

Planktonic phosphorus pool sizes and cycling efficiency in coastal and interior British Columbia lakes

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SUMMARY

1. Limnologists have long acknowledged the importance of phosphorus (P) in determining the organism biomass and productivity of lake ecosystems. Despite a relatively large number of studies that have examined P cycling in lake ecosystems, there remain several substantial methodological issues that have impeded our understanding of P cycling in limnetic plankton communities. Two critical issues confronting ecologists are (1) a lack of precise measurements of the dissolved inorganic phosphorus (PO_4^{3-}) and (2) accurate or complete measurements of dissolved P regeneration rates by plankton communities.
2. Here, we examine patterns of epilimnetic planktonic P pool sizes and turnover rates in eight lakes in British Columbia, Canada over a 2-year period. We determine the concentrations and turnover times of P in various planktonic compartments (dissolved and various planktonic size fractions), using recently developed methods for estimating phosphate concentration and planktonic regeneration rates.
3. The pico- and nanoplankton size fraction (0.2–20 μm) played a central role in planktonic P cycling in lakes examined by this study. On average across lakes, pico- and nanoplankton contained >60% of the planktonic P, accounted for >90% PO_4^{3-} uptake, and contributed 50% of the plankton community dissolved P regeneration rate.
4. PO_4^{3-} concentrations determined by steady state bioassays (ssPO_4^{3-}) were extremely low (87–611 pmol L^{-1}) and were 2–3 orders of magnitude less than simultaneously measured colorimetric soluble reactive phosphorus estimates. Lake ssPO_4^{3-} concentrations increased linearly with total phosphorus (TP), and the slope of this relationship was approximately 1, indicating that PO_4^{3-} remained a consistent proportion of the TP pool across a range of TP concentrations.
5. Turnover rates of the total planktonic P pool and the <20 μm pool became more rapid with increasing lake TP, indicating that, according to this metric, planktonic P cycling efficiency increased with TP concentrations. We also detected a significant relationship between particulate phosphorus (PP) <20 μm turnover time and seston N : P ratios, with PP <20 μm turnover times becoming slower with increasing seston N : P. These findings suggest that long-standing conceptual models of nutrient cycling that predict slower cycling rates and decreasing cycling efficiency with increasing TP concentrations require further empirical examination. We postulate that patterns in lake P turnover and cycling efficiency are a result of complex interactions between plankton biomass and composition, and the ratios of multiple nutrients (C, N, P), rather than solely a function of the TP pool.

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Introduction

The role of phosphorus (P) in lake ecosystems has received intensive study from limnologists and ecologists over the last 30+ years. Phosphorus has received much of this attention because it often limits the biomass and productivity of lake plankton communities (Dillon & Rigler, 1974; Smith, 1979; Mazumder, 1994; but see Elser, Marzolf & Goldman, 1990) and excessive P inputs have been implicated as a major cause of eutrophication (e.g. Schindler, 1977). The importance of P to freshwater plankton communities has led to the detailed examination of P cycling of individual lakes (Levine, Stainton & Schindler, 1986; Taylor & Lean, 1991) and the comparison of P fluxes in systems of contrasting productivity and plankton community structure (Mazumder *et al.*, 1992). These studies and many others have identified factors controlling the structure and function of planktonic food webs and have provided valuable insight into aquatic ecosystem nutrient cycling.

While the number of studies examining lake P cycling is extensive, limnologists have yet to resolve several fundamental issues central to P cycling in lake ecosystems (Tarapchak & Nalewajko, 1986; Taylor & Lean, 1991). A major challenge confronting limnologists is the lack of accurate methods for measuring the concentration of dissolved inorganic phosphorus (PO_4^{3-}) in lakes, especially lakes that are severely P-limited. The need for accurate PO_4^{3-} measurements is critical because PO_4^{3-} is considered the form of dissolved P most readily available to plankton. Limnologists have known for decades that traditional colorimetric methods used to measure PO_4^{3-} (soluble reactive phosphorus or SRP) overestimate concentrations by orders of magnitude (Rigler, 1966; Hudson, Taylor & Schindler, 2000; Hudson & Taylor, 2005) and even more sensitive methods, such as the Rigler bioassay or column chromatography, also greatly overestimate PO_4^{3-} concentrations (Taylor & Lean, 1991; Hudson *et al.*, 2000). Recently developed methods [steady-state bioassay of Hudson *et al.* (2000) and Hudson & Taylor (2005); MAGIC method of Karl & Tien (1992)] have estimated PO_4^{3-} concentrations in P-limited freshwater and marine systems in the pico- and low nanomolar range (Hudson *et al.*, 2000; Wu *et al.*, 2000; Hudson & Taylor, 2005), well below

the detection limits of traditional methods. Despite the implications of such low PO_4^{3-} concentrations, few studies have utilised either of these methods to assess whether phosphate concentrations in other water bodies are within the same range.

The ability to measure P regeneration by planktonic organisms and the relative importance of different plankton groups as suppliers of regenerated P is an unresolved and critical question. Recycled or regenerated nutrients from planktonic organisms can supply a substantial proportion of the nutrients required by plankton communities, especially during summer stratification (Sterner *et al.*, 1995; Hudson, Taylor & Schindler, 1999; Vanni, 2002). However, most methods used to estimate P regeneration from plankton communities have methodological limitations that may yield inaccurate or incomplete P regeneration rates. More commonly used methods for estimating P regeneration rates may violate critical methodological assumptions (Harrison & Harris, 1986), have ineffective or inconsistent treatments (Tremaine & Mills, 1987; Dodds, Priscu & Ellis, 1991), or only measure regeneration from a select planktonic group, such as zooplankton (Lehman, 1980). In addition, many of these methods rely upon colorimetric determination of SRP to calculate P regeneration rates, which, as previously noted, can greatly overestimate PO_4^{3-} concentrations. Regardless of methodological limitations, few studies have compared the absolute amounts and the relative contribution of different planktonic groups as recyclers of P (Dodds *et al.*, 1991; Taylor & Lean, 1991; Hudson & Taylor, 1996). Thus, in order to better understand P cycling in lake plankton communities, dissolved P regeneration rates and the relative importance of different planktonic groups requires further study.

Because of the aforementioned methodological issues, empirical models predicting trends of P cycling in lake ecosystems are based on problematic methods. Many of the current conceptual and empirically derived models propose that planktonic P dynamics is largely a function of the water column P concentration. For example, Harris (1986) and Capblancq (1990) predict that the cycling rates of epilimnetic P pools will become progressively slower as epilimnetic total phosphorus (TP) concentration

increases, indicating decreasing P cycling efficiency with increasing TP. However, recent empirical studies using more current analytical methods have called these predictions into question. Using a relatively new technique to measure planktonic P turnover, Hudson *et al.* (1999) found that the turnover rate of planktonic P pools did not significantly vary with TP concentration. Despite the implications of Hudson *et al.*'s (1999) findings, few studies have simultaneously examined the turnover and recycling of multiple planktonic compartments to determine which variables most influence patterns of P cycling across lake ecosystems.

In the study presented here, we examine patterns of planktonic P cycling (P pool sizes and turnover rates) in eight lakes over a 2-year period. We determine the concentrations and turnover of P in various planktonic compartments (dissolved and planktonic P pools of various size fractions) in lakes in coastal and interior regions of British Columbia, Canada. This study represents one of the first attempts to conduct a relatively broad examination of a variety of planktonic P pools and the turnover rates of these pools in a series of lakes that represent a range of TP. In addition, we use a number of recently developed methods for estimating planktonic regeneration (Hudson & Taylor, 1996) and phosphate concentration (Hudson *et al.*, 2000).

Methods

Study sites and lake sampling

The eight lakes examined in this study are located on the south coast and in the southern interior of British Columbia (Table 1; Spafard *et al.*, 2002). Lakes vary in their concentrations of TP, total nitrogen (TN) and chlorophyll *a* (Chl *a*), zooplankton biomass and bacterial abundance. Based upon nutrient concentrations and the densities and biomasses of organisms, the lakes represent a range in productivity from ultra-oligotrophic to mesotrophic (Wetzel, 2001; Kalff, 2002). While this range in TP is not extensive (i.e. from ultra-oligotrophic to hyper-eutrophic), it represents approximately an order of magnitude span in TP concentration (2–20 $\mu\text{g TP L}^{-1}$) and encompasses a substantial portion of the range of TP concentrations of lakes in the coastal and southern interior regions of British Columbia (British Columbia Ministry of

Table 1 Site abbreviations, number of times sampled in 2000 and 2001, the location within British Columbia, and morphometric and limnological characteristics of the eight lakes examined by this study. Values for nutrient concentrations, and plankton biomasses and densities are the mean (± 1 SD) of the May–October period in 2000 and 2001.

Site	Site Abbr.	N		Location	Lat, Long ($^{\circ}\text{N}$, $^{\circ}\text{W}$)	SA (ha)	Z_{mean} , Z_{max} (m)	TP ($\mu\text{mol L}^{-1}$)	TN ($\mu\text{mol L}^{-1}$)	Zooplankton ($\mu\text{g L}^{-1}$)	Chl <i>a</i> ($\mu\text{g L}^{-1}$)	Bacteria ($\times 10^6$ cells mL^{-1})
		2000	2001									
Council Lake	COL	5	5	Coastal	48°31', 123°40'	16	5.2, 17	0.12 (± 0.04)	6.9 (± 2.0)	15.8 (± 16.9)	0.9 (± 0.3)	0.8 (± 0.5)
Cusheon Lake	CUL	5	5	Coastal	48°48', 123°28'	31	4.4, 9.5	0.52 (± 0.22)	29.8 (± 10)	51.9 (± 44.0)	5.9 (± 8.8)	1.9 (± 0.5)
Elk Lake	ELL	5	5	Coastal	48°31', 123°23'	246	7.7, 19.4	0.57 (± 0.17)	28.7 (± 3.9)	51.1 (± 57.9)	3.0 (± 1.0)	2.0 (± 0.8)
New Lake	NEL	3	2	Interior	49°30', 115°50'	27	3.0, 10.4	0.33 (± 0.13)	76.9 (± 14.2)	19.7 (± 8.2)	2.3 (± 1.4)	2.4 (± 0.5)
Phillips Reservoir	PHR	3	2	Interior	49°27', 115°42'	30	7.7, 17.4	0.24 (± 0.11)	7.2 (± 2.2)	24.9 (± 12.1)	0.7 (± 0.4)	1.3 (± 1.0)
Shawnigan Lake North	SHL-N	5	12	Coastal	48°37', 123°38'	360	14.3, 53	0.16 (± 0.06)	11.0 (± 1.9)	10.7 (± 15.9)	1.6 (± 0.4)	0.7 (± 0.3)
Shawnigan Lake South	SHL-S	5	12	Coastal	48°37', 123°38'	60	11.9, 27	0.15 (± 0.06)	11.0 (± 1.7)	12.2 (± 11.3)	1.7 (± 0.5)	0.8 (± 0.3)
Sooke Lake	SOL-N	4	8	Coastal	48°33', 123°41'	430	23.2, 70	0.10 (± 0.05)	5.6 (± 0.9)	12.6 (± 22.6)	0.8 (± 0.4)	0.7 (± 0.3)
Reservoir North												

Environment, Lands and Parks & Environment Canada, 2000).

Lakes were sampled throughout the summers of 2000 and 2001, and each lake was sampled a minimum of two times during the May–October stratification period (Table 1). Water temperature was measured with a YSI Model 58 (Yellow Springs Instruments, Yellow Springs, OH, U.S.A.), and the epilimnion was defined as the upper portion of the water column that did not exhibit temperature change ≥ 1 °C m^{-1} water depth.

The lakes examined in this study occupied different bioclimatic regions of southern British Columbia (coastal versus the interior near the Rocky Mountains), and regional climate differences could lead to systematic differences in thermal regimes between lakes. Because many of the rate processes examined by this study (plankton PO_4^{3-} uptake and regeneration) may be sensitive to variation in water temperature, we examined whether there were systematic temperature differences between lakes in these two regions. We compared mean summer epilimnetic temperatures of lakes in both regions by calculating mean summer epilimnetic temperature for each coastal lake (COL, CUL, ELL, SHL-S, SHL-N, SOL-N) and each interior lake (NEL, PHR) in each year (2000 and 2001). We then compared mean summer temperatures in coastal ($n = 12$) and interior ($n = 4$) lakes with one-way ANOVA. Mean (± 1 SD) summer epilimnetic temperature for coastal (17.69 ± 0.38) and interior (16.21 ± 1.10) lakes did not significantly differ ($F_{1,14} = 2.75$, $P = 0.119$).

Epilimnetic water was collected with an integrated sampler made of a 5-cm diameter weighted Tygon tube at the deepest point in each lake basin. Integrated epilimnetic samples were collected from 0 to 5.5 m, or to the bottom of the epilimnion if the bottom of the layer was < 5.5 m. On each sampling date, triplicate water samples were collected to measure TP, TN, Chl *a* and bacteria abundance. In addition, approximately 20 L of epilimnetic water was collected in a large dark-coloured plastic container that had been cleaned and thoroughly rinsed with distilled and deionised water prior to field collection. Water from the 20-L container was used for estimation of particulate phosphorus (PP), particulate N and C (PN and PC), $^{32}PO_4^{3-}$ uptake bioassays, and dissolved P regeneration rate estimates. Immediately prior to collection, all containers were rinsed a minimum of three times

with water from the site. Within 3–4 h of collection, water was transported to the lab for analyses. Zooplankton were collected by triplicate vertical tows of a 80- μm Wisconsin plankton net and preserved in 4% sugar-formalin.

Plankton and nutrient analyses

Chlorophyll *a* was estimated by filtration onto Whatman GF/F filters and extracted in 95% ethanol at 4 °C overnight in the dark. Absorption was measured on a Pharmacia Ultraspec[®] 2000 spectrophotometer (Amersham Biosciences, Buckinghamshire, England) with a 10-cm quartz cell and Chl *a* concentration ($\mu g L^{-1}$) was calculated according to Wintermans & De Mots (1965). Bacteria density was estimated by DAPI staining, filtration onto black Nuclepore 0.2- μm filters and counted under UV light (Kemp *et al.*, 1993). Crustacean zooplankton were counted under a dissecting microscope with the aid of software (Z-Count) and biomass was estimated by measuring at least 150 individuals or by measuring all individuals in at least 10% of the total sample volume. Length measurements were converted to biomass using published length–mass relationships (Bottrell *et al.*, 1976; Rosen, 1981; Culver *et al.*, 1985; Yan & Mackie, 1987).

Total phosphorus was determined by potassium persulphate digestion in an autoclave and analysed as PO_4^{3-} on a Lachat autoanalyzer (QuickChem[®] 8000; Zellweger Analytics, Lincolnshire, IL, U.S.A.). PP determination was performed by serial filtration (Mazumder *et al.*, 1988; Taylor & Lean, 1991) and we determined PP concentration in five plankton size classes: > 200 (mesoplankton), 200–41 (large microplankton), 41–20 (small microplankton), 20–3 (nanoplankton) and 3–0.2 μm (picoplankton). Particulates were collected on 50 \times 50 mm acid-rinsed squares of Nitex netting (200- and 41- μm) or 47-mm diameter Nuclepore polycarbonate filters (20-, 3- and 0.2- μm). We determined that brief acid rinsing did not change aperture size of Nitex by measuring apertures before and after rinsing with acid. A total of 2–6 L of lake water was used in the serial filtration process, depending upon the concentration of particles in the epilimnion at the time of sampling. After serial filtration, Nitex screens and Nuclepore filters were placed into acid-washed screw-cap test tubes and digested with potassium persulphate in an autoclave. P contained in each plankton size class was

determined as in TP analyses and corrected for Nitex and Nuclepore blanks. The sum of concentrations in all size fractions was considered the concentration of total PP (TPP). Total dissolved phosphorus (TDP) was calculated by subtracting TPP from simultaneously measured TP; therefore, TDP was composed of forms of P that can pass through a 0.2- μm filter, which generally includes very small particles (<0.2 μm), colloidal P, small molecular weight organic P and PO_4^{3-} (Lean, 1973).

Total nitrogen was determined by autoclaving samples with alkaline potassium persulphate and measured as nitrate with the cadmium reduction method [American Public Health Association (APHA), 1998]. PN and PC were measured by collecting particulates on ashed GF/F filters (0.7 μm nominal pore size), and analysed on a Costech CHN analyzer (Valencia, CA, U.S.A.) with a Delta^{plus} Advantage mass spectrometer (Thermo Electron Corporation, Waltham, MA, U.S.A.) as the detector. Acetanilide standard curves were generated for each analysis run.

Estimation of PO_4^{3-} concentration

PO_4^{3-} concentration was measured using two methods: the colorimetric SRP method and the steady-state bioassay method (ss PO_4^{3-}) developed by Hudson *et al.* (2000). In the colorimetric method, undigested filtered lake water (<0.2 μm) was analysed as PO_4^{3-} on a Lachat autoanalyzer.

To estimate PO_4^{3-} concentration with the Hudson *et al.* (2000) steady-state bioassay method, the $^{32}\text{PO}_4^{3-}$ uptake constant (k) and the planktonic dissolved P regeneration rate (see PO_4^{3-} uptake and turnover and Plankton regeneration and turnover below) are utilised in the following equation

$$k \times [\text{PO}_4^{3-}] = \text{dissolved P regeneration rate}$$

This method assumes that, in systems with rapid PO_4^{3-} uptake and turnover (i.e. P is deficient) uptake and regeneration of dissolved inorganic P by the plankton community are tightly coupled and essentially equal in the short-term. In nutrient limited pelagic systems, the uptake and regeneration of dissolved inorganic nutrients by plankton communities are approximately equal and the balance of uptake and regeneration control the short-term steady state concentrations of inorganic nutrients (Dodds, 1993; Harrison, 1993). This method also assumes that

dissolved P regenerated by the plankton community is mostly in the form of phosphate or low molecular weight organic compounds that are quickly hydrolysed by phosphatases (Peters & Lean, 1973; Lean & Nalewajko, 1976; Taylor & Lean, 1981). Based upon these assumptions, the method of using the plankton dissolved P regeneration rate and the PO_4^{3-} uptake constant to estimate the concentration of PO_4^{3-} is applicable to systems that exhibit rapid uptake and turnover of the PO_4^{3-} pool (Hudson *et al.*, 2000).

PO_4^{3-} uptake and turnover

To determine the uptake and turnover of PO_4^{3-} pools in the study lakes, we conducted $^{32}\text{PO}_4^{3-}$ uptake bioassays (Lean & White, 1983; Mazumder *et al.*, 1988). Uptake bioassays for each lake on each sampling date were conducted in duplicate or triplicate. Carrier-free $^{32}\text{PO}_4^{3-}$ (Sigma-Aldrich, St. Louis, MO, U.S.A.) was added to 100 mL of whole lake water (final activity 900–3200 Bq mL⁻¹) in an acid-washed high-density polyethylene (HDPE) beaker. Two-millilitre subsamples were removed at 0.5, 1, 2, 4, 6 and 10 min after addition of $^{32}\text{PO}_4^{3-}$ and passed through 25-mm diameter 0.2- μm Nuclepore filters and the filtrate was placed in scintillation vials. After 15 min, 5-mL subsamples were collected and passed through 0.2-, 3- and 20- μm Nuclepore polycarbonate filters, filters were then placed into scintillation vials and dissolved with 100 μL of methylene chloride : ethanol amine (10 : 1). Scintillation fluor (10 mL, Scintiverse II; Fisher Scientific, Waltham, MA, U.S.A.) was added to vials containing filtrate and filters. For all uptake incubations, water was maintained at epilimnetic temperature. ^{32}P activity of samples was determined using a Beckman LS6000IC or Wallac 1410 liquid scintillation counter. ^{32}P activities on the three different filters (0.2-, 3- and 20- μm) taken at the end of bioassays were used to estimate per cent $^{32}\text{PO}_4^{3-}$ uptake by different size classes of plankton (0.2–3, 3–20 and >20 μm). Assuming activity on the 0.2 μm filter represented the total assimilated $^{32}\text{PO}_4^{3-}$ by all size classes of the plankton community, the $^{32}\text{PO}_4^{3-}$ taken up by the 0.2–3 μm fraction was calculated by subtracting the activity of the 3- μm from the 0.2- μm filter, and the uptake of the 3–20 μm fraction was estimated by subtracting the activity of the 0.2–3 μm size class and the >20 μm size class (activity on the 20 μm filter).

To estimate the turnover time of the lake PO_4^{3-} pool, the natural log (ln) of the per cent $^{32}\text{PO}_4^{3-}$ left in solution was plotted as a function of time (minutes) and ordinary least-squares regression was used to estimate the slope. The absolute value of the slope was the $^{32}\text{PO}_4^{3-}$ uptake constant (k , min^{-1}) and the reciprocal ($1/k$) was the turnover time in minutes (Lean, 1973).

Plankton regeneration and turnover

To measure the dissolved P regeneration by the plankton community, we used the method of Hudson & Taylor (1996). Discussion of methodological and theoretical considerations of this method is outside the scope of this paper, but these topics are thoroughly considered elsewhere (Hudson & Taylor, 1996, 2005). Briefly, immediately after bringing lake water into the lab, 4 L were gently decanted into acid-washed HDPE square-sided bottles and carrier-free $^{32}\text{PO}_4^{3-}$ was added (final activity 225–800 Bq mL^{-1}). Bottles were incubated at epilimnetic temperature for 28–36 h to label the plankton community. After the incubation period, 200–400 mL volumes were removed to determine the dissolved P release rates of the entire plankton community and of different plankton size fractions. We determined dissolved ^{32}P release rates from the unfractionated plankton community (whole water), plankton >200, 200–41, 41–20 and <20 μm . To size-fractionate samples, water was collected in acid-washed HDPE beakers after passing through 200-, 41- or 20- μm Nitex screens, using gravity. To determine the dissolved ^{32}P activity at time zero, 5-mL subsamples were immediately removed and passed through 25-mm diameter 0.2- μm syringe filters (Sarstedt Filtropur[®] polyethersulfone filters Sarstedt AG & Co., Nümbrecht, Germany) into scintillation vials. 'Cold' $^{31}\text{PO}_4^{3-}$ was then added to each beaker to a final concentration of 24 $\mu\text{mol P L}^{-1}$ (750 $\mu\text{g P L}^{-1}$) to act as a competitive inhibitor for ^{32}P (Hudson & Taylor, 1996). Over the following 5–8 h, four to seven subsamples (5 mL) were removed from each beaker and passed through 0.2- μm syringe filters into scintillation vials. Beakers containing the unfractionated plankton community and the various size fractions were performed in duplicate.

To estimate ^{32}P release rates of the various size fractions, dissolved ^{32}P activity (dpm L^{-1} in <0.2 μm filtrate) from each size-fractionated water sample was

plotted as a function of time (starting at time zero), and the slope was estimated using ordinary least-squares regression. The slope was considered to be equivalent to the dissolved ^{32}P release rate by plankton within each size fraction. To estimate dissolved P release rate ($\text{ng P L}^{-1} \text{h}^{-1}$) from each size fraction, the total ^{32}P added at the beginning of the initial incubation period and the P concentration were used in the following equation:

$$\text{P release rate} = \frac{(^{32}\text{P release rate} \times [\text{TP}])}{\text{total initial } ^{32}\text{P activity}}$$

The P regeneration rate of each plankton size class (unfractionated plankton community, >200, 200–41, 41–20 and <20 μm) was estimated by using TP concentration and the total activity of ^{32}P added to the initial 4-L sample (Hudson & Taylor, 1996, 2005).

We calculated the turnover time (in days) of the TPP pool and the various planktonic PP size fractions by using PP concentrations and daily regeneration rates. The turnover time for the TPP pool was calculated as the concentration of TPP divided by the unfractionated plankton community daily regeneration rate ($\text{nmol L}^{-1} \text{day}^{-1}$) and the turnover times of plankton size fractions were calculated by dividing the concentration of PP in a specific size class (>200, 200–41, 41–20 and <20 μm) by the daily regeneration rate of the size fraction.

Data analysis

For nutrient concentrations, plankton biomass and densities, and rate measurements (TP, PP, TN, PC, PN, ssPO_4^{3-} , SRP, Chl *a*, zooplankton biomass, bacterial abundance, regeneration rates and turnover times) we calculated a mean of the summer thermally stratified period for each lake across both years ($n = 8$). We used the summer mean for each lake instead of using individual time series measurements in analyses, because we wished to describe the average summer conditions and rate processes within each lake during the stratified period and avoid pseudoreplication issues (Hurlbert, 1984). While use of summer means does not allow for examination of seasonal variability or trends, seasonal or annual means are commonly used to describe lake conditions and processes (Mazumder, 1994; Fee *et al.*, 1996; Knoll, Vanni & Renwick, 2003).

To examine patterns in P cycling across the set of lakes, we *a priori* examined P pool sizes, flux rates and turnover times in relation to epilimnetic TP concentration. We performed this analysis first because several conceptual models of pelagic nutrient cycling propose that nutrient pool sizes and turnover rates are a function of the water column nutrient concentration (Reynolds, 1984; Harris, 1986; Capblancq, 1990). In addition, a limited number of studies that have utilised relatively new methods of estimating PO_4^{3-} concentration and planktonic P turnover rates have found that these measures exhibit significant relationships with TP concentration (Hudson *et al.*, 1999, 2000). Concentrations of TDP, TP, SRP, ssPO_4^{3-} and the various plankton PP size fractions were regressed as a function of lake TP concentration using ordinary least-squares regression. Dissolved P regeneration rates of the various size fractions and the turnover rates of the various P pools (PO_4^{3-} , TPP and PP size fractions) were also examined as a function of TP. In addition to TP, we also examined P pool sizes and turnover rates in relation to a number of other biological and nutrient variables (bacteria density, Chl *a* and zooplankton biomass, TN, TN : TP, $\text{PO}_4^{3-} : \text{NH}_4^+$, particulate N (PN), PN : PP, particulate C (PC), PC : PP and PC : PN). Statistical analyses were performed with SPSS version 8.0 (SPSS, Inc., Chicago, IL, U.S.A.). Significance for all analyses was set at $\alpha \leq 0.05$.

Results

Lake TP, PP and TDP concentrations

Mean summer TP of lakes ranged from 0.097 to 0.548 $\mu\text{mol L}^{-1}$ (3.0–16.9 $\mu\text{g L}^{-1}$). Several limnological variables exhibited a significant relationship with TP (Table 2). TN increased with TP; however, one lake (NEL) had much higher TN concentrations than others (Table 1). When NEL was included in a TP–TN regression, TN exhibited a non-significant relationship with TP, but the relationship was significant when NEL is excluded (Table 2). Bacteria density, Chl

a and zooplankton biomass significantly increased with TP (Table 2), indicating that the plankton community was likely limited by P (Davies, Nowlin & Mazumder, 2004).

Mean summer particulate P in the planktonic pool ranged from 0.07 to 0.33 $\mu\text{mol L}^{-1}$ and on average (± 1 SD) 73 (± 35) % of TP in lakes was in particulate form (Table 3). The concentration of TPP increased linearly with TP (Fig. 1); however, as TP increased the TP–TPP regression line increasingly deviated from a 1 : 1 line. This trend indicates that the TDP fraction became an increasingly larger fraction of the TP pool as TP increased. In several lakes, TPP concentrations were approximately equal to TP, indicating TDP was extremely low or undetectable (Table 3); in these lakes, lake TP concentrations were relatively low ($\leq 0.11 \mu\text{mol L}^{-1}$; Fig. 1).

Within the plankton PP pool, the picoplankton contained 42–58% ($\bar{x} = 50\%$) and the nanoplankton contained 12% of PP (Table 3); thus, the pico- and nanoplankton ($< 20 \mu\text{m}$) contained the majority of PP (62%) in all lakes. The mesoplankton contained the second largest proportion of the PP pool ($\bar{x} = 18\%$). The small and large microplankton, on average contained 13% and 8% of PP, respectively.

PO_4^{3-} concentration

PO_4^{3-} concentrations measured as SRP ranged from 0.024 to 0.083 $\mu\text{mol L}^{-1}$ (0.75–2.58 $\mu\text{g L}^{-1}$). There was little variation in SRP concentration between lakes (mean SRP ± 1 SD = 0.048 \pm 0.02 $\mu\text{mol L}^{-1}$) and SRP was not a significant function of TP ($r^2 = 0.036$, $P = 0.990$).

Steady state bioassay PO_4^{3-} measurements (ssPO_4^{3-}) ranged from 87 to 611 pmol L^{-1} , and ssPO_4^{3-} concentration increased linearly with both TP and TDP (Fig. 2a & b). Across lakes, ssPO_4^{3-} made up a small percentage of the TP and TDP pools, on average composing $< 0.001\%$ of TP and $< 0.24\%$ of TDP (excluding lake data points where TDP was undetectable). When ssPO_4^{3-} and simultaneously measured

Variable	Equation	r^2	F	d.f.	P -value
TN ($\mu\text{mol L}^{-1}$) – with NEL	$y = 69.91x + 3.6$	0.27	2.23	1, 6	0.186
TN ($\mu\text{mol L}^{-1}$) – without NEL	$y = 49.6x + 1.5$	0.88	86.54	1, 5	0.001
Bacteria ($\times 10^6$ cells mL^{-1})	$y = 3.2x + 0.5$	0.70	14.15	1, 6	0.009
Chl <i>a</i> ($\mu\text{g L}^{-1}$)	$y = 7.4x + 0.1$	0.64	10.72	1, 6	0.017
Zooplankton ($\mu\text{g L}^{-1}$)	$y = 83.2x + 3.8$	0.84	32.6	1, 6	0.001

Table 2 Relationship between lake total phosphorus (TP) ($\mu\text{mol L}^{-1}$) and total nitrogen (TN), bacteria abundance, chlorophyll *a* concentration (Chl *a*) and zooplankton biomass of the study lakes

Table 3 Concentration of particulate phosphorus (PP), the percentage of the total phosphorus (TP) pool in the particulate form (% TP) and the percentage of TPP in the various plankton size fractions of the eight study lakes. Mean summer values (± 1 SD) for 2000 and 2001 are given for each lake.

Site	PP ($\mu\text{mol L}^{-1}$)	% TP	% TPP 0.2–3 μm	% TPP 3–20 μm	% TPP 20–41 μm	% TPP 41–200 μm	% TPP >200 μm
COL	0.09 (± 0.01)	100 (± 0)	46 (± 7)	13 (± 6)	17 (± 7)	10 (± 4)	15 (± 1)
CUL	0.27 (± 0.09)	50 (± 11)	45 (± 11)	9 (± 1)	6 (± 1)	21 (± 3)	19 (± 11)
ELL	0.33 (± 0.10)	61 (± 23)	42 (± 10)	11 (± 6)	4 (± 1)	13 (± 6)	31 (± 10)
NEL	0.19 (± 0.01)	57 (± 12)	58 (± 6)	11 (± 1)	6 (± 1)	12 (± 5)	14 (± 1)
PHR	0.18 (± 0.03)	74 (± 4)	53 (± 4)	14 (± 4)	4 (± 1)	11 (± 4)	18 (± 11)
SHL-N	0.10 (± 0.01)	61 (± 18)	49 (± 5)	14 (± 5)	8 (± 1)	12 (± 1)	19 (± 8)
SHL-S	0.11 (± 0.01)	99 (± 1)	51 (± 9)	14 (± 1)	10 (± 1)	13 (± 4)	13 (± 6)
SOL-N	0.08 (± 0.02)	83 (± 24)	57 (± 1)	12 (± 4)	5 (± 1)	10 (± 1)	18 (± 4)
All Sites	–	73 (± 35)	50 (± 1)	12 (± 4)	7 (± 4)	13 (± 8)	18 (± 3)

COL, Council Lake; CUL, Cusheon Lake; ELL, Elk Lake; NEL, New Lake; PHR, Phillips Reservoir; SHL-N, Shawnigan Lake North; SHL-S, Shawnigan Lake South; SOL-N, Sooke Lake Reservoir North.

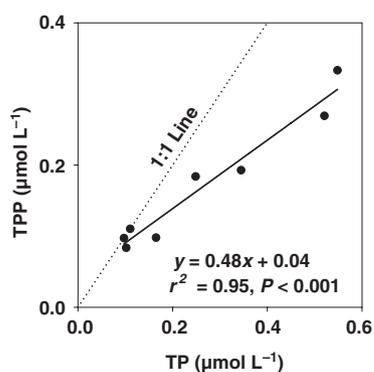


Fig. 1 Relationship between total phosphorus (TP) and the concentration of total particulate phosphorus (TPP). Note that as TPP concentration increases with TP concentration, there is an increasing deviation from a 1 : 1 line.

SRP concentrations were compared, ssPO_4^{3-} estimates were 2–3 orders of magnitude less than SRP concentrations. We further examined the relationship between ssPO_4^{3-} and SRP measurements and found that estimates of PO_4^{3-} concentration by both methods were not significantly correlated with one another (Fig. 2c; Pearson $r = 0.300$, $F_{1,6} = 0.593$, $P = 0.30$).

We compared our ssPO_4^{3-} measurements with those of Hudson *et al.* (2000), who first reported PO_4^{3-} concentrations in freshwater systems using the steady state bioassay method. Hudson *et al.* (2000) present their data in a $\log_{10} \text{ssPO}_4^{3-}$ – $\log_{10} \text{TP}$ relationship and found a significant positive linear relationship between the two measures (Hudson, Taylor & Schindler's regression equation: $\log_{10} [\text{ssPO}_4^{3-}] = 0.745 (\log_{10} [\text{TP}]) - 2.11$). When we transform our data and plot $\log_{10} \text{ssPO}_4^{3-}$ in pmol L^{-1} as a function of $\log_{10} \text{TP}$ in

pmol L^{-1} ($\log_{10} [\text{ssPO}_4^{3-}] = 1.05 (\log_{10} [\text{TP}]) - 3.30$, $r^2 = 0.91$, $P < 0.001$) alongside the relationship from Hudson *et al.* (2000), three things are apparent (see inset figure in Fig. 2a). First, our measures of ssPO_4^{3-} fall within the range of values reported by Hudson *et al.* (2000). Secondly, our ssPO_4^{3-} measurements are consistently higher than those of Hudson *et al.* (2000) at a given TP (i.e. our regression line is elevated above theirs). Finally, the slope of the $\log \text{ssPO}_4^{3-}$ – $\log \text{TP}$ relationship presented by Hudson *et al.* (2000) is 0.745, indicating that the proportion TP occupied by PO_4^{3-} declined with increasing TP. In contrast, the slope of our relationship is approximately 1, indicating ssPO_4^{3-} remained a consistent proportion of TP across lakes.

PO_4^{3-} uptake and turnover

$^{32}\text{PO}_4^{3-}$ uptake constants varied from 0.119 to 0.164 min^{-1} , suggesting that plankton communities in lakes were strongly P-deficient (Table 4). The picoplankton size fraction (0.2–3 μm) overwhelmingly dominated the uptake of $^{32}\text{PO}_4^{3-}$, accounting for 77–91% of $^{32}\text{PO}_4^{3-}$ uptake (Table 4). Nanoplankton (3–20 μm) accounted for a smaller portion of the $^{32}\text{PO}_4^{3-}$ uptake (8–21%); therefore, plankton >20 μm only accounted for 3–6% of $^{32}\text{PO}_4^{3-}$ uptake (Table 4).

The turnover time of PO_4^{3-} pools during the summer stratification period (calculated from $^{32}\text{PO}_4^{3-}$ uptake constants) was extremely rapid and varied from 5.6 to 8.4 min ($\bar{x} = 6.9$ min; Table 4). $^{32}\text{PO}_4^{3-}$ turnover time did not vary significantly with TP ($P = 0.720$), ssPO_4^{3-} ($P = 0.550$), SRP ($P = 0.841$) or TDP ($P = 0.561$). In addition, PO_4^{3-} turnover time was not

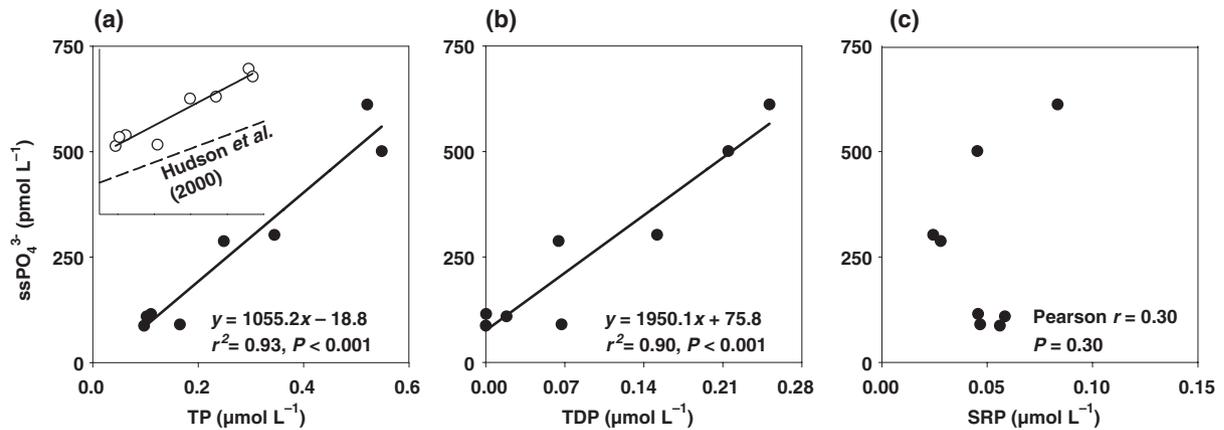


Fig. 2 Relationship between steady state bioassay measurements of PO_4^{3-} concentration (ssPO_4^{3-}) and (a) total phosphorus (TP), (b) total dissolved phosphorus and (c) soluble reactive phosphorus. The smaller figure inset in panel (a) is the $\log_{10} [\text{ssPO}_4^{3-}] - \log_{10} [\text{TP}]$ relationship for lakes in the present study and the ordinary least squares regression line. The dashed line in the inset figure in panel (a) is the $\log_{10} [\text{ssPO}_4^{3-}] - \log_{10} [\text{TP}]$ relationship from Hudson *et al.* (2000). See text for the regression equations for both $\log_{10} [\text{ssPO}_4^{3-}] - \log_{10} [\text{TP}]$ relationships.

related significantly to $\text{ssPO}_4^{3-} : \text{NH}_4^+$ ($P = 0.255$), TPP concentration ($P = 0.914$), TN ($P = 0.499$), TN : TP ($P = 0.622$), particulate N (PN; $P = 0.549$), PN : PP ($P = 0.814$), particulate C (PC; $P = 0.293$), PC : PP ($P = 0.428$), PC : PN ($P = 0.353$), Chl *a* ($P = 0.459$) or bacteria abundance ($P = 0.539$). Water temperature can affect planktonic rate-dependent process, such as PO_4^{3-} uptake and turnover, thus we also examined whether PO_4^{3-} turnover times in lakes were a function of mean epilimnetic temperatures (Table 4); PO_4^{3-} turnover times were not significantly related to mean epilimnetic temperature ($P = 0.370$).

Planktonic P regeneration

Regeneration rates of unfractionated (whole) plankton communities ranged from 2.0 to 16.5 $\text{nmol P L}^{-1} \text{h}^{-1}$

(60–511 $\text{ng P L}^{-1} \text{h}^{-1}$) and increased linearly with TP (Fig. 3a). The regeneration rate of mesoplankton (>200 μm), small microplankton (200–41 μm) and pico- and nanoplankton (<20 μm) had a significant positive relationship with TP (Fig. 3b–e). The relationship between TP and the regeneration rate of pico- and nanoplankton was much stronger than the relationships between TP and the other plankton size fractions.

We compared the relationship between regeneration rates and TP from our lakes to the regeneration rate–TP relationship of Hudson *et al.* (1999). Regeneration rates and TP concentrations were \log_{10} transformed to facilitate this comparison (see insert in Fig. 3a). The slope of our log-transformed regression line [$\log_{10} \text{Regeneration Rate (ng L h}^{-1}) = 1.153 \log_{10} \text{TP (}\mu\text{g L}^{-1}) + 1.240$] was similar to that of Hudson, Taylor & Schindler [$\log_{10} \text{Regeneration Rate$

Site	Temperature ($^{\circ}\text{C}$)	k (min^{-1})	% 0.2–3 μm	% 3–20 μm	% >20 μm
COL	17.6 (11.3–20.5)	0.125 (± 0.03)	78 (± 5)	19 (± 4)	4 (± 1)
CUL	19.3 (13.0–23.0)	0.178 (± 0.02)	85 (± 3)	11 (± 2)	5 (± 1)
ELL	19.6 (13.9–23.1)	0.119 (± 0.02)	81 (± 9)	14 (± 5)	6 (± 4)
NEL	16.6 (14.4–19.0)	0.164 (± 0.01)	91 (± 1)	8 (± 2)	2 (± 1)
PHR	14.5 (10.3–17.3)	0.175 (± 0.02)	90 (± 1)	8 (± 1)	2 (± 1)
SHL-N	17.6 (12.0–21.4)	0.156 (± 0.02)	79 (± 7)	18 (± 7)	3 (± 1)
SHL-S	17.9 (11.6–21.6)	0.146 (± 0.01)	77 (± 4)	21 (± 4)	3 (± 1)
SOL-N	16.7 (9.7–21.9)	0.129 (± 0.01)	79 (± 1)	13 (± 6)	3 (± 1)
All Sites	17.7 (9.7–23.1)	0.149 (± 0.04)	83 (± 5)	14 (± 6)	4 (± 1)

COL, Council Lake; CUL, Cusheon Lake; ELL, Elk Lake; NEL, New Lake; PHR, Phillips Reservoir; SHL-N, Shawnigan Lake North; SHL-S, Shawnigan Lake South; SOL-N, Sooke Lake Reservoir North.

Table 4 Lake water temperatures, $^{32}\text{PO}_4^{3-}$ uptake constants (k), and the percent uptake of PO_4^{3-} by plankton size fractions during $^{32}\text{PO}_4^{3-}$ uptake bioassays. Mean lake water temperatures for summers 2000 and 2001 and the absolute range in temperatures across both summer are presented for each lake. k and percentage uptake values for each plankton size fraction presented for each lake are the summer means (± 1 SD) of 2000 and 2001

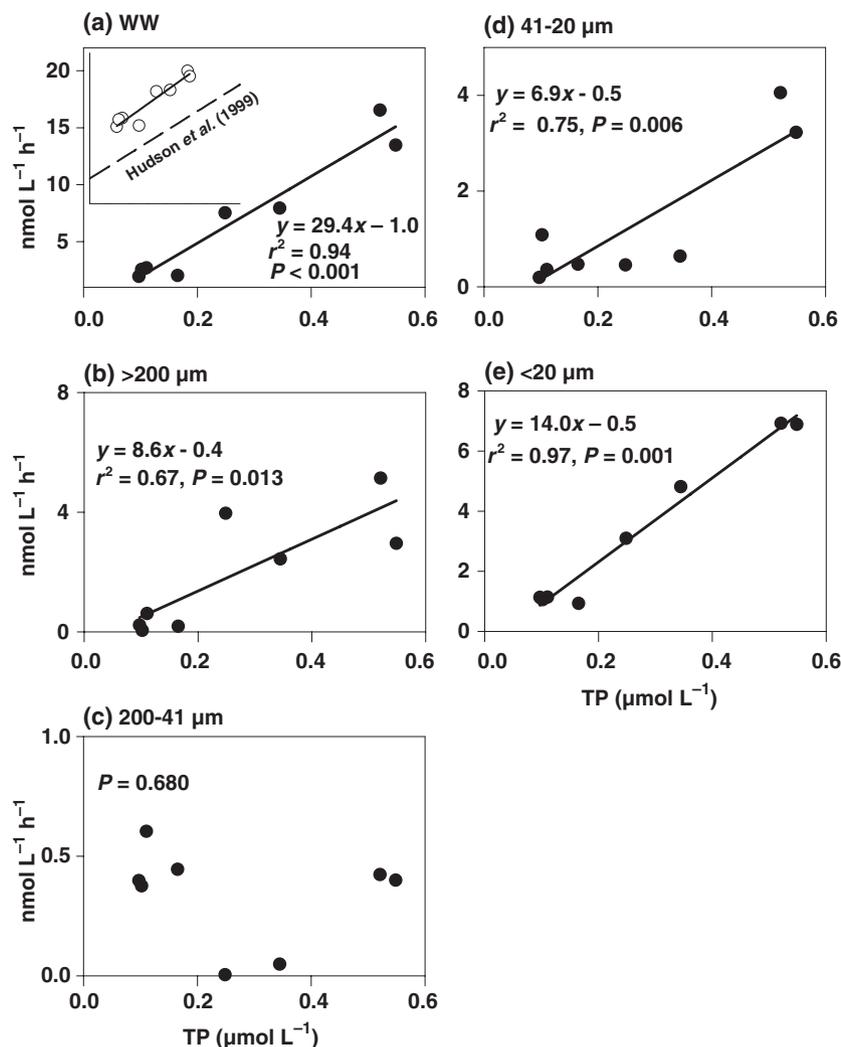


Fig. 3 Dissolved phosphorus regeneration rates ($\text{nmol L}^{-1} \text{h}^{-1}$) of the various size fractions of the plankton community as a function of TP. (a) The whole water (WW) unfractionated plankton community, (b) the $>200 \mu\text{m}$ mesoplankton, (c) the $200\text{--}41 \mu\text{m}$ large microplankton, (d) the $41\text{--}20 \mu\text{m}$ small microplankton and (e) the $<20 \mu\text{m}$ pico- and nanoplankton. The smaller figure inset in panel (a) is the \log_{10} Regeneration Rate– \log_{10} [TP] relationship for the lakes in the present study and the ordinary least squares regression line. The dashed line in the inset figure in panel (a) is the \log_{10} Regeneration Rate– \log_{10} [TP] relationship from Hudson *et al.* (1999). See text for the regression equations for both \log_{10} Regeneration Rate– \log_{10} [TP] relationships.

(ng L h^{-1}) = $1.0077 \log_{10} \text{TP} (\mu\text{g L}^{-1}) + 0.7206$]; however, both our slope (1.153) and intercept (1.240) were greater than Hudson, Taylor & Schindler (slope = 1.0077, intercept = 0.7206), indicating that plankton community P regeneration rates in our lakes were consistently higher per unit TP and that regeneration rates increased more rapidly with TP than the lakes studied by Hudson *et al.* (1999).

The pico- and nanoplankton size fraction ($<20 \mu\text{m}$) was typically the largest contributor to community regeneration rates (Table 5), accounting for 41–57% of the community regeneration rate ($\bar{x} = 49\%$). Mesoplankton ($>200 \mu\text{m}$) were also relatively important contributors to community regeneration rates, accounting for 3–45% ($\bar{x} = 22\%$) of the community regeneration rate (Table 5). The per cent contribution

of large and small microplankton was more variable among lakes (large microplankton = 1–23%, small microplankton = 8–26%).

Planktonic P turnover and cycling rates

Plankton community regeneration rates were rapid enough to recycle TPP pools every 0.7–2.1 days ($\bar{x} = 1.3$ days) and TPP turnover rates exhibited a significant linear relationship with TP concentration, turning over more rapidly as TP concentration increased (Fig. 4a). Within the PP pool, the turnover rate of the mesoplankton pool ($>200 \mu\text{m}$) exhibited a similar significant relationship with increasing TP ($r^2 = 0.228$, $F_{1,6} = 1.8$, $P = 0.026$); however, the large microplankton ($41\text{--}20 \mu\text{m}$; $F_{1,6} = 0.002$, $P = 0.966$) and small

Table 5 Per cent contribution of plankton size fractions to the community dissolved P regeneration rate. Values presented for each lake are the summer mean (± 1 SD) of 2000 and 2001

Site	% <20 μm	% 20–41 μm	% 41–200 μm	% >200 μm
COL	57 (± 13)	8 (± 2)	20 (± 17)	16 (± 6)
CUL	45 (± 2)	22 (± 4)	6 (± 6)	28 (± 4)
ELL	53 (± 8)	19 (± 2)	7 (± 10)	21 (± 1)
NEL	55 (± 3)	12 (± 14)	2 (± 3)	31 (± 20)
PHR	45 (± 24)	10 (± 14)	1 (± 1)	45 (± 38)
SHL-N	42 (± 3)	26 (± 4)	23 (± 8)	9 (± 2)
SHL-S	41 (± 14)	15 (± 2)	19 (± 6)	25 (± 6)
SOL-N	51 (± 6)	31 (± 20)	15 (± 14)	3 (± 1)
All Sites	49 (± 6)	18 (± 8)	11 (± 9)	22 (± 13)

COL, Council Lake; CUL, Cusheon Lake; ELL, Elk Lake; NEL, New Lake; PHR, Phillips Reservoir; SHL-N, Shawnigan Lake North; SHL-S, Shawnigan Lake South; SOL-N, Sooke Lake Reservoir North.

microplankton (41–20 μm ; $F_{1,6} = 2.79$, $P = 0.146$) did not exhibit a significant relationship with TP. The turnover rate of the pico- and nanoplankton fraction (<20 μm) was significantly related to TP concentration, exhibiting shorter turnover times with increasing TP concentration (Fig. 4b; $r^2 = 0.795$, $F_{1,6} = 23.27$, $P = 0.003$). Of the plankton size fractions, the pico- and nanoplankton exhibited the strongest relationship between turnover rate and TP concentration (Fig. 4b), and on average, the pico- and nanoplankton fraction contained 62% of planktonic P. Thus, the turnover of the nano- and picoplankton appears to strongly contribute to the significant relationship between TPP turnover rates and TP. Interestingly, the mean ± 1 SD turnover rates of the mesoplankton (3.17 ± 5.48 days),

the large microplankton (6.76 ± 14.71 days), the small microplankton (1.07 ± 1.55 days) and the nano- and picoplankton (3.01 ± 1.74 days) were similar and within the same order.

The turnover rate of TPP was not a significant function of PC : PP ($P = 0.295$), PC : PN ($P = 0.967$) or PN : PP ($P = 0.102$). Turnover rates of PP <20 μm were not significantly related to PC : PP ($P = 0.234$) or PC : PN ($P = 0.936$), but were a significant function of PN : PP (Fig. 5; $r^2 = 0.508$, $F_{1,6} = 6.194$, $P = 0.047$), with PP <20 μm turnover rates becoming progressively slower as planktonic PN : PP ratios increased.

Discussion

In the present study, we examined patterns of planktonic P cycling in coastal and interior British Columbia lakes and found that the pico- and nano-plankton size fraction (<20 μm) played a principal role in planktonic P recycling across a diversity of lakes, by containing a substantial proportion of the P in plankton and accounting for much of the dissolved P regeneration. We also found that PO_4^{3-} concentrations determined by steady state bioassays were orders of magnitude lower than concurrently measured SRP measurements. Furthermore, turnover rates of planktonic TPP and PP <20 μm pools became more rapid in systems with higher TP concentration. This study represents one of the first attempts to examine the sizes and turnover rates of a variety of planktonic P pools in a diversity of lakes, utilising recent methods for measuring phosphate

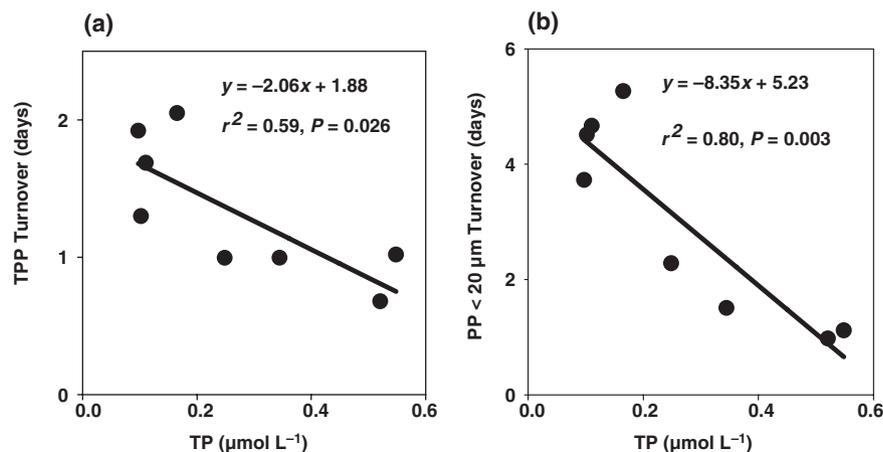


Fig. 4 Relationship between total phosphorus concentration and the turnover time (in days) of the (a) total particulate pool (TPP) and (b) the pico- and nanoplankton phosphorus pool (PP < 20 μm).

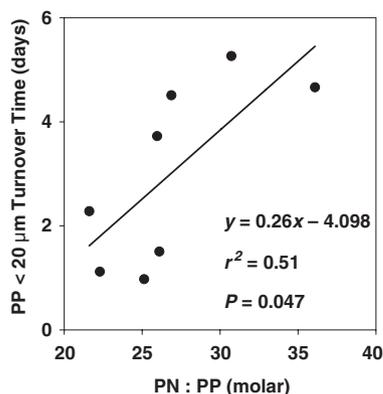


Fig. 5 Relationship between seston N : P ratios and the turnover time (in days) of the PP < 20 μm pool.

concentration, planktonic P regeneration and planktonic P turnover.

Planktonic organisms <20 μm were relatively important in P cycling within the lakes examined by this study, containing a substantial portion of the planktonic P pool and dominating the *in situ* uptake of PO_4^{3-} . The pico- and nanoplankton size fraction contained the largest portion of planktonic PP (>60%), a finding consistent with a number of studies (Mazumder *et al.*, 1988; Vadstein *et al.*, 1988; Taylor & Lean, 1991). When we examine the *in situ* PO_4^{3-} uptake by different plankton size classes, the picoplankton (<3 μm), in particular, consistently accounted for >80% of $^{32}\text{PO}_4^{3-}$ uptake in our lakes. Others have also noted that picoplankton can be primarily responsible for *in situ* PO_4^{3-} uptake across a wide range of productivity, biomass and plankton community structure (Currie & Kalff, 1984; Mazumder *et al.*, 1988; Suttle & Harrison, 1988a). It is hypothesised that PO_4^{3-} uptake partitioning among different plankton size fractions is principally a function of the severity the P-deficiency of the plankton community (Currie, Bentzen & Kalff, 1986). Under strongly P-deficient conditions with low *in situ* PO_4^{3-} concentrations, smaller plankton with larger surface area to volume ratios are competitively superior to larger cells, exhibiting more rapid PO_4^{3-} uptake. In addition to a size advantage by bacterioplankton in the uptake and assimilation of PO_4^{3-} , bacterioplankton frequently exhibit strong P-deficiency (Toolan, Wehr & Findlay, 1991; Coveney & Wetzel, 1992; Cotner *et al.*, 1997; Zohary & Robarts, 1998; DeBruyn *et al.*, 2004; Sterner *et al.*, 2004), which may be a function of their relatively high P requirements (Vadstein *et al.*, 1988). All lakes

in our study exhibited PO_4^{3-} turnover times ≤ 10 min, indicating strong P-deficiency of the plankton community. In addition, when we performed nutrient limitation assays (i.e. P-debt assays) in many of our study lakes, the <3 μm plankton size fraction often accounted for the largest proportion of the plankton community P-deficiency (Davies *et al.*, 2004).

In the lakes of the present study, the pico- and nanoplankton (<20 μm) size fraction and the larger mesoplankton (>200 μm) fraction maintained large roles in epilimnetic P recycling. The pico- and nanoplankton contributed, on average, 50% of the community dissolved P regeneration rate and the mesoplankton (composed of mostly zooplankton) accounted for approximately 20% of the plankton community regeneration rate. The relatively large per cent contribution by mesoplankton to the community regeneration rate reinforces the importance of zooplankton in P cycling (Lehman, 1980; Sterner, 1986; Vanni, 2002). While the role of zooplankton in epilimnetic nutrient recycling has received considerable attention from aquatic ecologists, a smaller number of studies have indicated that smaller planktonic organisms also play a significant role in nutrient recycling. Harrison (1983) examined the relative importance of microplankton (microzooplankton, phytoplankton and bacteria) in ocean sites of varying productivity and found that microplankton provided 50–100% of the SRP requirements for primary production. Hudson & Taylor (1996) found that plankton 0.8–40 μm accounted for 58% of the plankton community dissolved P regeneration rate and that plankton <0.8 μm contributed an additional 19% of the community regeneration rate in two oligo-mesotrophic lakes. Dodds *et al.* (1991) determined that plankton <3 μm accounted for 69% of the regenerated P in oligotrophic Flathead Lake.

In our study, both the <20 μm and the >200 μm size fractions were consistently the largest contributors to pelagic P recycling, but it is likely that they contribute to the community regeneration rate through different recycling pathways. The pico- and nanoplankton fraction release dissolved P through cell membrane leakage, cell death and viral lysis. In contrast, the mesoplankton fraction probably grazes pico- and nanoplankton and egests or excretes dissolved P. While the amount of P released by these alternate recycling pathways is of similar magnitude in our study lakes (both fractions contributed on

average 20–50% of the total plankton community regeneration rate), it is possible that the quality of the dissolved P released by these pathways may differ, leading to differential uptake and use of dissolved P sources by phytoplankton and bacterioplankton. It has been postulated that zooplankton excrete P as inorganic phosphate or low molecular weight organic compounds, while algae and bacteria release organically bound P across membranes or through cell lysis (Peters & Lean, 1973; Wetzel, 2001). In the current study, we did not assess differences in the quality of dissolved P sources from different planktonic groups and we do not know if any potential differences in quality of regenerated dissolved P sources affected phytoplankton and bacterioplankton dynamics.

In our study lakes, PO_4^{3-} measurements based upon steady state bioassays were low (87–611 pmol L^{-1}) and in the same range found by Hudson *et al.* (2000), who also used the steady state bioassay method. Our ssPO_4^{3-} measurements were approximately 2.5 orders of magnitude lower than contemporaneous colorimetric SRP estimates, approximately the same difference observed by Hudson *et al.* (2000) who compared ssPO_4^{3-} and SRP measurements from 14 lakes. In our study, we did not observe a significant correlation between ssPO_4^{3-} and SRP, indicating that, while SRP methods overestimate *in situ* PO_4^{3-} concentrations, they do not consistently overestimate PO_4^{3-} concentration. We also observed that TP– ssPO_4^{3-} and TP–SRP relationships were markedly different; ssPO_4^{3-} concentrations increased as a linear function of TP, whereas SRP did not exhibit a significant relationship with TP. Our data and that of others imply that the use of SRP methods to examine P dynamics under P-deficient conditions can be inappropriate and potentially misleading (Rigler, 1966; Taylor & Lean, 1991; Hudson *et al.*, 2000).

ssPO_4^{3-} concentrations increased linearly with TP and ssPO_4^{3-} was a consistent proportion of the TP pool across the range of TP in this study (i.e. the $\log_{10} [\text{TP}] - \log_{10} [\text{ssPO}_4^{3-}]$ slope ≈ 1). Hudson *et al.* (2000) also found that ssPO_4^{3-} concentrations increased linearly with TP in 56 North American lakes, but unlike our findings, the proportion of PO_4^{3-} as TP declined with TP ($\log_{10} [\text{TP}] - \log_{10} [\text{ssPO}_4^{3-}]$ slope < 1). Reviews of nutrient cycling in aquatic ecosystems (Harris, 1986; Capblancq, 1990) predict that PO_4^{3-} concentration will increase with TP and that PO_4^{3-} will become an

increasingly larger proportion of the TP pool in more P-rich lakes (e.g. the slope of $\log_{10} \text{TP} - \log_{10} \text{PO}_4^{3-}$ relationship would be > 1). This prediction is likely based upon studies that utilised colorimetric determinations of SRP to estimate PO_4^{3-} (Harris, 1986; Capblancq, 1990). Reagents used with SRP methods lead to the release of substantial amounts of P bound in other dissolved compounds, thus leading to potential overestimates of *in situ* PO_4^{3-} concentrations (Baldwin, 1998). Interestingly, in our study, the concentration of TDP increased with TP (inferred from the TP–TPP relationship in Fig. 1). When $\log_{10} \text{TDP}$ is regressed as a function of $\log_{10} \text{TP}$, the slope is > 1 ($\log_{10} [\text{TDP}] = 2.88 \log_{10} [\text{TP}] - 10.93$; $r^2 = 0.56$; $P = 0.055$), indicating that TDP becomes an increasing fraction of the TP pool as TP increases. Our observed relationship between TDP and TP follows the predicted relationship between PO_4^{3-} and TP (Harris, 1986), suggesting that methodological limitations may have had a role in the predicted relationships of some theoretical models. Given recent advances in the ability to accurately measure PO_4^{3-} concentrations across a diversity of lake systems, it is imperative that aquatic ecologists begin to empirically re-evaluate theoretical predictions.

The turnover of the PO_4^{3-} pool in all lakes in the present study was rapid and not a function of dissolved or particulate nutrient concentrations and ratios. Likewise, Currie (1990) examined PO_4^{3-} turnover in 36 north temperate lakes and determined that a diversity of water chemistry measurements explained relatively little variance in PO_4^{3-} uptake constants and turnover times. Mazumder *et al.* (1988) found that PO_4^{3-} turnover times in experimental lake enclosures were a function of plankton size distribution and biomass rather than ambient phosphate concentrations. In contrast to these studies, White *et al.* (1982) and Prepas (1983) found that PO_4^{3-} turnover rates in lakes was a function of *in situ* PO_4^{3-} concentration. White *et al.* (1982) and Prepas (1983) estimated PO_4^{3-} concentrations with column chromatography and colorimetric SRP analyses, and both methods tend to overestimate ambient PO_4^{3-} concentrations in P-deficient systems (Taylor & Lean, 1991; Hudson *et al.*, 2000). In our study, PO_4^{3-} turnover times were always rapid (< 10 min) and the range of PO_4^{3-} concentrations (87–611 pmol L^{-1}) were less than White *et al.* (1982) and Prepas (1983); the greatest ssPO_4^{3-} concentrations we observed in our lakes are

almost an order of magnitude lower than the smallest PO_4^{3-} concentrations reported by White *et al.* (1982) and Prepas (1983). It is entirely possible that our range in PO_4^{3-} concentration was not great enough to include systems in which PO_4^{3-} concentrations became large enough to influence PO_4^{3-} turnover time. However, Hudson *et al.* (2000) also observed PO_4^{3-} turnover times consistently ≤ 10 min in a set of lakes with TP and ssPO_4^{3-} concentrations that encompassed a far greater range than our study (TP = 0.06–4.5 $\mu\text{mol L}^{-1}$; PO_4^{3-} = 27–16,800 pmol L^{-1}).

Plankton P-deficiency remained severe (PO_4^{3-} turnover time ≤ 10 min) in our study, even when up to 50% of TP was in the dissolved fraction (TDP). PO_4^{3-} is considered the most easily utilised form of dissolved P for plankton, but a large portion of TDP in lakes is in the form of dissolved organic compounds (Lean, 1973). Dissolved organic P (DOP) is accessible by osmotrophic plankton with the aid of non-specific phosphomonoesterases (Feder, 1973), thus a significant portion of the DOP pool may be usable by plankton (Chróst *et al.*, 1986). Therefore, the size of the DOP pool (and potentially the TDP pool) may affect the severity of P-deficiency of the plankton community. The lack of a relationship between PO_4^{3-} turnover time and TDP concentration in our study may derive from the rapid uptake and assimilation of a diversity of dissolved P sources by P-deficient picoplankton ($< 3 \mu\text{m}$). It is hypothesised that in severely P-limited plankton communities, larger phytoplankton ($> 3 \mu\text{m}$) will use DOP sources to meet P requirements, because picoplankton consistently out-compete larger plankton for PO_4^{3-} under P-deficient conditions (Currie *et al.*, 1986; Currie, 1986). Evidence supporting the hypothesis of the segregated uptake of dissolved P sources by different size fractions of the plankton community is equivocal (Berman, 1988; Tarapchak & Moll, 1990; Bentzen & Taylor, 1991; Bentzen, Taylor & Millard, 1992; Cotner & Wetzel, 1992). Bentzen & Taylor (1991) and Bentzen *et al.* (1992) found that bacteria dominated the uptake of labile organic P and the turnover of the organic P pool was relatively rapid (≤ 40 min). Berman (1988) and Tarapchak & Moll (1990) found that bacterioplankton utilise DOP sources to a greater extent than algae in Lake Kinneret and Lake Michigan, respectively. Cotner & Wetzel (1992) similarly found that the bacterial size fraction of the Third Sister Lake plankton community took up greater amounts of organic P at ambient PO_4^{3-} concen-

trations, but as PO_4^{3-} levels were progressively increased above ambient levels, phytoplankton took up a greater proportion of the DOP source. In the present study, we did not examine the concentration of DOP sources in the TDP pool or the turnover and cycling of DOP compounds, therefore we are unable to address specific questions regarding the role of TDP and DOP in P cycling. Currently, data on freshwater DOP cycling and the availability of different DOP sources to plankton are relatively rare (but see Benitez-Nelson & Buesseler, 1999; Benitez-Nelson & Karl, 2002 for marine ecosystems); thus, future studies should address links between the cycling and utilisation of DOP and size-fractionated plankton P-deficiency in freshwater ecosystems.

Lake PP pools turned over rapidly (every 1.1–6.8 days) and the turnover time of the TPP and PP $< 20 \mu\text{m}$ pools linearly decreased with increasing TP concentration. We also found that the turnover times of the various plankton size fractions were similar to one another (every 1–7 days). Our planktonic PP pool turnover times were generally within the range of planktonic P pool turnover times of reported by other investigators (Taylor & Lean, 1991; Fisher & Lean, 1992; Hudson & Taylor, 1996; Hudson *et al.*, 1999) and Taylor & Lean (1991) similarly found that the P turnover times of the different plankton size fractions in Jacks Lake (pico-, nano-, micro- and mesoplankton) were similar and within the same order (2.9–4.5 days). While there is general agreement between our PP turnover times and those reported in the literature, our turnover times tend to be comparatively rapid. For example, our TPP turnover times were consistently more rapid (every 0.7–2 days) than those reported by Hudson *et al.* (1999) (every 3.5–7 days). We utilised the same method as Hudson *et al.* (1999) to estimate TPP turnover times, but our planktonic P regeneration rates were consistently greater than theirs (Fig. 5), leading to comparatively faster PP turnover times. Our greater plankton regeneration rates (and thus more rapid PP turnover times) may be a result of abiotic or biotic conditions unique to our study lakes. However, we cannot omit the possibility the relatively rapid PP turnover times we observed are a methodological artefact, stemming from our sampling procedures, sample handling, or incubation conditions.

It has been hypothesised that P cycling within the plankton community in oligotrophic lakes is more

efficient than in eutrophic lakes. The turnover of P by plankton in low TP systems has been likened to a 'rapidly spinning wheel' that gradually slows as TP concentration increases (Harris, 1986). Several empirical studies examining nutrient cycling efficiency in lakes have failed to support the hypothesis that nutrient cycling efficiency should decline in more nutrient-rich systems (Baines & Pace, 1994; Hudson *et al.*, 1999). In a study examining the relationship between lake productivity and sedimentation in 15 lakes, Baines & Pace (1994) found the proportion of phytoplankton primary production lost to sedimentation declined with lake productivity, opposite of theoretical predictions (Reynolds, 1984; Harris, 1986). Hudson *et al.* (1999) examined the turnover rate of the PP pool in a number of North American lakes of varying TP content and found that PP turnover did not significantly vary with TP. Results from our study also do not appear to support the predictions of Harris (1986). Contrary to predictions, the turnover of planktonic P became more rapid as TP concentration increased, indicating nutrient cycling efficiency increased with TP. It is again critical to note that the range of TP observed by our study is limited (ultra-oligotrophic to mesotrophic); however, despite the limit in the range in TP in our study, the contradiction between nutrient cycling paradigms and empirical studies (including ours) suggests that long-standing conceptual models of nutrient cycling require further empirical examination.

Phosphorus dynamics in lakes are influenced by a diversity of biotic and abiotic factors. The cycling rate of both dissolved and particulate forms of P are dependent upon P concentration relative to other potentially limiting nutrients. For example, PO_4^{3-} uptake rates by plankton communities may be sensitive to the concentration of other dissolved nutrients (i.e. $\text{PO}_4^{3-} : \text{NH}_4^+$ supply ratios; Suttle & Harrison, 1988b). The turnover rates and regeneration of P from specific zooplankton taxa can be dependent upon the P concentration in ingested food sources relative to the nutrient composition of the animal's body (ecological stoichiometric theory; Elser & Urabe, 1999; Sterner & Elser, 2002). The size distribution of grazer communities (small versus large bodied grazer communities) can also affect PO_4^{3-} turnover times and the concentration of P in different plankton size classes (Mazumder *et al.*, 1988). Given these and other factors, it is likely that patterns in lake P turnover and

cycling efficiency are a result of complex interactions between plankton biomass and composition, zooplankton grazing and the ratio of nutrients (C : N : P). In the present study, we observed a significant relationship between PP <20 μm turnover times and the seston PN : PP ratios, with turnover times becoming slower with increasing seston PN : PP. Particulate nutrient ratios are often used to infer plankton nutrient limitation and nutrient status (Hecky, Campbell & Hendzel, 1993; Davies *et al.*, 2004) and plankton may recycle or preferentially retain specific nutrients in relation to the interaction between nutrient supply ratios and metabolic demands. The relationship between PP <20 μm turnover time and seston N : P suggests that as N becomes more abundant relative to P, the cycling efficiency of planktonic P pools may decline. Therefore, we suggest that future studies should address potential relationship between P cycling efficiency and the status of multiple nutrients within plankton communities, rather than a simple function of the pool size of a single nutrient.

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