection limit depending on analyte sensitivity and matrix interference

† Method detection limit equaled  $10 \text{ ng}\cdot\text{L}^{-1}$ . Geosmin was the only taste and odour compound detected in the samples. Although it was present below the LOQ, we were able to estimate concentrations above  $10 \text{ ng}\cdot\text{L}^{-1}$ .

‡ In Oil, comparable to 2t,6t-nonadienal (Kochhar 1993)

§ Fl = flavour threshold

Figure 5.1: Relationship between organic carbon and FPA (a), TP and FPA (b) and chlorophyll and FPA (c) for all study lakes and reservoirs.

Error bars represent the standard error of TOC, DOC and chl *a* measurements respectively. POC was calculated as the difference between TOC and DOC.

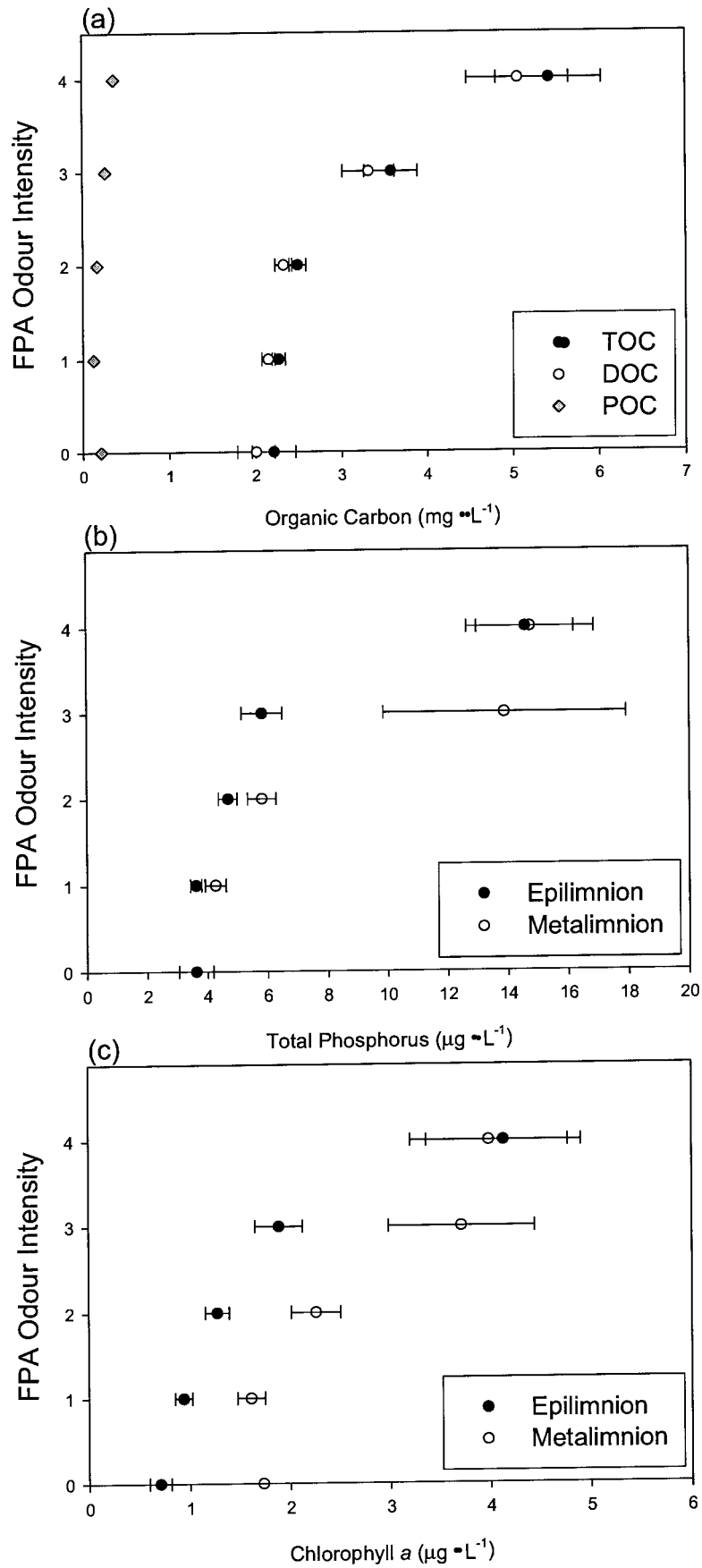


Figure 5.2: Weber-Fechner curves for geosmin (a), dimethyl sulfide (b), and hexenol (c).

Error bars as in Figure 5.1.

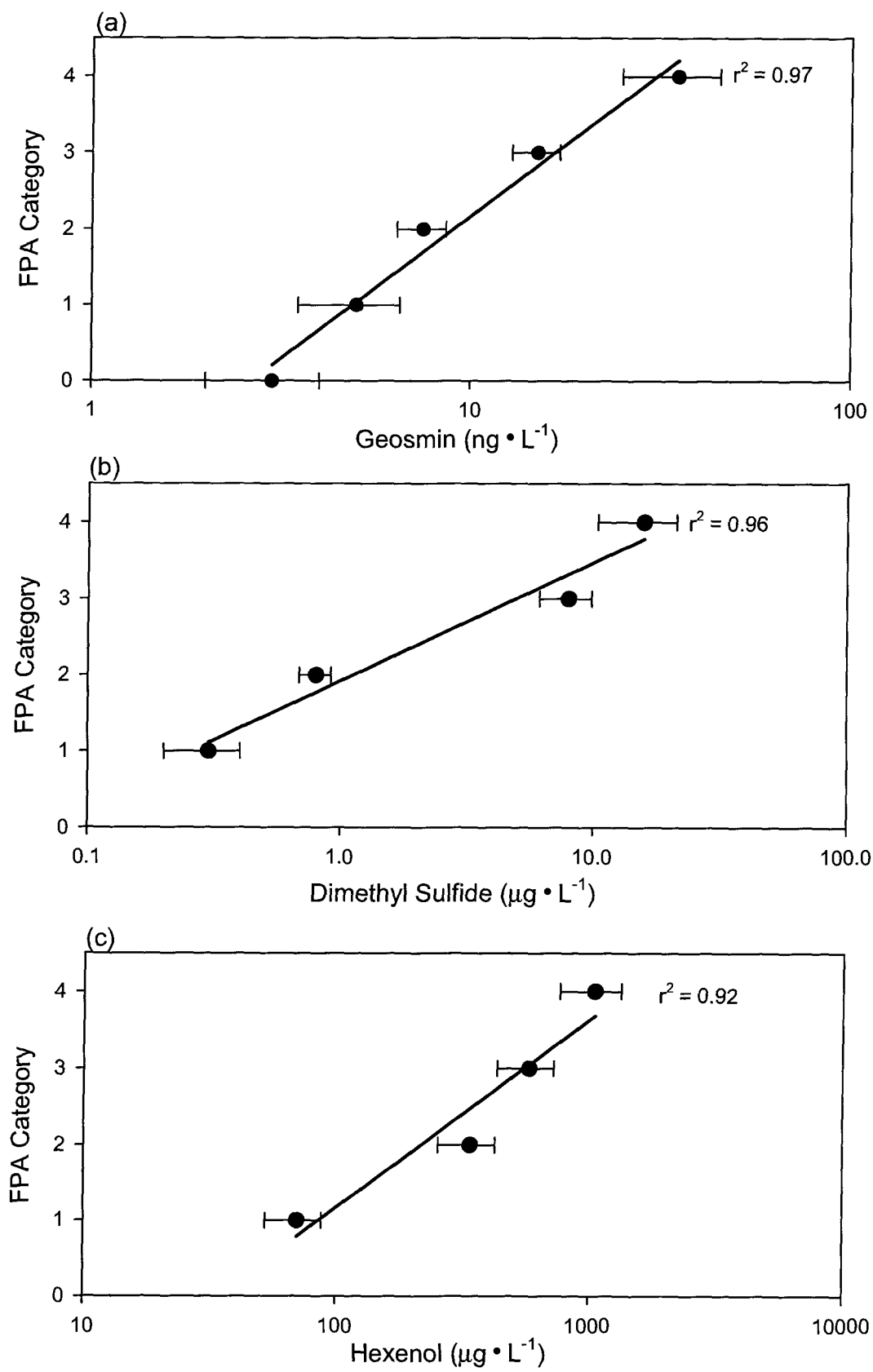


Figure 5.3: Chl *a* vs. FPA for sites and dates with either earthy, green vegetation+grassy or decomposing vegetation odours.

FPA values have been corrected for concentrations based on Weber-Fechner relationships (Figure 2). The numbers above points in represent the number of points used in that average and highlight that metalimnetic samples were only analyzed during the summer of 2001. Error bars as in Figure 5.1.

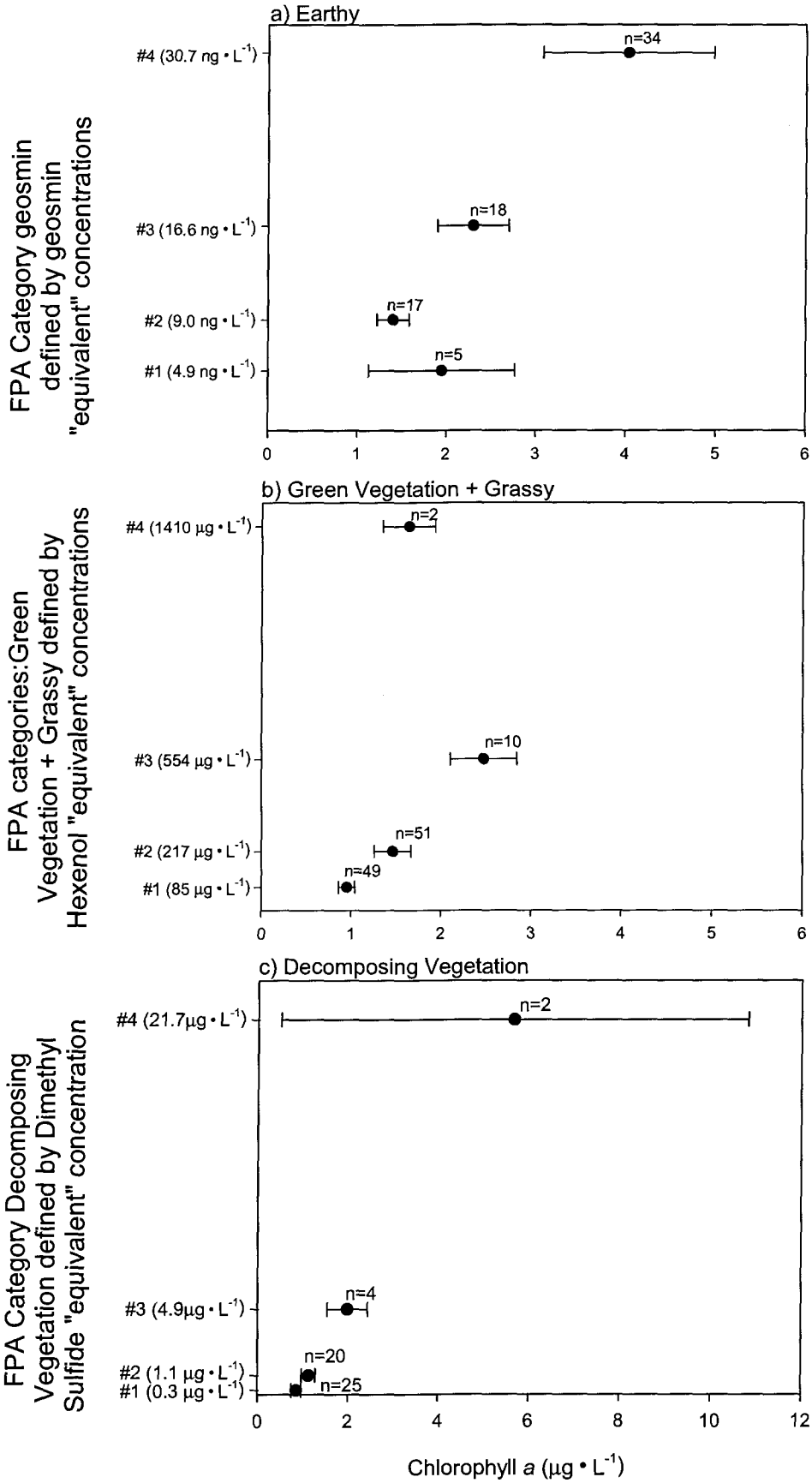


Figure 5.4: Comparison of geosmin concentrations determined from GC-ITMS and FPA values for selected lakes (see text).

GC-ITMS analysis was conducted between June and October 2001 (indicated by the vertical dotted lines). Black circles represent geosmin concentrations above the detection limit, grey circles were used when geosmin was found in the water, but couldn't be quantified. Mean FPA values are given with standard error estimates as determined from Weber-Fechner plot (Figure 5.2a). FPA can be read off either y-axis.

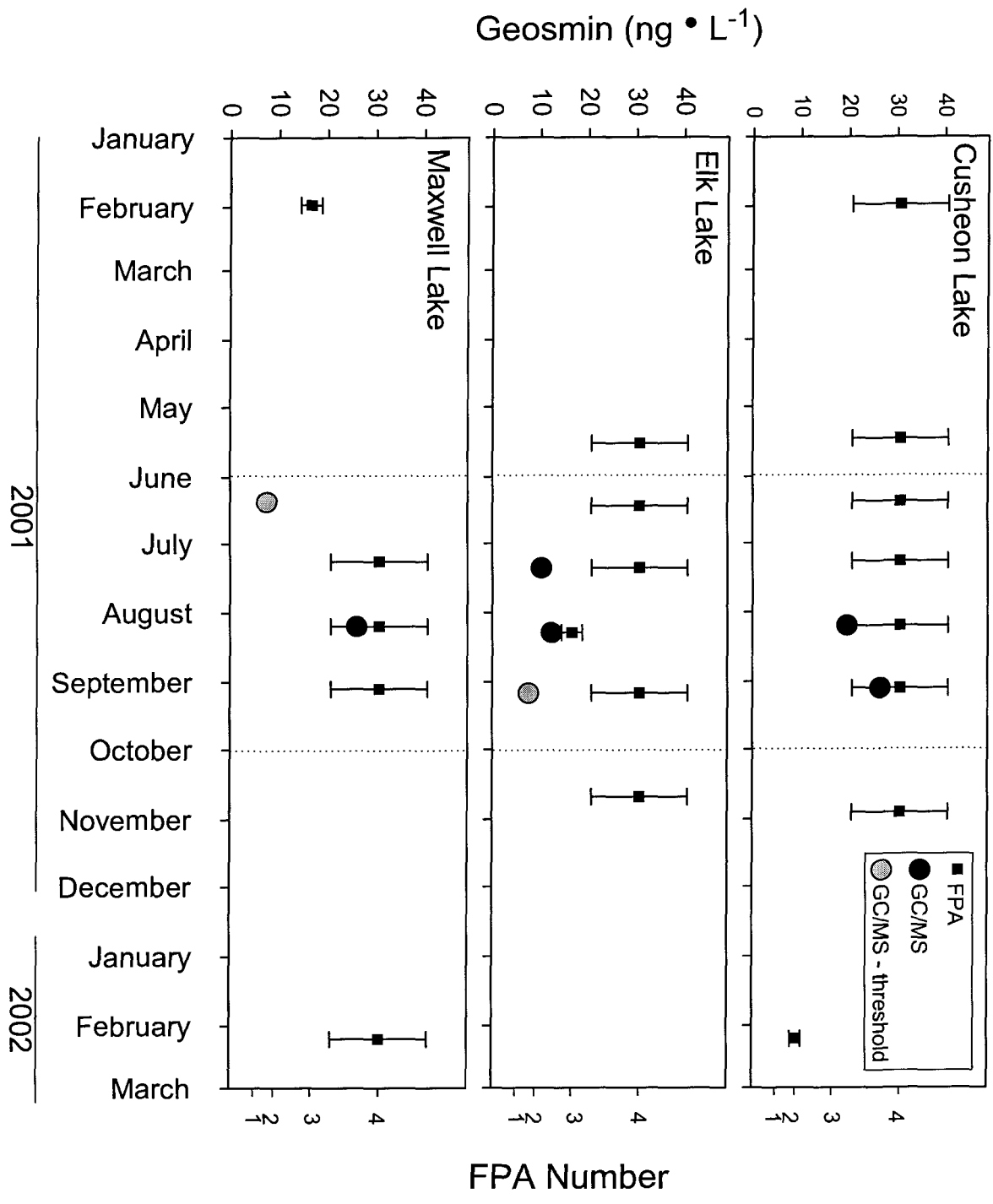


Figure 5.5: Water supply for the CRD (Victoria).

Black points represent sampling points for distribution analysis of FPA.

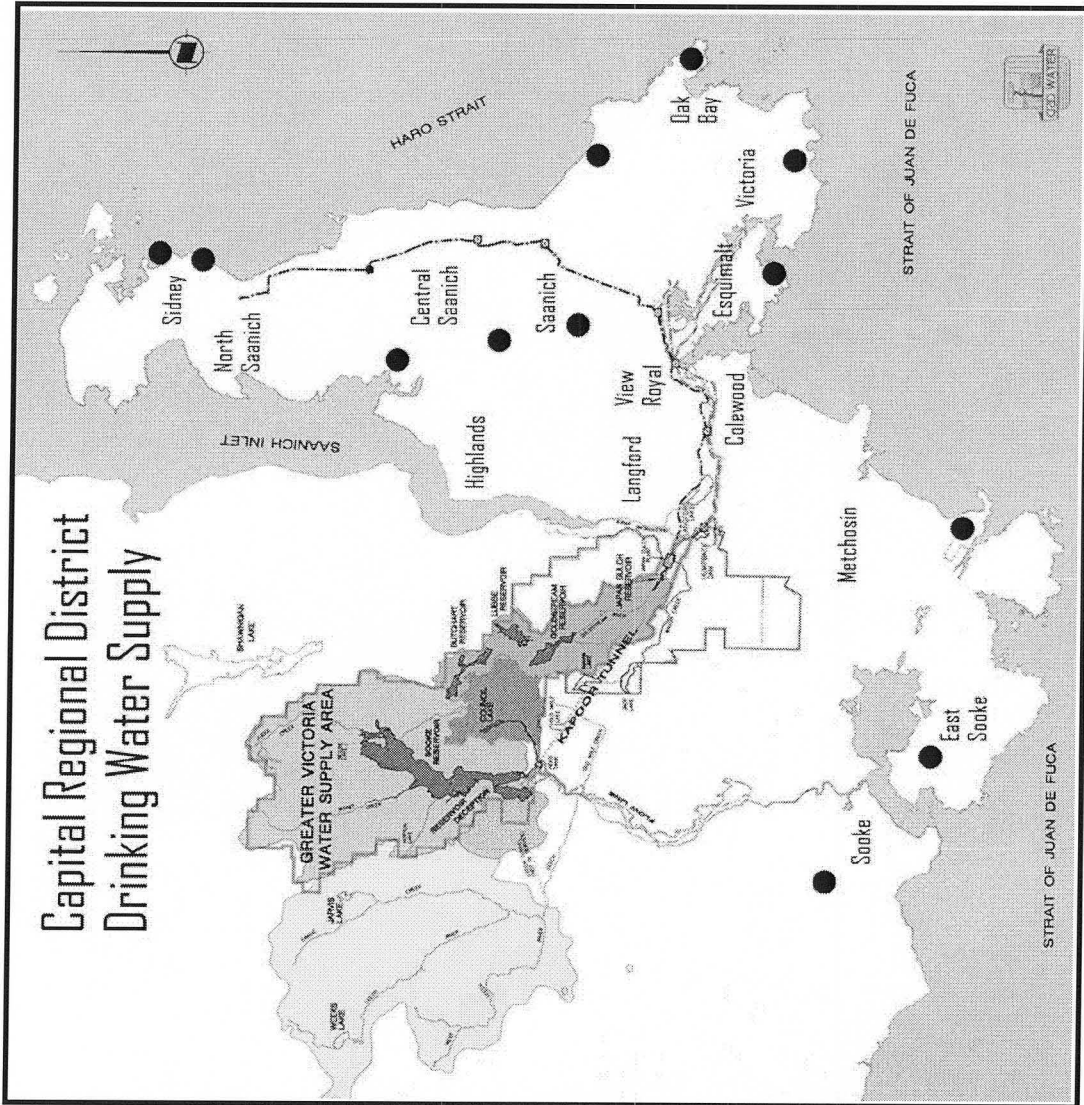
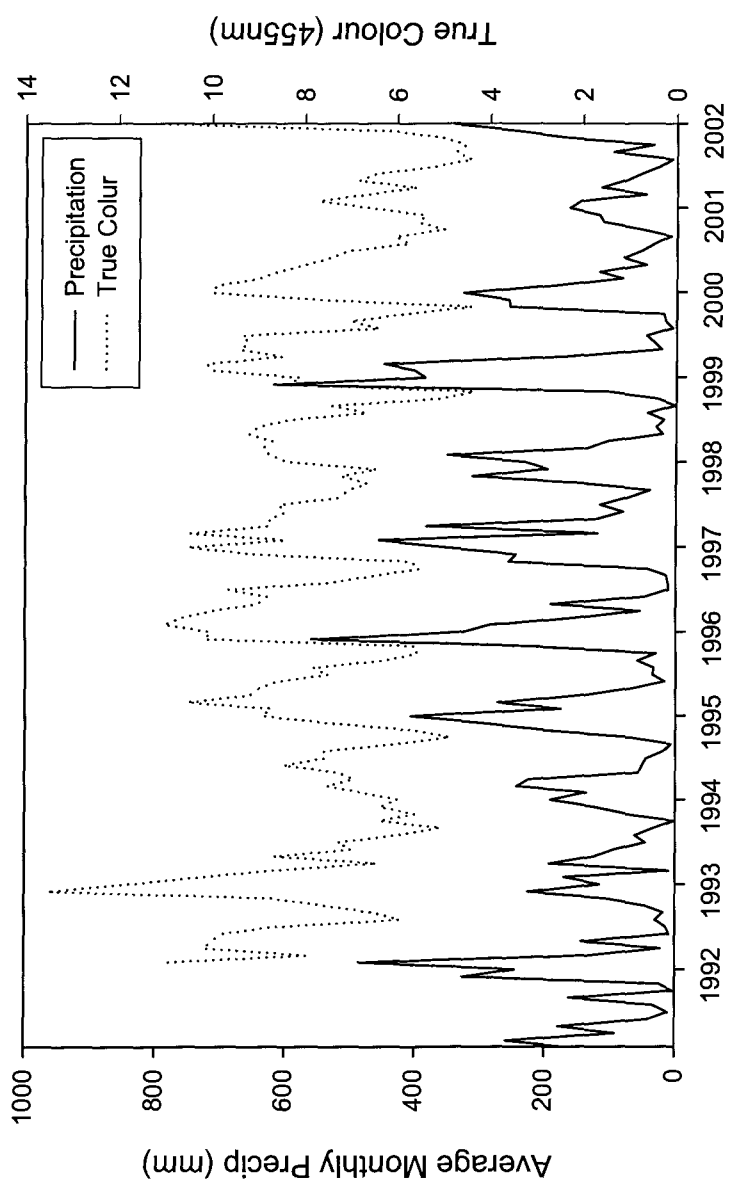


Figure 5.6: Comparison of monthly average precipitation and true colour in the south basin of Sooke Lake Reservoir between 1992 and 2002.

Colour was most strongly correlated with precipitation after approximately a two month lag ( $r=0.56$  at +2 lag).



**Chapter 6: Health and Environmental Policy Issues in Canada: The Role of  
Watershed Management in Sustaining Clean Drinking Water Quality at  
Surface Sources**

***Abstract***

Sustaining clean and safe drinking water sources is increasingly becoming a priority because of global pollution. The means of attaining and maintaining clean drinking water sources requires effective policies that identify, document, and reduce watershed risks. These risks are defined by their potential impact to human health. Health and risk are, therefore, indelibly linked because they are in part defined by each other. Understanding pathogen ecology and identifying watershed sources remains a priority because of the associated acute risks. Surface water quality changes resulting from inputs of human waste, nutrients and chemicals are associated with higher drinking water risks. Nutrient input can increase primary production and the resulting increase of organic matter results in greater disinfection byproduct formation or requires greater treatment intensity. Many drinking water disease outbreaks have resulted from breaches in treatment facilities, therefore, even with greater treatment intensity poor source water quality intrinsically has greater associated health risks. Government and international agencies play a critical role in developing policy. The goal of maintaining water supplies whose availability is maximized and risks are minimized (i.e. sustainable) should be a vital part of such policy. Health risks are discussed in the context of a multi-barrier perspective and it is concluded that both passive (protection) and active (prescriptive management) management is necessary for sustainability. Canadian aboriginal water systems, British Columbian water policy and U.S. EPA policies are given as examples. The basis for developing effective policies includes a strong reliance on sound science and effective instrumentation with careful consideration of stakeholders' interests. Only with such directed policies can the future availability of clean drinking water sources be ensured.

### *Introduction*

Nowhere does the link between human health and the environment manifest itself more strongly than our reliance on fresh clean drinking water. Management and treatment of waste generated from human activities including industrialization, agriculture, logging, and urbanization, have largely been insignificant in preventing pollution from affecting surface water quality on both local and global scales (Abu-Zeid 1998). All levels of governments (local, provincial/state, and federal) bear the responsibility for setting policies to ensure the protection of our water resources and for providing instruments for the attainment of these policies. The policies of governments and international agencies directly impact environmental and human health, and the economic, social and cultural facets of our lives. Water resource management should be based on our scientific understanding of health and environmental risks, associated financial costs, and societal acceptance of these risks and costs.

Canada is bestowed with an estimated 9% of the world's renewable supply of fresh water and lakes account for roughly 7.5% of its inland surface area (Can. Gov. 2001). These surface sources, for example, provide at least three quarters of British Columbian (BC) residents with their drinking water (BC Auditor General 1999). Given its natural abundance Canadians are accustomed to water that is both plentiful and inexpensive. On a per capita basis Canadians use water at one of the highest rates in the world, while paying the least amount for it (McKanna 2000). Despite having a wealth of water, drinking water sources are usually proximately located to areas they supply. Thus, impacts of industrialization, agriculture, and urbanization are closely linked to drinking water supplies. It is a critical goal to create a sustainable framework for human

utilization of the environment and specifically for the protection of drinking water supplies so as to ensure human and environmental health.

Challenges toward achieving sustainable water supplies include a lack of recognition for the role of strong watershed management and discontinuity between policy makers, policy instrumentation, managers and scientists. The goal of this paper is to demonstrate the need for strong policies to direct source water management and the critical role of integrating the traditionally unique areas of policy, management, health and the environment. Given its sensitivity to both short- and long-term pollution, and its prevalence in Canada and elsewhere as a source supply, I examined health and environmental implications of policy regarding surface-sources of drinking water. Water quality is defined from a human health perspective and the major diseases and environmental risks to human health are outlined. From this perspective, Canadian and BC policies are reviewed and specific examples are given. The end of the paper builds on the requirements of sound scientific knowledge and strong policies by stressing the critical importance of effective policy instrumentation and sound risk management. I underscore the philosophy that many managers are acceding to, that drinking water quality issues need to evolve from a strict treatment-based approach (of both raw drinking water and sewage treatment) toward a watershed management approach (Aust. NHMRC 2002). Only then can we ensure the short and long-term attainment of cost effective, high quality drinking water (Foran et al. 2000).

### ***Water Quality***

Herein, surface-source water is defined as untreated or unfiltered (i.e. raw) water from lakes, streams, and rivers that water utilities or individuals use for drinking.

Finished water is that which is delivered to consumers after receiving treatment. Usually, minimum treatment includes disinfection. Quality drinking water is ultimately defined as that which is safe for drinking and cooking (Gadgil 1998). Subjectivity associated with such a holistic definition has led to the functional separation of water quality into three measurable criteria; 1) water free of disease causing organisms, 2) water with harmful chemicals below defined thresholds and physical parameters within acceptable ranges, and 3) water with radioactive compounds below defined thresholds (Health Canada 1996). Other sub-classifications can include aesthetically pleasing aspects, which is of concern to water purveyors because of consumer water safety perceptions. Governments and agencies, including Environment Canada, the U.S. Environmental Protection Agency (EPA) and the World Health Organization (WHO) have established guidelines that specify acceptable concentrations and limits (e.g. MCL: maximum contaminant levels; MAC: maximum acceptable concentrations) for many microbiological components, chemical/physical parameters and radiological amounts.

Establishing scientifically-based limits for each of these components has been, and is, a vital objective for providing safe drinking water. Once specific water quality objectives are established it is necessary to determine the appropriate solutions for both short- and long-term attainment of these goals. Water utilities in developed nations have traditionally relied on treatment of water immediately prior to and during distribution. However, the old adage that "an ounce of prevention is worth a pound of cure" is establishing itself in progressive water utilities where multi-faceted protection and treatment plans are being developed to decrease costs, treatment requirements, and health risks associated with drinking water (Chichilnisky and Heal 1998; Aust. NHMRC 2002).

### *Health and Water Quality*

The relationship between surface-source and finished water quality, in its simplest form, is that cleaner source water requires less intense water treatment and has lower associated acute and chronic health risks. Common health risks of drinking water include enteric pathogens, disinfection by-products, chemical contamination, and other toxic compounds, such as those produced by cyanobacteria. The importance of identifying and breaking pathogen cycles to prevent waterborne illnesses was established in the 19<sup>th</sup> century (cf. Evans 1987; Brody et al. 2000). More recent studies in developing nations have demonstrated that breaking pathogen transmission cycles through proper sanitation and sewage management improve health benefits more than simple provision of clean drinking water, although both are desirable (Esrey 1996). Understanding the reasons for human disease outbreaks include: knowing the human infectious dose of an organism required to produce a disease, knowing the morbidity (or mortality) associated with an infection of the organism, understanding how immunity develops within a population, knowing that if population immunity develops there is less likelihood of disease occurring, and understanding the lifecycles and ecology of human pathogens.

The route of drinking water can be grouped into three main categories , 1) source water environment, 2) treatment, and 3) distribution and delivery (Figure 6.1). It is recognized that no single means of treatment is infallible, ideally redundancies within each category should be used to reduce health related risks. Each of these categories, and processes within, that identify and ameliorate risks are considered a barrier in a multi-barrier protection plan. Furthermore treatment intensity is dependent on source water quality. Pristine source water requires only disinfection, while other sources require

further treatment (Figure 6.1). Regardless of how developed a watershed is, management strategies should be aimed at reducing health risks in drinking water. Policies, especially of governments, provide specific direction for management strategies.

Environment and human health are inextricably linked at both proximate and extended time scales (Figure 6.2). Environmental degradation/negligence can lead to both acute and chronic human health problems. Long-term human health issues are mostly related to chemicals and physical agents (e.g. Radon). Those of particular interest include disinfection byproducts and arsenic. Drinking water guidelines are based on our best understanding of the available science, however, this area of science is complex. Inferences based on toxicological studies (microorganisms and animals) regarding the health effects of drinking approximately 1.5L over approximately 70 years is not exact and relies on consensus building among scientists and arbitrary safety factors when there is scientific uncertainty. Ignoring long-term issues, especially when implications can be predicted, is not the most efficient policy for sustaining human and environment health. Foresight to implement integrated source water management has the potential to significantly reduce future costs and risks of providing drinking water. Therefore, it should be a priority to create a balance between both short- and long-term policies, as opposed to having a disproportionate amount of policy aimed at solving short-term issues (Figure 6.2). There are, of course, many factors and variables to consider, including the major health issues outlined below.

#### *Enteric Pathogens:*

The intimate link between availability and abundance of safe, clean drinking water and human health has defined economic and social progress in developed nations

(WHO 1999). One of WHO's (cited in WHO 1997) primary goals is to ensure that clean water is available for all humans. It is estimated that half the population of the developing world is inflicted with the major microbial diseases associated with water supply and sanitation. Over 3 million children under 5 years of age die every year as a result of contracting diarrheal diseases (WHO 1996). Infection by pathogenic bacteria, protozoa, and viruses are the most prevalent global health risks associated with drinking water. Common organisms in drinking water that have been identified as posing major threats to human health include 1) bacteria: enteropathogenic *Escherichia coli* (notably *E. coli* 0157:H7), *Vibrio cholerae*, *Shigella*, *Campylobacter jejuni*, *Salmonella*, *Yersinia enterocolitica*, 2) protozoans: *Giardia lamblia*, *Cryptosporidium parvum*, *Entamoeba histolytica*, *Toxoplasma gondii*, *Balantidium coli*, and 3) viruses: Norwalk and Norwalk-like, Rotavirus, Hepatitis A and E; (Matsunaga and Okochi 1998; Ford and Mac Kenzie 2000; Haas 2001).

#### *Disinfection:*

In developed nations, acute health risks from microbiological pathogens have largely been mitigated through the disinfection of potable water. The goal of water disinfection is to inactivate waterborne pathogens (Braghetta et al. 1997) primarily by some form of chlorination, ozonation, or more recently ultra-violet radiation (UV). Filtration is often used prior to disinfection to physically remove particles and pathogens. Plotkin and Plotkin (1988) suggest that safe drinking water [as obtained primarily through disinfection] has been the most important historical mode for reducing human mortality, and Craun (1994a) claims Abel Wolman stated no single chemical has saved as many lives as chlorine. Regardless of water purveyor size, chlorine remains the least

expensive and most effective treatment for microbiological pathogens (Clark and Adams 1993).

*Disinfection Resistant Pathogens:*

Despite the benefits of disinfection, several pathogens are resistant to traditional chlorination processes. Two of these pathogens, *Cryptosporidium* and *Giardia*, have been subject to increased research over the past ten years because their oocysts and cysts are not wholly inactivated by chlorination or ozonation or completely removed by filtration. They also have a low infective dose and are common in surface water (LeChevallier et al. 1991a; LeChevallier et al. 1991b; Kramer et al. 1995; DuPont et al. 1995; Goldstein et al. 1996; Isaac-Renton 1996). In Milwaukee, 1993, the largest recorded waterborne outbreak in the U.S. occurred when over 400 000 people became very ill and more than 100 people died after being infected with *Cryptosporidium* originating from the drinking water supply (Mac Kenzie et al. 1994).

Users of surface-derived drinking water are at a higher risk for infection by *Giardia* and *Cryptosporidium*. Exposure to *Cryptosporidium* and *Giardia*, as reflected by human antibodies, was examined in three BC communities (Isaac-Renton et al. 1999). Residents whose communities were served by ground water supply had significantly lower exposures to both pathogens than communities supplied with either a protected or non-protected surface source.

The prevalence of surface water as a source may be a contributing factor to BC having higher reported enteric diseases compared to the rest of Canada (BC Auditor General 1999). LeChevallier et al. (1991a) found either one of, or both *Cryptosporidium* and *Giardia* in 97% of 66 surface source-drinking water supplies in 14 U.S. states and 1

Canadian province (AB). Rose (1988) found *Cryptosporidium* in 72% of surface water samples in western U.S, and Isaac-Renton et al. (1996) found 69% of source drinking water supplies in BC tested positive for the presence of *Giardia* cysts. LeChevallier et al. (1991a) found *Cryptosporidium* and *Giardia* distribution to be positively correlated with each other and with other water quality parameters including turbidity, faecal coliform and total coliform bacteria. In a summary of four BC watersheds, it was found that water supplied from a protected (restricted public access) forested watershed had the lowest mean *Giardia* cyst concentration, whereas the mean cyst abundance in a protected (peripheral fencing) agricultural watershed was slightly higher (Ong et al. 1996). Both protected watersheds had lower mean cyst concentrations than the two unprotected watersheds. The study also linked higher cyst numbers in one of the watersheds to a cattle ranch. In a different source water comparison study LeChevallier et al. (1991a) found fully protected watersheds had lower *Giardia*, but not *Cryptosporidium* cyst concentrations in watersheds of limited access, compared to those with recreational and agricultural activities, or those with sewage and industrial discharge.

Identifying and mediating specific sources of *Cryptosporidium* and *Giardia* contamination in watersheds may provide as much risk protection as being able to control and limit all activities in a watershed. Future studies need to examine the ecology of important drinking water pathogens. This will provide insight into why their abundance increases at certain times and thereby provide water managers with a better understanding of how to resolve problems at the source supply.

The prevalence in surface drinking water sources, persistence in treatment, and health risks make *Cryptosporidium* and *Giardia* especially important to consider when

developing policy and strategies for source water management. Presence of these pathogens is especially critical for individuals with compromised immune systems. While most filtration and disinfection processes reduce the number of viable cysts (Trussel 1993), UV seems to be the best treatment option (Craik et al. 2001). Because most water utilities do not have UV disinfection plants a multi-barrier approach that focuses on reducing high-risk activities within the watershed may provide the most effective means of reducing transmission risks. A multi-barrier approach also offers protection against treatment facilities that have inadequate or interrupted disinfection. Craun (1988) attributed 13-14% of waterborne disease outbreaks in the U.S. to such systems (1971 to 1985). In order to effectively lower risks, watershed programmes should move beyond monitoring for source water pathogens towards an understanding of their ecology and population dynamics.

Recent guidelines established in Australia focus on a multi-barrier process approach. This involves adaptive management aimed at specific critical control points within each drinking water system, including the origin of source pathogens. These points represent system vulnerabilities to specific risks within all stages of the water delivery (Figure 6.1) and aims to ameliorate risks where they occur. This differs philosophically from the technologically driven end-of-tap treatment of water pollutants and pathogens common in much of the developed world.

*Disinfection By-Products:*

While acute infection risks are significantly lowered by disinfection processes, the disinfectant (e.g. chlorine, chloramine, chlorine dioxide, ozone) reacts with organic compounds in water to produce secondary compounds known as disinfection by-products

(DBPs). Health Canada (1995) divides DBPs into three main categories; 1) substances that may cause deleterious toxic, carcinogenic, or genotoxic effects, 2) assimilable organic carbon that stimulates bacterial growth in distribution systems and 3) compounds of objectionable taste and odour. Numerous DBPs have been identified (Health Canada 1995; Richardson 1998). The best known and most well studied are total trihalomethanes (TTHM - including: chloroform, bromodichloromethane, dibromochloromethane, and bromoform), and haloacetic acids. Since trihalomethanes (THMs) were first discovered in chlorinated drinking water (Bellar et al. 1974), research has investigated potential health problems associated with these compounds. Concentration of TTHMs in treated water is a function of temperature, the chlorine demand, total organic carbon concentration (Symons et al. 1975) and contact time. Source water with less organic carbon is considered to be of higher quality because, all else being equal, it has a lower chlorine demand and fewer DBPs will form when the water is treated (Symons et al. 1975; Craun 1993). Growth of bacteria in distribution systems is a function of several factors, but dissolved organic carbon (DOC) (Niquette et al. 2001) and perhaps phosphorus (Sathasivan and Ohgaki 1999) are the most important. Greater DOC concentration increases bacterial regrowth, and therefore residual distribution disinfection demand, and DBP formation. Understanding the relationship between DOC and bacterial growth in distribution systems is important, especially when employing new disinfection technologies like UV or ozone treatments.

Since their discovery, medical researchers have examined potential health implications of DBPs. Most studies have examined the health effects of DBPs in drinking water, however, it should be noted that drinking water is not the only means of

exposure to DBPs. Weisel and Chen (1994) found heated water previously treated with chlorine posed a 50% increase to exposure via inhalation and dermal contact. This can be especially important after bathing or showering (Miles et al. 2002). Risk from inhalation and dermal exposure varies for each DBP, some have lower, similar, or higher calculated cancer risks. Hutcheson et al. (1994) calculated the cancer potential factor of chloroform from heated water to be 13 times greater from inhalation compared to oral ingestion.

Epidemiological and toxicological studies have been used to elucidate relationships between DBPs and health (e.g. primarily cancer). Descriptive epidemiological studies compare groups of people exposed to different water sources (often ground water is used because of its significantly lower DBP content) and rates of cancer within each of those groups. Odds ratios (i.e. the ratio of the odds of a person having a disease in an exposed group to the odds of a person having the same disease in the unexposed group) are commonly used to draw conclusions about exposure to DBP. Studies have shown no increased association of cancer to DBP in drinking water (Lawrence et al. 1984; Young et al. 1987), increased association of cancer to DBP in drinking water (Cantor et al. 1987; Zierler et al. 1988; Fagliano et al. 1990; Doyle et al. 1997) and association to some cancers, but not others (Bull et al. 1995). Odds ratios in such studies are usually less than 2, meaning that the strength of association between DBPs and cancer is undetectable (1.0 – 1.2), weak (1.2 – 1.5) or at best moderate (1.5 – 3.0) (Monson 1990). Detection of weak associations requires more rigorous experimental studies because of natural variability and confounding bias in such studies (Monson 1990).

Toxicological studies have mainly focused on associations between THMs and cancer risk (Boorman et al. 1999). Many DBP compounds have been demonstrated to produce carcinogenic or mutagenic effects. Implications for human exposure, however, remain largely unknown because these studies typically test compounds on animals for shorter periods and at levels several magnitudes higher than typical human exposures. Results are often linearly extrapolated to human exposure (Craun et al. 1994b). Despite weaknesses of both epidemiological and toxicological studies a growing number of researchers are finding increased health risks associated with exposure to DBPs suggesting, but not proving, that DBPs increase incidence of certain cancers (Bull 1993).

A recent review (Graves et al. 2001) of both epidemiological and toxicological studies examining effects of DBP on human reproduction and development found no evidence of association for many parameters (including: neonatal death, low birth weight, pre-term delivery, and congenital, cardiac, gastrointestinal, genital, integument, musculoskeletal and chromosomal abnormalities) and only a few with suggestive or positive associations (*in utero*- growth retardation, urinary tract defects). There were mixed or inconsistent results for studies examining the relationship of DBP with still birth/foetal death, spontaneous miscarriage, all central nervous system anomalies, and congenital abnormalities/birth defects. ILSI's (1998) report on toxicity of exposure to DBPs concluded that on-going and continued interdisciplinary research with epidemiologic, toxicologic, and mechanistic foci is necessary to further our understanding of risks associated with DBPs. Regardless of the relationship between exposure to DBPs and human health, one assured approach to mitigate this risk is to reduce organic precursors in raw source water.

*Chemical Contamination:*

It could be argued that the use of chemicals and their introduction into the environment results in the most obvious alteration/contamination of surface waters. Of the chemicals listed in the Canadian Drinking Water Guidelines (Health Canada 1996) most are used in agriculture or industry. Numerous organic chemicals were found in a U.S. national survey of streams susceptible to contamination (Kolpin et al. 2002). Chemical introduction into surface waters either directly, or indirectly through deposition in watersheds or the atmosphere, decreases the quality of the surface water. For example inorganic nutrients from fertilizers, especially nitrogen and phosphorous, can stimulate aquatic productivity (OECD 1982). Numerous studies have examined the causes and effects of excessive nutrients on eutrophication. Eutrophication results in unfavorable changes to water quality, including: higher dissolved organic and particulate carbon concentrations, higher bacterial numbers, shifts in phytoplankton species composition and formation of algal blooms, deoxygenation of hypolimnetic waters, unpleasant tastes and odours, and changes in food web structure and fish species composition (OECD 1982).

Algae and algal exudates (DOC) are important precursors of THM production (Hoehn et al. 1980; Karimi and Singer, 1991). Studies, such as those linking algae and DOC to THMs, underscore the critical link between source water quality parameters and health risks. Such studies also provide directives for the management of both surface sources and water treatment. For example, an experiment with chlorine as a primary treatment found that there was an increase in DOC concentration from ozone pretreated algal cultures. This resulted in higher chloroform levels compared to cultures without

ozone pretreatment (Plummer and Edzwald 2001). The particulate biomass, however, was still attributed as being the major precursor of DBPs (ca. 70%). This emphasizes that source water quality and treatment methods are both important variables in the overall quality of water.

The introduction of agricultural and domestic pesticides, industrial and domestic cleaners, and private and industrial waste into surface waters pose an increased risk to human health because many of these chemicals are considered toxic, carcinogenic, or genotoxic (Health Canada 1996). Water quality criteria for drinking water supplies for many of these compounds have been established. The risks associated with chemicals can most effectively be abated by reducing their use and managing their disposal at the source. If multi-barrier critical control point policy of surface source drinking water is established then specific strategies regarding both point-source and non-point source pollution would be a priority and would result in lower risks of pollutants in drinking water.

*Cyanobacteria:*

The abundance of cyanobacteria (blue-green algae) in source drinking water is an important health issue. For centuries people have associated cyanobacterial blooms with poor water quality, however, only in the past couple of decades have we begun to understand and appreciate the importance of toxins and taste and odour production from these algae (Chorus 2001). Other algal groups are known to produce toxins (e.g. Prymnesiophyceae and Dinophyceae), however, cyanobacteria represent the greatest risk in freshwater (Carmichael and Falconer 1993). Toxigenic species occur in at least 18 genera (Skulberg et al. 1993) although species of *Anabaena*, *Aphanizomenon*, *Nodularia*,

*Oscillatoria*, *Microcystis* are the principal ones associated with health risks (Carmichael and Falconer 1993). Prolific cyanobacterial growth is associated with nutrient rich waters, warm temperatures and sufficient light (Reynolds 1984b; Carmichael 2001).

Cyanobacteria are known to produce acute hepatotoxins, cytotoxins, neurotoxins, and gastrointestinal disturbances and respiratory and allergic reactions (Falconer 1999; Carmichael 2001). The principal cyanobacterial toxin considered in drinking water guidelines is microcystin-LR. Falconer (1999) reported a provisional drinking water guideline of 1 µg/L for the U.S (Falconer 1999), while Canada recently approved a guideline concentration of 1.5 µg/L. Microcystin-LR specifically targets the liver, kidney and small intestine (Falconer 1993) and involves acute hepatotoxicosis. Livestock deaths, (Puschner et al. 1998), and occasionally human deaths have been attributed to cyanobacterial-derived toxins. In 1996 the deaths of 76 people were attributed to cyanotoxin-contaminated water used for treatment in a Brazilian dialysis centre (Carmichael et al. 2001). In addition to microcystin, Australia has guidelines for anatoxin-a, saxitoxin and cylindrospermopsin.

Acute large-scale impacts are not common because people generally avoid consuming water with obvious bloom formations. Health consequences of consuming sublethal toxin concentrations are therefore an important health issue. While cyanotoxin toxicological tests clearly demonstrate these toxins have adverse health effects, epidemiological studies are more complicated because of the influence of other variables including: enteric bacteria, protozoa, viruses, DBPs, seasonality of cyanobacterial abundance, and variance in toxin consumption. A sudden release of toxins can occur when cyanobacterial blooms die. This occurs when environmental conditions become

unfavorable for the bloom or if algaecides are used to “improve” water quality. Because human intake of toxins is higher under such conditions their effects are more pronounced in the short term, for example, increased liver damage was associated with the treatment of a *Microcystis* bloom with copper sulphate (Falconer et al. 1983). Liver damage and tumor growth are two of the primary health problems associated with the consumption of cyanotoxins (Falconer 1991, 1993). The few long-term epidemiological studies on the effects of human health are suggestive, but not conclusive, that cyanobacteria in source water is associated with greater health risks (Shun-Zhang 1989).

Cyanotoxins normally pass through water treatment processes and are resistant to boiling (Falconer et al. 1989). Therefore, the prevention of cyanobacterial blooms is a more effective means of reducing toxins than is the typical water treatment process (Bischoff 2001). Eliminating the ecological competitive advantages of cyanobacteria by lowering nutrient discharge, especially phosphorous (Downing et al. 2001), or disrupting water column stability should be the primary goal for reducing both acute and chronic health risks associated with cyanobacterial blooms. Such strategies eliminate infrastructure costs associated with treating drinking water, reduce risks by reducing or eliminating the toxin source, contribute to other improved water quality parameters (e.g. reduced organic carbon) and in multipurpose lakes improve the aesthetic value.

#### *Radionuclides:*

Although radionuclides have the potential to seriously affect health, I will not discuss them at length. Radionuclides that occur in drinking water include Radon-222 and Radium, both of which occur primarily in ground water. Uranium and up to 200 human-made radionuclides that are potential surface water contaminants can be found in

both ground and surface water, (Lowry and Lowry 1988). The presence of harmful radioactive elements in surface-source drinking water is, to a large extent based upon the past and current policies of government nuclear programmes. Nuclear programmes of all governments and organizations should consider both short- and long-term risks to human health.

### ***Policy***

#### *Background – Canadian and BC Water Policy:*

The process of establishing safeguards and guidelines necessary for the continued attainment of high drinking water quality primarily depends on government policy. Policies are instruments through which governments can wield their power and provide directives for action (Elmore 1987), such as establishment of specific procedures and rules, which in turn are used by regional, and local governments and water purveyors. Policies should be based on the best available information and result in action based on these principals (Forsberg 1998). The Canadian Federal Water Policy has two principal goals, 1) to protect and enhance the quality of water, and 2) to promote the wise and efficient management and use of water (Env. Can. 1987). It includes a specific policy statement regarding safe drinking water stating that it will continue to establish safe drinking water guidelines (e.g. the Canadian Drinking Water Quality Guidelines), to aid jurisdictions, conduct research, promote public awareness, and consider legislation relating to federal jurisdiction. Health Canada also serves as Secretariat to the Federal-Provincial Subcommittee on Drinking Water – a committee with representatives from the provinces, territories and Environment Canada that reviews and proposes new drinking water guidelines.

In Canada there is a division between the federal and provincial governments' roles and jurisdictions in protecting drinking water. The provinces, under the Constitution Act, have proprietary rights to water resources (surface and ground water) and are responsible for authorization and use of water, development relating to water, flow regulations, and they have the authority to legislate water supply and pollution control (Env. Can. 1987). The federal government maintains jurisdiction involving navigation and fisheries (e.g. section 35 under the Fisheries Act), national parks, and aboriginal reservations.

Recent changes in BC drinking water policy are used to highlight the importance of policy in shaping management, the need for strong instrumentation (see below) and the need to improve our scientific understanding of watershed processes. British Columbia passed the Safe Drinking Water Regulation (SDWR) under the Health Act in 1992 (BC Gov. 1992). The SDWR placed the responsibility of safe drinking water provision on water purveyors subject to approval of Medical Health Officers and set the microbiological limits of bacteria in finished water. The mechanism of enforcement in BC has, for example, involved putting conditions on operating water permits. In April 2001 the Drinking Water Protection Act (DWPA) was enacted and it, in part, outlined development of drinking water protection plans within BC (BC Gov. 2001a). If a watershed area was established, the plan was designed to prohibit contaminant introduction or anything that would result in a health hazard. In its present form the DWPA is only a framework. In September 2001, under a new provincial government, this Bill was placed under review by the Minister of newly named Ministry of Water, Land and Air Protection [WLAP; formerly named Ministry of the Environment Land and

Parks (MELP). NB: due to the change in government in 2001, I have chosen to differentiate NDP and Liberal initiatives by using both the old (MELP) and new (WLAP) environment ministry acronyms]. The review concluded that BC “urgently needs the consolidated legislation that the DWPA provides” (Marshall 2002). A complicating political issue is that protection of surface-source drinking water also falls under the jurisdiction of any Ministry in contact with watersheds, lakes, and rivers, including Ministries of Environment, Forests, Health, Energy and Mines, and Transportation and Highways (BC Auditor General 1999). Therefore, if the recommendations of the DWPA review panel are followed and a new Drinking Water Protection Agency is formed, each of these ministries would be required to relinquish some of their current jurisdictional power. BC Ministry of Health Services has recently been designated as the lead agency responsible for the safety of drinking water but no Drinking Water Protection Agency has been formed.

Regulations that are needed to make the DWPA enforceable are currently being drafted, although the administrative and policing resources have not yet been allocated. This new legislation will further strengthen the ability to improve water quality by means of enforcement (Table 6.1). If all the legislation and policies are implemented, BC will have one of the strongest drinking water regulations in the country (including source water protection). Regulations do not necessarily equate with compliance, but they should be a manifestation of water policy objectives and provide an enforceable structured framework for those objectives.

MELP recently established a policy regarding the protection of freshwater (BC MELP 1999a). This MELP report outlines strategies related to water pollution control,

water-use planning, fish protection, bulk water transport, non-point source pollution, water education and stewardship, flood safety, dam strategies and drinking water protection. The overlying goal is “healthy aquatic ecosystems, assured human health and safety, sustainable social, economic, and recreational benefits of water”. The process by which the government of BC plans to attain these goals is, by nature, an on-going process. One key component of developing effective policies is public participation. A recent consultation process conducted by MELP (BC MELP 2001) suggests that the BC public wants enactment of strong legislation, including raw and finished water standards, increased research on drinking water issues, public education, and a greater emphasis on watershed protection. This sentiment, reflects some of the legislation in the DWPA and such directives will further establish links between source water quality, drinking water quality, and health in BC

*Policy Instruments:*

Instruments represent the means by which policy goals are attained. Categories of policy instrumentation include 1) regulatory, 2) market and incentive-based, and 3) information provision. Each have affirmative (e.g. prescriptive, subsidies, encouragement) and negative (e.g. fines, fees or taxes, warnings) variants (Bemelmans-Videc and Vedung 1998). The achievement of policies depends primarily on the effectiveness, efficiency, legality, legitimacy, and underlying democracy of these instruments (Bemelmans-Videc 1998).

Ecological studies have identified nitrogen and phosphorous as key elements in eutrophication, so present day policy reflects this knowledge and focuses on reducing the loading of these elements into water bodies (Forsberg 1998). This is primarily conducted

through legislation on discharge of point-source pollution (Johns 2001) that includes public and corporate information campaigns, provision of incentives and financial assistance to achieve compliance, and the dispensing of fines. Without more comprehensive policy instruments water quality objectives will not be attained. If left unabated non-point source pollution, increased urbanization, intensive agriculture practices, increased anthropogenic nitrogen fixation and large-scale phosphorous consumption will continue to pollute surface waters (Forsberg 1998).

At worst policies are politically motivated gestures, at best they generate specific strategies, define instruments of implementation, identify local action plans and, ultimately, meet their mission statements. A fundamental shift in focus to economic and health values associated with protection of surface waters is necessary to provide incentive and justification for the protection of surface-source waters (Newsome and Stephen 1999). Policies in both Canada and BC have been established and outline a commitment for the assurance of both quality finished drinking water and the protection of surface waters. It is therefore imperative that we understand impacts on the sustainability of both water quantity and quality, the relationship between quality of surface-source water and the quality of finished water, the relationships between finished water and consumer health, and both the short- (Harrington et al. 1989) and long-term costs (Havelaar et al. 2000) of protecting our drinking water supplies (World Bank 1993). Such knowledge forms the foundation for effective policy instrumentation because, in combination with public participation, the instruments will be legitimate, legal, and democratic.

*Policy Examples:*

*Canada:* Since the development of a Water Policy in 1987, the Canadian federal government has played an active role in establishing and updating drinking water guidelines. Pressing water quality issues, however, do remain within its legal jurisdiction. Moore (1999) examined drinking water systems in aboriginal communities across Canada and found that 25% posed health and safety risks. As both the responsible and regulatory agency, the federal government needs to maintain incentive for managing these systems.

*British Columbia:* British Columbian water sources were identified by the Auditor General as being strained (BC Auditor General 1999). Enteric diseases in BC are higher than in the rest of Canada, in part due to lack of filtration, predominance of surface water as a source (as opposed to ground water), and the presence of *Cryptosporidium* and *Giardia*. Smaller communities and towns are at a greater risk because they lack the finances to build extensive water treatment facilities. A key conclusion of the Auditor General was that BC lacks an effective and integrated approach to land-use management with respect to protection of drinking water sources. This concern was intended to be addressed by the DWPA.

The importance of non-point source pollution is recognized by the BC government and it is the only province in Canada to develop an action plan to address this issue (BC MELP 1999b). Ecological directives for drinking water management will continue to face challenges because environmental problems are usually not identified conclusively and disagreements on the impacts and practicalities of solutions will always exist. Further complications can arise; for example, when solutions such as nutrient

reduction are identified then questions regarding specific targets (e.g. nitrogen or phosphorus – which one and how much?) will remain (Sims et al. 1999). Understanding the detailed relationships between environment and health is important because changes in policies and regulations will negatively affect some stakeholders. This action plan (BC MELP 1999b) represents a promising step towards managing surface water quality because it identifies educational, on-going assessments, economic incentives and legislation as being key instruments to successfully reaching water quality objectives.

Within BC small systems are considered most at risk for waterborne illness because they lack the financial resources to construct and maintain filtration plants and for the most do not have protected watersheds. If situated in the U.S. these systems would not comply with the non-filtration rule (see below). Establishing sound partnerships and agreements with land owners and users requires effective instrumentation by the government and backed by enforceable legislation.

*United States:* Due to both its global influence and proximity U.S. regulations are often used for comparison in Canada. The U.S. Safe Water Drinking Act (SWDA) amendments of 1986 and 1996 are important because (among other things) they recognizes the relationship between treatment intensity and water sources. One of the requirements of the SWDA is that filtration of surface sources are required unless water suppliers can demonstrate that their source water is of high quality. Maintaining non-filtration includes meeting raw water coliform bacteria requirements, turbidity requirements, having an appropriate disinfection system using a minimum of two disinfectants, meeting protozoan and viral disinfection criteria, and maintaining an effective watershed control programme. Effective watershed programmes are designed

to minimize risks from transportation (especially spills), residential (especially sewage/septic), industrial (primarily waste), agriculture and forestry (nutrients, chemicals, roads), recreational (public access & the concomitant risks of enteric pathogen introduction), and natural (animals, landslides) sources (Leland and Berg 1988). New York city has decided to invest in a watershed management programme to maintain high water quality from its source water so as to avoid filtration costs in the future (Chichilnisky and Heal 1998; Foran 2000).

The U.S. principally relies on treatment to reach water quality objectives and only when treatment intensity is less than that required (e.g. filtration) do the strict watershed rules apply. Thus, the U.S. primarily depends on technology to ensure that pathogens and pollutants remain below guideline levels. Development of policies to adopt a multi-barrier plan and focus on risk amelioration at critical control points from source to tap (e.g. Aust. NHMRC 2002) would serve to reduce immediate risks and long-term sustainability of drinking water resources.

### ***Risk Assessment and Cost***

Health risks associated with drinking water can be reduced to the following equation: risk equals the likelihood of an event occurring (probability) multiplied by the consequence (the measurable effect). Health risks of contracting a disease from untreated drinking water is high in probability and high in consequence, whereas health risks of contracting a disease from disinfected water are lower in probability and, in the immunocompetent, lower in consequence. Drinking water that is free of pathogens and harmful chemicals is not a practical or desirable goal, rather water purveyors should ensure that water is free of human pathogens and any chemicals present are below the

concentrations where science suggests they are not of health concerns. Water treatment plants and water strategies are not 100% effective. In developed nations water-borne diseases and chemical contamination from drinking water occur every year (viz. Haas 2001). The practice of water treatment to disable pathogens decreases the health risks, but does not eliminate them. Recent examples, including the *Cryptosporidium* outbreaks in Milwaukee (1993) and Nevada (1994) occurred despite water treatment using filtration and chlorination. Communities and governments need to assess their priorities and balance risks with costs when developing surface-source drinking water policies. The potential economic costs associated with drinking water-borne illnesses were assessed in a commissioned paper by Livernois (2002) following an outbreak of *E. coli* in Walkerton Ontario that resulted in seven people's death and 2300 people becoming ill. In this small Canadian community the tangible economic impact was estimated to be \$64.6 million (Livernois 2002).

Health risks in unprotected watersheds are considered to be greater than those in protected watersheds because there are fewer checks-and-balances. Risks include the introduction of chemicals and nutrients (through industrial, personal, or accidental means), unknown handling of sewage (human and agricultural), and increased sediment load from land-clearing and road building. Leaching of human and animal wastes into source waters increases the acute risks of infection by enteric pathogens. Disposal of toxic, carcinogenic or mutagenic chemicals increases the risk of long term health problems. Increased nutrient loads cause higher biomass in source water and may result in either increased DBPs or require increased treatment levels (i.e. filtration) and can

cause shifts to less desirable potentially toxic algal species. All these activities have the potential to degrade water quality parameters.

There still exists great uncertainty linking the degradation of water quality to health. Activities should be audited and their effects estimated (Fewtrell et al. 2001) so that risks are better understood and policies are directed towards the goal of sustaining water supplies, maximizing health, and minimizing costs. For example, the BC Auditor General (1999) estimated that adding filtration systems to the smaller water systems outside Vancouver and Victoria would initially cost around \$CDN700 million with an additional \$30 million for annual maintenance. Other estimates suggest initial costs may be as high as \$2 billion (BC Gov. 2001b). For economic reasons this investment isn't likely to be made soon, so municipalities along with the provincial government need to find other means of reducing health risks. It is in BC's interest to conduct studies examining source-water supply on human health so that watershed effects can be estimated and risks better understood. While short term costs may be lower, unchecked development can lead to increased risk through environmental degradation and therefore increase costs in the future.

### ***Role of Science***

Implicit in this paper is the requirement for sound science in all aspects of drinking water management. For example scientific studies form the basis of treatment development, setting MCLs/MACs and understanding source water and watershed ecology. The effectiveness of management and policy development is dependent upon a concerted scientific effort to understand all aspects of drinking water systems. Sound science forms the basis of the legitimacy, equitability and legality of implementing water

management strategies. A major struggle in developing science-based policy is the lack of effective mechanisms to integrate current science into policy.

***Conclusion - Health, Policy & the Environment***

Ultimately drinking water management must be based on sound science, strong policy, effective policy instrumentation and a clear understanding of risk. The current crux of quality drinking water focuses on the end product, however, water quality is a consequence of water source, treatment, and distribution. The attainment of safe drinking water should employ several strategies (Table 6.2).

Ideally drinking water protection should focus on raising the quality of source water rather than increasing the sophistication of treatment (Gostin et al. 2000). Treatment alone is not failsafe (Craun 1988; Goldstein et al. 1996) but rather should be used as part of a multi-barrier approach. Protecting surface source waters can reduce the number of pathogens and organic matter entering treatment facilities, thus reducing acute enteric risks, lowering DBPs and reducing dissolved organic carbon substrates that promotes bacterial growth in distribution systems. Protection and management can also serve to reduce cyanobacterial toxins. The link between source water quality and need for treatment is recognized by the US SWDA. Assessments have demonstrated that effective management can reduce, delay, or avoid substantive costs associated with water treatment beyond disinfection (Chichilnisky and Heal 1998). It is especially critical to understand the ecology of human pathogens and manage them by prevention of source-water contamination.

In certain systems more direct management may be desirable, such as construction of pre-impoundments to trap sediment and phosphorous, wet-land

restoration and construction, and lake mixing and aeration (Straškraba 1996). Settling in pre-impoundments can reduce biomass and pathogens in reservoirs, for example by reducing protozoan cysts (Isaac-Renton 1996). More actively managed watersheds effectively use natural ecosystems to do work (that would otherwise require more intensive treatment facilities) and should be considered financial assets.

Few water utilities have the resources to implement comprehensive watershed programmes without assistance. This is especially true of utilities servicing small populations with watersheds owned and used by numerous stakeholder. Since BC has relatively abundant and clean surface sources it should protect these in a sustainable manner. The US SWDA specifies that filtration is needed unless the water supply is of high quality. BC needs to formally recognize the critical role of source water protection by providing instrumentation to protect the health of water and, whenever possible, avoid constructing filtration plants. Watershed environmental degradation costs need to be evaluated so that higher value is placed on maintaining quality water sources. It is also important to examine watershed stakeholders whose use may not be assessed at the appropriate resource cost. Higher short term user costs to develop integrated watershed programmes can lower both risk and long-term human and environment health costs. The disparity between policies outlining watershed protection and the ability of water utilities to implement watershed programmes underlies the critical role of government. Policy must move beyond statements and provide instruments for the practical attainment of policy objectives.

The recent BC provincial health officer's report on drinking water quality (BC Gov. 2001b) outlines, in a more comprehensive manner than the 1999 Auditor General's

report, specific steps required to attain water quality objectives. Governments must develop strategic plans and site-specific solutions and ultimately issue permits and licenses. In BC policy for source-water protection has been developed (BC MELP 1999a, 1999b; BC Gov. 2001a) and some key principals established (BC Gov. 2002). It is still too early to know the specific instrumentation to be employed or to assess the effectiveness of these strategies.

All countries need to consider both the short and long term implications of water resource management. Effective management of drinking source water is especially critical for systems that do not have the financial resources to build and maintain treatment facilities other than the use of chlorine as a disinfectant. However, even those systems with greater financial resources benefit by having cleaner source water. Long term water quality implications of emphasizing treatment alone compared to surface source water protection as part of a multi barrier approach are unknown. However, there is greater risk of disease outbreak from source water that is of lower quality, there are increased costs associated with treating lower quality water and a greater prevalence of treatment by-products that can affect health. Prevention of water pollution seems a more wise and prudent management directive than does cleaning our water sources in the future. Water is essential for sustainability, so policies should necessarily be aimed to manage and use water in a sustainable manner.

Table 6.1: Comparison of current\* water quality regulatory limits for British Columbia, Canada, the US and the World Health Organisation.

Criterion	BC Regulations	Canada Guidelines	USEPA Regulations	WHO Guidelines
See following page for table annotations				
Microbiological				
Faecal coliform	✓	✓	✓	✓
Giardia	✗	0 is desirable	✓	✓
<i>Cryptosporidium</i>	✗	0 is desirable	✓	✓
Chemical				
Specific Chemical Targets	NOEL-DMO**	✓	✓	✓
Turbidity	NOEL-DMO**	some guidelines	✓	✓
Disinfection byproducts	NOEL-DMO**	TTHM guidelines	✓	✓
Algal Exudates				
Microcystin-LR	✗	✓	✓	✓
Taste & Odour	✗	suggested, no standard	odour Threshold 3: federally non-enforceable, states can	suggested, no standard
Source Water Protection				
Legislated Enforceable	✓ *** not directly***	only in federal jurisdictions, often indirect <sup>†</sup>	✓	some countries use and enforce WHO guidelines
Focus on Multibarrier Protection <sup>†</sup>	✗	✗	BMP codes of practices <sup>§</sup>	suggested

Table 6.1 annotations.

- \* Note: at time of writing. Enforceable standards and legislation can change rapidly.
- \*\* NOEL–DMO: No Official Enforceable Legislation - at the Discretion of the regional Medical Officer. BC will soon be set to have stronger standards, however, whether the regulations necessary for enforcement will be written for the standards is, as of yet, unknown.
- \*\*\* The DWPA (see text) is legislated but in its present form is a framework only. Regulations needed to make the DWPA enforceable are being drafted. In the interim source water protection can only be maintained through less direct means such as pollution legislation and, for example, the Forestry Practice Code's designation of community watersheds.
- † e.g. under the Fisheries Act the federal government has jurisdiction over fisheries which includes the destruction of habitat or addition of substances deemed deleterious to fish. Fisheries destruction, either physical or resulting from the addition of deleterious substances could, in theory, be used as enforceable legislation in drinking water source protection. However, because the provinces have proprietary rights to both the land and water resources, any action the federal government might take would undoubtedly interfere with provincial jurisdictions.
- ‡ Although certain jurisdictions have source water protection, in general the only mandated drinking water quality objective is at the tap. Note, in order to be exempt from filtration during treatment in the US watershed management is a requirement. Recent Australian drinking water policies adopt a process approach and identify critical control points in an ecosystem framework that include the source watershed, the source water, the treatment process and the distribution system. These policies are currently among the most integrated multibarrier approaches.
- § Best Management Practice. Focus on end-of-tap objectives quantity and quality objectives for individual water parameters, e.g. Maximum Contaminant Levels

Table 6.2: Strategies for attainment of sustainable clean drinking water sources.

Prevention and Treatment

- source-water and treatment are both important for determining water quality;
- inactivation/removal of acute enteric pathogens is a priority;
- disinfection has inherent health risks, including DBP formation and resistant pathogen transmission (e.g. *Cryptosporidium* and *Giardia*);
- presence of anthropogenically derived chemicals (including agricultural and domestic pesticides and industrial products) increases the health risk of drinking water;

Source Water Ecology

- understanding pathogen ecology is necessary for effective environmental management;
- addition of nutrients decreases surface source water quality;
- cyanobacteria can produce dangerous toxins and may present an important health risk;

Policy, Risk Assessment and Sustainability

- multi-barrier approach with adaptive management of critical control points;
- effective policy requires effective instrumentation;
- risk and cost assessment provide a means of prioritizing management strategies;
- strategies for water provision should contain objectives for long-term sustainability.

Figure 6.1: Schematic of surface source drinking water, treatment and distribution.

Water sources are impacted by many natural and anthropogenic activities. Each watershed activity must be considered for water quality risk and stakeholder interest. Less pristine water requires greater treatment to obtain the same water quality. End-of-tap water quality is the principal variable considered for all drinking water policy.

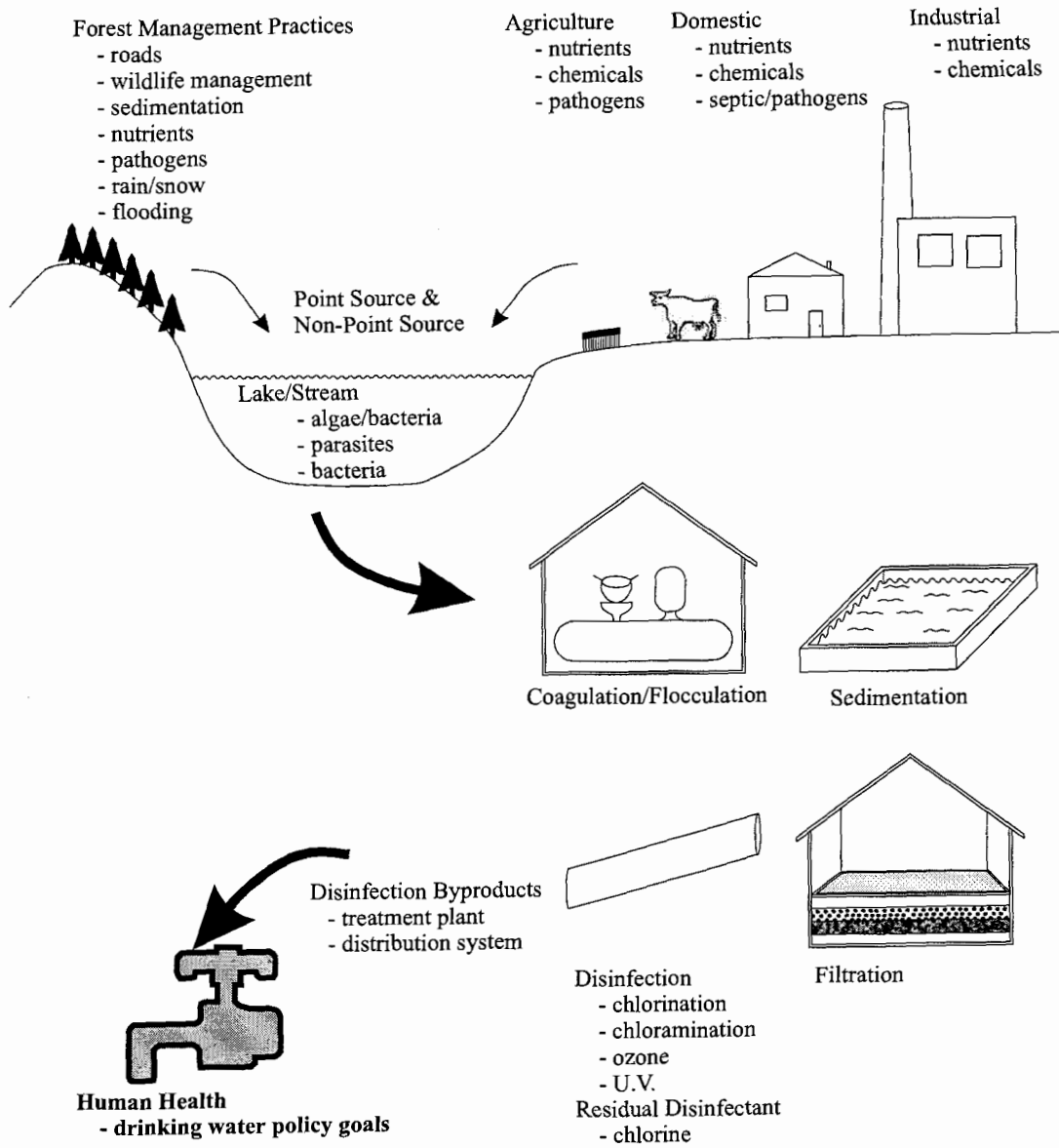
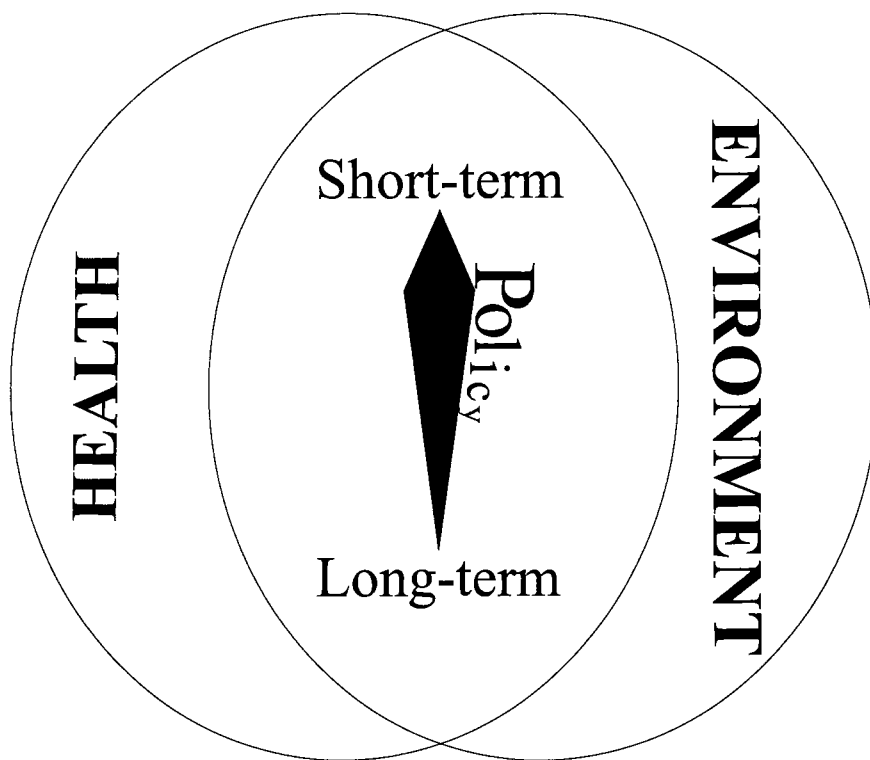


Figure 6.2: Relationship between policy, health, and the environment.

Policy is a driving force for dealing with both health and environmental issues.

Human health and environment health are strongly related. Both human and environmental health should be considered in the context of temporal scale, moving from the immediate to the long-term



**Chapter 7: Conclusions – summary and synthesis**

### ***Research Objectives and Themes***

The objective of this study was to understand the growth and distribution of phytoplankton and to examine the link between phytoplankton and drinking water quality in lakes and reservoirs. Nutrients play a vital role in shaping plankton communities and remain one of the most commonly managed components of lakes. Definitions of spatial and temporal nutrient deficiency and limitation are established in *Chapter 2*. Seasonal productivity of plankton communities is assessed in *Chapter 3*. Successional patterns and associations between phytoplankton species and nutrient indicators are demonstrated in *Chapter 4*. The origin of odour compounds in drinking water systems is addressed in *Chapter 5*. Health issues relating to surface waters and the role of science and policy for management are emphasized in *Chapter 6*.

### ***Summary of Major Findings***

#### *Nutrient Dynamics:*

The lakes in this study were considered to be phosphorus limited as revealed by high TN:TP ratios. P-debt bioassays and rapid  $\text{PO}_4^{3-}$  turnover (Nowlin 2003) and perpetually low SRP values in the epilimnia of the coastal oligotrophic lakes also suggest general P-deficiency. However, nitrogen is an important nutrient affecting growth rates during the stratified period and there was a pronounced decline of DIN concentrations from the winter to the summer. N-debt was greatest in the summer and relaxed in the winter, as was the AER bioassay, which was only measured in the second year.

Size fractionated nutrient bioassay studies suggest that the role of N in affecting phytoplankton growth and species composition may be of more important in size fractions greater than 3  $\mu\text{m}$ , whereas P-deficiency is dominated by the < 3  $\mu\text{m}$  fraction.

These findings are especially relevant to the coastal oligotrophic lakes that are known to have mixotrophic algae. Bacterivorous algae can obtain nutrients from phagotrophy thereby out-competing strict autotrophs (Caron et al. 1993; Bergström et al. 2003). A study conducted in a Swedish humic lake found that nutrient addition of nitrogen in an N-limited lake decreased the mixotrophic to autotrophic ratio by half (Bergström et al. 2003). In my study lakes it is probable that mixotrophy plays an important role in mineral nutrition, and should be considered in future studies and management decisions that evaluate causes and consequences of blooms of specific algal species (e.g. *Dinobryon* spp.).

Phytoplankton most closely associated with conditions of nutrient deficiency were chrysophytes and centric diatoms (Figure 4.6), whereas cyanobacteria and several pennate diatoms were associated with both high total nutrients and high dissolved nutrients. Several chrysophytes are known to be mixotrophic, and may dominate low nutrient conditions. Changes in nutrient loading may change species composition by decreasing the predominance of mixotrophic algae. Changes in species composition occur at short (i.e. intra-annually) and long time scales (e.g. decadal) and it is unknown what the sensitivity of this balance is.

#### *Seasonal Productivity:*

Measurements of productivity demonstrated a high degree of seasonal variability among coastal lakes in this region. Despite low light and temperatures, and complete water column mixing, plankton communities in the winter were active. Maxwell Lake algal biomass and integral productivity were highest in the winter. Winter productivity near the surface of Council Lake was similar to summer productivity, suggesting the

principal factor limiting photosynthesis in the lake was light. In both Council and Maxwell lakes, ambient nutrient concentrations were low throughout the year and P-turnover time was rapid (*Chapter 3*). Maxwell and Council were the smallest and shallowest oligotrophic lakes, so their mean mixing depth was smaller. Thus, during winter mixing these lakes should be more conducive to higher rates of productivity. The unique seasonal pattern observed in Maxwell Lake was, in part, attributed to the small size structure of the crustacean zooplankton community. Future research should examine lakes with variable zooplankton community structure to determine if the size structure is a principal cause of the high wintertime productivity.

*Seasonal Succession:*

The lack of a wintertime “senescence” of plankton communities is argued to be a primary factor affecting interannual species composition. Successional progression of biomass and species of these coastal monomictic lakes did not clearly fit the pattern of successional models (Sommer et al. 1986). The observed variability of species between years corroborates findings that Parks (1995) made in his ten-year study of southern coastal BC lakes. However, seasonal shifts of phytoplankton species were not found to be random (i.e. there was an observable progression). Seasonal patterns of nutrient deficiency were observed, notably in lakes of higher trophic status. The fall bloom in Cusheon and Elk constitute a predictable event because the magnitude of influence is greater than autogenic successional forces and stochastic allogenic forces (e.g. weather).

*Source Water Odour Compounds:*

Total phosphorus was the best single predictor of odour type and intensity. There was a clear difference in the relationship between odour and chlorophyll in the

epilimnion and metalimnion. However, the difference in odour intensity between the epilimnion and metalimnion was smaller when related to total phosphorus. This data suggests that metalimnetic phytoplankton have more chlorophyll per unit biomass, in agreement with theory about low-light adaptation. Since phosphorus ultimately limits the biomass in these lakes it is the biomass itself that is the strongest predictor of odour, not chlorophyll *per se*.

Water purveyors' concerns over taste and odour problems are rarely associated with water sources as oligotrophic as Sooke Lake Reservoir. The low biomass and high species diversity typical of these systems results in a multitude of organoleptic algal byproducts at low concentrations. Flavour Profile Analysis (FPA) has a clear advantage when evaluating odour compounds in oligotrophic lakes – notably a composite picture of odour production can be easily obtained and my observation that TP is closely correlated with FPA intensity may become a popular index for evaluating taste and odor events. Many of these compounds would be missed if analyzed using standard analytical procedures. The situation of oligotrophic lakes contrasts with that in mesotrophic to hyper-eutrophic water sources where a single species producing one or a few compounds can be the principal cause of a taste and odour event. In this latter situation it becomes prudent to understand the causes of these species and the triggers for the production of the odour compounds. However, dramatic changes in seasonal succession or management strategies can affect oligotrophic lakes. At the end of this study the water level in Sooke Lake Reservoir was raised by 2 m, cleared forest was inundated for the first time (during the winter of 2002 – 2003). Average summertime phosphorus and chlorophyll concentrations increased during the summer of 2003 (Nowlin 2003) and a fall

taste and odour event attributed to a *Gloeotrichia* bloom occurred in the late summer (S. Irwin, CRD pers. comm.). This stresses the importance of understanding phytoplankton dynamics as they relate to management strategies, even in high quality water sources.

### ***Towards the Integration of Science and Management***

The need to apply management strategies in the context of surface drinking water sources is suggestive of either a failure of systems to provide adequate water supply or water quality (*sensu* Moss 1999) or a desire to improve safety and quality of water to meet ever growing human consumption. Science-based studies can provide the basis for both management tools and political direction since the management goal of science is to provide predictions for various management strategies (Reynolds 1999). Reynolds (1999) suggests that the key to reaching this goal is to “to identify and quantify the principal regulatory processes” of each system. The primary focus of my thesis was to examine process in lakes of the Coastal and Insular Mountain Region of British Columbia (Northcote and Larkin 1956), and specifically to address the fundamental phytoplankton regulatory processes of several drinking water lakes and reservoirs. The preceding chapters summarize the major findings of my work, however, several emerging themes about the functioning of these systems have emerged.

- 1) Plankton biomass in these lakes is ultimately limited by phosphorus, and phosphorus deficiency is prevalent, however, phosphorus modelling alone cannot explain how these lakes function. Smaller plankton demonstrated the greatest phosphorus deficiency.
- 2) Nitrogen is a seasonally important nutrient, and is hypothesized to be important for shaping species composition, especially for large phytoplankton.

- 3) Future understanding of nutrient dynamics requires a recognition and study of mixotrophic strategies for nutrient acquisition. The resilience of mixotrophic populations to nutrient pulses (*sensu* Bergström et al. 2003) may offer some explanations for observed seasonal and interannual shifts in community composition.
- 4) A regional coherence among lakes emerged from this study. Most notable were the annual patterns of soluble reactive phosphorus (SRP),  $\text{PO}_4^{3-}$  turnover time (Nowlin 2003), and productivity. Unlike the wintertime “slumber” presumed for most temperate lakes, the plankton communities remain active throughout the year.
- 5) Seasonal peaks in biomass and productivity in eutrophic lakes were dominated by nutrient events in the fall in Cusheon Lake and in the spring and fall in Elk Lake, whereas oligotrophic lakes did not demonstrate such a clear pattern. Interannual variability in oligotrophic lakes was attributed to stochastic events. Long-term studies are needed to fully appreciate sources of interannual variability from stochastic events (de Hoyos and Comin 1999). Two years is insufficient to consider interannual changes, so I was unable to fully evaluate the role of stochastic events. However, the data point to wintertime flushing and nutrient loading as being important.

Comprehensive management of drinking water sources requires an integrated approach and relies on identifying critical control points as part of multibarrier plan. Ecological management relies on the ability to predict changes in the environment and the subsequent effect on drinking water quality. This includes not only understanding aquatic processes but also incorporating an integrated approach to evaluate watershed

perturbations (Carignan and Steedman 2000). One of the challenges in reaching this goal is to include complexities that arise from natural stochastic events since it remains a principal source of short-term natural variability in lakes (Moss 1999). Moving towards an understanding of lake functioning and watershed interactions allows for the capacity to understand how water quality will change with the occurrence of perturbations or stochastic events.

TP was the most parsimonious predictor of odour intensity and it was argued that despite species composition, biomass was the major underlying factor of source water odour. However, species composition was important and as TP concentrations increased the type of odour detected changed. Although the principal origins of taste and odour in tap water originating from Sooke Lake Reservoir (CRD) were derived from treatment processes and the distribution systems, algal blooms can cause taste and odour events in the CRD water supply. No seasonal pattern was detected to allow for the evaluation of susceptibility to such blooms in Sooke Lake Reservoir but changes in nutrient pulses and shifts in nutrient supply ratios in combination with stable meteorological conditions increase the possibility of such blooms. While this work demonstrates that phosphorus is a major driving force in defining productivity and growth of phytoplankton in a majority of coastal BC lakes and reservoirs, the next challenge is to define or model the assimilative capacity of these ecosystems, such as defining phytoplankton compositional resistance to P and N loading.

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