

**PHOSPHOROUS SIZE-DISTRIBUTION AND CYCLING IN THE PLANKTON OF
TWO OLIGOTROPHIC LAKES WITH CONTRASTING FISH COMMUNITIES**

by

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ABSTRACT

The effect of food web structure on phosphorus (P) cycles in two oligotrophic lakes was investigated. In 1993, Mouse Lake contained planktivorous fish, while Ranger Lake contained piscivorous and planktivorous fish. In late 1993 and early 1994, the majority of piscivores were removed from Ranger and placed in Mouse L.

Total P (TP) concentrations changed slightly in Mouse Lake; TP was higher when planktivorous fish were abundant. TP declined during summer stratification each year, but the rate of decline was unaffected by treatment. Meso- and nanoplankton size fractions of P responded inversely to each other in Ranger L., but not in Mouse L. The size-distribution of particulate P (PP) did not respond to treatment.

Turnover time of PO_4^{-3} did not respond to treatment and was always rapid. However, PO_4^{-3} concentrations and uptake velocities showed a weak response, particularly in Mouse L.; PO_4^{-3} and uptake velocities were greater when piscivores were abundant. By comparison with regeneration rates, ambient uptake and PO_4^{-3} concentrations appeared to be overestimated by the modified Rigler radiobioassay.

A technique was developed to measure release of dissolved phosphorus (DP) from plankton communities. Plankton are radiolabeled with phosphate and then re-assimilation is competitively blocked with $^{31}\text{PO}_4^{-3}$. The increase in dissolved radioactivity is then assayed. Initial ratio of ^{31}P to radioactivity and increase in dissolved radioactivity are used to estimate release of DP. Mean epilimnetic regeneration was $41 \text{ ng P l}^{-1} \text{ h}^{-1}$ and mean turnover time of TP was 7 days. The size fraction less than $40 \mu\text{m}$ accounted for 78% of total regeneration. Protists

and bacteria appear to be the major regenerators of phosphorus in the plankton of these lakes.

Total P regeneration and specific regeneration were greatest when piscivores were abundant. Zooplankton ($>40\ \mu\text{m}$) regeneration and specific regeneration were also greatest when piscivores were abundant. Protist ($0.8\text{--}40\ \mu\text{m}$) regeneration and specific regeneration responded to treatment in Ranger L., but not in Mouse L. Bacterial rates ($<0.8\ \mu\text{m}$) did not respond to treatment. Zooplankton and protists rates were often inversely related. Phosphorus regeneration does not seem to be the primary mechanism structuring the pelagic communities in these biomanipulated lakes.

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Chapter 1

GENERAL INTRODUCTION

“Our investigations are clear enough to indicate that the biocenotic relations between the fish stock and the plankton (i.e., the biotic environment of plankton) are at least as important as the influence of the physical and chemical (abiotic environment) factors for the formation of the plankton association, as far as, indeed, the physical or chemical factors do not act by their extreme values as absolutely limiting factors” Hrbacek et al. (1961, p 13). Although fisheries biologists were already cognizant of the top down effect that fish often had on the structure of food webs in lakes, Hrbacek and coworkers are credited for bringing it to the attention of the limnological community (Benndorf 1995).

Later, Brooks and Dodson (1965) presented the size-efficiency hypothesis to explain the effects of fish on zooplankton communities as demonstrated by their own research and that by Hrbacek and coworkers. They proposed that lakes with abundant, visually feeding, planktivorous fish would be characterized by small zooplankton because of the size selective impact of fish. However, in lakes with abundant piscivorous fish, the abundance of planktivorous fish would be reduced, lessening the predation pressure on large zooplankton which in turn would become abundant and decrease the abundance of small zooplankton through competitive interactions. This pattern was also observed in lakes lacking fish.

The management of eutrophic lakes by manipulating fish assemblages was advocated and became known as biomanipulation (Shapiro et al. 1975). According to the biomanipulation hypothesis, which is an extension of Brooks and Dodson’s hypothesis, the

removal of planktivorous fish or the introduction of large numbers of piscivores into lakes would increase the biomass of large herbivores which would reduce phytoplankton biomass, to create enhanced water clarity. The theoretical equivalents to the biomanipulation model are the models developed by Hairston et al. (1960), Fretwell (1977) and Oksanen et al. (1981). These models predict that plant biomass increases strongly in response to increasing resource availability in odd-link ecosystems (e.g. algae, zooplankton and planktivorous fish), but not in even-link ecosystems (Mazumder 1994a).

The concept of biomanipulation is straightforward and more economical to implement than competing restoration measures for lakes (Benndorf 1995). However, in the late 1960s and 1970s limnological interest was still strongly directed by Lindeman's (1942) trophic-dynamic concept, as witnessed by the International Biological Program which emphasized physiochemical controls and upward transport in food chains (Carpenter et al. 1991). This was the time when the role of phosphorus in the eutrophication of lakes was being debated. Total phosphorus-chlorophyll *a* models and nutrient reduction techniques were being developed and tested (e.g., Vollenweider 1968; Schindler 1974). The successful restoration of Lakes by the reduction of phosphorus loads, (e.g., Lake Washington, Edmondson 1977), was thought to have distracted the limnological community from the utility of the biomanipulation approach.(pers. comm. Shapiro, University of Minnesota, Minneapolis, Minnesota). However, by the late 1970's the high residual variance in the relationship between phosphorus and algal parameters did not appear to be improving (e.g., Schindler 1978) and limnologists started looking for explanations beyond the physiochemical realm.

The size efficiency hypothesis and the biomanipulation approach to lake restoration fostered a large body of research in subsequent years (e.g., see reviews by Northcote 1988 and Gophen 1990). During this time, two noteworthy hypotheses were developed to describe trophic level biomass regulation in freshwater pelagic ecosystems: the trophic cascade hypothesis (Carpenter and Kitchell 1985 and 1988) and the top-down bottom-up hypothesis (McQueen et al. 1986). Biomanipulation, the trophic cascade, and top-down bottom-up hypotheses are probably more similar than they are different, particularly the former two. To an extent, they all recognize that nutrient availability and food web structure function together to establish the biomass at each trophic level; for example, when nutrients rise, the potential biomass and production of each trophic level should also rise (McQueen et al. 1989; Kitchell and Carpenter 1993a). The trophic cascade hypothesis states “a rise in piscivore biomass brings decreased planktivore biomass, increased herbivore biomass, and decreased phytoplankton biomass” Carpenter and Kitchell (1985, p 634). The difference between the trophic cascade hypothesis and biomanipulation hypothesis is subtle: the biomanipulation approach emphasizes grazing as the main vector that alters algal biomass (e.g. see Shapiro and Wright, 1984), while the trophic cascade hypothesis emphasizes both grazing and changes to nutrient availability, resulting from grazing, as vectors structuring the algal community. The top-down bottom-up hypothesis states “top-down control should be strong at the top of the food web and should weaken towards the bottom. The model also predicts that bottom-up control should be strong at the bottom of the food web and should weaken towards the top” McQueen et al. (1989, p 302). Top down refers to predator regulation, while bottom up refers to nutrient and prey regulation. The top-down

bottom-up hypothesis puts more emphasis on bottom up effects (e.g., nutrient availability), than the other two. It implies that the impact of fish manipulations will dampen out as they cascade down the food web so that there will be minimal effects on algal biomass and productivity.

Along a productivity gradient, the top-down bottom-up hypothesis also predicts that top-down control will be greatest on lower trophic levels in oligotrophic systems and weakest in eutrophic systems (McQueen et al. 1986; see also Oksanen 1981). This is part of the rationale for the Dorset Lakes Biomanipulation Study in oligotrophic Mouse and Ranger Lakes which complements the earlier work by McQueen et al. (1986 and 1989) in eutrophic Lake St. George. However, since the inception of the Dorset Lakes Biomanipulation Project in 1990, a variety of review papers have been published that did not find any evidence to support the prediction that top-down control is greatest on lower trophic levels in oligotrophic systems and weakest in eutrophic ones (Carpenter et al. 1991; DeMelo et al. 1992; Sarnelle 1992; Mazumder 1994a).

Both hypotheses (i.e., the trophic cascade or top-down bottom-up) are supported in the literature and the magnitude of response to biomanipulation became a focus of debate (Carpenter and Kitchell 1992; Demelo et al. 1992; Hunter and Price 1992; Menge 1992; Power 1992; Strong 1992). Limnologists moved beyond the debate and started to ask which factors modulate resource and predator limitation in different systems (Hunter and Price 1992; Power 1992). One important development from the debate was the recognition that ecosystem ecology had to be incorporated into population and community ecology, if mechanisms underlying food web interactions were to be identified (McQueen et al. 1990;

Carpenter et al. 1991; Carpenter and Kitchell 1993; Bengtsson et al. 1996; Sterner et al. 1996). In particular, this has meant a return to research on nutrient budgets and nutrient cycles in temperate lakes. For example, McQueen et al. (1990 p 618) summarize “The dilemma for biomanipulation practitioners is that for phytoplankton, the effects of predation and nutrient availability are confounded..... We must therefore ask ourselves whether algae disappear because they are eaten, or because there are less nutrients available....until proper biomanipulation based nutrient dynamic and energy flow experiments are published..... This work must be one of the priorities of future biomanipulation research...” Carpenter and Kitchell (1993 p 331) echo the same message in their conclusions “Analysis of trophic cascades in the context of whole-lake nutrient cycles (through models, empiricism, and ecosystem experiments) will be an important step toward integrating nutrient and food web effects on lakes ecosystems.” Phosphorus cycles have been emphasized, instead of nitrogen cycles, in recent biomanipulation studies, because P is recognized as the most common growth-limiting nutrient of algae in temperate lakes (Horne and Goldman 1994).

However, recent efforts to model the cycling of P in biomanipulation studies are mainly based on indirect measures (e.g., Carpenter et al. 1992; He et al. 1993; Schindler et al. 1993) and must be interpreted cautiously. An exception, is the study by Mazumder et al. (1992), where P concentrations (total, particulate and dissolved), P sedimentation, turnover time and percent uptake, along with grazing and fish growth were examined in mesocosms in eutrophic Lake St. George. Here, a partial picture of the P cycle was modeled. Contrary to the trophic cascade (Carpenter et al. 1985) and the top-down bottom-up (McQueen et al.

1990 p 618) hypotheses, Mazumder and coworkers estimated that P regeneration declined when abundant planktivorous fish were present.

My objective was to examine the distribution and cycling of phosphorus in two oligotrophic lakes that had contrasting fish communities. Results from this study will be compared to the predictions of the trophic cascade. However, reference will also be made to the biomanipulation and top-down bottom-up hypotheses and the results of other studies. When piscivorous fish are abundant, the trophic cascade hypothesis predicts that the biomass in planktivorous fish will be reduced through piscivory (Fig. 1.1). Reduced planktivore biomass is then expected to reduce planktivory on zooplankton, permitting zooplankton biomass to increase. An increase in zooplankton biomass is predicted to enhance herbivory, and cause a reduction in phytoplankton biomass. The phosphorus content in plankton will be examined (chapter 2) to determine if these inverse biomass patterns occur under contrasting fish communities. For example, when piscivores are abundant, greater concentrations of particulate phosphorus (PP) should be seen in zooplankton and less PP in phytoplankton.

The trophic cascade hypothesis also predicts that nutrient supply will be greater to phytoplankton when planktivores are abundant (Fig. 1.1.). Zooplankton biomass and mean zooplankton size are expected to decline (Carpenter and Kitchell 1985 and 1988; Kitchell and Carpenter 1993a). This reduction in zooplankton size is predicted to enhance phosphorus regeneration. The authors emphasize allometric theory to support this prediction. Phosphate uptake (chapter 3) and phosphorus regeneration (chapter 5) will be

measured to see if there is support for this prediction. Total planktonic P regeneration was measured with a new technique (chapter 4).

Study Lakes

This study was part of a larger research effort that was coordinated by Dr. D. McQueen at York University. Although my study concentrated on ecosystem parameters (P cycles), the main thrust of the other participants was on biotic parameters (fish, zooplankton, benthic invertebrates and phytoplankton). Papers have recently been published on the ecology of the lakes (e.g., Tsalkitzis et al. 1994; Visman et al. 1994; Ramcharan et al. 1995; Demers et al. 1996; Demers 1996). The paper by Ramcharan et al. (1995) is the introductory paper that describes the study lakes in detail. A brief description based on Ramcharan et al. (1995) and Demers (1996) is provided below.

Mouse (45° 11' N, 78° 51' W) and Ranger (45° 09' N, 78° 51' W) lakes are situated on the southern tip of the Precambrian Shield, on the lands of the Leslie M. Frost Natural Resources Centre, which is located south (12 km) of Dorset, Ontario. These lakes were selected because they have similar physical, chemical and morphometric characteristics (Table 1.1). Both are brown water, oligotrophic, Precambrian Shield lakes, and small enough that their abiotic and biotic properties may be documented with adequate resolution. Yet they are large enough to stratify and have the habitats found in larger lakes. They are single-basin lakes with small littoral zones with limited macrophyte beds. Each lake is in a separate drainage basin that has not recently been affected by agriculture, industry or forestry. Public access to the lakes is restricted. However, there is a small Junior Ranger

Camp on Mouse L. and a field house on Ranger L. Sewage from both residences is collected in septic systems.

Mouse lake has been free of obligate piscivores for much of its recent history. The Ontario Ministry of Natural Resources had attempted to stock the lake with brook or rainbow trout (*Salvelinus fontinalis*, *Oncorhynchus mykiss*, respectively), but these attempts were unsuccessful. In 1991, the lake contained populations of white sucker, (*Catostomus commersoni*), yellow perch (*Perca flavescens*), pumpkinseed (*Lepomis gibbosus*), creek chub (*Semotilus atromaculatus*), finescale dace (*Phoxinus neogaeus*), golden shiner (*Notemigonus crysoleucas*) and brown bullhead (*Ictalurus nebulosus*). Ranger L. has had large populations of smallmouth bass (*Micropterus dolomieu*) and largemouth bass (*Micropterus salmoides*) for at least 30 years. Ranger L. also contains white sucker, yellow perch, pumpkinseed, creek chub, golden shiner, redbelly dace (*Phoxinus eos*), brook stickleback (*Culea inconstans*) and common shiner (*Luxilus cornutus*) (Demers 1996).

The removal of the largemouth and smallmouth bass from Ranger L. and their introduction into Mouse L. is referred to as biomanipulation. Bass removal from Ranger L. commenced in late August of 1993 and continues each year. The majority of bass removed in 1993 and 1994 were placed into Mouse L. Since 1994, the bass populations in Mouse L. have been self-recruiting and further additions from Ranger L. are no longer conducted. Bass are still present in Ranger L., but their biomass has been greatly reduced (Table 1.2). The effect of the fish transfer is still being evaluated, and may continue to be monitored for some additional years. Examination of the Secchi depth data (Fig. 1.2, data from D.

McQueen, York University, Ontario, Canada) suggests, that Mouse L. has become slightly clearer than Ranger L in the postmanipulation period (late summer 1993 onward). Although my intention is not to evaluate the success of the biomanipulation (i.e. if water clarity is enhanced when piscivores are abundant), a change in the lakes appears to have occurred (as illustrated in Secchi depth) and may be reflected in the P cycle.

Table 1.1. Morphometric, physical and chemical characteristics of Mouse and Ranger Lakes (1991-92). Interannual means and standard errors are presented for chemical data (from Demers 1996).

Characteristic	Mouse L.	Ranger L.
Catchment area (ha)	190	260
Surface area (ha)	9.0	11.3
Volume ($\text{m}^3 \times 10^5$)	4.3	6.3
Flushing rate (yr^{-1})	2.4	2.3
Maximum depth (m)	9.0	13.0
Mean depth (m)	4.9	5.6
Shoreline length (km)	1.6	1.6
Total phosphorus ($\mu\text{g P l}^{-1}$)	7.29 (0.47)	5.92 (0.25)
Total nitrogen ($\mu\text{g l}^{-1}$)	279 (9)	287 (8)
Chlorophyll-a ($\mu\text{g l}^{-1}$)	2.59 (0.30)	2.66 (0.27)
Dissolved organic carbon (mg l^{-1})	4.42 (0.11)	4.98 (0.12)
Dissolved inorganic carbon (mg l^{-1})	0.545 (0.110)	0.515 (0.051)
pH	5.67 (0.03)	6.14 (0.04)

Table 1.2. Mean abundance and biomass estimates for largemouth and smallmouth bass populations in Mouse and Ranger Lakes from 1991-95 (Data from Demers 1996).

Year and Month	Mouse Lake Abundance (ha ⁻¹)	Biomass (kg ha ⁻¹)	Ranger Lake Abundance (ha ⁻¹)	Biomass (kg ha ⁻¹)
Largemouth Bass				
May 1991			41.5	5.16
May 1992			38.2	3.42
May 1993			41.1	3.75
May 1994	20.1	4.39	26.1	0.86
May 1995	75.7	5.27	48.0	0.85
Smallmouth Bass				
May 1991			21.8	1.55
May 1992			14.1	0.98
May 1993			20.3	1.98
May 1994	5.6	1.28	5.3	0.68
May 1995	8.8	2.33	11.0	0.33

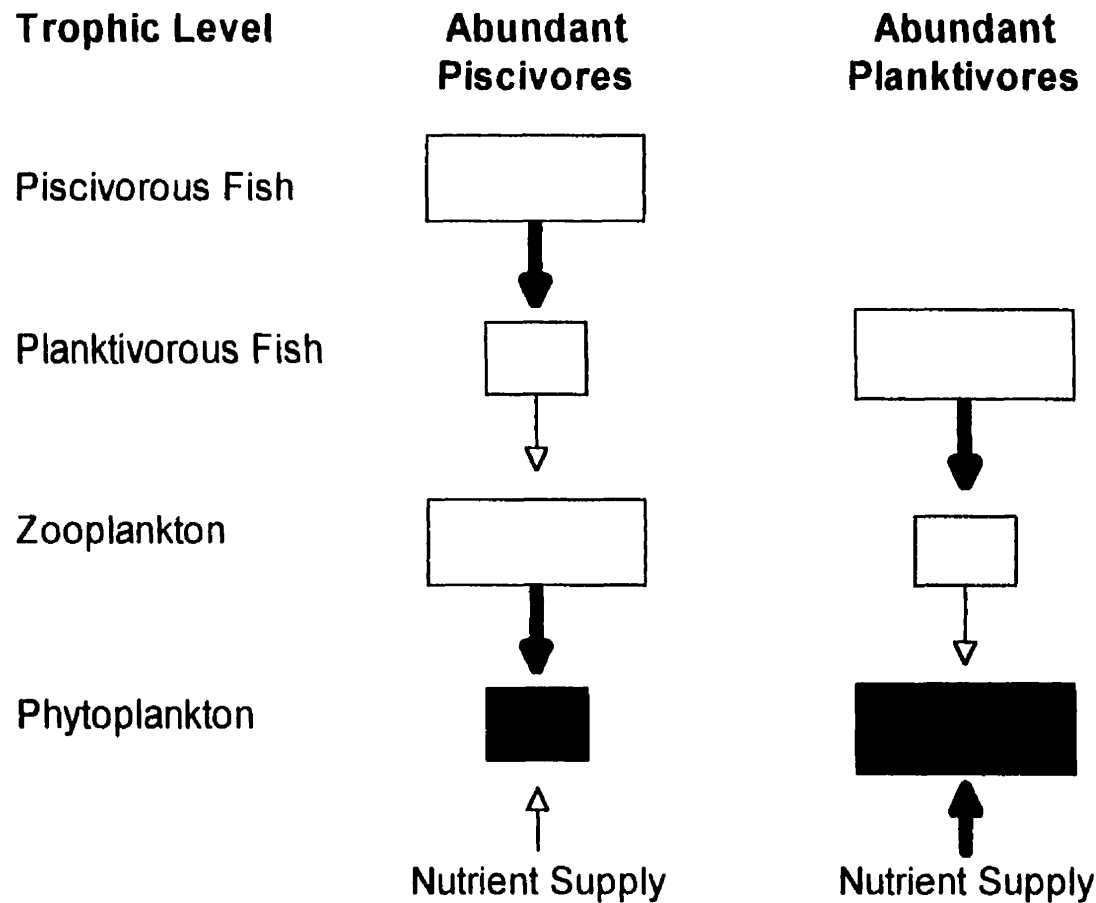


Figure 1.1. Graphic depiction of the trophic cascade hypothesis. Two simplified pelagic food webs serve as an example. One example represents a food web when piscivorous fish are abundant and another when planktivorous fish are abundant. Each box, represents biomass at a particular trophic level. Inverse patterns in biomass are presented. Arrows pointing down depict predation or grazing activities (top down processes). Darker arrows represent enhanced predation or grazing activities. Arrows pointing up depict nutrient supply to phytoplankton (bottom up processes). The darker arrow pointing up represents enhanced nutrient supply. Solid black boxes represent phytoplankton.

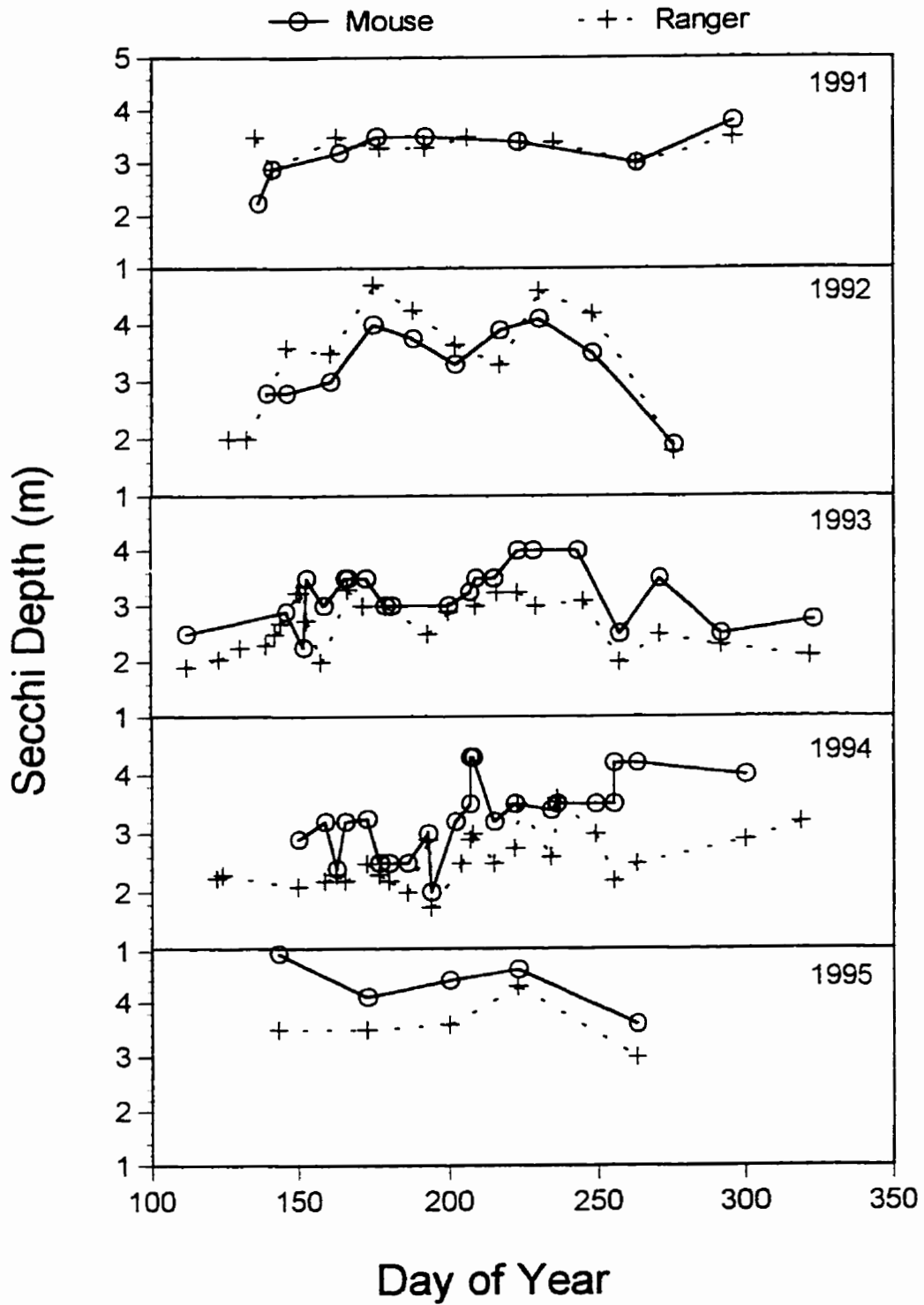


Figure 1.2. Secchi Disk depth (m) in Mouse and Ranger Lakes from 1991 to 1995 (from Dr. D. McQueen, York University, North York, Ontario, Canada).

Chapter 2

THE EFFECT OF PISCIVORE REMOVAL AND INTRODUCTION ON TOTAL PHOSPHORUS AND PARTICULATE PHOSPHORUS FRACTIONS

Introduction

Total phosphorus concentrations have been observed to decline when lakes or enclosures have been treated by removing all fish or adding piscivores. For example, in 1980 when planktivorous fish were abundant in Round Lake, mean TP concentration was $\sim 50 \mu\text{g P l}^{-1}$ (Shapiro and Wright 1984). The abundance of planktivorous fish was greatly reduced in the fall of 1980. Then piscivorous fish were introduced into the lake. By 1982, the TP concentration had decreased to $\sim 30 \mu\text{g P l}^{-1}$. In a mesocosm study in Lake St. George, TP was elevated (mean $23.6 \mu\text{g P l}^{-1}$) in mesocosms with planktivores and reduced in fishless mesocosms (mean $17.6 \mu\text{g P l}^{-1}$) (Mazumder et al. 1990). Similar effects of biomanipulation on TP have been observed in other studies (e.g., see Northcote 1988; Reinertsen et al. 1990; Lazzaro et al. 1992; Kasprzak et al. 1993; Hambright 1994). Shapiro and Wright (1984) and Dini et al. (1987) suggested that the diel migrations of *Daphnia* transported P out of the epilimnion to bring about the decline in TP. Reinertsen et al. (1990) suggested that the decline in TP in Lake Haugtjern resulted from enhanced sedimentation rates of large zooplankton and reduced P regeneration from sediments. Other studies that have directly addressed this question (Mazumder et al. 1989; Guy et al. 1994; Larocque et al. 1996), suggest that the loss of P from the epilimnion is a result of changed particle-size distribution and greater sedimentation velocities. Regardless of the mechanism, we may

anticipate a more rapid decline in TP concentration from spring to late summer and a reduced mean TP concentration when piscivores are abundant in either Mouse or Ranger L.

The trophic cascade hypothesis (Carpenter et al. 1985) states that biomasses at adjacent trophic levels are expected to be inversely related, while biomasses at trophic levels separated by an intermediate trophic level are expected to be directly related. These expectations are not always realized in biomanipulations (Benndorf and Miersch 1991; DeMelo et al. 1992; Reynolds 1994) even if water clarity is enhanced. However, the expectation of inverse biomass patterns presents a useful model from which questions about biomanipulated food webs may be posed (Carpenter and Kitchell 1993). For example, when piscivores are abundant, is zooplankton biomass enhanced, and phytoplankton biomass reduced? In turn, when planktivorous fish are abundant, is zooplankton biomass reduced and phytoplankton biomass enhanced?

Traditionally, these questions have been addressed with a taxonomically based approach, where the abundance and biomass of populations and communities are examined. This is a convenient method for fish, but very time consuming for examining the zooplankton and microbial components of food webs (Mazumder et al. 1988; Almond et al. 1996). Furthermore, this approach is difficult to integrate with microbial rate measurements. For example, isolating taxonomic groups of microorganisms (e.g., microautotrophs from microheterotrophs) for rate measurements may not be possible. Therefore, for convenience and to integrate rate measurements with biomass measurements, I elected to use physical size fractionation of plankton in Mouse and Ranger L.

The biomass of mesozooplankton and nanoplankton are expected to be inversely related before and after biomanipulation (Carpenter and Kitchell 1993). In addition, when planktivores are abundant, P has been observed to be low in the mesoplankton and dissolved pools and to be high in nanoplankton pools (e.g., Lazzaro et al. 1992; Mazumder et al. 1992; Mazumder and Lean 1994). Therefore, I will concentrate analyses on these size fractions (i.e., mesoplankton $>250 \mu\text{m}$, nanoplankton $2\text{-}20 \mu\text{m}$ and dissolved $<0.2 \mu\text{m}$) to determine if the concentration of P in each fraction was equal before and after the fish transfer.

A potentially useful index for characterizing the size distribution of particulate phosphorus in a water column was developed by Mazumder et al. (1988). This approach considers the proportion of PP (PP in a fraction/total PP) as a function of filter pore size (log size). The negative slope of this relationship summarizes the distribution of PP in the water column (for example, see Fig. 2.1). If a large proportion of PP is in small particles then the slope of this relationship will be steep (e.g., -0.3). However, if the majority of PP is in large particles then the slope will be gentle (e.g., -0.2). If there are equal quantities of PP in each 10-fold size range then the slope will be intermediate (approximately -0.25). Under planktivory, the mean size of planktonic communities often declines and planktonic biomass is shifted from large to small organisms (Mazumder et al. 1988; Carpenter and Kitchell 1993; Almond et al. 1996; Proulx et al. 1996). Therefore, steep slopes are expected when planktivorous fish are abundant and gentle slopes when piscivorous fish are abundant in Mouse and Ranger Lakes.

Methods

Epilimnetic water was collected (20 l) from the middle of the epilimnion (station 4) on each lake with a Van Dorn sampler once every two weeks from June to August (1993-94). Additional, but less frequent, samples were also taken in May, September and October. In 1995, samples were collected once a month from May to September. Water was transported to the laboratory and 3 to 6 subsamples (35 or 100 ml) were removed for total phosphorus measurements (TP). These samples were supplemented by other TP samples that were collected one to two days later for P regeneration experiments (see Chapter 4). All vessels used for P measurements had been autoclaved with persulfate solution prior to being filled. TP samples were returned to University of Waterloo where phosphorus concentrations were determined as outlined by Parsons et al. (1984).

The rates of decline of TP over the open water season were compared among years and between lakes to isolate treatment effects (statistical model $TP = \text{constant} + \text{Day} + \text{Year} + \text{Lake} + \text{Day} * \text{Year} + \text{Day} * \text{Lake} + \text{Lake} * \text{Year} + \text{Day} * \text{Year} * \text{Lake}$). If the expected inverse patterns from treatments were effective (i.e., steep slopes of decline when piscivores were abundant and less steep slopes when planktivores were abundant), then the third order interaction term (i.e., $\text{Lake} * \text{Year} * \text{Day}$) of this comparison would be significant. Normality and heterogeneity of variance assumptions of the ANOVA model were examined graphically (i.e., with a normal probability plot of residuals, and with a plot of studentized residuals versus expected values). Total P was the most frequently sampled parameter in this study. No evidence of autocorrelation over time was found (Durbin-Watson D statistic 2.008, and first order autocorrelation -0.005). Therefore, observations were treated as

independent. Other parameters in this study were measured less frequently, and were assumed to be independent over time.

The remainder of the mid-epilimnetic water was used for particulate phosphorus (PP) analysis. One to six liters of lake water was poured through an apparatus which consisted of three nitex screens (250, 80 and 40 μm) that were stacked one above the other. Material caught on each screen was vigorously backwashed into a graduated cylinder with deionized water, made up to 100 ml, with deionized water, and then decanted into leached glass or plastic sample bottles. Samples were corrected with blanks of deionized water.

Additional water (7 l) was gently poured into a leached ($\sim 0.1 \text{ N HCl}$) column (1.5 m tall and made of potable grade polyvinylchloride). This column, which I refer to as a gravity filtration tower, had a series of outlets at its base to which clean 47 mm syringe filter holders were attached. Each syringe holder contained either a polycarbonate filter of the pore size 0.2, 0.8, or 2.0 μm , or a nitex screen of the size 20 or 40 μm . Syringe holders with filters were bled before filtrate (35 ml) was collected. During filtrate collection, the water column was stirred gently and continuously with a stir bar to reduce heterogeneity of particles along the length of the column. An enlarged filter holder (6 times the surface area of a 47 mm syringe holder) was constructed and used in 1994 and 1995 to augment the rate at which 0.2 μm filtrate was collected. The gravity filtration column was constructed to prevent the destruction of delicate microorganisms during filtration; a problem common to traditional filtration techniques (e.g., Goldman and Dennett 1985; Taylor and Lean 1991). Phosphorus concentrations were estimated for the following size classes <0.2, 0.2-0.8, 0.8-2.0, 2.0-20, 20-40, 40-80, 80-250, and >250 μm . Many of these size classes were chosen to

facilitate comparisons between studies on the Dorset lakes (Ramcharan et al. 1995) and in other lakes (e.g., Mazumder et al. 1988).

The mesozooplankton fraction ($>250\ \mu\text{m}$), nanoplankton fraction ($2\text{-}20\ \mu\text{m}$) and the dissolved fraction ($<0.2\ \mu\text{m}$) are expected to be inversely related before and after biomanipulation (e.g., Lazzaro et al. 1992; Mazumder et al. 1992; Carpenter and Kitchell 1993; Mazumder and Lean 1994). Therefore, each of these fractions was examined separately (model 1 2x2 ANOVA) to determine if the concentration of P was equal before and after biomanipulation in each lake. In this analysis, the second order interaction term was of particular interest because it would capture the inverse patterns that would be expected to occur between each fraction before and after treatment. To simplify the interpretation of the analysis, both postmanipulation years (1994-95) were pooled into one treatment year. In order to meet homogeneity of variance assumptions, PP concentrations were transformed (\log_{10}).

Slope parameters which characterize the distribution of PP in each lake, were tested to see if the distribution of PP was equal before and after the fish transfer (model 1 2x2 ANOVA), where lake and year (1993 and 1994-95) were the main effects. Again, the interaction term in this analysis was of particular interest for reasons described above. All statistical analyses here and in following chapters were carried out using SYSTAT, version 5 (Wilkinson et al. 1992).

Results

Mean TP estimates from Ramcharan et al. (1995) for the open water periods of 1991 and 1992 were combined with mean TP estimates (day 130 to 238 for years 1993-95) from this study (Table 2.1). Mean concentrations in Mouse L. were slightly greater in the premanipulation years (1991-93) than in postmanipulation years (1994-95). Over the same period, mean TP in Ranger L. does not appear to have changed. Mean concentrations were not analyzed further (i.e., with a model I 2x2 ANOVA) because concentrations in Ranger L. did not respond to treatment and the 95 % C.I. overlapped between years and lakes.

The rate of decline of TP from spring to fall within the epilimnion of a lake tends to be exponential (e.g., Guy et al. 1994); however, I did not find any evidence for rejecting a linear model in Mouse and Ranger Lakes (Fig. 2.2). Therefore, the decline in TP was modeled with a linear function. The earliest date of sampling was on day 130 (May 10th, 1995). Each season was marked by a decline in TP until destratification in late summer or early fall. Then TP started to rise as the epilimnion deepened. The start of destratification was not consistent across years (Fig 2.2). The latest date just before TP began to rise was found to be day 238 (August 26th). Only TP concentrations up to and including day 238 were used to fit each model. Otherwise, inconsistencies in the time of destratification would introduce biases into the calculated rates of decline of TP from each model. For unknown reasons, one TP value was extremely high in Mouse L. in 1993 (day 182), and was not included in the statistical analyses.

The rate of decline in TP was 0.021 to 0.068 $\mu\text{g P l}^{-1} \text{d}^{-1}$ (Table 2.2) resulting in a decline of 28-61% from initial TP. Rates were significantly different from zero in both lakes over all years. Total phosphorus concentrations in both lakes declined more rapidly before biomanipulation (1993) than they did after biomanipulation (Fig. 2.2 and Table 2.2). The rate of decline of TP was significantly different between lakes (Day*Lake interaction, $P=0.02$) with Ranger L. steeper, and over years (Day*Year interaction, $P=0.01$), since certain years were steeper than other years. But treatment effects were not significant (Lake*Year*Day interaction, $P=0.85$), the rate of decline of TP was less in postmanipulation years in both lakes (Table 2.3).

Phosphorus in the mesoplankton fraction ($>250 \mu\text{m}$) was greatest under piscivory (1993) and reduced under planktivory (1994-95) in Ranger L. (Fig. 2.4 and 2.5). An inverse pattern in Mouse L was not evident (Fig. 2.3 and 2.5), and therefore, the second order interaction term was not significant for this fraction ($P=0.39$, Table 2.4). Phosphorus in the nanoplankton fraction (2-20 μm) increased slightly from 1993 to 1995 in Ranger L., but again, a reverse trend was not noted in Mouse L. Therefore the treatment effect was not significant ($P=0.63$, Table 2.4). The dissolved fraction of P ($<0.2 \mu\text{m}$) was significantly greater before the fish transfer (year effect, $P=0.004$) in both lakes and significantly different between lakes ($P<0.001$). That is, the dissolved P was greater in Ranger L. However, the effect of biomanipulation did not produce significant inverse patterns in the lakes (interaction term $P=0.94$, Table 2.4).

Subtle patterns in the phosphorus fractions are noticeable in Figure 2.5, when the mesoplankton fraction is compared with smaller fractions from each lake. In Ranger L.

positive patterns are visible between mesoplankton ($>250\ \mu\text{m}$) PP and the following fractions: 20-40, 0.8-2, 0.2-0.8 and $<0.2\ \mu\text{m}$. Negative patterns exist between the mesoplankton fraction and the following fractions: 80-250 and 2-20 μm . In Mouse L. these subtle patterns are also visible between the mesoplankton group and some of the smaller fractions; positive patterns exist with the 40-80, 2-20, and 0.8-2 μm fractions and negative relationships with the 0.2-0.8 and the $<0.2\ \mu\text{m}$ fractions.

The distribution of PP appears to be less variable in 1995 than in 1993-94 (Figs. 2.3 and 2.4). This is caused by the different sampling frequencies between years; $n=5$ in 1995 compared to $n=10$ in 1993 and 1994. The more frequent sampling program of 1993-94 captured more of the variability in lake plankton over the course of the open water season.

The slope parameters, estimated from the relationship between the proportion of PP retained on filters and the logarithm of filter size, are presented in Figure 2.6 for each sampling date and the mean slope of each season is summarized in Table 2.5. Slopes overlap between years in both lakes. Some of the overlap is derived from rapid changes in adjacent slope parameters in 1994 in Mouse L. and in 1993 in Ranger L. These rapid changes are created by shifts in the distribution of PP in the plankton fractions (Figs. 2.3 and 2.4). For example, on day 173 in Mouse L. (1994) the majority of PP was in the 0.8 to 40 μm size fractions (Fig. 2.3) and subsequently, on day 200 the mesoplankton fraction contained much of the PP. In Ranger L. there is less overlap of slopes (Fig. 2.6) during the summer months (day 182-243). During summer 1993 in Ranger L. the slope parameters were less steep because there was a greater proportion of P in the large size fractions.

However, due to the great variability in slope response, the overall effect of treatment was non-significant (interaction term $P=0.56$, Table 2.6).

Discussion

The slight difference in TP concentrations between lakes in 1991-92 (Table 2.1) supports the observation that lakes with similar physical features will have higher concentrations of TP when planktivorous fish assemblages are dominant (Shapiro and Wright 1984; Wright and Shapiro 1984; Mazumder et al. 1990; Reinertsen et al. 1990; Lazzaro et al. 1992; Kasprzak et al. 1993; Hambright 1994). In the final year before the lakes were biomanipulated (1993), mean TP concentration (between day 130 and 238) was slightly lower in Ranger than Mouse (6.4 versus $6.8 \mu\text{g P l}^{-1}$). As observed in other studies, piscivore introduction into Mouse L was followed with a decline in mean TP to $6.4 \mu\text{g P l}^{-1}$ in 1994 and then to $5.2 \mu\text{g P l}^{-1}$ in 1995. After piscivore reduction in Ranger L., mean TP concentration, as expected, increased to $6.9 \mu\text{g P l}^{-1}$ in 1994, but then, unexpectedly, declined to $6.2 \mu\text{g P l}^{-1}$ in 1995.

The TP response to the fish transfer was weak, as illustrated by the overlap of 95% C.I. before and after treatment (Table 2.1). Changes in TP in other biomanipulated lakes (see citations above) have been more pronounced, but these studies were conducted in mesotrophic and eutrophic lakes, where relative changes in TP may be more detectable (e.g., Shapiro and Wright 1984). In lakes as oligotrophic as Ranger and Mouse, such changes in mean TP concentrations may be masked by analytical error, seasonal variation,

and spatial heterogeneity. For example, in a biomanipulation study in oligotrophic Lac Croche, water clarity was enhanced, but a change in TP was not detectable (Proulx et al. 1993). In addition, as reviewed by Benndorf and Miersch (1991), an increase in water clarity after biomanipulation is not always associated with a decrease in TP concentration.

TP concentrations declined 28-61% between spring and late summer. This range is similar to those described for other lakes in the region (Guy et al. 1994). When planktivorous fish are abundant in mesocosms, the rate of P sedimenting per day is reduced and the rate of decline of TP is reduced (Mazumder et al. 1989; Mazumder and Lean 1994). Similarly, Bossard and Uehlinger (1993) found that the mean residence time of TP in mesocosms in L. Lucerne was increased when planktivorous fish were present. This response was evident in Ranger L. (Fig. 2.2 and Table 2.2), which had a steep decline in TP when piscivores were abundant (1993) and a reduced decline in TP when planktivores became the dominant fish (1994-95) after piscivore reduction. In 1993, the observed rate of decline in both lakes did fit the predicted pattern: Ranger had a steeper decline than Mouse. However, the rates of decline in Mouse L. after biomanipulation were the reverse of what was expected: the rate of TP decline was reduced after piscivores were introduced. In fact, the pattern of decline in TP between both lakes before and after the biomanipulation was similar, resulting in a non-significant third order interaction (Table 2.3).

The response of the rate of decline in TP in Mouse L. may be explained by climatic and physiographic factors. The immediate topography surrounding Mouse L. is noticeably steeper than that of Ranger L. (personal observation, and see Energy, Mines and Resources Canada topographic map 31 E/2). Therefore, Mouse L. may be less affected by wind

induced mixing, especially in spring, and turnover may be less or incomplete in this lake in comparison to Ranger L. I commenced sampling of the lakes a month or more after the ice-out period (on June 4, May 11 and May 10 in 1993, 1994 and 1995, respectively), which occurs in April. Therefore, the period of TP decline may have been partly missed and the rates of decline in TP concentrations in Mouse L. may have appeared less steep than they actually were. The same result would develop if Mouse L. was experiencing incomplete turnover in the spring of 1994-95.

Phosphorus profiles do not exist for the early spring periods just before (March) and after (April) ice-out; however, oxygen profiles for 1993 and 1994 are available (unpublished data from Dr. Don McQueen, Department of Biology, York University, North York, Ontario, Canada). Both lakes were more completely mixed (Fig 2.7), particularly Ranger L., in May of 1993 than in May of 1994. At 6 m depth on May 9, 1994, Mouse lake had an oxygen concentration of only $\sim 2.5 \text{ mg l}^{-1}$ compared to $\sim 5 \text{ mg l}^{-1}$ on May 18 1993. More complete mixing in the spring of 1993 may have led to elevated TP levels, and, therefore, steeper slopes of decline in TP concentrations throughout the spring period, particularly in Ranger L. The comparison of oxygen profiles for March and May of 1994 suggests that both lakes, particularly Mouse L., did not turn over that spring; the March and May profiles of each lake are almost identical. If there was incomplete turnover in 1994 (and possibly 1995) then water column P concentrations may not have been enhanced with P regenerated from the sediments. This would explain the reduced rates of decline in TP for 1994 compared to 1993 in both lakes (Figure 2.2). Therefore, food web structure and the decline in TP may be confounded by the timing and degree of spring turnover in these lakes. For

example, in a multi-lake study, the relationship between the rate of decline of TP and community structure was not significant (Guy et al. 1994). The authors suggested that the variable influence of spring concentrations of dissolved P (DP) may have obscured such a relationship. The response of TP decline to biomanipulation may require re-evaluation.

The size distribution of PP in the plankton of Ranger L. (Fig. 2.4 and 2.5) matches the pattern that is expected by the trophic cascade hypothesis (Carpenter et al. 1985). Mesoplankton biomass (in this case PP > 250 μm fraction) was greater (Fig. 2.4 and 2.5) and nanoplankton biomass PP (2-20 μm fraction) was less when piscivores were abundant (1993). When piscivore abundance was greatly reduced (1994-95) and planktivorous fish remained, the inverse pattern developed. The distributions of TDP (Fig. 2.4 and 2.5) also follow the expected pattern described by Mazumder (1994b). That is, TDP concentrations are elevated under piscivore or fishless treatments and reduced under planktivore treatments. However, the size distribution of PP and TDP did not follow any particular pattern in Mouse L, and the overall interaction between both lakes was non-significant.

The well documented negative relationship between microplankton and mesozooplankton (e.g., Gilbert 1988; Christoffersen et al. 1993; Pace and Vaque 1994) is evident in Ranger L. (PP in 80-250 μm fraction). Under intense planktivory, zooplankton biomass is often concentrated in the microplankton fraction, but when planktivory is relaxed, microzooplankton biomass is often reduced and the biomass of large herbivores becomes important (Brooks and Dodson 1965; Mazumder et al. 1992; Christoffersen et al. 1993; Mittelbach et al. 1995; Almond et al. 1996). Although large mesozooplankton are capable of grazing bacteria (Jeppesen et al. 1996), the 0.8-2 μm fraction of PP, and to a

lesser extent the 0.2-0.8 μm fraction of PP, fluctuated positively with the mesoplankton fraction of PP over the study period. These two fractions may be responding negatively to the grazing activities of the nanoplankton fraction or positively to the dissolved fraction. A review by Pace and Cole (1994) found that bacterial biomass and productivity often demonstrated nutrient limitation (bottom up control) instead of consumer regulation (top down control).

The expected inverse patterns in PP and DP are also present between Mouse and Ranger Lakes before biomanipulation (see 1993 in Fig. 2.5); Mouse L. had less P in the mesoplankton and the DP fractions, and slightly more in the nanoplankton fraction. The between lake pattern in mesoplankton was also observed in 1991 and 1992; zooplankton biomass in Ranger L. was dominated by large individuals, mainly *Daphnia catawba*, and zooplankton biomass in Mouse L. was dominated by small individuals, mainly *Leptodiatomus minutus* (Ramcharan et al. 1995).

However, postmanipulation PP and DP in Mouse L. did not appear to respond to the introduction of piscivores (Fig 2.3 and 2.5). Inverse biomass patterns were not evident between the mesoplankton PP or the nanoplankton PP. In fact, the relationship between these two fractions is weakly positive. The relationship between TDP and the mesoplankton PP is the reverse of what would be expected from the review by Mazumder (1994b). Only the microplankton fraction (80-150 μm fraction) appears to have responded to the biomanipulation in the expected manner; that is, high biomass under planktivory and reduced biomass under piscivory. The lack of response of the P fractions in Mouse L. to the fish transfer resulted in non-significant interaction terms for the overall study (Table 2.4).

There are potential explanations for the lack of response in the P fractions to the biomanipulation. Although filter size fractionation of PP is a convenient technique for examining food webs, it does have shortcomings. For instance, slight shifts in the nanoplankton size distribution which may enhance water clarity, may go unnoticed. For example, in mesocosms containing planktivorous fish, Proulx et al. (1996) observed significant increases in the algal biomass in the 2-10 μm fraction and significant decreases in the 10-20 μm .

Planktivory (by fish or invertebrate planktivores) must be drastically reduced before improvements in water clarity can be achieved (Benndorf 1995). Fish population data have recently become available for Ranger and Mouse L. for the period of 1991-95 (Demers 1996). With the high abundance of piscivorous bass in Mouse L. during 1994-95, the resident planktivorous fish (e.g., perch and pumpkinseed) were expected to decline rapidly. Instead, yellow perch biomass rapidly increased from 3.6 kg ha^{-1} in May to 19 kg ha^{-1} in August of 1994. Total planktivorous fish biomass (yellow perch, pumpkinseed, golden shiner and creek chub; white sucker not included) exceeded all previous (1991-94) estimates of planktivore biomass by more than 40%. The potentially high rate of planktivory associated with this biomass of planktivores may be the main reason that mesoplankton PP did not increase and nanoplankton PP did not drop in 1994 (Figure 2.5).

Interestingly, in Mouse L. in 1994, between the days 159-186 (June 8-July 5), zooplankton PP (> 40 μm fraction) and bacterial PP (0.2 to 0.8 μm) were very low, while PP in the protist fractions (0.8-2, 2-20 and 20-40 μm) was extremely high. For instance, on day 173 (June 22) 92% of the PP was in the protist fractions and only 8% was in the

zooplankton fractions, and the amount of PP in the bacterial fraction was undetectable. This resembles a small trophic cascade that may have developed from intense planktivory from perch fry. A decline in water clarity is associated with this event (see Fig. 1.2, days 175-205). Young perch can impart strong top down effects resulting in significant reductions in mesozooplankton biomass and water transparency, while enhancing algal biomass (Kurmayer and Wanzenbock, 1996).

Bass piscivory did not impact upon the Mouse L. planktivore populations until 1995; by August 1995, perch biomass had declined by 78%, and total planktivorous fish biomass dropped far below (<16%) premanipulation levels (Demers 1996). The gradual increase of PP in the mesoplankton fraction (Fig 2.3), the reduced PP in the nanoplankton fraction (compared to 1994), and the enhanced water clarity (Fig. 1.2), are probably a response to declining planktivory throughout 1995. As observed here and elsewhere (e.g., Benndorf 1995), the effect of piscivory on planktivorous fish is not always immediate. For example, Findlay et al. (1994) did not observe an effect by northern pike on resident perch populations until two years after the introduction of pike.

Slope parameters of the PP size distribution (Fig. 2.6) are similar to slopes found in other studies for Ontario lakes (Mazumder et al. 1988; Guy et al. 1994; Almond et al. 1996). Mean slope parameters (Table 2.5) for both lakes are approximately -0.25, which indicates that both lakes were characterized by intermediate PP distributions; that is, they were not dominated by large or small plankton. The inverse pattern of biomass between trophic levels (Carpenter et al. 1985) that was anticipated with biomanipulation was not found with this approach (interaction term, $P=0.56$, Table 2.6); slope parameters often

overlapped between years. Although a less negative slope parameter was expected for Mouse L. in 1994, a mean slope of -0.27 was obtained (Table 2.5). This may reflect the high degree of planktivory on mesozooplankton that probably occurred in Mouse L. in 1994 when perch were extremely abundant (as discussed above). In fact, the steepest slope parameters (<-0.3) of this study were found in June and early July, when young of the year perch may have been most abundant. Some of these slopes exceed any of the slopes reported for other lakes in Ontario (Mazumder et al. 1988; Guy et al. 1994; Almond et al. 1996). As the planktivore populations in Mouse L. declined throughout 1995, the slope parameter gradually rebounded to become less negative than 1993 slopes (Fig. 2.6).

There is less overlap of slope parameters in Ranger L., especially during the summer period (between days 180-250) and this separation is consistent with the expectations of the trophic cascade (Carpenter et al. 1985). However, there is considerable overlap at either end of each season. The relevance of early spring and fall slope parameters is questionable. The deepening of the epilimnion and the eventual turnover of the lakes in late summer and early fall may obscure some of the food web effects that characterize biomanipulations. Stratified conditions may be necessary to observe food web effects, otherwise, the impact of biomanipulation on sedimentation rates may be overridden by physical mixing (Mazumder et al. 1989; Proulx et al. 1993 and 1996; Larocque et al. 1996). The time of spring turnover and the degree of mixing (e.g., Fig. 2.7) may also complicate or override food web effects (as discussed at the beginning of this section). For example, in years when spring turnover is complete, high concentrations of TDP in the water column may buffer PP losses from grazing and sedimentation. Therefore, food web interactions determined by PP analysis

would go unnoticed (Guy et al. 1996). Future studies may consider restricting sampling to the stratified season or the summer period when algal biomass is more determined by grazing and nutrient regulation (see Carpenter et al. 1991 for similar arguments).

Particulate P in Ranger L. responded weakly to piscivore removal. As in Mouse L., the response may be related to the level of planktivory in the lake. The mean biomass of planktivorous fish (common shiner, creek chub, redbelly dace, pumpkinseed and yellow perch) after biomanipulation (1994-95) only doubled from premanipulation levels. This biomass of planktivores was still only ~ 25% of the biomass of planktivores in Mouse L. before manipulation. Furthermore, in 1994 and 1995, mean bass biomass in Ranger L. was still ~20-25% of the mean premanipulation biomass (years 1991-1993). Although adult piscivorous bass were greatly reduced in the lake, their continued presence might still prevent the planktivorous fish from foraging in the pelagic zone (e.g., via behavioural interactions, Carpenter et al. 1987). Therefore, the intensity of planktivory in Ranger L. was probably greater than it was in 1993, but less than the levels of planktivory in Mouse L. in premanipulation years. This may explain the weak response of Ranger L. to biomanipulation as observed by the PP analysis.

Table 2.1. Mean total phosphorus ($\mu\text{g l}^{-1}$) in Mouse and Ranger Lakes from 1991-95. Means are followed by 95% confidence intervals and sample size. Means for 1993-95 are based on concentrations measured between day 130 to day 238. Means for 1991 and 1992 are for the open water season and are from Ramcharan et al. (1995). Premanipulation years were 1991-93 and postmanipulation years were 1994-95.

Year	Mouse L.	Ranger L.
1991	6.8 \pm 1.7 (10)	5.9 \pm 0.84 (10)
1992	7.8 \pm 1.3 (10)	5.91 \pm 0.77 (10)
1993	6.8 \pm 1.4 (10)	6.4 \pm 1.5 (11)
1994	6.4 \pm 0.63 (13)	6.9 \pm 1.1 (13)
1995	5.2 \pm 1.3 (8)	6.2 \pm 1.9 (8)

Table 2.2. Linear regression analysis of the rate of decline of TP from spring to late summer (Day 130-238) each year (1993-95) in Mouse and Ranger Lakes. The rate of decline of TP ($\mu\text{g l}^{-1} \text{d}^{-1}$) is depicted by the slope of the line fitted to each curve in Figure 2.2. The standard error (S.E.) of the slope, sample size (n) and the probability that the slope is equal to zero (P) is provided with the coefficient of determination (R^2).

Lake and Year	Slope	S.E.	n	P	R^2
Mouse					
1993	-0.055	0.012	10	0.002	0.72
1994	-0.021	0.006	13	0.005	0.53
1995	-0.035	0.009	8	0.007	0.73
Ranger					
1993	-0.068	0.011	11	0.0002	0.81
1994	-0.045	0.008	13	0.0001	0.75
1995	-0.055	0.011	8	0.002	0.81

Table 2.3. Year to year and lake to lake comparison of the rates of decrease of total phosphorus from day 130 to 238.

Effect	<i>P</i>
Day	0.0000 ⁻
Year	0.0032
Lake	0.014
Day*Year	0.014
Day*Lake	0.018
Lake*Year	0.74
Lake*Year*Day	0.84

⁻ *P* < 0.00001

Table 2.4. Comparison of phosphorus concentrations ($\mu\text{g l}^{-1}$) in size fractions (<0.2, 2-20, or >250 μm) before (1993) and after (1994-95) biomanipulation (model I 2x2 ANOVA), where lake and year were the main effects and sampling dates within each year and lake were considered replicates. Concentrations from the two post-manipulation years were pooled into one treatment year.

Dependent Variable	Size Fractions	Year <i>P</i>	Lake <i>P</i>	Year*Lake <i>P</i>
Phosphorus Concentration	Mesoplankton (>250 μm)	0.066	0.14	0.39
	Nanoplankton (2-20 μm)	0.62	0.65	0.63
	Dissolved Phosphorus (<0.2 μm)	0.0040	0.00038	0.94

Table 2.5. Mean slopes of particulate phosphorus in Mouse and Ranger Lakes over the three years of study. Slopes were estimated from the relationship between proportions of PP retained on filters and logarithm of filter size. Means are determined from a complete sampling period in each year (May-Oct.). Means are followed by 95% confidence intervals and sample size.

Year	Mouse L.	Ranger L.
1993	-0.25 ± 0.026 (10)	-0.24 ± 0.042 (9)
1994	-0.27 ± 0.041 (10)	-0.25 ± 0.036 (10)
1995	-0.24 ± 0.043 (5)	-0.24 ± 0.047 (5)

Table 2.6. Comparison of slopes (model I 2x2 ANOVA) estimated from the relationship between the proportion of particulate phosphorus retained on a filter and the logarithm of filter size (see text for further explanation). Lake and year were the main effects and the slopes estimated on each sampling day of each year in each lake were considered replicates. Slopes from the two postmanipulation years (1994-95) were pooled into one treatment year.

Dependent Variable	Year <i>P</i>	Lake <i>P</i>	Year*Lake <i>P</i>
Slope of Particulate Phosphorus	0.41	0.15	0.56

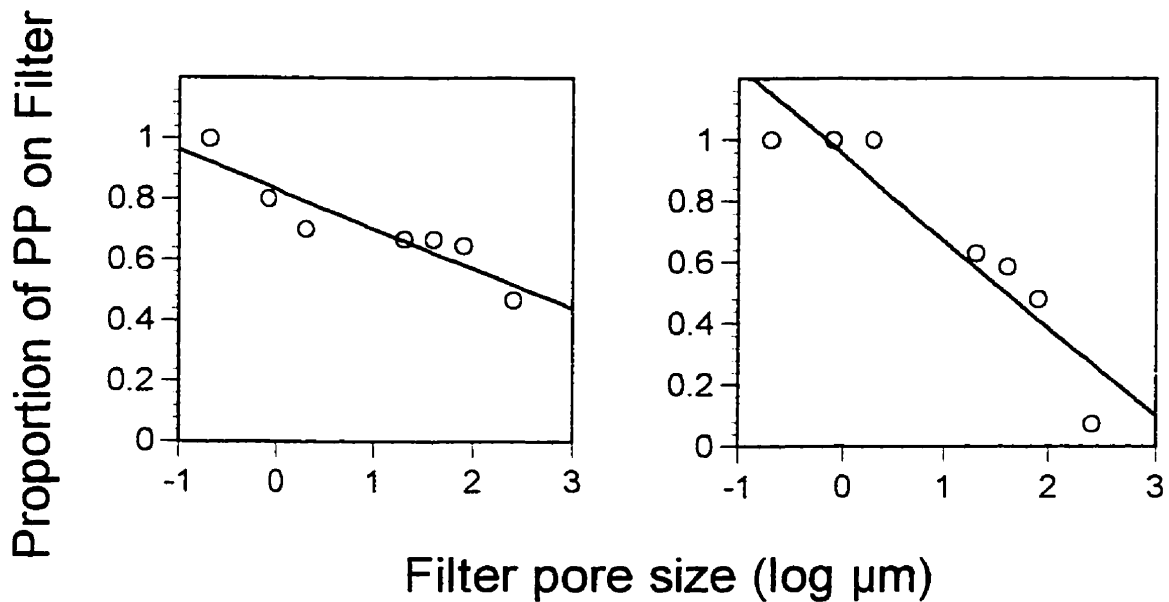


Figure 2.1. Two examples of the proportion of particulate phosphorus (PP/ Total PP) as a function of filter pore size (log size) on May 24 (left panel), and on August 16, 1994, in the epilimnion of Ranger Lake. On May 24, much of the PP is in larger particles resulting in a gentle slope (-0.13). On August 16, more of the PP is in smaller particles, resulting in a steep slope (-0.28).

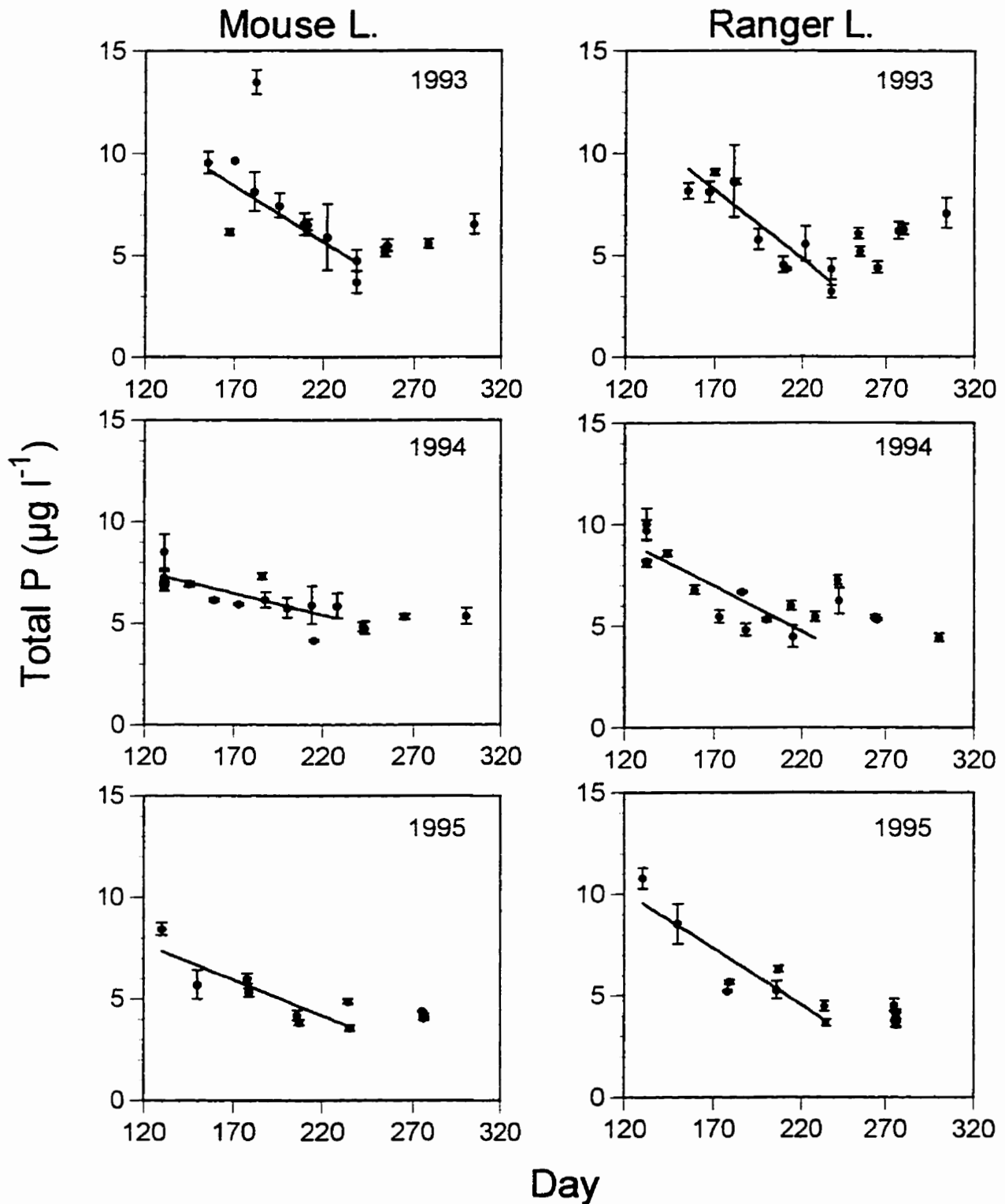


Figure 2.2. Decline of TP from spring to fall (1993-1995) in the epilimnia of Mouse and Ranger L. Error bars equal one standard error and represent analytical error associated with measuring TP on 2-6 subsamples. Linear regressions are fitted up to day 238, just before the epilimnia started to destratify. The outlier on day 181 (Mouse L. 1993) was not included in the regression. See Table 2.2. for regression statistics.

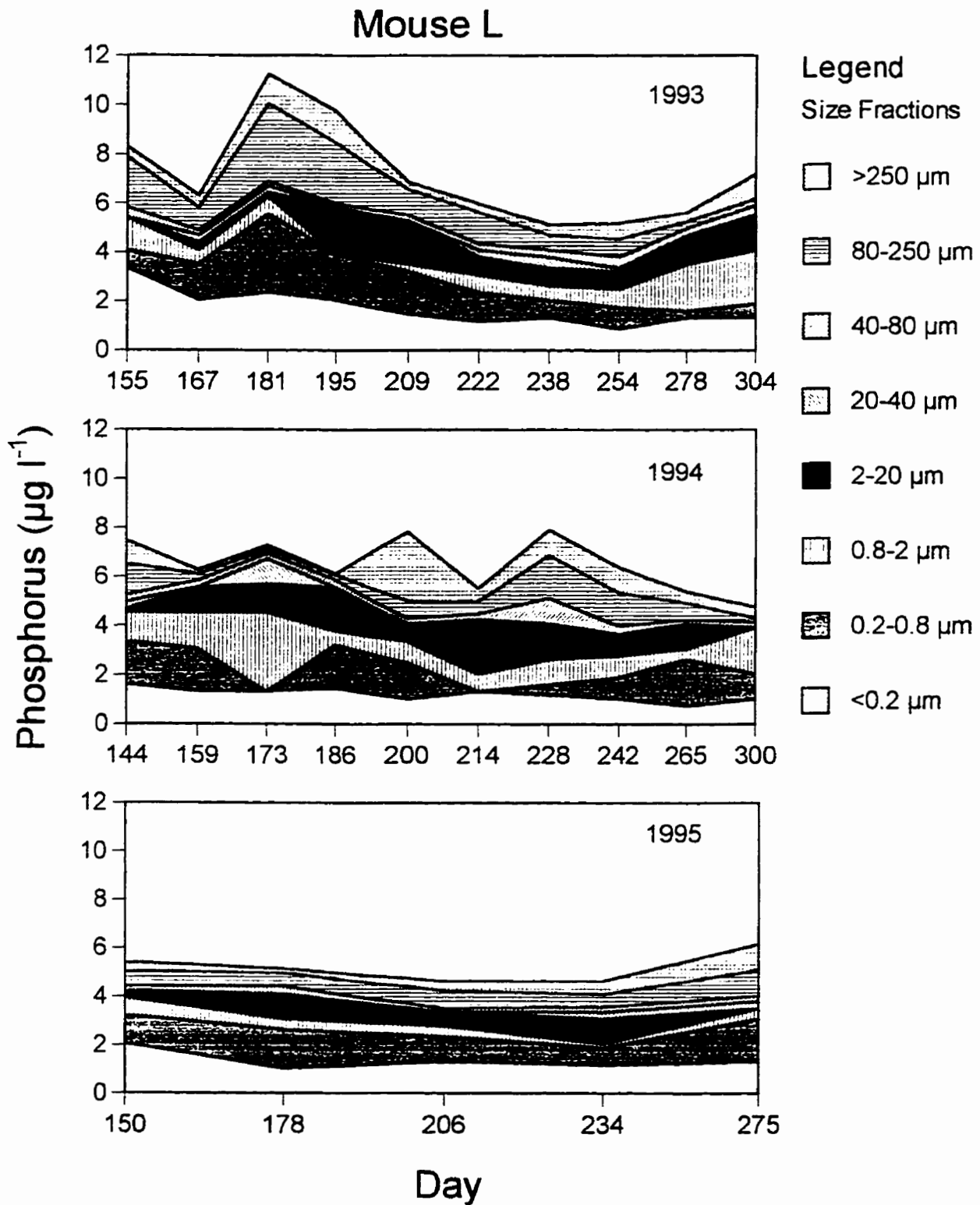


Figure 2.3. The distribution of phosphorus ($\mu\text{g l}^{-1}$) in size fractions of epilimnetic water from Mouse L. during the open water seasons of 1993-95. Note that sampling dates are not equidistant within or between charts.

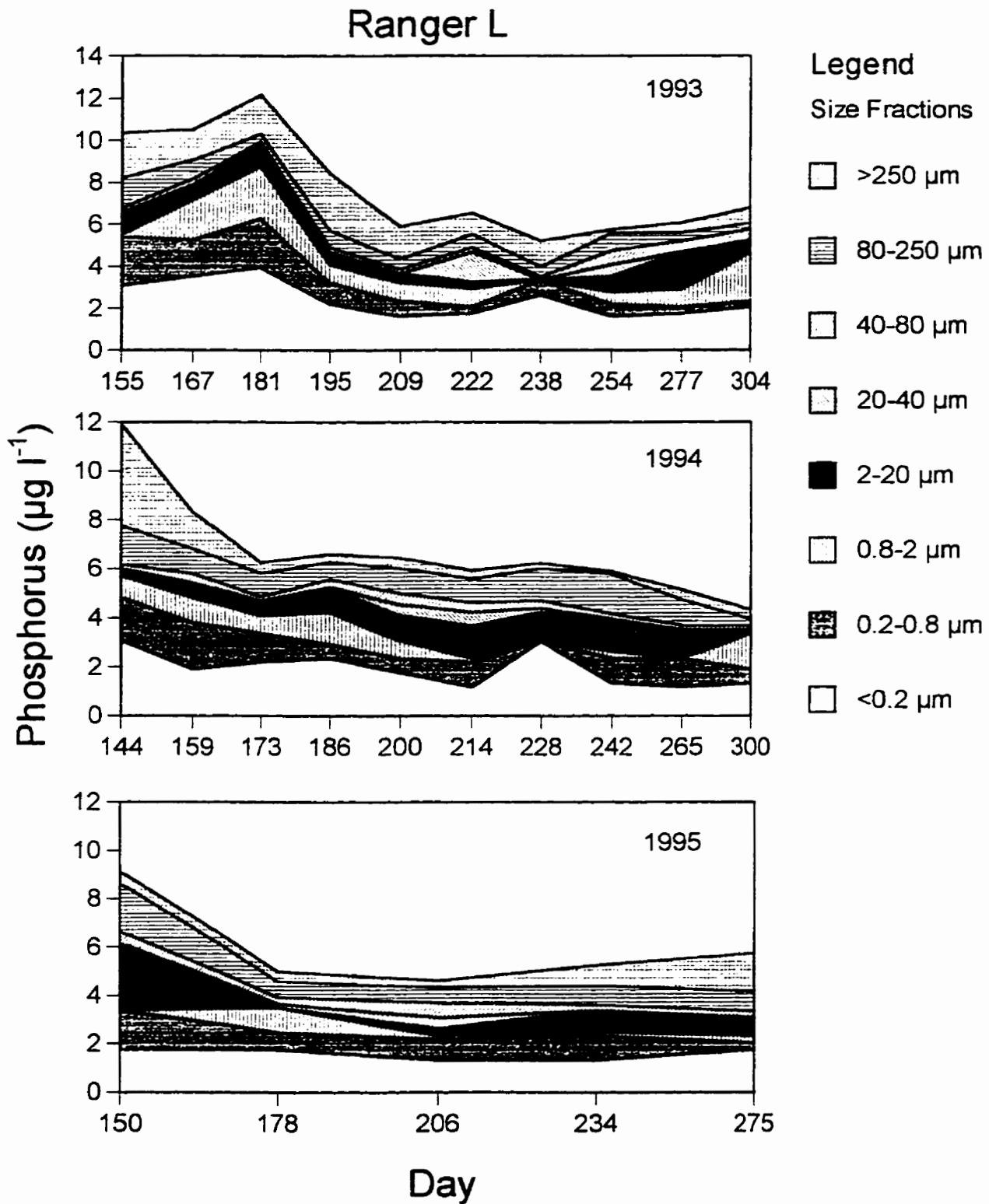


Figure 2.4. The distribution of phosphorus ($\mu\text{g l}^{-1}$) in size fractions of epilimnetic water from Ranger L. during the open water seasons of 1993-95. Note that sampling dates are not equidistant within or between charts.

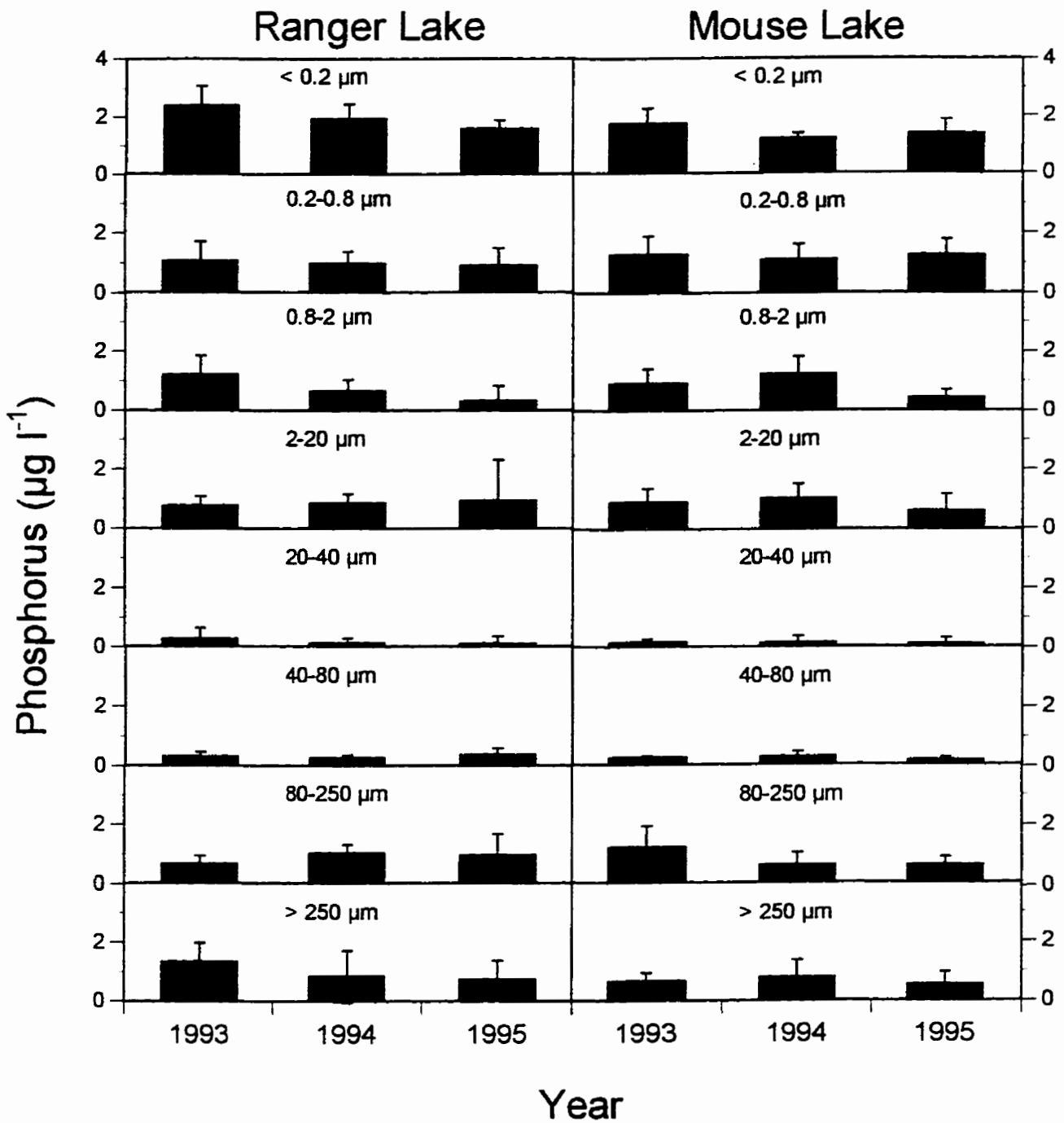


Figure 2.5. The mean concentration of phosphorus ($\mu\text{g l}^{-1}$) in each size fraction for each year for each lake. Error bars represent one standard error of the mean.

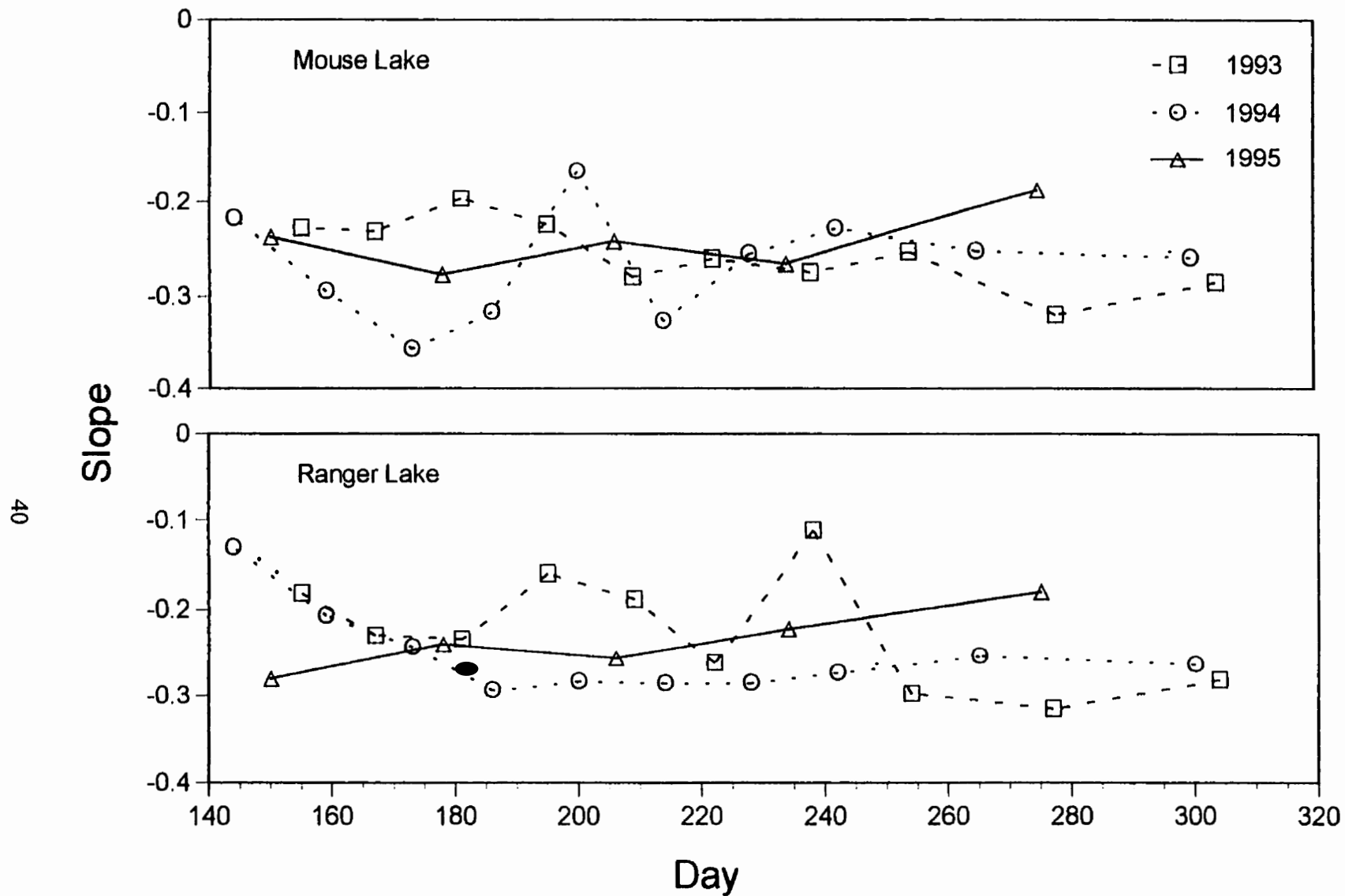


Figure 2.6. Slopes of particulate phosphorus during the open water season in each lake for years 1993-95. Slopes are estimated from the relationship between the proportion of particulate phosphorus retained on a filter and the logarithm of filter size. For example, if there is more particulate phosphorus in large fractions (e.g., >250 μm) then the slope will be more positive.

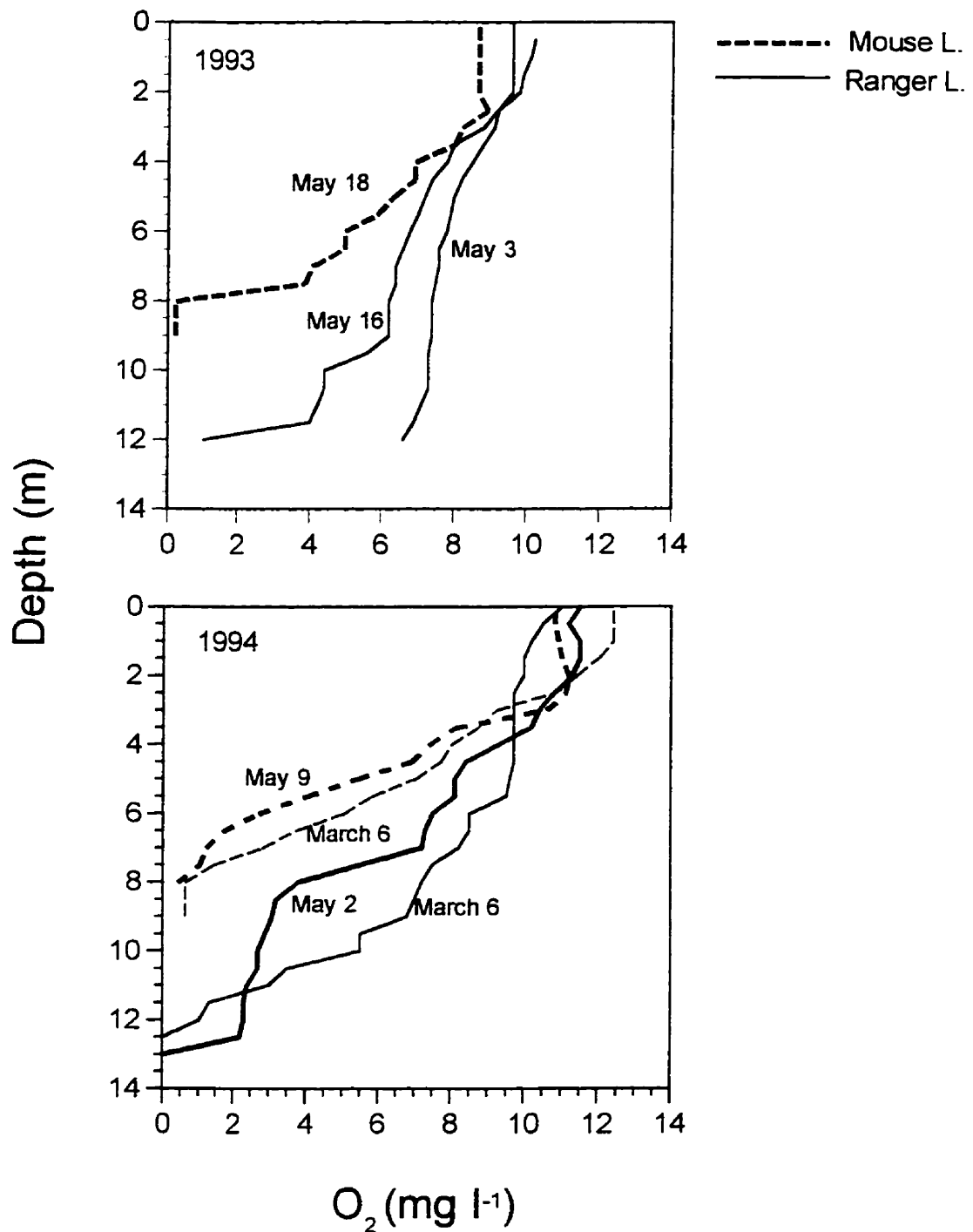


Figure 2.7. Oxygen profiles for Mouse and Ranger Lake in 1993 and 1994. Solid lines depict Ranger L. profiles and dotted lines depict Mouse L. profiles. In 1994, the profiles for both lakes are shown before (March) and after ice-out (May). Data used in these profiles were obtained from Dr. D. McQueen (Dep't. Biology, York University, North York, Ontario, Canada).

Chapter 3

THE EFFECT OF PISCIVORE REMOVAL AND INTRODUCTION ON PHOSPHATE CONCENTRATION AND UPTAKE

Introduction

The effect of biomanipulation on phosphate and phosphate uptake kinetics is poorly understood. This is surprising, as phosphate is considered the only form of P that is transported into planktonic microorganisms (Chrost 1990). Research in this area may have been discouraged by the methodological difficulties of measuring phosphate (e.g., see Bentzen and Taylor 1991; Fisher and Lean 1992). Despite the difficulties, the effect of biomanipulation on phosphate turnover and uptake has been investigated with radiophosphate in mesocosm and lake studies by Mazumder et al. (1988 and 1990). Other studies have taken less direct routes to assess the effect of biomanipulation on PO_4^{3-} and its uptake kinetics. Most often, dissolved P (DP) or fractions of DP (e.g., soluble reactive phosphorus) are used as a surrogate estimate of P availability (e.g., Vanni and Temte 1990; Vanni et al. 1990; Christoffersen et al. 1993; Hanson and Butler 1994). Other studies have used alkaline phosphatase activity as a measure of P limitation (e.g., Bergquist and Carpenter 1986; Elser et al. 1986; Moegenburg and Vanni 1991).

The effect of biomanipulation on PO_4^{3-} concentrations and uptake have not produced consistent results. Mazumder et al. (1988 and 1990) and Mazumder and Lean (1994) reported reduced DP and decreased turnover times of PO_4^{3-} in mesocosms and lakes that had abundant planktivorous fish. They also observed that the uptake of $^{32}\text{PO}_4^{3-}$ was greater by the 0.2-1 μm fraction when planktivorous fish were present. These authors

suggested that PO_4^{-3} became more limiting in treatments with abundant planktivores. Additional biomanipulation studies (Hulbert et al. 1972; Vanni and Temte 1990; Vanni et al. 1990; Moegenburg and Vanni 1991; Christoffersen et al. 1993; Hanson and Butler 1994) that used less direct approaches to estimate P-limitation, support those of Mazumder et al. (1988 and 1990) and Mazumder and Lean (1994). However, the trophic cascade hypothesis predicts that P-limitation will be greatest in piscivore treatments (Kitchell and Carpenter 1993a; Kitchell et al. 1993). Nutrient limitation, combined with elevated grazing activities, is expected to reduce algal biomass and enhance water clarity in lakes with intense piscivory. In studies on Paul, Tuesday and Peter Lakes (Bergquist and Carpenter 1986; Elser et al. 1986; Carpenter et al. 1993), alkaline phosphatase measurements indicated that nutrient limitation was more severe when piscivores were abundant.

The effect of piscivore removal and introduction on PO_4^{-3} concentration and uptake kinetics will be examined in light of the two perspectives listed above. In oligotrophic Mouse and Ranger Lakes, nutrient limitation may be present in both lakes. Therefore, the question that I will address is, which lake under which treatment demonstrates the greatest P-limitation. Phosphate concentration (by a modified Rigler radiobioassay), turnover times (TT), and uptake velocities of PO_4^{-3} will be calculated to assess the effect of the fish transfer.

Methods

Epilimnetic water was collected (6-8 l) from the middle of the epilimnion (station 4) on each lake with a Van Dorn sampler once every two weeks from June to August (1993-94). Additional, but less frequent, samples were also taken in May, September and October. In 1995, samples were collected once a month from May to September. In 1993, water was returned to the laboratory at the university of Waterloo, and kept at ambient lake temperature. At Waterloo, processing of the water occurred on the day of collection or on the following day. In 1994 and 1995, water was taken to the nearby Dorset laboratory where the water was processed within hours of collection.

At the onset of each sampling season, all laboratory equipment that would hold lake water was initially washed (0.1% contrad-70), rinsed (ethanol), leached (0.1 N HCl) and stored in sealed polyethylene bags. Before each incubation this equipment was then quickly leached (0.1 N HCl) and rinsed with lake water. Methods of Bentzen and Taylor (1991) were followed for uptake measurements and radiobioassays.

Lake-water was shaken and decanted into a graduated cylinder. Then fifty ml were decanted into a clean (0.1 N HCl) polyethylene beaker. Carrier free radiophosphate ($^{32}\text{PO}_4^{-3}$ or $^{33}\text{PO}_4^{-3}$, $\sim 170 \text{ Bq ml}^{-1}$) was added to the water. Incubations were for 8 to 16 min. At various times into the incubation (e.g., 1, 3, 5, 8, 12 min), 1 ml aliquots of water were filtered through a 0.2 μm polycarbonate filter (25 mm, Poretics). Additional aliquots were taken on the final incubation time (e.g., 12 min) and filtered through 0.8, 2.0 and 12 μm polycarbonate filters. Then two 1 ml aliquots were taken from the incubation vessel to determine total radioactivity added. Filters were dissolved (in 100 μl of 10:1 methylene

chloride and ethanolamine) in glass minivials. Scintillation fluor was added (ICN Ecolume or Ecolite) and radioactivity on each filter was determined by liquid scintillation counting. Quenching of samples was not detected. Radioactivity was corrected for background counts. Radioactivity remaining in the dissolved fraction (i.e., total DPM minus filter DPM) over time was fitted to a trinomial (for example, see Fig. 3.1). The uptake constant (k) was determined by taking the first derivative at time zero and dividing by the mean radioactivity in the totals. The reciprocal of this uptake constant was equal to the turnover time of phosphate. The uptake constant for the 0.2 to 0.8 μm fraction and the % uptake by each fraction (i.e., 0.2-0.8, 0.8-2.0, 2.0-12 and >12 μm) were also calculated.

The uptake run described above was followed by a modified Rigler (1966) radiobioassay to determine phosphate concentrations in the lake water. Four beakers containing 50 ml of lake water were set up in a series and injected with an increasing amount of phosphate and radiophosphate. For example, beaker one would contain $1 \mu\text{g P l}^{-1}$ (final conc.) and 50 Bq ml^{-1} of radioactivity, while beaker four would contain $10 \mu\text{g P l}^{-1}$ and 500 Bq ml^{-1} . Incubations were usually run for 2 min. in beakers 1 and 2, and for 8-16 minutes in beakers 3 and 4. Incubations were terminated by filtering 1 ml from each beaker onto a 0.2 μm polycarbonate filter (25 mm). At the same time, 1 ml from each beaker was also filtered onto a 0.8 μm polycarbonate filter. The uptake rate constant ($k \text{ min}^{-1}$) for each filter was determined. Then the uptake rate constants for the 0.2-0.8 fraction were calculated (e.g., $k_{0.2-0.8 \text{ at } 2 \text{ min}} = k_{0.2 \text{ at } 2 \text{ min}} - k_{0.8 \text{ at } 2 \text{ min}}$) and plotted against the corresponding concentration of phosphate added (P_a). The uptake rate constant for the 0.2-0.8 fraction, described above ($P_a=0$), was also added to this plot. This plot was fitted to a nonlinear

model described by Bentzen and Taylor (1991) as the Li equation (after Li 1983):

$$k_i = V_m / (K_t + P_n) + P_{ai}$$

The Li equation is a modification of the Michaelis-Menten equation, where k_i is equal to the uptake constant at i th P addition, V_m is the maximum velocity of PO_4^{3-} uptake, K_t is the Michaelis-Menten half-saturation constant for P transport ($\mu\text{g P l}^{-1}$), P_n is the ambient phosphate concentration ($\mu\text{g P l}^{-1}$), and P_{ai} is the added phosphate ($\mu\text{g P l}^{-1}$) at the i th addition. There are a variety of advantages to using this model (see Bentzen and Taylor 1991). For example, I did not have to determine if V_{max} was reached with the concentrations of phosphate I added to the incubations. Therefore, by fitting the model, I obtained an upper estimate ($K_t + P_n$) of the phosphate concentration on each sampling date in Mouse and Ranger L. Using the uptake for the 0.2-0.8 μm size-fraction to fit the model provides the lowest estimates of $K_t + P_n$ (Bentzen and Taylor 1991).

Next, the velocity of phosphate uptake (v) for the whole community ($>0.2 \mu\text{m}$) was calculated by multiplying the uptake constant (min^{-1}) for the $>0.2 \mu\text{m}$ fraction by the upper estimate of phosphate ($K_t + P_n$). Uptake velocities for size fractions were also calculated. Therefore, turnover time, upper ambient phosphate concentration, and uptake velocities of phosphate for the whole community ($<0.2 \mu\text{m}$) and for size fractions (0.2-0.8, 0.8-2.0, 2.0-12 and $>12 \mu\text{m}$) were calculated. A model I, 2x2 ANOVA was used to test if inverse patterns were present in these variables. As in the previous chapter, a significant interaction term would indicate that inverse patterns were present. Parameters for both postmanipulation years (1994-95) were pooled into one year.

Results

Turnover times (TT) for PO_4^{-3} extended from 3 to 10 min. (Table 3.1). Mean TT time was greater in Ranger L. than in Mouse L. in 1993. However, in 1994 and 1995, there was little difference between TT in either lake. In fact, both lakes show a gradual increase in TT over the study period. In light of the similarities in TT between lakes, a statistical comparison (ANOVA) was not performed. In 1995, Day 151 TT were not estimated; radioactivity on the filters was very irregular. I forgot to rinse forceps after opening a fresh vial of $^{33}\text{PO}_4^{-3}$.

The estimated PO_4^{-3} concentrations were between 0.1 and 1.2 $\mu\text{g P l}^{-1}$ (Table 3.2). The mean concentrations suggest an inverse pattern was present between lakes; that is, Ranger lake had a greater mean PO_4^{-3} concentration than Mouse L. in 1993, but after the fish transfer (1994-95) Mouse L. had a greater mean PO_4^{-3} concentration than Ranger L. This inverse pattern was near significant (Table 3.4, 2nd order interaction term, $P=0.094$). The early season in 1995 was marked by high PO_4^{-3} concentrations, which declined rapidly to concentrations found in previous years. This wide range in mean PO_4^{-3} concentration and the small sample size resulted in wide 95% confidence intervals. As observed with the TT data, mean PO_4^{-3} concentrations gradually increased from 1993 to 1995.

Uptake velocity of ambient PO_4^{-3} by the entire community ($>0.2 \mu\text{m}$) was between 33 and 182 $\text{ng P l}^{-1} \text{min}^{-1}$ (Table 3.3). As observed with the mean PO_4^{-3} data (Table 3.2), inverse patterns between lakes are also present in mean uptake velocities. Mouse L shows the greatest changes between pre- and postmanipulation mean uptake velocities, while Ranger appears unaffected. Despite the pronounced changes in Mouse L., this inverse

pattern was not significant (Table 3.4, 2nd order interaction term, $P=0.19$). Again the means, especially in 1995, had broad 95% confidence intervals.

The picoplankton fractions (0.2-0.8 and 0.8-2.0 μm) had the greatest uptake velocities for PO_4^{3-} (Table 3.3). Uptake velocities for the $>12 \mu\text{m}$ were always less than 10 $\text{ng P l}^{-1} \text{min}^{-1}$. Uptake into this fraction was often so low that it was difficult to quantify. Consistent patterns in uptake velocity across size fractions before and after biomanipulation were not evident in Ranger Lake. However, in Mouse L. uptake velocity in all fractions (except the $>12 \mu\text{m}$) were always greater after piscivores were added.

Discussion

Turnover times (Table 3.1) were rapid and similar to those reported for other precambrian shield lakes (Rigler 1966; Currie 1990; Bentzen and Taylor 1991). TT has been recognized as an indicator of P-limitation in lakes (White et al. 1982; Lean et al. 1987). Turnover times under 10 minutes are indicative of high P deficiency (Lean et al. 1987). Mazumder et al. (1990) found that lakes and mesocosms that contained abundant planktivores had shorter TT than fishless mesocosms or lakes with piscivores. The enhanced algal biomass common to systems with abundant planktivores is believed to increase the demand for PO_4^{3-} and thus shorten TT (Mazumder 1994b; Mazumder and Lean 1994). However, TT of PO_4^{3-} was unaffected by piscivore removal. A treatment response in TT was expected in Ranger L. because the particulate (PP) and dissolved phosphorus (DP)

fractions did show a weak response to treatment (Fig. 2.5) and TT of PO_4^{-3} has been shown to be related to PP in other studies (White et al. 1982; Mazumder et al. 1990).

Turnover times of PO_4^{-3} should be positively related to the size of the dissolved phosphate pool (e.g., White et al. 1982). However, TT of phosphate was not significantly related to PO_4^{-3} ($P=0.113$, $n=38$, $r^2=0.068$) in Mouse or Ranger (pooled data from both lakes over 3 years). Turnover times of PO_4^{-3} in Mouse and Ranger L. appear to be disconnected from parameters to which they are usually related. TT of PO_4^{-3} does not appear to support either perspective discussed in the introduction (i.e., in which treatment P should be more limiting).

Phosphate concentrations in Mouse and Ranger L. (Table 3.2) are similar to concentrations measured elsewhere with the Rigler radiobioassay (Peters 1979; Bentzen and Taylor 1991; Dodds et al. 1991; Bentzen et al. 1992). Phosphate concentrations have been observed to decline under increasing P deficiency (Currie et al. 1986). Phosphate concentrations did show a weak treatment response (Table 3.4, $P=0.094$), particularly in Mouse L, where PO_4^{-3} concentrations were greater when piscivores were present (Table 3.2). This observation supports the interpretation that P limitation is more severe in lakes dominated by planktivores (Mazumder et al. 1988 and 1990; Mazumder and Lean 1994) and not in lakes dominated by piscivores (Bergquist and Carpenter 1986; Elser et al. 1986; Carpenter et al. 1993).

Mean uptake of radiophosphate was 50% in the 0.2-0.8 μm fraction, 29% in the 0.8-2.0 μm fraction, 18% in the 2.0-12 μm and 3% in the >12 μm fraction, during the 3 years of study. Currie et al. (1986) concluded that the partitioning of phosphate uptake in

favor of bacteria is a function of the severity of P deficiency. The majority of uptake in Mouse and Ranger L was by picoplankton (<2.0 μm). However, a treatment response was not evident. Picoplankton did not take up a greater percentage of radiophosphate under planktivore treatment. For example, mean percent uptake in picoplankton was 77, 86 and 73% in Mouse in 1993, 1994 and 1995, respectively, and 79, 79 and 73% in Ranger in 1993, 1994 and 1995, respectively. Mazumder et al. (1988 and 1990) did observe greater uptake into picoplankton under planktivore treatment. In addition, I did find a weak treatment response in PO_4^{-3} concentrations that supported nutrient limitation under the planktivore treatment (see above). The lack of treatment response in percent uptake is puzzling and does not support the results found elsewhere (Mazumder et al. 1988 and 1990).

Uptake velocities of phosphate (v) are calculated by multiplying the estimated phosphate concentrations (Rigler radiobioassay) by the uptake rate constant for the whole community (k). Uptake velocities (Table 3.3, >0.2 μm column) are similar to those for Jack's L. and Lake Ontario (Bentzen and Taylor 1991 and Bentzen et al. 1992). Uptake velocities responded weakly to treatment ($P=0.19$, Table 3.4); they were lower under planktivore treatment in either lake, particularly Mouse L. The lower phosphate concentrations found under planktivore treatments (Table 3.2) are driving this relationship. Systems with abundant planktivores have been observed to have greater pico-nanoplankton biomass, shorter turnover times (Mazumder et al. 1988 and 1990), and lower dissolved P or phosphate (Hulbert et al. 1972; Mazumder et al. 1990; Vanni and Temte 1990; Vanni et al. 1990; Hanson and Butler 1994; Mazumder 1994b; Mazumder and Lean 1994). Mazumder

et al. (1988 and 1990) also found a greater proportion of radiophosphate in fractions <1 μm in planktivore treatments. In this study, uptake velocities were greater in the small fractions (0.2-0.8 and 0.8-2.0 μm) under piscivore treatment (Table 3.3). Without uptake velocities from the above studies, comparisons are difficult to interpret.

Interestingly, whole water regeneration rates (Chapter 5, Table 5.2) were also greater when piscivores were abundant. Therefore, the trends in uptake velocity appear to be real, instead of being an artifact of analytical error or bias. Possibly, the microbial community under piscivore treatment had a greater uptake velocity of PO_4^{-3} without having a shorter TT than the microbial community under planktivore treatment because of the larger pool of PO_4^{-3} in the piscivore treatment (Table 3.2). Conceivably, the phosphate pool was greater because a different dynamic existed between top-down and bottom-up regulation of the microbial assemblage in the piscivore treatment. For example, an increase in grazer control may have permitted a larger pool of PO_4^{-3} to develop in the piscivore treatment.

The difference between whole water P regeneration and uptake velocities (>0.2 μm) is remarkable (compare Table 3.3 with 5.2). If we standardize rates to common units (e.g., $\text{ng P l}^{-1} \text{ h}^{-1}$), uptake velocities are ~100-fold greater than regeneration rates. If regeneration rates are accurate and uptake velocities are overestimated, then phosphate concentrations in natural waters are much smaller than previously thought. For example, the mean phosphate concentrations in Table 3.2 may be only ~5 ng P l^{-1} instead of ~500 ng P l^{-1} . This seems minute; however, other researchers have suggested that this may be correct. For example, Taylor and Lean (1991) concluded that PO_4^{-3} may have been under 1 nM in mesotrophic

Jacks L. Furthermore, the Rigler radiobioassay does overestimate PO_4^{-3} concentrations (e.g., Fisher and Lean 1992). However, the accuracy of the regeneration rates, estimated by the new technique in chapter 5, also requires confirmation.

In conclusion, TT of PO_4^{-3} did not respond to the fish transfer (e.g., as seen by Mazumder et al. 1990). However, PO_4^{-3} concentrations (calculated by radiobioassay) did respond weakly and indicate that PO_4^{-3} may be more limiting when planktivores are abundant. Another weak effect was found in the uptake velocities ($>0.2 \mu\text{m}$) of PO_4^{-3} . The interpretation of these measurements on P-limitation requires further confirmation and consideration before any conclusions may be drawn. Finally, in light of the regeneration rates and the results of other studies, upper ambient concentrations of PO_4^{-3} (as determined by the Rigler radioassay) appear to be grossly overestimated.

Table 3.1 Turnover time (min) of phosphate in Mouse and Ranger Lake (1993-95). Mean rates and confidence intervals (95% C.I.) are listed at the end of each year. Lakes were biomanipulated in fall, 1993.

Year	Day of Year	Turnover Time (min)	
		Ranger L.	Mouse L.
1993	155	6.18	6.86
	169	4.81	4.42
	183	5.95	4.26
	196	7.66	3.85
	211	5.52	3.45
	222	6.36	5.03
	239	4.96	5.01
	255	6.06	5.80
	280	7.37	4.58
	Mean ± 95% C.I.	6.34 ± 0.62	5.36 ± 0.90
1994	160	6.77	5.83
	174	6.54	7.02
	187	7.89	4.89
	201	4.82	5.80
	215	7.55	6.18
	229	6.12	6.25
	243	6.94	6.66
	266	8.48	8.55
	Mean ± 95% C.I.	6.89 ± 0.95	6.40 ± 0.90
1995	179	7.75	6.55
	207	6.42	6.34
	235	5.64	5.21
	275	8.14	7.67
	Mean ± 95% C.I.	6.99 ± 1.85	6.44 ± 1.61

Table 3.2 Modified Rigler radiobioassay estimates of phosphate concentrations in Mouse and Ranger L. (1993-1995). Mean concentrations and confidence intervals (95% C.I.) are listed at the end of each year. Lakes were biomanipulated in fall, 1993.

Year	Day of Year	Ranger L. ($\mu\text{g P l}^{-1}$)	Mouse L. ($\mu\text{g P l}^{-1}$)
1993	169	0.526	0.346
	183	0.464	0.333
	196	0.442	0.334
	211	0.311	0.194
	222	0.378	0.280
	239	0.183	0.259
	255	0.533	0.344
	Mean \pm 95% C.I.	0.405 \pm 0.116	0.299 \pm 0.053
1994	160	0.463	0.427
	174	0.540	0.665
	187	0.556	0.738
	201	0.390	0.694
	215	0.455	0.608
	229	0.404	0.506
	243	0.226	0.270
266	0.289	0.631	
	Mean \pm 95% C.I.	0.4154 \pm 0.0956	0.567 \pm 0.131
1995	151	1.112	0.742
	179	0.900	1.189
	207	0.318	0.500
	235	0.358	0.430
	275	0.335	0.268
	Mean \pm 95% C.I.	0.604 \pm 0.464	0.626 \pm 0.445

Table 3.3. Uptake rates of phosphate ($\text{ng P l}^{-1} \text{min}^{-1}$) by size fractions in each Lake (1993-1995). Mean concentrations and confidence intervals (95% C.I.) are listed at the end of each year. Lakes were biomanipulated in fall, 1993.

Year	Day of Year	Uptake Rate ($\text{ng P l}^{-1} \text{min}^{-1}$) by Size Fraction by Lake											
		>0.2 μm		0.2-0.8 μm		0.8-2 μm		2-12 μm		>12 μm			
		Ranger L.	Mouse L.	Ranger L.	Mouse L.	Ranger L.	Mouse L.	Ranger L.	Mouse L.	Ranger L.	Mouse L.	Ranger L.	Mouse L.
1993	169	109.21	78.17	64.69	51.22	28.51	18.25	8.77	0.00	7.24	9.21		
	183	77.95	78.15	46.39	55.49	15.20	16.18	14.82	5.53	1.54	0.95		
	196	57.70	86.82	35.55	45.41	11.36	23.31	8.82	16.30	1.97	1.80		
	211	56.34	56.07	32.29	25.84	14.07	17.56	9.05	10.64	0.93	2.03		
	222	59.39	55.66	24.17	27.17	16.82	13.94	16.64	11.62	1.76	2.92		
	239	36.86	51.64	15.72	14.94	10.11	17.74	8.74	15.07	2.29	3.89		
	255	87.97	59.37	25.58	23.99	28.97	20.03	27.38	12.88	6.03	2.46		
	Mean	69.35	66.55	34.91	34.87	17.86	18.14	13.46	10.29	3.11	3.32		
	\pm 95% C.I.	22.24	12.99	15.09	14.43	7.18	2.73	6.44	5.29	2.28	2.55		
1994	160	68.37	73.24	48.11	50.80	11.31	19.73	4.29	1.27	4.66	1.44		
	174	82.55	94.72	41.72	56.80	25.42	28.96	13.81	6.96	1.59	2.00		
	187	70.40	150.91	40.20	65.75	17.17	49.06	11.33	32.72	1.70	3.38		
	201	81.03	119.62	32.22	83.18	25.05	28.25	21.01	6.01	2.75	2.17		
	215	60.30	98.37	35.07	50.03	12.78	28.79	11.12	17.90	1.33	1.64		
	229	66.05	80.85	29.02	41.24	25.00	26.34	10.44	11.75	1.58	1.52		
	243	32.56	40.55	13.70	19.45	10.91	12.45	7.32	7.90	0.62	0.75		
266	34.09	73.76	14.24	39.02	12.26	27.32	6.54	5.45	1.05	1.97			
	Mean	61.92	91.50	31.79	50.78	17.49	27.61	10.73	11.25	1.91	1.86		
	\pm 95% C.I.	15.99	27.80	10.43	15.87	5.54	8.70	4.32	8.34	1.06	0.63		
1995	179	115.89	181.65	46.59	59.64	38.41	77.09	27.34	38.74	3.55	6.18		
	207	49.54	78.83	21.72	36.99	15.54	14.26	9.62	22.63	2.67	4.96		
	235	63.57	82.59	23.73	24.17	19.87	27.06	18.30	28.60	1.67	2.76		
	275	41.19	34.88	15.40	21.78	15.74	8.90	9.32	3.81	0.73	0.39		
		Mean	67.55	94.49	26.86	35.64	22.39	31.83	16.14	23.44	2.15	3.57	
	\pm 95% C.I.	53.34	98.66	21.68	27.57	17.29	49.52	13.60	23.36	1.95	4.06		

Table 3.4. Comparison of phosphate concentrations and phosphate uptake velocities ($>0.2 \mu\text{m}$) before (1993) and after (1994-95) fish transfer (for each variable a model I 2x2 ANOVA), where lake and year were the main effects and sampling dates within each year and lake were considered replicates. Concentrations and uptake velocities from the two post-manipulation years were pooled into one treatment year.

Dependent Variable	Year <i>P</i>	Lake <i>P</i>	Year*Lake <i>P</i>
Phosphate Concentration	0.72	0.0084	0.094
Phosphate Uptake Velocity	0.21	0.54	0.19

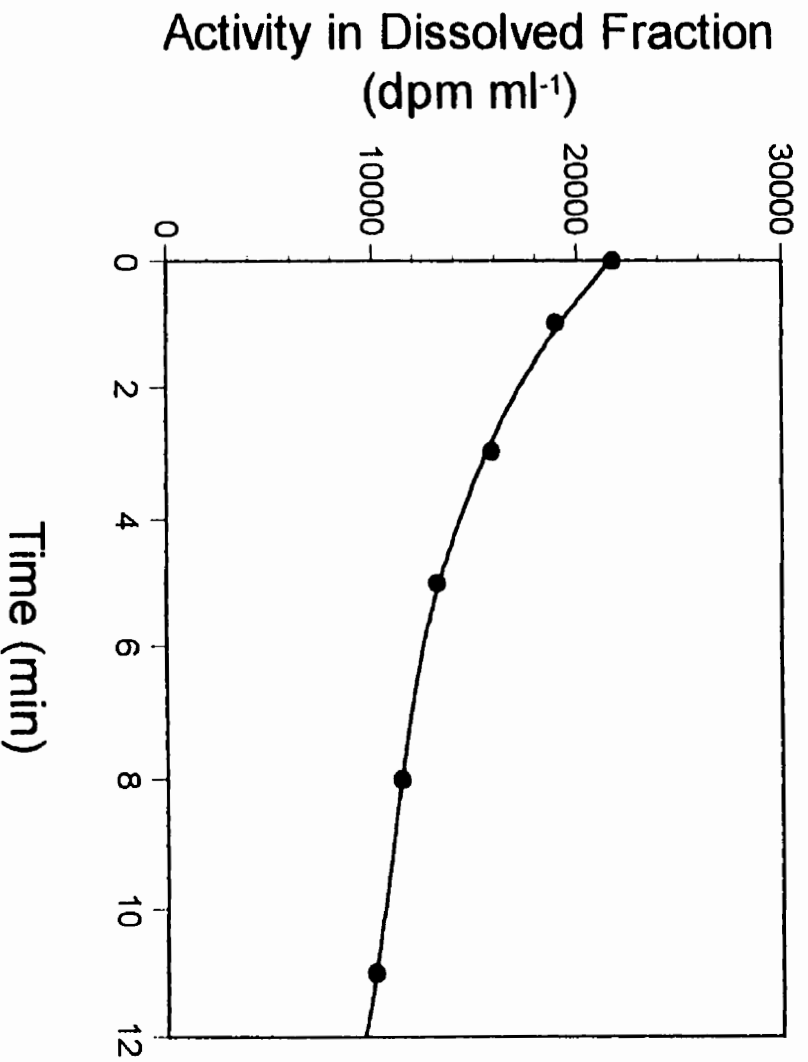


Figure 3.1. Example of a time course of radiophosphate uptake. Conducted on Oct. 2nd, 1995 with Ranger Lake epilimnetic water. Radiophosphate is added to the lake water at time = zero, then the radiophosphate is quickly taken up by the particulate pool. The time course is fitted to a trinomial. The uptake constant (k) is equal to the first derivative at time = 0 divided by the total radioactivity added. The turnover time of phosphate is equal to the reciprocal of the uptake constant.

Chapter 4

MEASURING PHOSPHORUS REGENERATION IN PLANKTONIC COMMUNITIES

Introduction

"Effects of zooplankton on phytoplankton biomass and productivity are not intuitively clear because they result from countervailing processes (grazing vs. nutrient recycling) and potentially compensatory allometric relationships" (Carpenter et al. 1985 p. 635). Today, a decade later, the above statement is still pertinent; progress towards understanding the mechanisms behind successful biomanipulations are still unclear. The role of nutrient cycling has been poorly addressed (Lyche et al. 1996). The importance of various groups to phosphorus regeneration has been claimed (e.g., Moegenburg and Vanni 1991; Kraft 1993; Findlay et al. 1994), but not put into the context of total planktonic regeneration. Furthermore, techniques to measure total planktonic regeneration of P are absent or are inadequate (Stockner and Porter 1988; Sterner 1989b).

For example, isotope dilution approaches (e.g., Harrison 1983) have been used to estimate P regeneration, but assume that incorporated label is not recycled over the course of an incubation (Glibert and Capone 1993). More importantly, the isotope dilution method depends on the accurate measurement of PO_4^{-3} as SRP, even though SRP measurements grossly overestimate true PO_4^{-3} concentrations (Fisher and Lean 1992). In addition, regeneration rates of specific planktonic groups (e.g., microbial) are often overestimated such that they exceed total planktonic regeneration rates (Glibert et al. 1992; Dodds 1995). An approach by Dodds et al. (1991) relies on antibiotic inhibition, but antibiotic treatment may be ineffective (Tremaine and

Mills 1987). Therefore, to examine P regeneration in the Dorset Lakes Biomanipulation Project, I developed a technique to measure phosphorus release by entire or size-fractionated plankton communities.

Methods

Water samples were removed from the middle of the epilimnion of each lake with a Van Dorn sampler on three occasions during each summer. Water was placed in 4-liter clear and collapsible polyethylene containers that had been washed (0.1% contrad-70), rinsed (ethanol), and leached (0.1 N HCl). Each sample was incubated with carrier-free radiophosphate ($^{32}\text{PO}_4^{-3}$ or $^{33}\text{PO}_4^{-3}$, 230-3,200 Bq ml⁻¹, ICN Biomedicals) for 17 to 76 h to label the planktonic community (Fig. 4.1). At the start of each incubation, subsamples were taken to determine the ratio of total phosphorus (Parsons et al. 1984) to total radioactivity. During summer 1993, water was transported to the laboratory and incubated at $24\pm 2^\circ\text{C}$ (epilimnion) and $11\pm 2^\circ\text{C}$ (metalimnion) under artificial fluorescent light (12:12 L/D). During summer 1994 and 1995, incubations were conducted at depth in the lakes.

At the end of each incubation, 40-300 ml subsamples were extracted gently from each container through a 40- μm nylon screen (Nitex) or a 0.8- μm polycarbonate filter (Poretics) via syringe filtration to produce three size fractions of lake water: <0.8 μm ; <40 μm ; and whole water. The <0.8- μm filtrate was considered a bacterial fraction. Protist release was estimated as the release rate for the <40- μm fraction minus the <0.8- μm fraction release rate. The

zooplankton release rate was considered equal to the whole water release rate minus the <40- μm fraction release rate.

A subsample (1 to 8 ml) from each fraction was then gently pushed through a 0.2- μm syringe filter (polysulfone acrodisc, Gelman). This filtrate was radioassayed for dissolved ^{33}P and considered a zero time measurement for the postincubation period (Fig. 4.1). Then each fraction was flooded with $^{31}\text{PO}_4^{-3}$ to competitively inhibit further ^{33}P uptake. The injection of competitive inhibitor marked the start of the postincubation period. A final concentration of competitive inhibitor of $100 \mu\text{g P liter}^{-1}$ was used in 1993 and a concentration of $1,000 \mu\text{g P liter}^{-1}$ was used during 1994 and 1995. Additional subsamples (1-8 ml) of dissolved ^{33}P were taken from each fraction for 1 to 7 h after the start of the postincubation period (Fig. 4.1).

This dissolved ^{33}P radioactivity was plotted against postincubation time for each fraction. The slope of this line (Fig. 4.1) was used as an estimate of the release rate of dissolved ^{33}P . This estimate was converted to rates of phosphorus release using the ratio of total ^{33}P radioactivity to total phosphorus in each 4-liter container at the beginning of the incubation:

$$\text{P release} = ^{33}\text{P release} \times \text{total P} \div \text{total } ^{33}\text{P}.$$

My working definition for phosphorus regeneration was the transfer of phosphorus from the particulate pool ($>0.2 \mu\text{m}$) to the dissolved pool ($<0.2 \mu\text{m}$) over time. The dissolved ^{33}P released can be characterized by column chromatography or other techniques (e.g., Taylor and Lean 1981), but I do not do so here. Existing evidence suggests that the majority of regenerated dissolved P may be PO_4^{-3} (Peters and Lean 1973; Taylor and Lean 1981; Jurgens and Gude 1990), and organic P compounds may also be rapidly mineralized by phosphatases.

Aqueous samples for radioassays were placed in Ecolume or Ecolite (ICN Biomedicals) and counted on a liquid scintillation counter (LKB Rackbeta). Radioassays were corrected for background counts and radioactive decay. Quenching of samples was not detected.

Assumptions

There are five critical assumptions to the technique: incubation conditions do not affect the regeneration rate; the ratio of $^{31}\text{PO}_4^{-3}$ and ^{33}P is the same in the incubation vessel and in the released material; the concentration of competitive inhibitor ($^{31}\text{PO}_4^{-3}$) is adequate to block re-incorporation of released ^{33}P during the postincubation period; the addition of competitive inhibitor ($^{31}\text{PO}_4^{-3}$) does not lead to elevated ^{33}P release; and last, that the initial regeneration rates in the postincubation period can be estimated, e.g., with a linear fit. These assumptions are addressed below.

The length of time required for the injected ^{33}P to become distributed among all compartments in a manner similar to ^{31}P is long relative to the incubation with ^{33}P . If the entire community has not reached steady state, the phosphorus release rate of certain fractions (e.g., zooplankton) may be underestimated. Therefore, once in summer 1994 and again in 1995, I examined the release rate of dissolved ^{33}P as a function of incubation length with ^{33}P . Specifically, 4-liter containers of epilimnetic water from Ranger Lake were injected simultaneously with $^{33}\text{PO}_4^{-3}$ and then incubated for different lengths of time (0.18 to 70 h) before addition of the competitive inhibitor.

The effective concentration of competitive inhibitor ($^{31}\text{PO}_4^{-3}$) that will prevent re-incorporation of released ^{33}P will vary with factors that influence the maximum uptake velocity of PO_4^{-3} , such as postincubation length, biomass of plankton, ambient phosphorus concentration, and water temperature. Too low a concentration will result in release rates being underestimated. Therefore, the effect of competitive inhibitor concentration on re-incorporation was examined.

Two containers (4 liters each) of epilimnetic Ranger Lake water were incubated at mid epilimnetic depth, but, unlike other samples used for measuring phosphorus release, they were not injected with ^{33}P initially. At the end of the incubation period these samples were retrieved and injected with competitive inhibitor ($^{31}\text{PO}_4^{-3}$, 100 and 1,000 $\mu\text{g P liter}^{-1}$ final conc'n.). Then, 5 min after competitive inhibitor injection, these two containers were injected with $^{33}\text{PO}_4^{-3}$. The activity of dissolved ^{33}P in these two samples was assayed (as described earlier) over an extended length of time, e.g., 10 h. This experiment was repeated with metalimnetic water at both concentrations of competitive inhibitor. A decline in dissolved ^{33}P over time in these samples would indicate that the competitive inhibitor was insufficient to block incorporation of ^{33}P . In this event, release rates of phosphorus would be underestimated if they were not corrected for re-incorporation.

A possible concern with the technique is that the addition of a high concentration of $^{31}\text{PO}_4^{-3}$ may displace $^{33}\text{PO}_4^{-3}$ from organisms that exhibit no net regeneration of P under normal circumstances. However, I have some evidence that "chasing" does not occur. Occasionally, labeled fractions do not release ^{33}P after the addition of competitive inhibitor. If chasing was occurring, these fractions would be expected to release label after the addition of $^{31}\text{PO}_4^{-3}$. Additional evidence that radiophosphorus cannot be displaced from plankton by phosphate may

be found in other studies (e.g., Lean and Cuhel 1987). I addressed the problem of “chasing” further with epilimnetic water from Ranger L. in 1995. One-liter clear, collapsible polyethylene containers were filled with lake water and incubated with $^{33}\text{PO}_4^{-3}$ for ~14 h. Then competitive inhibitor was injected at either 10, 100, 500, 1,000, 5,000, or 10,000 $\mu\text{g } ^{31}\text{P liter}^{-1}$ (final conc'n). Each concentration was replicated two to four times. The release of dissolved ^{33}P was assayed over an 8 h postincubation period in each container and dissolved P regeneration was calculated as described above.

The accumulation of ^{33}P in the dissolved pool (Fig. 4.1) was fitted to a linear function to estimate dissolved P release. I have no evidence to reject a linear model of release. However, I reasoned that the appropriate function would become evident if the rate of release was examined over an extended postincubation period. Therefore, two experiments (one in 1994 and another in 1995) were set up as described earlier, except that the postincubation period extended for 46–49 h, instead of the usual 1 to 7 h.

Results and Discussion

We expected to see a positive relationship between release rate and incubation time (Fig. 4.2). I estimated that the length of time required to completely label the plankton would be >70 h, particularly the zooplankton fraction. In one experiment, I found that the zooplankton fraction had only reached 48% isotopic equilibrium after 46 h of incubation with ^{33}P . However, maximum release rates were observed within the first hour of incubation (Fig. 4.2), suggesting that the internal pools and pathways responsible for the majority of P release have rapid turnover

times. Such pathways probably include egestion processes in protists and zooplankton, which typically have gut passage times less than 1 h (Peters 1984). Sloppy feeding may also contribute substantially to release rates. Therefore, striving to obtain isotopic equilibrium in animals responsible for regeneration may not be necessary or advantageous in order to obtain the best estimate of P release. Shorter incubations (e.g., 12-24 h) will also lessen the effects of disturbance artifacts. The results for 1995 in Fig. 4.2 suggest a diel pattern of release. This outcome warrants further investigation.

A final concentration of 100 or 1,000 $\mu\text{g } ^{31}\text{P liter}^{-1}$ prevented significant uptake of dissolved ^{33}P in the epilimnion of Ranger L. (Fig. 4.3). However, 70% of the dissolved ^{33}P was incorporated into the particulate pool of metalimnetic water from Ranger L. when the concentration of the competitive inhibitor was 100 $\mu\text{g } ^{31}\text{P liter}^{-1}$. Here, release rates may be underestimated. If the appropriate concentration of competitive inhibitor is unknown, then I recommend conducting the above experiment with the same concentration of $^{31}\text{PO}_4^{-3}$ used in the release experiments as a check. If re-incorporation is found to be significant, then greater concentrations of inhibitor would be required.

The regeneration rate is underestimated when only 10 $\mu\text{g P liter}^{-1}$ is used to prevent re-incorporation of ^{33}P (Fig 4.4), as one might expect from Fig. 4.3. However, as also seen in Fig. 4.3, 100 to 1,000 $\mu\text{g P liter}^{-1}$ is adequate to prevent re-incorporation in Ranger L. epilimnetic water. More important, the estimate of the regeneration rate remains constant over this range of competitive inhibitor (Fig. 4.4). When very high concentrations of competitive inhibitor were used ($\geq 5,000 \mu\text{g liter}^{-1}$) the regeneration rate increased, which may be indicative of “chasing” or simply osmotic shock and cell leakage. I conclude that the concentration of competitive inhibitor

(100 to 1,000 $\mu\text{g P liter}^{-1}$) used to estimate release rate of dissolved P in these lakes is adequate to block uptake of ^{33}P , but not sufficient to “chase” label from cells. Note that a 1,000-fold (10 to 10,000 μg) increase in competitive inhibitor concentration only resulted in a 3.5-fold increase in apparent regeneration rate. This demonstrates the high affinity that plankton have for previously bound P.

During the two extended postincubation experiments, the accumulation of dissolved ^{33}P over time fit a linear function ($R^2 \geq 0.98$) in both years (Fig. 4.5). When a linear model is appropriate, samples of the dissolved pool need only be taken at the start and end of the postincubation period. If other patterns are found, it may be necessary to fit a nonlinear function and solve for the derivative at the time of injection of competitive inhibitor.

On average, bacteria ($<0.8 \mu\text{m}$ fraction) accounted for 21% of the whole water rate in both lakes over the three year study (Table 4.1). Protists ($<0.8\text{--}40 \mu\text{m}$) contributed the greatest (50%) to total planktonic regeneration. Zooplankton rates ($>40 \mu\text{m}$) were the most variable, and on average accounted for 32% of the whole water rate. The zooplankton fraction, on one occasion, contributed 100% to the whole water release rate. The rates indicate that the microbial component of these lakes is the greatest contributor of dissolved regenerated P.

The release rate in whole water (unfractionated) was 16 to 84 $\text{ng P liter}^{-1} \text{h}^{-1}$. These rates were sufficient to recycle the total epilimnetic phosphorus pool in 2.6 to 15 d. Similar turnover times have been estimated for the epilimnion of a mesotrophic precambrian shield lake (Taylor and Lean 1991; Fisher and Lean 1992). Estimating turnover times for the particulate pool via release rates avoids the errors associated with using the Rigler bioassay to derive uptake

velocities for PO_4^{3-} (e.g., for errors see Tarapchak and Herche 1988 and 1989; Bentzen and Taylor 1991; Fisher and Lean 1992).

We did not expect to see significant release of dissolved ^{33}P from the bacterial fraction since bacteria may compete with algae for phosphate in oligotrophic freshwaters (Rothhaupt 1992; Vadstein et al. 1993) and the level of P-deficiency was great in the two study lakes (see chapter 3). I suggest that the bacterial release rates may be accounted for by bacterial cell autolysis or lysis from viral infections (e.g., see Middelboe et al. 1996). For example, 10 to 20% of marine heterotrophic bacteria were estimated to be lysed daily by viruses (Suttle 1994). In August 1994, in Ranger and Mouse Lakes, the bacterial fraction (0.2 to 0.8 μm) contained 0.8 to 1.0 $\mu\text{g P liter}^{-1}$. If 10 to 20% of this phosphorus was being released into the dissolved pool daily (i.e., through cell lysis), I would expect regeneration rates of 80 to 200 $\text{ng P liter}^{-1} \text{d}^{-1}$ or 3 to 8 $\text{ng P liter}^{-1} \text{h}^{-1}$. Therefore, viral lysis of bacterial cells may account for the observed rates from the $<0.8 \mu\text{m}$ fraction. Alternatively, plankton greater than 0.8 μm , i.e., bacterivores, may have entered the bacterial fraction due to inaccurate pore sizes in polycarbonate filter membranes.

It is often difficult to justify new techniques, because the data they generate are novel and not readily comparable to other research. In addition, the results from other studies are often obtained from different environments, which further complicates meaningful comparisons. However, techniques can be compared based on the assumptions they make, the degree to which they manipulate communities, their breadth of applicability and their convenience of use. There are a handful of techniques that have been used to measure directly the release rates in planktonic

communities; their shortcomings will be examined in light of this technique which has already been described. I do not intend review all approaches, but only those that I deem typical.

The method used by Taylor and Lean (1991) would be useful for direct comparisons with the technique described here, especially for zooplankton fractions; however, their technique requires considerable effort to separate and measure release rates of each zooplankton taxon and cannot be extended to nanoplankton and bacteria except by mass balance. The technique is further complicated by the need to determine the abundance of the taxa. The isotope dilution method employed by Harrison (1983), based on the dilution of tracer in the dissolved pool, assumes that incorporated ^{33}P is not released from the particulate pool over the course of an incubation (Glibert and Capone 1993). Harrison and Harris (1986) questioned this assumption and found considerable regeneration of label. In my incubations, maximal release rates of ^{33}P have been reached within minutes (Fig. 4.2). Therefore, release rates may be seriously underestimated in isotope dilution experiments if the recycling of label is not considered (Harrison 1993). Additionally, the isotope dilution method depends on the accurate measurement of PO_4^{-3} as SRP, even though SRP measurements grossly overestimate true PO_4^{-3} concentrations (Fisher and Lean 1992). The technique also assumes that regeneration products will be released as SRP, although other forms of dissolved P may be released. Furthermore, in size fractionation studies, plankton must be separated before incubation with label; a procedure that may be responsible for inflated release rates in smaller size fractions (Harrison 1993). With my method, size fractionation occurs after incubation with label. Another approach by Dodds et al. (1991) depends on the effectiveness of antibiotic treatments to prevent uptake of P by pico- and nanoplankton. However, as indicated by their own results and those of others (Tremaine and

Mills 1987), antibiotic treatment is often not effective. Additional techniques for measuring P regeneration rates are available (e.g., Lehman 1980; Korstad 1983), but are limited to the measurement of zooplankton release rates.

I have presented a technique that measures dissolved phosphorus release from entire planktonic communities. Release rates can be used as an alternative or as a compliment to uptake rates for examining the dynamics of P in planktonic communities, and can be quantified without having to estimate PO_4^{-3} concentration. Although not described here, this technique may be applicable to the measurement of regeneration of other nutrients.

Table 4.1. Mean regeneration rates (n=9) of dissolved phosphorus (ng P liter⁻¹ h⁻¹) in the epilimnion of Mouse and Ranger L. The range for each mean is in brackets. Rates were measured three times each summer (1993 to 1995).

<u>Mean and range of release rates by size fraction (ng P liter⁻¹ h⁻¹)</u>				
Lake	Bacteria <0.8 μm	Protists 0.8-40 μm	Zooplankton >40 μm	Whole water
Ranger L.	7.0 (0-29)	14 (0-26)	15 (0-60)	35 (27-60)
Mouse L.	9.6 (3.7-15)	24 (11-42)	9.6 (0-40)	43 (16-84)

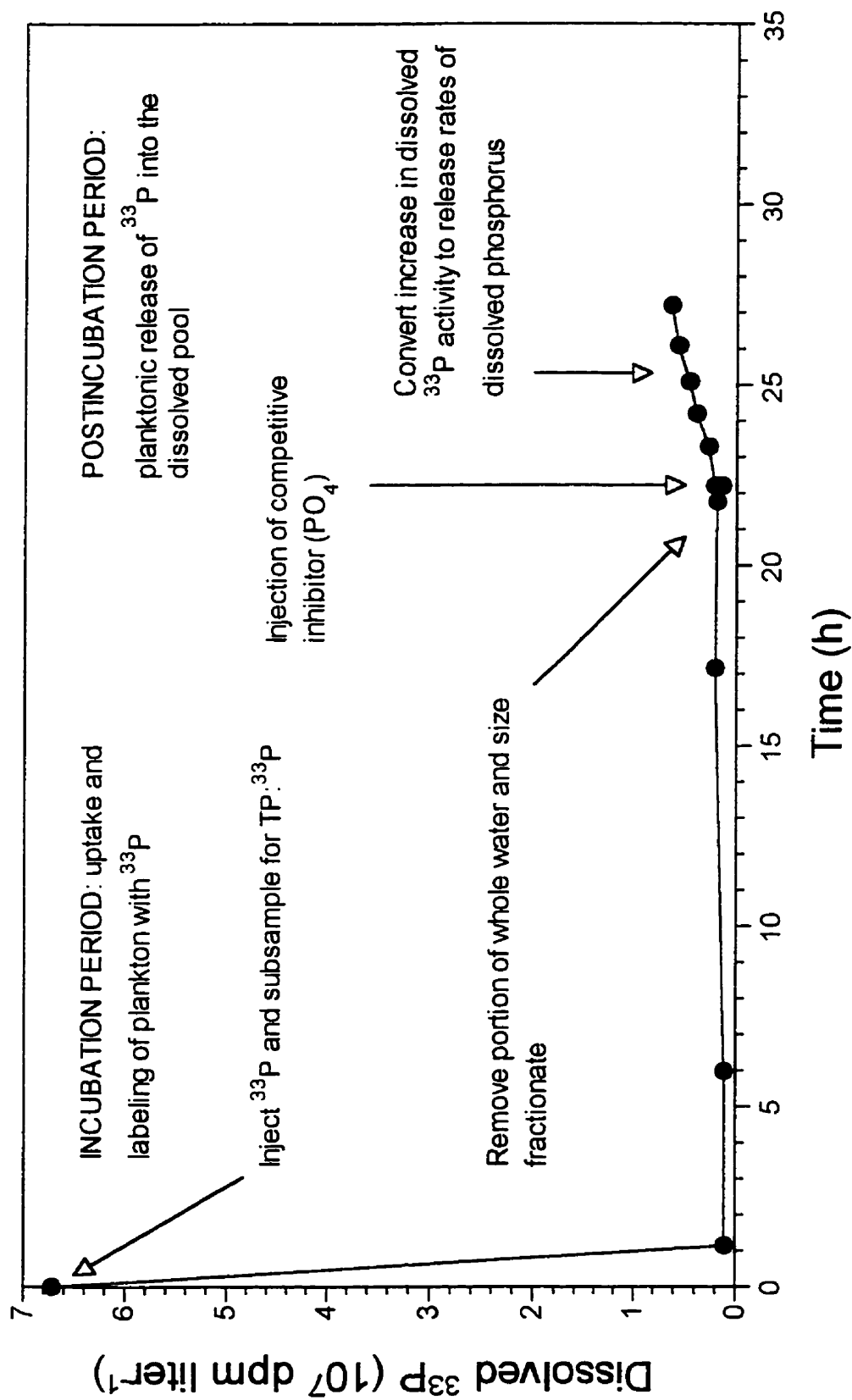


Fig. 4.1. An example of the uptake and release of ^{33}P in unfractionated epilimnetic water from Lake Wilcox (a lake not described in this study), conducted in September of 1994. Labeling occurs in the first 22 h (incubation period). Then further uptake of dissolved ^{33}P is blocked with the addition of competitive inhibitor (postincubation period). Dissolved ^{33}P activity increases as it is released over the postincubation period. This increase in ^{33}P is converted to dissolved phosphorus release.

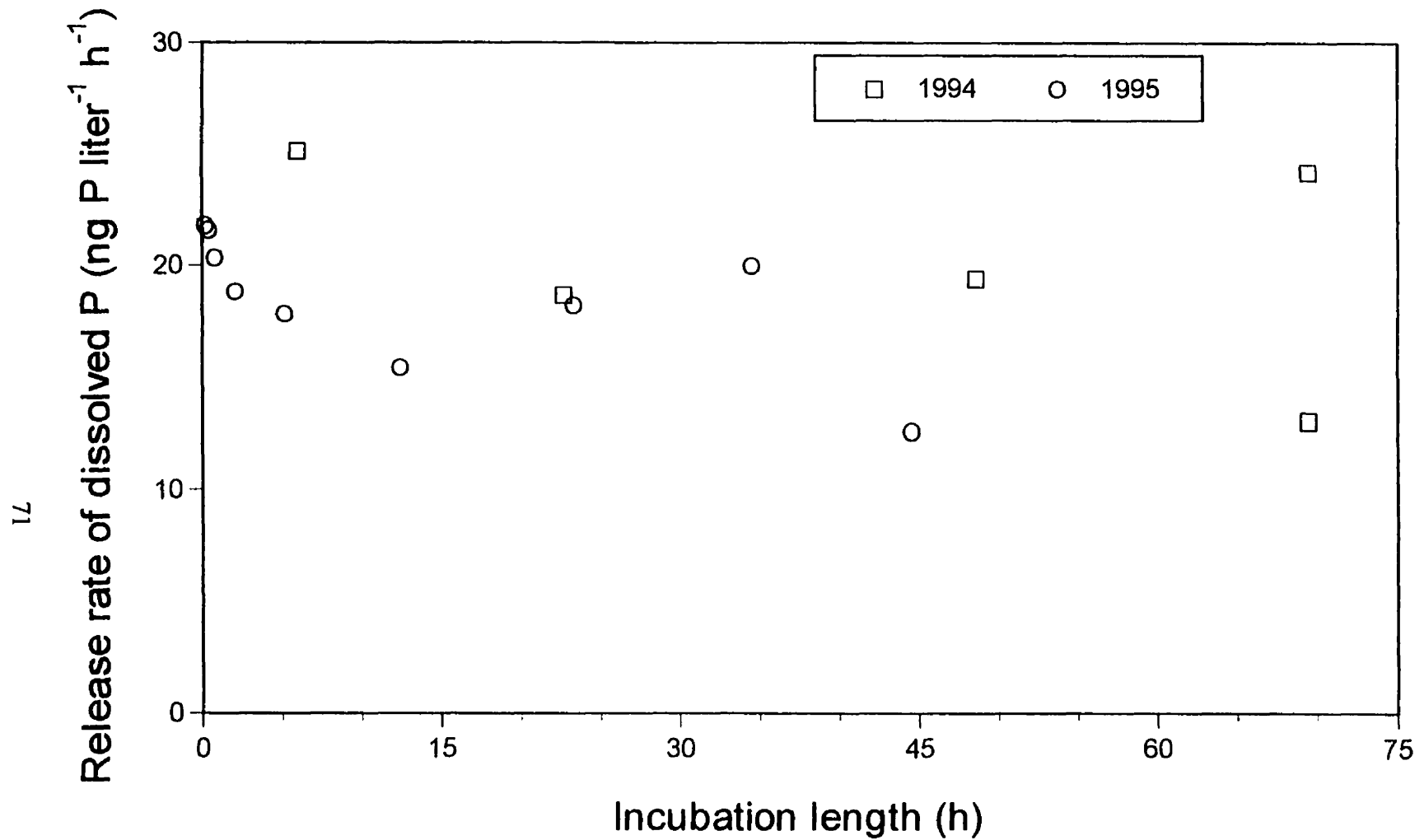


Fig. 4.2. Release rate of dissolved P as a function of incubation length (0.18 to 69.4 h). Conducted once in 1994 and once in 1995 on unfractionated epilimnetic Ranger L. water. The X axis is the time the water was incubated before being injected with competitive inhibitor. The Y axis is the rate of release of dissolved P (ng P liter⁻¹ h⁻¹). For example, with only a 3-h incubation with ³³P, a release rate of 21 ng P liter⁻¹ h⁻¹ was measured in the plankton community in 1994.

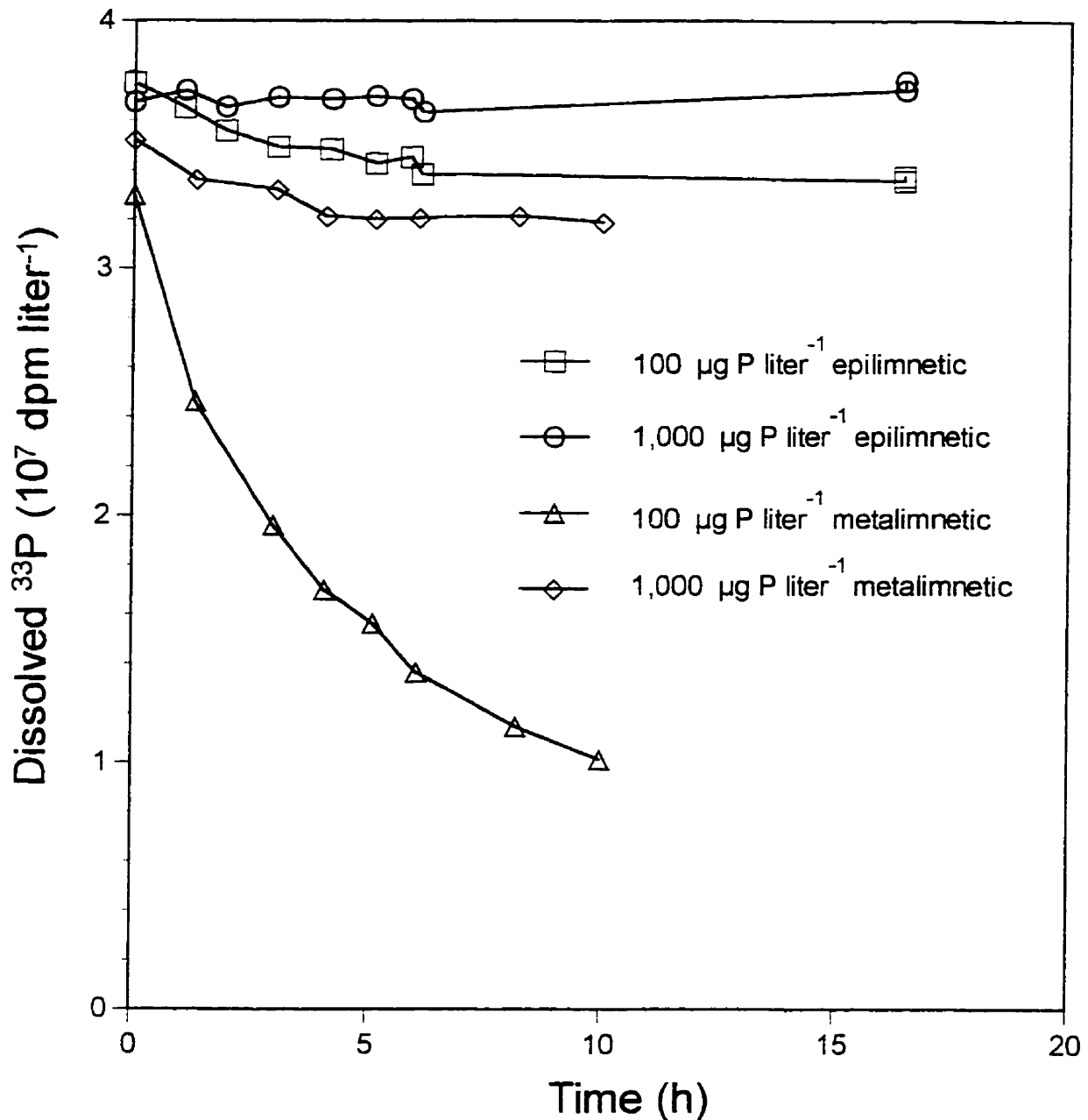
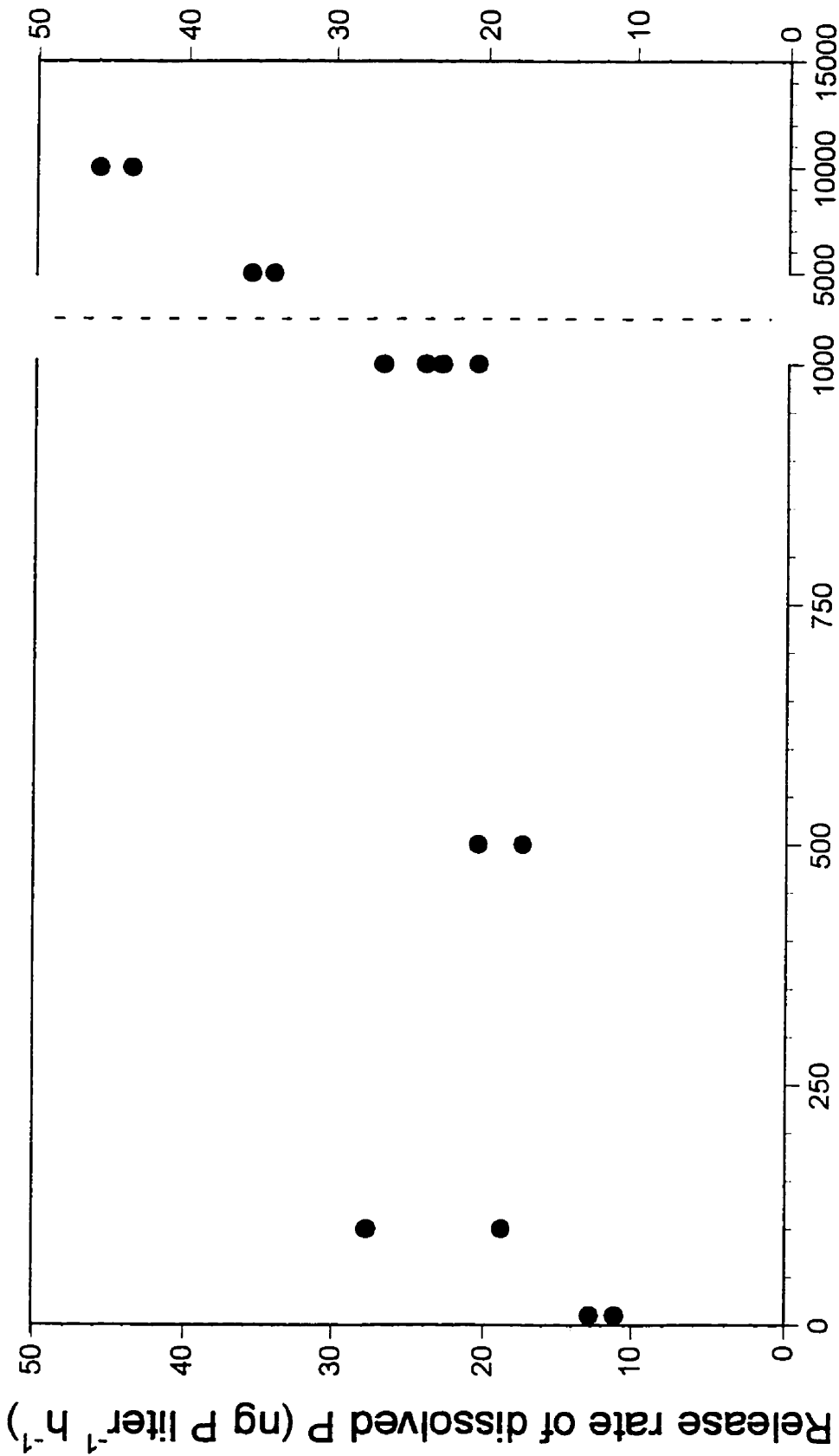


Fig. 4.3. Determination of the degree of re-incorporation of released ^{33}P over time after competitive inhibitor (PO_4) addition. Conducted with competitive inhibitor concentrations of 100 and 1,000 $\mu\text{g P liter}^{-1}$ on Ranger L. epilimnetic water and at 100 and 1,000 $\mu\text{g P liter}^{-1}$ on metalimnetic water. Little incorporation occurred in epilimnetic water. However, there was considerable incorporation of ^{33}P in metalimnetic water with a competitive inhibitor concentration of 100 $\mu\text{g P liter}^{-1}$. In this last experiment, the rate of dissolved phosphorus release would have been underestimated.



Competitive inhibitor concentration ($\mu\text{g P liter}^{-1}$)

Fig. 4.4. The effect of competitive inhibitor (PO_4) concentration on the release rate of dissolved P. Conducted once in 1995 on unfractionated epilimnetic water from Ranger L. Note the break in the X axis between 1,000 and 5,000 $\mu\text{g P liter}^{-1}$. Release rates remain consistent over a wide range of competitive inhibitor concentration (<100 to >1,000 $\mu\text{g P liter}^{-1}$).

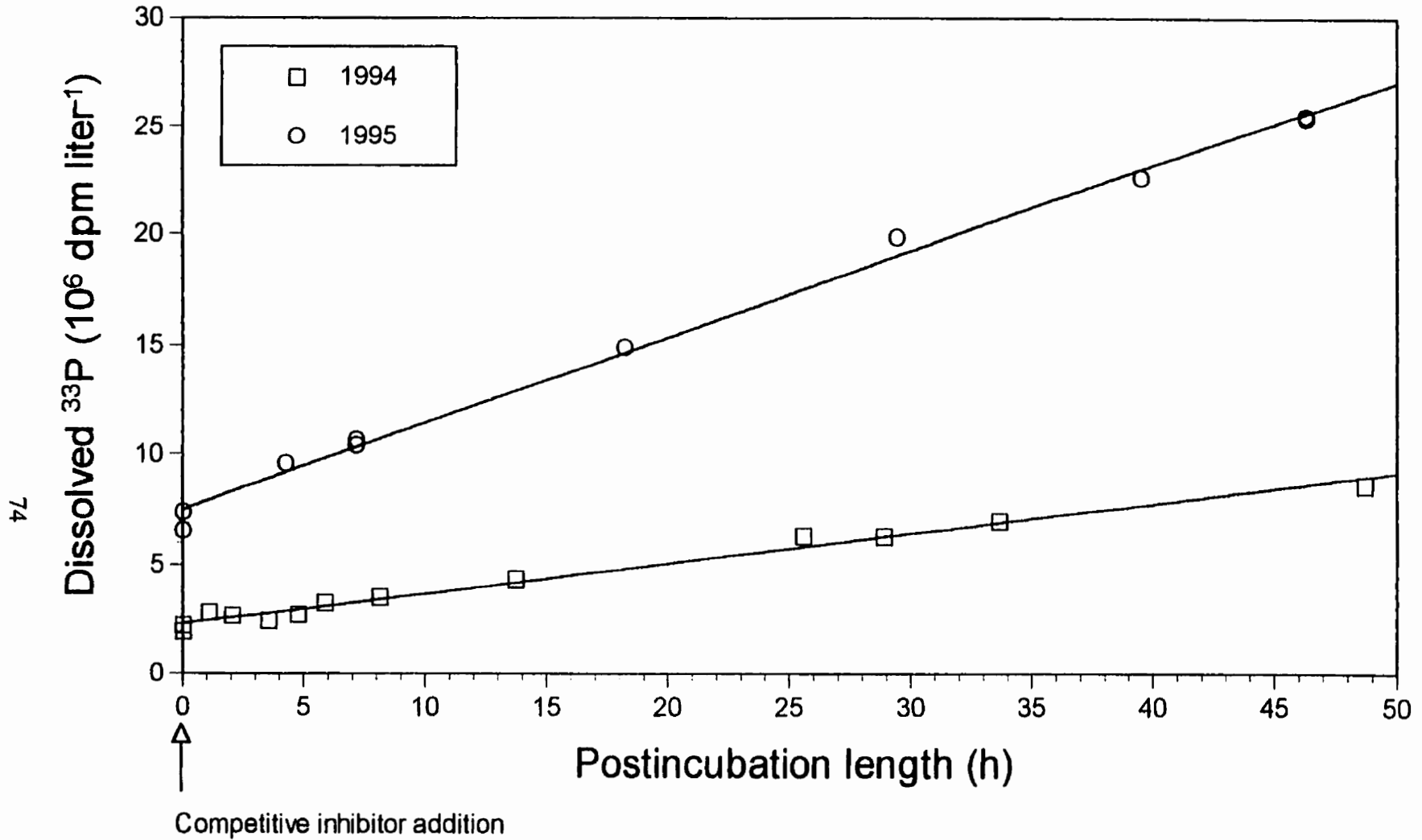


Fig. 4.5. Release of dissolved ^{33}P from Ranger L. epilimnetic water over two extended post-incubation periods (46 and 48 h). Conducted once in 1994 and again in 1995. Release is fitted to a linear model (in 1994 $R^2 = 0.98$, $n = 13$ and in 1995 $R^2 = 0.99$, $n = 10$).

Chapter 5

THE EFFECT OF PISCIVORE REMOVAL AND INTRODUCTION ON PHOSPHORUS REGENERATION

Introduction

The potential for consumers to affect phytoplankton communities indirectly via nutrient regeneration was recognized early in biomanipulation research (Kitchell et al. 1979; Bartell 1981; Shapiro and Wright 1984). Despite this early recognition, nutrient regeneration has largely been ignored in experimental (e.g., Carpenter et al. 1987; Carpenter and Kitchell 1993; Mazumder et al. 1988; McQueen et al. 1989; Persson et al. 1993) and theoretical studies that examine the effect of predation on food webs (e.g., see models by Hairston et al. 1960; Fretwell 1977; Oksanen et al. 1981; and Persson et al. 1988). The emphasis on direct effects (i.e., predation and grazing) in biomanipulation studies has been criticized (Cyr and Pace 1992; McQueen et al. 1992; Stone and Weisburd 1992; Bengtsson et al. 1996; Polis and Strong 1996; Vanni 1996). Recently, models have appeared that incorporate nutrient regeneration into simplified food chains (e.g., see Benndorf 1992; Carpenter et al. 1992; DeAngelis 1992; Stone and Weisburd 1992; He et al. 1993). Experimental data are required to confirm the accuracy of these models, but experimental studies on complete food webs have been slower to follow (Lyche et al. 1996).

Nonetheless, P regeneration by fish has been measured directly (e.g., Brabrand et al. 1990; Mather et al. 1995), or indirectly with bioenergetic models (e.g., Kraft 1993). In addition, regeneration rates of zooplankton (e.g., Peters 1975; Lehman 1980; Korstad 1983; Taylor, 1984; Moegenburg and Vanni 1991) and protists (e.g., Taylor 1984) have

also been measured. Food web components (e.g., fish or zooplankton or both) have also been manipulated in mesocosms in an attempt to separate regeneration effects from grazing effects on phytoplankton biomass (e.g., Threlkeld 1987; Brabrand et al. 1990; Vanni and Findlay 1990; Mazumder et al. 1992; Schindler 1992; Burns and Schallenberg 1996; Perez-fuentetaja et al. 1996). Other studies have attempted to measure P regeneration by entire planktonic communities in lakes (Fisher et al. 1988; Dodds et al. 1991; Taylor and Lean 1991). These studies provide insight into the importance of the various food web groups to P regeneration.

Despite the multitude of studies addressing P regeneration, there is still confusion about how biomanipulation will affect P regeneration and how P regeneration will affect food web structure. There are a variety of reasons for this confusion. For example, the contributions of different food web components are rarely put into the context of total P regeneration, or at least total planktonic regeneration, and therefore, the relevance of any component to total regeneration is difficult to ascertain. Furthermore, total P regeneration has not been measured in the context of lake biomanipulation (i.e., premanipulation and postmanipulation comparisons of rates are not available) or in a comparison of lakes with different planktivore to piscivore ratios (Lyche et al. 1996). In fact, the impact of biomanipulation on total P regeneration is largely speculative, despite the plethora of literature that has indirectly addressed it (e.g., see the review by Northcote 1988). Therefore we are still at the stage of asking rudimentary questions about how P regeneration may structure limnetic communities or how community structure determines P regeneration.

With a P regeneration technique available, the Dorset Lakes Biomanipulation Project (DLBP) provided an excellent opportunity to examine the role of regeneration in food webs undergoing biomanipulation. This inquiry may be directed by asking the following question. Is reduced P regeneration the mechanism responsible for enhanced water clarity in biomanipulated communities?

Successful biomanipulations are dependent on drastic reductions in planktivore biomass. This enables large zooplankton grazers to increase, and to reduce nanoplankton biomass (Shapiro and Wright 1984; Northcote 1988; Reynolds 1994; Benndorf 1995). However, modification of nutrient regeneration, brought about by changes in food web structure, has also been proposed to explain the observed response of phytoplankton (Bartell 1981; Northcote 1988; Sterner 1989a; Vanni and Findlay 1990; Cyr and Pace 1992; Schindler 1992; Kitchell et al. 1993; Perez-Fuentetaja et al. 1996). For example, the mean size of organisms in planktonic food webs has been observed to decline under intense planktivory (Mazumder et al. 1988; Carpenter and Kitchell 1993; Almond et al. 1996; Proulx et al. 1996). Allometric theory predicts that mass-specific physiological rates, including grazing and nutrient regeneration, decrease with increasing body size (Peters 1983; Peters and Downing 1984). Therefore, a shift to a smaller size spectrum of plankton may result in a more metabolically active food web with enhanced P regeneration (Bartell 1981; Northcote 1988; Carpenter and Kitchell 1984; Vanni and Findlay 1990; Cyr and Pace 1993) to support the observed increases in algal biomass that are characteristic of planktivore treatments. Or, under intense piscivory, a reduction in P regeneration may bring about a decline in algal biomass so that, water clarity is enhanced. Therefore, as proposed

by the trophic cascade hypothesis, did total planktonic P regeneration (whole water regeneration) decrease in Mouse L. and increase in Ranger L. in response to the fish transfer?

Analysis of the regeneration rates ($\text{ng P l}^{-1}\text{h}^{-1}$) and specific regeneration rates (regeneration rates/PP, in units day^{-1}) of planktonic size fractions may provide further insight into the mechanisms at work. For example, mesozooplankton biomass is expected to decline or shift to microplankton and nanoplankton biomass under intense planktivory. Will this result in enhanced nutrient regeneration as proposed by Carpenter and Kitchell (1993)? Under intense planktivory, will protist biomass increase and account for the majority of P regeneration?

Methods

Sampling protocol and schedules were described in chapter 4. Specific regeneration rates were calculated with PP concentrations collected 1-2 days (see chapter 2) before regeneration samples. Statistical analyses of the rates for each fraction were performed with a model I 2x2 ANOVA, where lake and year were the main effects and sampling dates within each year and lake were considered to be replicates. Rates from both post-manipulation years (1994 and 1995) were pooled into one treatment. This design was used to test for significant ($P \leq 0.05$) interactions between year and lake treatments. Certain rates were transformed (\log_{10}) to meet normality and homogeneity of variance assumptions.

No transformation was found adequate for the bacterial rates. Therefore, bacterial rates were ranked before analysis.

I also wanted to examine the relationship between zooplankton (>40 μm fraction) and protist (0.8-40 μm fraction) rates because zooplankton and algal biomass are expected to covary negatively (Shapiro and Wright 1984; Mazumder et al. 1988; Carpenter et al. 1985 and 1993). A repeated measures analysis of variance design (RMANOVA) was used because these two fractions were not independent of each other, that is, they were taken from the same water sample and calculated by subtraction from whole water rates (see methods in chapter 4). Assumptions concerning compound symmetry were not applicable to this analysis because the within subjects only consisted of two levels (i.e., zooplankton and protists). If zooplankton and protists rates did covary negatively, then a significant within subjects third order interaction term would result.

Results

Although variable, whole water regeneration rates were greatest in Ranger L. when it contained piscivores (Fig. 5.1 and 5.2). The zooplankton fraction (>40 μm) contributed the most to P regeneration during the premanipulation period (up to 100% of the whole water rate on day 255 in 1993). Bacterial (<0.8 μm) and protist fractions (0.8-40 μm) contributed the most to P regeneration during the postmanipulation period (1994-95) in Ranger L. Regeneration by bacteria was not detected in 1993.

Whole water regeneration rates in Mouse L. followed an inverse pattern over time to those in Ranger L. (Fig. 5.1 and 5.2). Mean whole water or total planktonic regeneration was before the introduction of piscivores (compare 1993 with 1994-95). High rates of whole water regeneration are found in early summer in Mouse L. (Fig. 5.1). Zooplankton contributed more to P regeneration after piscivores were introduced (1994-95). Protist fractions do not follow a particular pattern over the three years. Bacterial rates were slightly lower before piscivores were added. Unlike Ranger L., the sum of bacterial and protist rates (microbial group) exceeded zooplankton rates before and after fish manipulations.

Specific regeneration rates (i.e., regeneration rate/PP concentration) of TPP are similar between years in Ranger L., regardless of treatment (Fig 5.3 and 5.4). However, among the size fractions there are considerable differences between treatments. The zooplankton fraction has greater specific regeneration rates before piscivore removal (1993), while the protist and bacterial fractions have greater specific regeneration rates after piscivore removal (1994-95).

Unlike Ranger L., specific regeneration rates of TPP in Mouse L. are substantially different after treatment (Fig 5.4); rates are greater after piscivore addition (1994-95). Furthermore, an inverse pattern is visible between the lakes over time with regard to the zooplankton fractions, which have high rates with piscivores (1994-95) and reduced rates with planktivores (1993). In Mouse L. protist rates seem unaffected by treatment, while bacterial rates increase in the piscivore treatment, with considerable variability in 1994. This variability stems from the specific rate on day 215. Here, bacterial regeneration was measurable (see Fig. 5.1), but the quantity of PP in this fraction was not detectable (Fig. 2.3

in PP section); therefore, the calculation of a specific regeneration rate was not possible. The spike in whole water regeneration rates at the beginning of each season in Mouse L. (Fig 5.1) is not present in the specific regeneration of TPP. Instead, the zooplankton or protist fractions, or both, start each season with a high specific regeneration rate (Fig 5.3).

Comparison of rates for each individual fraction between lakes and between years (Table 5.1) revealed some significant differences ($P < 0.05$). Patterns were visible between lakes over time (Fig. 5.2 and 5.4), but they were not significant (see lake by year interaction terms). However, certain interaction terms (for whole water and zooplankton rates) were near significant ($0.06 < P < 0.18$). Comparisons between zooplankton ($>40 \mu\text{m}$ fraction) and protists ($0.8\text{--}40 \mu\text{m}$ fraction) rates between lakes and years were significant ($P \leq 0.04$, see the third order interaction term in Table 5.2).

Discussion

A reduction in P regeneration under piscivory and enhanced P regeneration under planktivory are processes believed to affect water clarity (Bartell 1981; Carpenter et al. 1984; Vanni and Findlay, 1990; Perez-Fuentetaja 1996). However, whole water (total planktonic) regeneration rates in Mouse and Ranger Lakes (Fig 5.2) do not support this interpretation, in fact, the rates follow an opposite pattern. Under planktivory, P regeneration was reduced, and, under piscivory, P regeneration was enhanced. Specific regeneration rates of total PP do not support this interpretation either, especially in Mouse L. Phosphorus was cycled faster through the particulate phase after piscivore introduction

(1994-95) into Mouse L. (Fig. 5.4). Furthermore, a comparison of mean rates between lakes in each year (e.g., compare Ranger 1993 with Mouse 1993) illustrates that whole water regeneration and TPP specific regeneration rates were always greater when piscivores were abundant (Fig. 5.2 and 5.4). However, the interaction terms that depict these patterns were not significant (Table 5.1, $P=0.18$ and $P=0.074$). More measurements are required before these observed patterns may be accepted with confidence.

However, there is additional evidence to support these observations. Phosphate estimates (by Rigler radiobioassay) were greater when piscivorous fish were abundant (chapter 3). For example, whichever lake contained the greatest biomass of piscivores, also had the greatest concentration of PO_4^{-3} in that year. Similar results have been seen elsewhere. Fishless mesocosms (I consider comparable to piscivore treatments) have elevated concentrations of TDP (Lazarro et al. 1992; Mazumder 1994b) or soluble reactive phosphate (Lynch and Shapiro 1981; Christoffersen et al. 1993) compared to mesocosms with planktivorous fish. If phosphorus regeneration was limiting algal biomass and production, then one would expect a small pool of PO_4^{-3} or TDP in the piscivore treatment not a larger pool. Mazumder et al. (1992) also reported that phosphate turnover times were much shorter in planktivore treatments. Again, if P regeneration was expected to limit algal biomass and production in fishless mesocosms, then P turnover times should be shorter in fishless treatments not planktivore treatments. In addition, Mazumder et al. (1992) found that large algal cells were contributing to a substantial proportion of phosphate uptake in the fishless mesocosms. If algae were nutrient limited in fishless treatments, the majority of

label should be taken up in the picoplankton, not the microphytoplankton (Currie et al. 1986).

Further evidence to support my results is available if we revisit the allometric scaling literature. The observation that the mean planktonic size spectrum shifts to a smaller size distribution under planktivory has been well documented (e.g., Mazumder et al. 1988; Carpenter and Kitchell 1993; Almond et al. 1996; Proulx et al. 1996); however, whether this shift in size structure enhances nutrient regeneration (Bartell 1981; Carpenter and Kitchell 1984 and 1993; Northcote 1988; Vanni and Findlay 1990) remains to be documented. Much of the literature does not support this interpretation. For example, zooplankton communities with larger mesozooplankton have been found to have higher community grazing rates than those communities dominated by microzooplankton (Knoechel and Holtby 1986; Vanni 1987; Mazumder et al. 1990; Vanni and Temte 1990; Cyr and Pace 1993). In a survey of zooplankton communities in 28 North American lakes, a positive relationship was found between biomass and mean zooplankton body size (e.g., Cyr and Pace 1993). Therefore, zooplankton communities dominated by large zooplankton may be characterized by high biomass and grazing rates. This alone should equate to enhanced regeneration of nutrients in piscivore treatments, if community grazing rates covary with nutrient regeneration. Furthermore, in an examination of regeneration rates for zooplankton in L. Ontario, Taylor (1984) did not find evidence to support the hypothesis that microzooplankton communities regenerate P at a greater rate than mesozooplankton communities. Therefore, there is support for the whole water and specific regeneration rates observed here (Tables 5.2 and 5.4).

I have not considered the regeneration of nutrients by fish. If I had included fish regeneration in the analysis, would whole water P regeneration follow the same pattern? Certain studies have concluded that fish regeneration of P is significant and may influence phytoplankton biomass (e.g., Brabrand et al. 1990; Vanni and Findlay 1990; Schindler 1992; Kraft 1993; Mather et al. 1995; Perez-fuentetaja et al. 1996; Vanni 1996). Other studies (e.g., Kitchell et al. 1975; Nakashima and Leggett 1980; den Oude and Gulati 1988; Boers et al. 1991; Mazumder et al. 1992; Proulx et al. 1996) have suggested that regeneration of P by fish was not significant. Two of the most recent studies that suggest that fish regeneration is significant are examined below in light of whole water regeneration rates in Mouse and Ranger Lakes. In this comparison, the importance of fish regeneration in Mouse and Ranger Lakes may be illustrated.

In Wolf L. (TP=6-10 $\mu\text{g l}^{-1}$), small (mean mass 1.87 g) redbreast sunfish (*Lepomis auritus*) were added at a final biomass of 30 kg ha⁻¹ to a mesocosm experiment (Perez-Fuentataja 1996). Excretion rates for the fish in the mesocosms were estimated with a bioenergetics model and were equal to $\sim 100 \text{ ng P l}^{-1}\text{d}^{-1}$, which is equivalent to 11% of whole water regeneration in Mouse and Ranger L. These rates may also be an overestimate for Mouse and Ranger Lakes. The biomass of fish per hectare in these mesocosms is approximately equivalent to the total fish biomass in either Mouse or Ranger Lakes (Ramcharan et al. 1995; Demers 1996). However, not all the fish in Mouse and Ranger are small planktivorous fish (i.e., under 2 g). Allometric scaling indicates that these young fish would excrete more nutrients than the natural fish assemblages found in Mouse and Ranger Lakes.

Another study by Vanni (1996) examined the P regeneration of gizzard shad (*Dorosoma cepedianum*). Gizzard shad, which are facultative detritivores, dominate the fish assemblage in eutrophic Acton L. They are presumed to act as a nutrient pump supplying phytoplankton with N and P. Vanni estimates the lake population at 100 kg ha^{-1} and calculates P excretion (bioenergetics approach) by gizzard shad to be between 47 and 200 $\text{ng P l}^{-1}\text{d}^{-1}$. If I reduce the biomass of gizzard shad in Acton lake to total fish biomass in Mouse and Ranger Lakes ($\sim 30 \text{ kg ha}^{-1}$) then we would expect regeneration rates of ~ 14 to $60 \text{ ng P l}^{-1}\text{d}^{-1}$. This only represents 2 and 6% of whole water regeneration in Mouse and Ranger Lakes, respectively. Furthermore, fish rates reported by Vanni and Findlay (1990) are considered to be overestimates by Mazumder et al. (1992). Brabrand et al. (1990) acknowledged that their fish regeneration rates may not represent more than a tenth of zooplankton regeneration rates and even less of microbial rates. Finally, Mouse and Ranger Lakes both contained fish assemblages (piscivore-planktivore, or just planktivore), so fish make some contribution to regeneration in both systems. Therefore, fish contribute, but their contribution would not alter the observed patterns for whole water regeneration in Mouse and Ranger Lakes.

Regeneration of P by fish may be significant for other reasons. Through feeding activities fish may be important for transferring new P to the pelagic zone from benthic and littoral habitats (Vanni 1996 and references within). This process may slow the epilimnetic decline of TP. However, fish may also move P out of the pelagic zone (Vanni 1996).

Although P regeneration is essential in maintaining food webs in lakes, the results do not support P regeneration as a primary mechanism structuring biomanipulated food webs.

Therefore, do the results support grazing as a primary mechanism structuring biomanipulated food webs? This question would be more appropriately answered by examining the effect of grazing on phytoplankton biomass and productivity. However, to my knowledge, total planktonic grazing rates, which include protists, have not been measured. We may turn to the size fractionated regeneration and specific regeneration rates to provide some insight. If elevated herbivory by zooplankton is the mechanism creating enhanced water clarity (references cited above), then we would expect higher regeneration rates and specific regeneration rates by the zooplankton fraction under piscivory. In turn, we may expect the protist group to demonstrate decreased rates under piscivory, since the biomass (PP) in this group may be grazed down, and shunted into zooplankton biomass and regeneration. The bacterial fraction ($<0.8 \mu\text{m}$) may or may not be affected by the fish transfer. Pace and Cole (1994) found that bacterial biomass and productivity was limited primarily by resources (bottom up control) and not by predators, albeit with exceptions, particularly when large *Daphnia* are abundant (Jeppesen et al. 1996).

The results in Figures 5.2 and 5.4, and the interaction terms from Table 5.1 ($P < 0.08$), indicate that the zooplankton regeneration rates were elevated under piscivory and reduced under planktivory. A reverse trend to this would be expected in the protist fraction. The reverse trend is detectable in Ranger Lake protists; rates are reduced under piscivory (1993) and enhanced under planktivory, especially in 1995 (Figs. 5.2 and 5.4). However, in Mouse Lake only a weak trend is detectable in regeneration (Fig. 5.2) and specific regeneration rates (Fig. 5.4). With the weak response from Mouse L., and the high

variability overall in the protist rates, the interaction term for the protists is not significant ($P \geq 0.23$).

However, examining each fraction independently may miss the broader pattern between fractions (zooplankton and protists). Significant interactions between zooplankton and protists rates were detected ($P \leq 0.04$, third order interaction terms, Table 5.2) and support the prediction that inverse patterns in rates are present between these two fractions. This is one of the few instances, where rate measurements between zooplankton ($>40 \mu\text{m}$ fraction) and protists (0.8–40 fraction) have been measured, and have demonstrated a treatment effect. Rate measurements of phosphate uptake and grazing have also been effective for detecting treatment effects (Mazumder et al. 1992).

The treatment effect on rate measurements may need to be reconciled with the non-significant outcome found with the PP fractions (Table 2.4). Patterns in static measurements of communities (e.g., biomass and abundance) are not always reflected in the patterns of dynamic measurements (e.g., regeneration, production, and turnover). For example, in a biomanipulation study in Lac Croche, mesocosms containing planktivorous fish had elevated rates of primary production without a detectable increase in algal biomass (Perin et al. 1996). Other researchers (Elser et al. 1987; Sterner 1989a) also indicate that the positive (nutrient regeneration) and negative (grazing) impact of zooplankton on algae is difficult to detect if biomass is the only parameter measured. Therefore, a lack of treatment effect on the observed PP fractions in Mouse L. would not necessarily predict no effect on rate measurements.

The high recruitment of perch in 1994 could be considered a factor that dampened the treatment effect in Mouse L.

The significance of protist and bacterial P regeneration has been overlooked in the freshwater biomanipulation literature. Occasional reference is made to the regeneration of these groups (e.g., Brabrand et al. 1990). However, much of the discussion about regeneration in biomanipulation studies concerns the impact of metazoans (e.g., Bergquist and Carpenter 1986; Sterner 1989a; Brabrand et al. 1990; Carney 1990; Elser et al. 1990; Vanni and Findlay 1990; Moegenberg and Vanni 1991; Carpenter et al. 1992 and 1993; He et al. 1993; Schindler et al. 1993; Perez-fuentetaja et al. 1996). This is a surprising development, in light of the early marine literature that highlighted the importance of microorganisms in nutrient regeneration (e.g., Pomeroy 1974; Azam et al. 1983; Harrison 1983). Here, I have demonstrated that microbial regeneration is significant and usually exceeds metazoan regeneration, especially when the lakes are dominated by planktivorous fish. Conclusions drawn from the above studies may require re-evaluation.

In conclusion, the pattern of whole water P regeneration changed with the removal and introduction of bass. However, these changes do not support the assertion that P regeneration is a primary mechanism enhancing water clarity. In fact, the results point to direct effects, i.e., grazing. This study is the first of its kind, in the sense that regeneration rates have been measured directly over the course of a biomanipulation. There is considerable variability in the rates, and treatment effects were often weak. In order to reduce the variability in estimating mean rates, I recommend that future studies should concentrate on more frequent measurements of regeneration during each season and

conduct the investigation over a longer time frame (e.g., 3 years before and 3 years after manipulation). In addition, the size fractions under consideration may require modification. For example, narrowing the size of fractions to focus on large zooplankton ($>200\ \mu\text{m}$), microplankton ($20\text{-}200\ \mu\text{m}$) and nanoplankton ($2\text{-}20\ \mu\text{m}$) may help isolate the effects on P regeneration.

Table 5.1. Comparison of regeneration rate and specific regeneration rate for each fraction (whole water, zooplankton, protists or bacteria). *P* values from a model I 2x2 ANOVA, where lake and year were the main effects and sampling dates within each year and lake were considered replicates. The interaction term (Year*Lake) is of particular interest. Rates from both post-manipulation years (1994-95) were pooled.

Variable	Planktonic Size Fraction	Year <i>P</i>	Lake <i>P</i>	Year*Lake <i>P</i>
Phosphorus Regeneration Rates				
	Whole Water	0.65	0.79	0.18
	Zooplankton (>40 μm)	0.62	0.12	0.08
	Protists (0.8-40 μm)	0.58	0.05	0.55
	Bacteria (<0.8 μm)	0.02	0.09	0.50
Phosphorus Specific Regeneration Rates				
	Total PP	0.03	0.52	0.07
	Zooplankton PP(>40 μm)	0.93	0.18	0.06
	Protist PP (0.8-40 μm)	0.31	0.09	0.23
	Bacterial PP (<0.8 μm)	0.01	0.06	0.66

Table 5.2. Comparison of regeneration rate and specific regeneration rate between zooplankton and protists. *P* values are presented from a repeated measures analysis of variance (RMANOVA), where lake (Mouse and Ranger Lakes) and year are the between subjects (not shown) and fractions (zooplankton and protists) are the within subjects. Sampling dates within each year are considered replicates. The third order interaction term (Fraction*Year*Lake) is of particular interest in this analysis. Rates from both postmanipulation years (1994-95) were pooled.

Variable	Within Subjects	<i>P</i>
Phosphorus Regeneration Rates		
	Fraction	0.03
	Fraction*Year	0.29
	Fraction *Lake	0.02
	Fraction*Year*Lake	0.04
Phosphorus Specific Regeneration Rates		
	Fraction	0.05
	Fraction*Year	0.56
	Fraction*Lake	0.04
	Fraction*Year*Lake	0.03

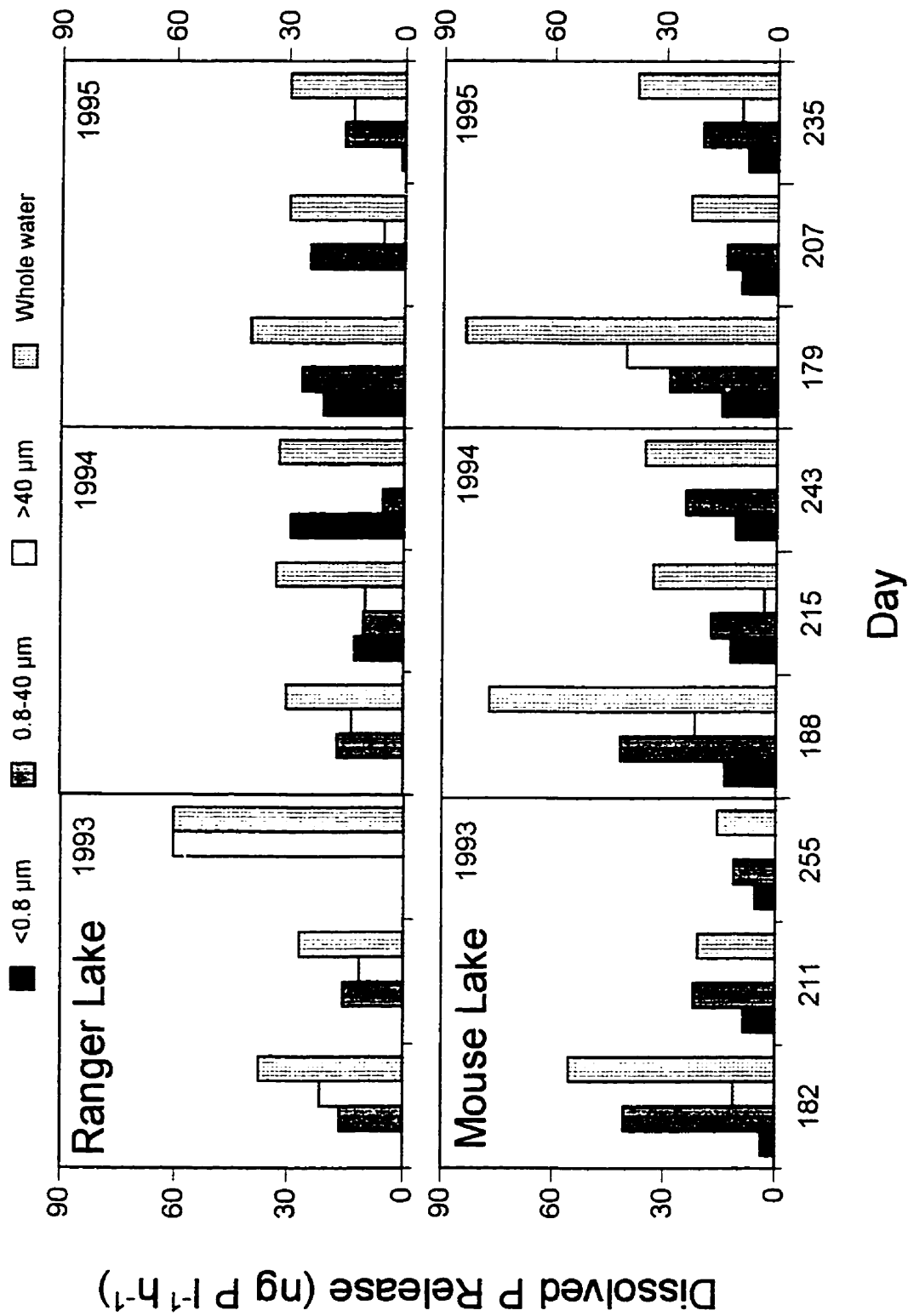


Figure 5.1. Release of dissolved P ($\text{ng l}^{-1}\text{h}^{-1}$) by each size fraction on each sampling date (julian date) for each year in the epiimnion of each lake. Note, certain fractions did not regenerate detectable levels of P.

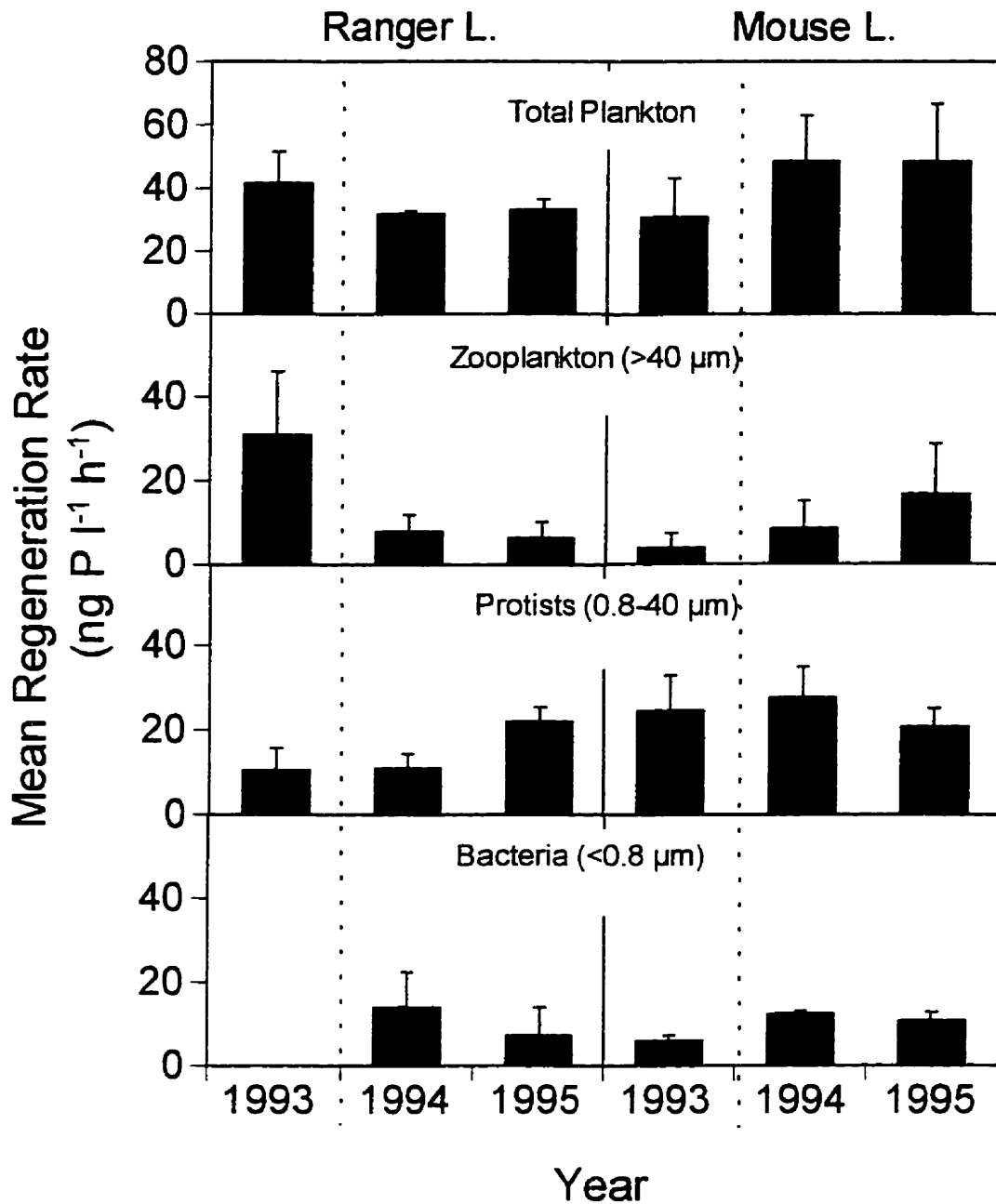


Figure 5.2. Mean regeneration rates of dissolved P ($\text{ng P l}^{-1} \text{h}^{-1}$) for total planktonic regeneration and for each size fraction in the epilimnion of each lake for each season (1993-1995). Error bars represent one standard error of the mean. The fish transfer is depicted by the dotted line in each lake. Bacterial regeneration was below detection in Mouse Lake in 1993.

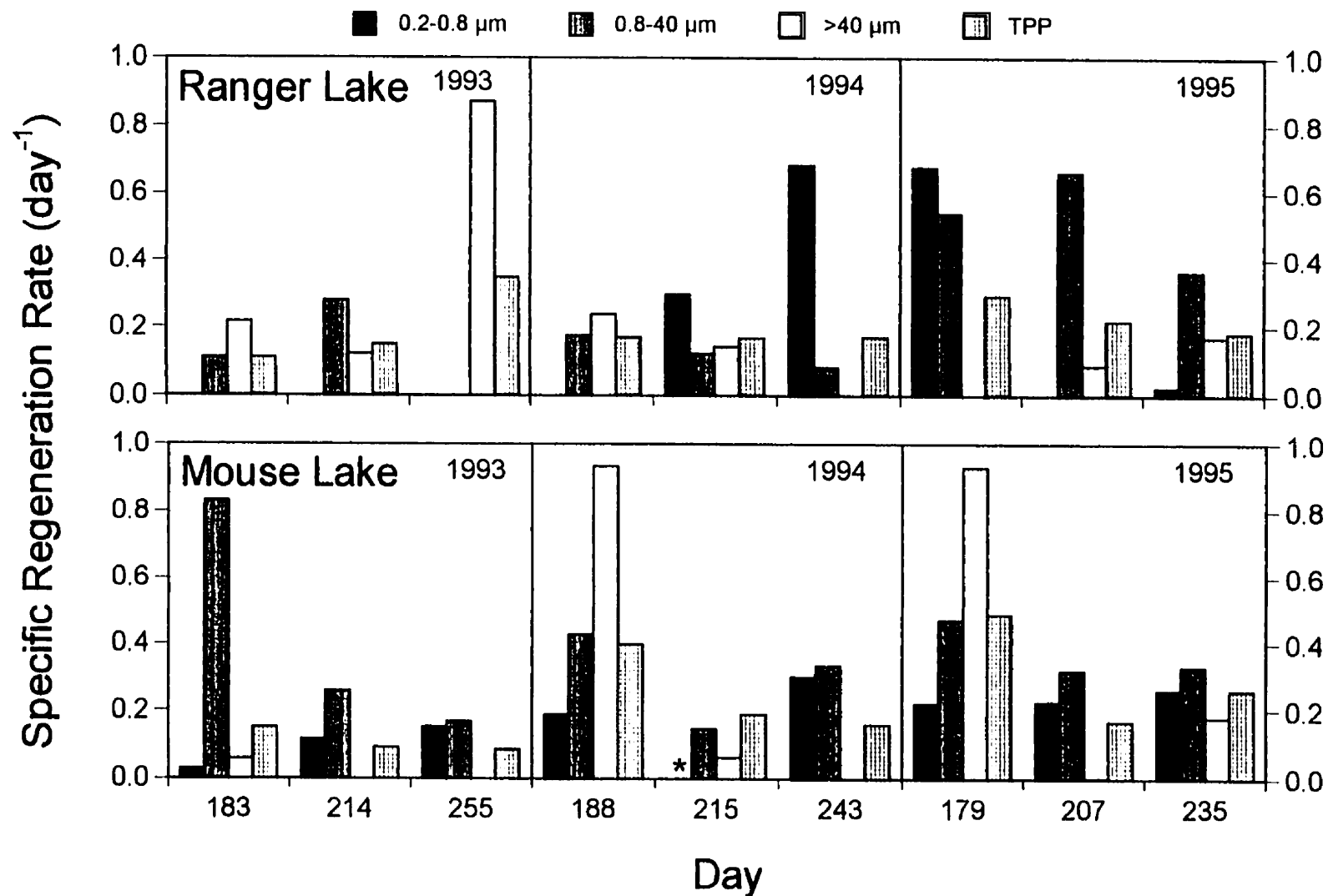


Figure 5.3. Specific regeneration rates (release rate/particulate P) for TPP and size fractions of PP for each sampling date (julian date) of each year in the epilimnion of each lake. The symbol (*) for day 215 (1994) in Mouse L. is a 0.2-0.8 μm fraction with undetectable PP. Specific regeneration rate of this fraction could not be calculated.

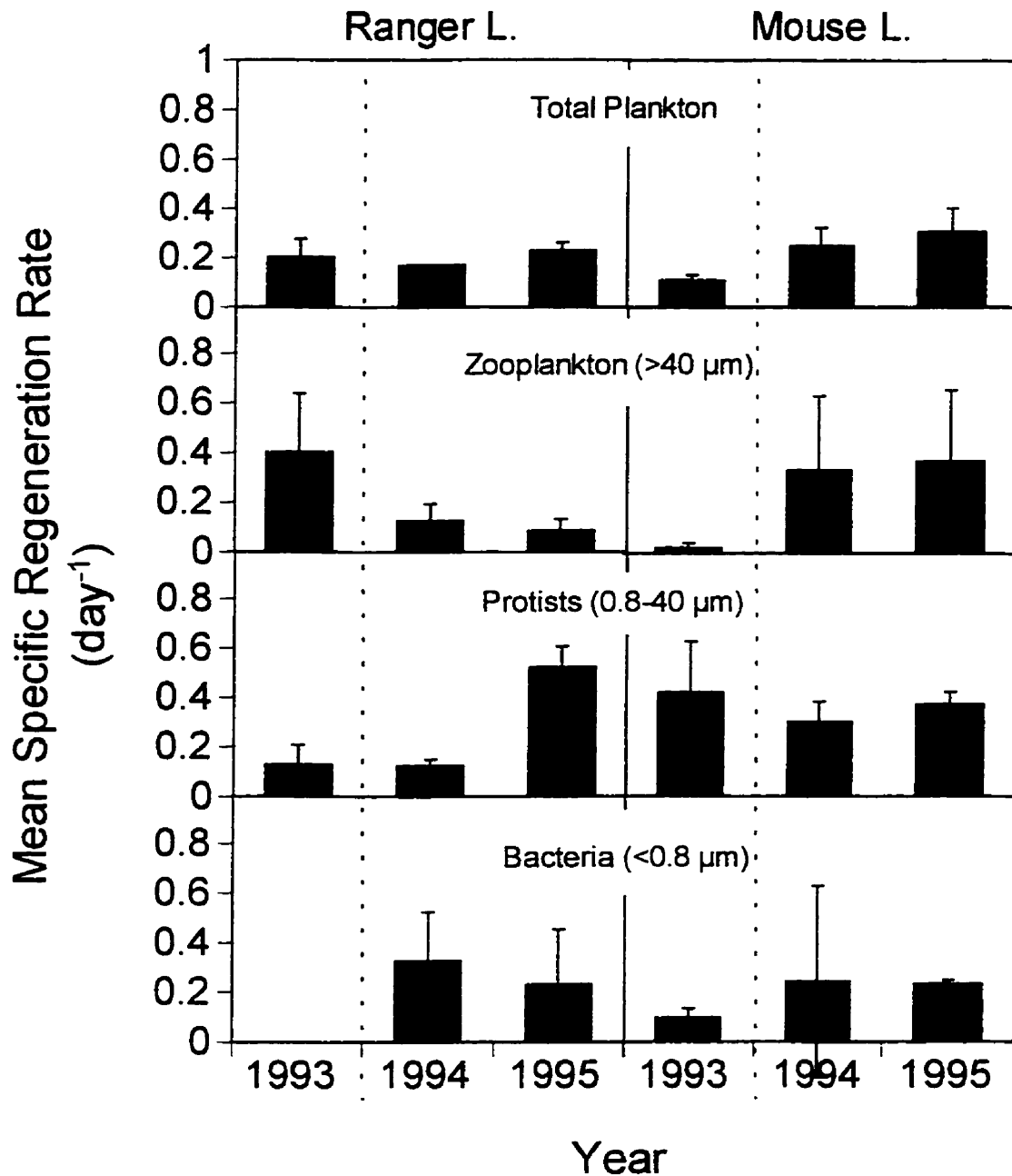


Figure 5.4. Mean specific regeneration rates of dissolved P (regeneration/PP) for total planktonic regeneration and for each size fraction in the epilimnion of each lake for each season (1993-1995). Error bars represent one standard error of the mean. The fish transfer is depicted by the dotted line in each lake. Bacterial regeneration was below detection in Mouse Lake in 1993.

Chapter 6

GENERAL SUMMARY

The relationship of phosphorus distribution and cycling to food web structure in Mouse and Ranger Lakes was subtle. Mean TP concentrations showed a very weak pattern of reduction under piscivory and enhancement under planktivory in Mouse Lake; a pattern which was more pronounced in other studies (e.g., Shapiro and Wright 1984). The rate of decline of TP during stratification followed the predicted pattern (e.g., see Guy et al. 1994; Mazumder and Lean 1994), except after bass introduction into Mouse L. The observed pattern may have been complicated by the timing and degree of spring turnover in each lake. Particulate P size fractions in Ranger L. demonstrated a slight treatment effect, but those in Mouse L. did not. The overall P size-distribution, as summarized by the slope parameters for PP did not follow the pattern documented by Mazumder et al. (1988). Again, the predicted response, particularly in Ranger L. was complicated by seasonal effects; trophic interactions in P-limited lakes appear to be strongest during summer stratification (Carpenter et al. 1991; Proulx et al. 1993). Episodic top-down effects on PP were evident, especially in June of 1994.

Turnover times in Mouse lake followed the pattern previously described by Mazumder et al. 1992. Turnover times were short under planktivory and longer under piscivory. In Ranger L. turnover times were longer under piscivory as predicted, but did not become shorter under planktivory. Phosphate concentrations (estimated by the Rigler radiobioassay) are predicted to be lower when planktivores are abundant. Phosphate

concentrations in Mouse L. conformed to this prediction, but phosphate concentrations in Ranger L. did not follow the predicted pattern after piscivore reduction. Uptake velocities were expected to be greatest in planktivore treatments, where algal biomass is expected to be greater and P-limitation more severe (Mazumder et al. 1988). However, uptake velocities were greatest when piscivores were abundant, particularly in Mouse Lake. This pattern was supported by P regeneration rates which were also greatest when piscivores were abundant.

A lack of suitable methods to directly measure total planktonic P regeneration prompted me to develop a technique (Chapter 4). The approach avoids the problems of measuring phosphate concentration (Fisher and Lean 1992) in order to estimate P regeneration. Short incubations (24 h or less) appear to provide maximal estimates of released P. Radiophosphorus bound to the particulate fraction does not appear to become dislodged (chased) when high concentrations of unlabelled phosphate (competitive inhibitor) are used to terminate incubations. The regeneration of P appears to be linear over time. Turnover time of PP (PP/regeneration) was similar to that estimated for another precambrian shield lake (Taylor and Lean 1991; Fisher and Lean 1992). Size fractionated regeneration does not appear to create inflated regeneration rates in the microbial fractions, as seen with isotope dilution approaches (Glibert et al. 1992; Dodds 1995). Regeneration by the <40 μm size fraction accounted for an average of 77% of the total.

Regeneration rates and specific regeneration rates (whole water and size fractionated) responded in certain instances to treatment; total planktonic P regeneration was greatest when piscivores were abundant. The zooplankton fraction and protist fraction

were often inversely related (i.e., zooplankton regenerated more P when piscivores were abundant, and protists regenerated more P when planktivores were abundant). The estimated phosphate concentrations and uptake velocities (chapter 3) add support to these observations. Similar results have been found elsewhere. In mesocosms, in eutrophic Lake St. George, Mazumder et al. (1992) measured P sedimentation, P distribution (TP, TDP, PP), zooplankton grazing and fish growth. They modeled the flux of P in their mesocosms and estimated that the P regenerated by fish and zooplankton in planktivore treatments was much less than the P regenerated by zooplankton in their fishless treatments.

Although this is an interesting development, the results from Mouse and Ranger Lakes should be interpreted cautiously. First, P parameters measured here were not always affected by treatment, or when an effect was present, it was often weak. This outcome may reflect the poor recruitment of planktivorous fish in Ranger L. after the majority of piscivores were removed, and the high abundance and biomass of planktivorous fish in Mouse L. after piscivores were introduced. I discuss this in some detail in chapters 2 and 5. Second, the effect of the fish transfer in Mouse and Ranger Lakes is still under evaluation. The overall evaluation may have a bearing on how the P results are further interpreted. For example, data from two reference lakes should be examined soon to determine which effects in Mouse and Ranger L. may be attributed to treatment effects and which may be attributed to external factors like climatic events. For example, patterns in the rate of TP decline (chapter 2) and turnover time (chapter 3) both illustrate potential climatic effects; that is, similar patterns in each lake over time, regardless of treatment.

Insight gained from this study may be useful for future studies. For example, food web effects were weak during spring and fall (also see Carpenter et al. 1991 and Proulx et al. 1993) and future studies may concentrate on summer sampling when stratification is well established. Increased replication on each sampling date would have improved precision in TP, PP and DP measurements. Regeneration rates were highly variable despite their apparent sensitivity to treatment. A schedule that included more replication on each sampling date and more frequent sampling over the summer would increase precision. Size fractions that isolate mesozooplankton, microplankton and nanoplankton regeneration may be more effective at capturing the effects. A concomitant measure of grazing rates, while measuring regeneration rates, would help isolate mechanisms. Since the effects of fish transfers are often not realized immediately, an extended sampling program may be more informative (e.g., 3 years before and 3 years after biomanipulation). Finally, the need to continue biomanipulation studies in oligotrophic lakes may be re-evaluated in light of results by others (Carpenter et al. 1991; DeMelo et al. 1992; Sarnelle 1992; Mazumder 1994a).

There are a number of points from this research that are worth highlighting. A technique is now available to measure total planktonic P regeneration, without having to measure phosphate. P regeneration estimates appear reasonable (compare with Taylor and Lean 1991; Fisher and Lean 1992). Interestingly, uptake constants and regeneration rates may all be estimated within the same regeneration experiment. If regeneration rates and uptake constants are entered into the two compartment model of Rigler (cited in Lean and Fisher 1992), we can calculate phosphate concentrations without having to use a Rigler radiobioassay. Preliminary calculations from chapter 3 suggests that the Rigler

radiobioassay overestimated phosphate concentrations in Mouse and Ranger L. by two orders of magnitude. Indeed, if phosphate is this low (e.g., 5 ng P l⁻¹), the technique may be adopted for measuring phosphate in addition to regeneration.

Contrary to recent reports (e.g., Rothhaupt 1992; Vadstein et al. 1993), bacteria were found to regenerate significant amounts of P. Nutrient regeneration by microorganisms was considered substantial decades ago, however, biomanipulation studies have ignored this group and have emphasized metazoan regeneration. What would our understanding be today, if P regeneration had been measured in a biomanipulation study 15 years ago? Isotope dilution techniques were available as early as 1979 to measure P regeneration (Blackburn 1979; Caperon et al. 1979).

This study addresses the debate about indirect effects (e.g., nutrient regeneration) and direct effects (e.g., grazing) in structuring food webs (see articles in Polis and Winemiller 1996). The trophic cascade hypothesis (Carpenter and Kitchell 1985) implies that enhanced water clarity is a function of intense grazing and reduced nutrient availability in piscivore treatments. McQueen (1990 p 618), also proposes that piscivore treatments are characterized by reduced nutrient regeneration. "During a biomanipulation experiment, planktivore abundance is decreased in order to increase zooplankton. This also reduces sediment bioturbation and nutrient recycling." However, Shapiro and Wright (1994) have emphasized that grazing was the mechanism that increased water clarity in their piscivore treatments. Regeneration rates measured directly in Mouse and Ranger L., and indirectly in mesocosms in Lake St. George (Mazumder et al. 1992), indicate that P regeneration is enhanced in piscivore or fishless treatments, not planktivore treatments. Therefore, the

results from Mouse and Ranger L. do not support the proposal that regeneration is a mechanism enhancing water clarity in piscivore treatments. In fact, the results point to grazing as the mechanism enhancing water clarity and, therefore, appear consistent with the biomanipulation model of Shapiro and coworkers.

REFERENCES

- Almond, J. R., E. Bentzen, and W. D. Taylor. 1996. Size structure and species composition of plankton communities in deep Ontario lakes with and without *Mysis relicta* and planktivorous fish. *Canadian Journal of Fisheries And Aquatic Sciences* 53:315-325.
- Azam, F., T. Fenchel, J. G. Field, J. S. Gray, L. A. Meyer-Reil, and F. Thingstad. 1983. The ecological role of water-column microbes in the sea. *Marine Ecology Progress Series* 10:257-263.
- Baca, R. M., and R. W. Drenner. 1995. Do the effects of piscivorous largemouth bass cascade to the plankton? *Hydrobiologia* 316:139-151.
- Bartell, S. M. 1981. Potential impact of size-selective planktivory on phosphorus release by zooplankton. *Hydrobiologia* 80:139-145.
- Bengtsson, J., H. Setälä, and D. W. Zheng. 1996. Food webs and nutrient cycling in soils: interactions and positive feedbacks. Pages 30-38 *in* G. A. Polis and K. O. Winemiller, editors. *Food Webs*, 1st Edition. Chapman and Hall, New York.
- Benndorf, J., and U. Miersch. 1991. Phosphorus loading and efficiency of biomanipulation. *Verhandlungen der Internationalen Vereinigung fuer Theoretische und Angewandte Limnologie* 24:2482-2488.
- Benndorf, J. 1992. The control of the indirect effects of biomanipulation. Pages 82-93 *in* D. W. Sutcliffe and J. G. Jones, editors. *Eutrophication: research and application to water supply*, 1st Edition. Freshwater Biological Association, Ambleside.
- Benndorf, J.. 1995. Possibilities and limits for controlling eutrophication by biomanipulation. *Internationale Revue der Gesamten Hydrobiologie* 80:519-534.

- Bentzen, E., and W. D. Taylor. 1991. Estimating Michaelis-Menten parameters and lake water phosphate by the Rigler bioassay: importance of fitting technique, plankton size and substrate range. *Canadian Journal of Fisheries And Aquatic Sciences* 48:73-83.
- Bentzen, E., W. D. Taylor, and E. S. Milliard. 1992. The importance of dissolved organic phosphorus to phosphorus uptake by limnetic plankton. *Limnology and Oceanography* 37:217-231.
- Bergquist, A. M., and S. R. Carpenter. 1986. Limnetic herbivory: effects on phytoplankton populations and primary production. *Ecology* 67:1351-1360.
- Blackburn, H. T. 1979. Method for measuring rates of NH_4^+ turnover in anoxic marine sediments, using a ^{15}N - NH_4^+ dilution technique. *Applied and Environmental Microbiology* 37:760-765.
- Boers, P., L. Van Ballegooijen, and J. Uunk. 1991. Changes in phosphorus cycling in a shallow lake due to food web manipulations. *Freshwater Biology* 25:9-20.
- Bossard, P., and U. Uehlinger. 1993. The effect of herbivorous crustacean zooplankton on epilimnetic carbon and phosphorus cycling. *Hydrobiologia* 254:21-34.
- Brabrand, A., B. A. Faafeng, and J. P. M. Nilssen. 1990. Relative importance of phosphorus supply to phytoplankton production: fish excretion versus external loading. *Canadian Journal of Fisheries And Aquatic Sciences* 47:364-372.
- Brooks, J. L., and S. I. Dodson. 1965. Predation, body size, and composition of plankton. *Science* 150:28-35.
- Burns, C. W., and M. Schallenberg. 1996. Relative impacts of copepods, cladocerans and nutrients on the microbial food web of a mesotrophic lake. *Journal of Plankton Research* 18:683-714.

- Caperon, J., D. Schell, J. Hirota, and E. Laws. 1979. Ammonium excretion rates in Kaneohe Bay, Hawaii, measured by a ^{15}N isotope dilution technique. *Marine Biology* 54:33-40.
- Carney, H. J. 1990. A general hypothesis for the strength of food web interactions in relation to trophic state. *Verhandlungen der Internationalen Vereinigung fuer Theoretische und Angewandte Limnologie* 24:487-492.
- Carpenter, S. R., and J. F. Kitchell. 1984. Plankton community structure and limnetic primary production. *The American Naturalist* 124:159-172.
- Carpenter, S. R., J. F. Kitchell, and J. R. Hodgson. 1985. Cascading trophic interactions and lake productivity. *Bioscience* 35:634-639.
- Carpenter, S. R., J. F. Kitchell, J. R. Hodgson, P. A. Cochran, J. J. Elser, M. M. Elser, D. M. Lodge, D. Kretchmer, X. He, and C. von Ende. 1987. Regulation of lake primary productivity by food web structure. *Ecology* 68:1863-1876.
- Carpenter, S. R., and J. F. Kitchell. 1988. Consumer control of lake productivity. *Bioscience* 38:764-769.
- Carpenter, S. R., T. M. Frost, J. F. Kitchell, T. K. Kratz, D. W. Schindler, J. Shearer, W. G. Sprules, M. J. Vanni, and A. P. Zimmerman. 1991. Patterns of primary production and herbivory in 25 north american lake ecosystems. Pages 67-96 in J. Cole, G. Lovett and S. Findlay, editors. *Comparative analyses of ecosystems: patterns, mechanisms, and theories*, 1st Edition. Springer-Verlag, New York.
- Carpenter, S. R., and J. F. Kitchell.. 1992. Trophic cascade and biomanipulation: interface of research and management—a reply to the comment by Demelo et al. *Limnology and Oceanography* 37:208-213.

- Carpenter, S. R., C. E. Kraft, R. Wright, X. He, P. A. Soranno, and J. R. Hodgson. 1992. Resilience and resistance of a lake phosphorus cycle before and after food web manipulation. *The American Naturalist* 140:781-798.
- Carpenter, S. R., and J. F. Kitchell. 1993. Simulation models of the trophic cascade: predictions and evaluations. Pages 310-331 in S. R. Carpenter and J. F. Kitchell, editors. *The trophic cascade in lakes*, 1st Edition. Cambridge University Press, Cambridge.
- Carpenter, S.R., Morrice, John Soranno, Patricia A. Elser, James J., MacKay, Neil A, Amand, Ann St. 1993. Primary production and its interactions with nutrients and light transmission. Pages 225-251 in S. R. Carpenter and J. F. Kitchell, editors. *The trophic cascade in lakes*, 1st Edition. Cambridge University Press, Cambridge.
- Christoffersen, K., B. Riemann, A. Klynsner, and M. Sondergaard. 1993. Potential role of fish predation and natural populations of zooplankton in structuring a plankton community in eutrophic lake water. *Limnology and Oceanography* 38:561-573.
- Chrost, R. J. 1990. Microbial ectoenzymes in aquatic environments. Pages 47-78 in J. Overbeck and R. J. Chrost, editors. *Aquatic microbial ecology: biochemical and molecular approaches*, 1st Edition. Springer, New York.
- Currie, D. J., E. Bentzen, and J. Kalff. 1986. Does algal-bacterial phosphorus partitioning vary among lakes? A comparative study of orthophosphate uptake and alkaline phosphatase activity in freshwater. *Canadian Journal of Fisheries And Aquatic Sciences* 43:311-318.
- Currie, D. J. 1990. Phosphorus deficiency and its variation among lakes. *Canadian Journal of Fisheries And Aquatic Sciences* 47:1077-1084.
- Cyr, H., and M. L. Pace. 1992. Grazing by zooplankton and its relationship to community structure. *Canadian Journal of Fisheries And Aquatic Sciences* 49:1455-1465.

- Elser, J. J., N. C. Goff, N. A. MacKay, A. L. St.-Amand, M. M. Elser, and S. R. Carpenter. 1987. Species-specific algal responses to zooplankton: Experimental and field observations in three nutrient-limited lakes. *Journal of Plankton Research*. 9:699-717.
- Elser, J. J., and C. R. Goldman. 1990. Experimental separation of the direct and indirect effects of herbivorous zooplankton on phytoplankton in a subalpine lake. *Verhandlungen der Internationalen Vereinigung fuer Theoretische und Angewandte Limnologie* 24:493-498.
- Findlay, D. L., S. E. M. Kasian, L. L. Hendzel, G. W. Regehr, E. U. Schindler, and J. A. Shearer. 1994. Biomanipulation of Lake 221 in the Experimental Lakes Area (ELA): effects on phytoplankton and nutrients. *Canadian Journal of Fisheries And Aquatic Sciences* 51:2794-2807.
- Fisher, T. R., R. D. Doyle, and E. R. Peele. 1988. Size-fractionated uptake and regeneration of ammonium and phosphate in a tropical lake. *Internationale Vereinigung Fur Theoretische Und Angewandte Limnologie* 23:637-641.
- Fisher, T. R., and D. R. S. Lean. 1992. Interpretation of radiophosphate dynamics in lake waters. *Canadian Journal of Fisheries And Aquatic Sciences* 49:252-258.
- Fretwell, S. D. 1977. the regulation of plant communities by food chains exploiting them. *Perspectives in Biology and Medicine* 20:169-185.
- Gilbert, J. J. 1988. Suppression of rotifer populations by *Daphnia*: a review of the evidence, the mechanisms, and the effects on zooplankton community structure. *Limnology and Oceanography* 33:1286-1303.
- Gilbert, P. M., C. A. Miller, C. Garside, M. R. Roman, and G. B. McManus. 1992. NH_4^+ regeneration and grazing: interdependent processes in size-fractionated $^{15}\text{NH}_4^+$ experiments. *Marine Ecology Progress Series* 82:65-74.

- Glibert, P. M., and D. G. Capone. 1993. Mineralization and assimilation in aquatic, sediment, and wetland systems, p. 243-272. *In* R. Knowles and T. H. Blackburn [eds], Nitrogen isotopes techniques. Academic.
- Goldman, J. C., and M. R. Dennett. 1985. Susceptibility of some marine phytoplankton species to cell breakage during filtration and post-filtration rinsing. *Journal of Experimental Marine Biology and Ecology* 86:47-58.
- Gophen, M. 1990. Biomanipulation: retrospective and future development. *Hydrobiologia* 200:1-11.
- Guy, M., W. D. Taylor, and J. C. H. Carter. 1994. Decline in total phosphorus in the surface waters of lakes during summer stratification, and its relationship to size distribution of particles and sedimentation. *Canadian Journal of Fisheries And Aquatic Sciences* 51:1330-1337.
- Hairton, N. G., F. E. Smith, and L. B. Slobodkin. 1960. Community structure, population control and competition. *The American Naturalist* 94:421-425.
- Hambright, K. D. 1994. Morphological constraints in the piscivore-planktivore interaction: implications for the trophic cascade hypothesis. *Limnology and Oceanography* 39:897-912.
- Hanson, M. A., and M. G. Butler. 1994. Responses of plankton, turbidity, and macrophytes to biomanipulation in a shallow prairie lake. *Canadian Journal of Fisheries And Aquatic Sciences* 51:1180-1188.
- Harrison, W. G. 1983. Uptake and recycling of soluble reactive phosphorus by marine microplankton. *Marine Ecology Progress Series* 10: 127-135.

- Harrison, W. G., and L. R. Harris. 1986. Isotope-dilution and its effect on measurements of nitrogen and phosphorus uptake by oceanic microplankton. *Marine Ecology Progress Series* 27: 253-261.
- Harrison, W. G. 1993. Nutrient recycling in production experiments. *ICES Marine Science Symposium*. 197: 149-158
- He, X., J. F. Kitchell, S. R. Carpenter, J. R. Hodgson, D. E. Schindler, and K. L. Cottingham. 1993. Food web structure and long-term phosphorus recycling: a simulation model evaluation. *Transactions of the American Fisheries Society* 122:773-783.
- Home, A. J., and C. R. Goldman. 1994. *Limnology*, 2nd Edition. McGraw-Hill, Toronto.
- Hrbacek, J., M. Dvorakova, V. Korinek, and L. Prochazkova. 1961. Demonstration of the effect of the fish stock on the species composition of zooplankton and the intensity of metabolism of the whole plankton association. *Verhandlungen der Internationalen Vereinigung fuer Theoretische und Angewandte Limnologie* 14:192-195.
- Hulbert, S. H., J. Zedler, and D. Fairbanks. 1972. Ecosystem alteration by Mosquitofish (*Gambusia affinis*) predation. *Science* 175:639-641.
- Hunter, M. D., and P. W. Price. 1992. Playing chutes and ladders: Heterogeneity and the relative roles of Bottom-up and Top-Down Forces in Natural Communities. *Ecology* 73:724-732.
- Jeppesen, E., M. Sondergaard, J. P. Jensen, E. Mortensen, and O. Sortkjaer. 1996. Fish-induced changes in zooplankton grazing on phytoplankton and bacterioplankton: A long-term study in shallow hypertrophic Lake Sobygaard. *Journal of Plankton Research* 18:1605-1625.

- Jurgens, K and Hans Gude. 1990. Incorporation and release of phosphorus by planktonic bacteria and phagotrophic flagellates. *Marine Ecology Progress Series* 59:271-284
- Kasprzak, P., L. Krienitz, and R. Koschel. 1993. Changes in the plankton of Lake Feldberger Haussee (Germany, Mecklenburg-Vorpommern) in response to biomanipulation. *Archiv fur Hydrobiologie*. 128:149-168.
- Kitchell, J. F., J. F. Koonce, and P. S. Tennis. 1975. Phosphorus flux through fishes. *Verhandlungen der Internationalen Vereinigung fuer Theoretische und Angewandte Limnologie* 19:2478-2484.
- Kitchell, J. F., R. V. O'Neill, D. Webb, G. W. Gallepp, S. M. Bartell, J. F. Koonce, and B. S. Ausmus. 1979. Consumer Regulation of nutrient cycling. *Bioscience* 29:28-34.
- Kitchell, J. F., and S. R. Carpenter. 1993a. Cascading trophic interactions. Pages 1-14 in S. R. Carpenter and J. F. Kitchell, editors. *The trophic cascade in lakes*, 1st Edition. Cambridge University Press, Cambridge.
- Kitchell, J. F., and S. R. Carpenter. 1993b. Synthesis and new directions. Pages 332-350 in S. R. Carpenter and J. F. Kitchell, editors. *The trophic cascade in lakes*, 1st Edition. Cambridge University Press, Cambridge.
- Kitchell, J. F., S. R. Carpenter, J. G. Hodgson, X. He, and P. A. Soranno. 1993. Phosphorus in food webs: compensatory responses in experimental lakes. *Verhandlungen der Internationalen Vereinigung fuer Theoretische und Angewandte Limnologie* 25:344-348.
- Knoechel, R., and L. B. Holtby. 1986. Construction and validation of a body-length-based model for the prediction of cladoceran community filtering rates. *Limnology and Oceanography* 31:1-16.

- Korstad, J. 1983. Nutrient regeneration by zooplankton in southern Lake Huron. *Journal of Great Lakes Research* 9: 374-388.
- Kraft, C. E. 1993. Phosphorus regeneration by Lake Michigan alewives in the mid-1970s. *Transactions of the American Fisheries Society* 122: 749-755.
- Kurmayer, R., and J. Wanzenbock. 1996. Top-down effects of underyearling fish on a phytoplankton community. *Freshwater Biology* 36:599-609.
- Larocque, I., A. Mazumder, M. Proulx, D. R. S. Lean, and F. R. Pick. 1996. Sedimentation of algae: Relationships with biomass and size distribution. *Canadian Journal of Fisheries And Aquatic Sciences* 53:1133-1142.
- Lazzaro, X., R. W. Drenner, R. A. Stein, and J. D. Smith. 1992. Planktivores and Plankton Dynamics: effects of Fish Biomass and Planktivore Type. *Canadian Journal of Fisheries And Aquatic Sciences* 49:1466-1473.
- Lean, D. R. S., A. A. Abbott, and F. R. Pick. 1987. Phosphorus deficiency of Lake Ontario plankton. *Canadian Journal of Fisheries And Aquatic Sciences* 44:2069-2076.
- Lean, D. R. S., and R. L. Cuhel. 1987. Subcellular phosphorus kinetics for Lake Ontario plankton. *Canadian Journal of Fisheries And Aquatic Sciences* 44: 2077-2086.
- Lehman, J. T. 1980. Release and cycling of nutrients between planktonic algae and herbivores. *Limnology and Oceanography* 25: 620-632.
- Li, W. K. W. 1983. Consideration of errors in estimating kinetic parameters based on Michaelis-Menten formalism in microbial ecology. *Limnology and Oceanography* 28:185-190.
- Lindeman, R. L. 1942. The trophic-dynamic aspect of ecology. *Ecology* 23:399-418.

- Lynch, M., and J. Shapiro. 1981. Predation, enrichment, and phytoplankton community structure. *Limnology and Oceanography* 26:86-102.
- Lyche, A., T. Andersen, K. Christoffersen, D. O. Hessen, P. H. B. Hansen, and A. Klysner. 1996. Mesocosm tracer studies .1. Zooplankton as sources and sinks in the pelagic phosphorus cycle of a mesotrophic lake. *Limnology and Oceanography* 41:460-474.
- Mather, M. E., M. J. Vanni, T. E. Wissing, S. A. Davis, and M. H. Schaus. 1995. Regeneration of nitrogen and phosphorus by bluegill and gizzard shad: effect of feeding history. *Canadian Journal of Fisheries And Aquatic Sciences* 52:2327-2338.
- Mazumder, A., D. J. McQueen, W. D. Taylor, and D. R. S. Lean. 1988. Effects of fertilization and planktivorous fish (yellow perch) predation on size distribution of particulate phosphorus and assimilated phosphate: large enclosure experiments. *Limnology and Oceanography* 33:421-430.
- Mazumder, A., W. D. Taylor, D. J. McQueen, and D. R. S. Lean. 1989. Effects of fertilization and planktivorous fish on epilimnetic phosphorus and phosphorus sedimentation in large enclosures. *Canadian Journal of Fisheries And Aquatic Sciences* 46:1735-1742.
- Mazumder, A., W. D. Taylor, D. J. McQueen, D. R. S. Lean, and N. R. Lanfontaine. 1990. A comparison of lakes and lake enclosures with contrasting abundances of planktivorous fish. *Journal of Plankton Research* 12:109-124.
- Mazumder, A., W. D. Taylor, D. R. S. Lean, and D. J. McQueen. 1992. Partitioning and fluxes of phosphorus: mechanisms regulating the size-distribution and biomass of plankton. *Archiv fur Hydrobiologie, Ergebnisse der Limnologie Beih* 35:121-143.
- Mazumder, A. 1994a. Patterns of algal biomass in dominant odd- vs. even-link lake ecosystems. *Ecology* 75:1141-1149.

- Mazumder, A. 1994b. Phosphorus-chlorophyll relationships under contrasting zooplankton community structure: potential mechanisms. *Canadian Journal of Fisheries And Aquatic Sciences* 51:401-407.
- Mazumder, A., and D. R. S. Lean. 1994. Consumer-dependent responses of lake ecosystems to nutrient loading. *Journal of Plankton Research* 16:1567-1580.
- McQueen, D. J., J. R. Post, and E. L. Mills. 1986. Trophic relationships in freshwater pelagic ecosystems. *Canadian Journal of Fisheries And Aquatic Sciences* 43:1571-1581.
- McQueen, D. J. 1990. Manipulating lake community structure: where do we go from here. *Freshwater Biology* 23:613-620.
- McQueen, D. J., R. France, and C. E. Kraft. 1992. Confounded impacts of planktivorous fish on freshwater biomanipulations. *Archives fur Hydrobiologie* 125:1-24.
- Menge, B. A. 1992. Community regulation: Under what conditions are bottom-up factors important on rocky shores? *Ecology* 73:755-765.
- Middelboe, M., N. O. G. Jorgensen, and N. Kroer. 1996. Effects of viruses on nutrient turnover and growth efficiency of noninfected marine bacterioplankton. *Applied and Environmental Microbiology* 62:1991-1997.
- Mittelbach, G. G., A. M. Turner, D. J. Hall, J. E. Rettig, and C. W. Osenberg. 1995. Perturbation and resilience: a long-term, whole-lake study of predator extinction and reintroduction. *Ecology* 76:2347-2360.
- Moegenburg, S. M., and M. J. Vanni. 1991. Nutrient regeneration by zooplankton: Effects on nutrient limitation of phytoplankton in a eutrophic lake. *Journal of Plankton Research* 13:573-588.

- Nakashima, B. S., and W. C. Leggett. 1980. The role of fish in the regulation of phosphorus availability in lakes. *Canadian Journal of Fisheries And Aquatic Sciences* 37:1540-1549.
- Northcote, T. G. 1988. Fish in the structure and function of freshwater ecosystems: a "top-down" view. *Canadian Journal of Fisheries And Aquatic Sciences* 45:361-379.
- Oksanen, L., S. D. Fretwell, J. Arruda, and P. Niemela. 1981. Exploitation ecosystems in gradients of primary productivity. *The American Naturalist* 118:240-261.
- den Oude, P. J., and R. D. Gulati. 1988. Phosphorus and nitrogen excretion rates of zooplankton from the eutrophic Loosdrecht lakes, with notes on other P sources for phytoplankton requirements. *Hydrobiologia* 169:379-390.
- Pace, M. L., and J. J. Cole. 1994. Comparative and experimental approaches to top-down and bottom-up regulation of bacteria. *Microbial Ecology* 28:181-193.
- Pace, M. L., and D. Vaque. 1994. The importance of *Daphnia* in determining mortality rates of protozoans and rotifers in lakes. *Limnology and Oceanography* 39:985-996.
- Parsons, T. R., Y. Maita, and C. M. Lalli. 1984. A manual of chemical and biological methods for seawater analysis, 1st Edition. Pergamon Press, Oxford.
- Perezfuentetaja, A., D. J. McQueen, and C. W. Ramcharan. 1996. Predator-induced bottom-up effects in oligotrophic systems. *Hydrobiologia* 317:163-176.
- Perin, S., F. R. Pick, D. R. S. Lean, and A. Mazumder. 1996. Effects of planktivorous fish and nutrient additions on primary production of shallow versus deep (stratified) lake enclosures. *Canadian Journal of Fisheries And Aquatic Sciences* 53:1125-1132.

- Persson, L., G. Andersson, S. F. Hamrin, and L. Johansson. 1988. Predator regulation and primary production along the productivity gradient of temperate lake ecosystems. Pages 45-65 in S. R. Carpenter, editor. *Complex interactions in lake communities*, 1st Edition. Springer-Verlag, New York.
- Persson, L., L. Johannsson, G. Andersson, S. Diehl, and S. F. Hamrin. 1993. Density dependent interactions in lake ecosystems: whole lake perturbation experiments. *Oikos* 66:193-208.
- Peters, R., and D. Lean. 1973. The characterization of soluble phosphorus released by limnetic zooplankton. *Limnology and Oceanography* 18: 270-279.
- Peters, R. H. 1975. Phosphorus regeneration by natural populations of limnetic zooplankton. *Verhandlungen der Internationalen Vereinigung fuer Theoretische und Angewandte Limnologie* 19:273-279.
- Peters, R. H. 1983. *The ecological implications of body size*. Cambridge University Press, Cambridge.
- Peters, R. H. 1984. Methods for the study of feeding, grazing and assimilation by zooplankton, p. 336-412. In J. A. Downing and F. H. Rigler [eds.], *A manual on methods for the assessment of secondary production in fresh waters*. Blackwell.
- Peters, R. H., and J. A. Downing. 1984. Empirical analysis of zooplankton filtering and feeding rates. *Limnology and Oceanography* 29:763-784.
- Polis, G. A., and D. R. Strong. 1996. Food web complexity and community dynamics. *The American Naturalist* 147:813-846.
- Polis, G. A., and K. O. Winemiller, editors. 1996. *Food Webs: Integration of Patterns and Dynamics*, 1st Edition. Chapman and Hall, New York.

- Pomeroy, L. L. 1974. The ocean's food web, a changing paradigm. *Bioscience* 24:499-504.
- Power, M. E. 1992. Top-down and bottom-up forces in food webs: do plants have primacy. *Ecology* 73:733-746.
- Proulx, M., F. R. Pick, A. Mazumder, and D. R. S. Lean. 1993. Effects of planktivorous fish on phytoplankton biomass and community structure. *Verhandlungen der Internationalen Vereinigung fuer Theoretische und Angewandte Limnologie* 25:331-334.
- Proulx, M., F. R. Pick, A. Mazumder, P. B. Hamilton, and D. R. S. Lean. 1996. Effects of nutrients and planktivorous fish on the phytoplankton of shallow and deep aquatic systems. *Ecology* 77:1556-1572.
- Ramcharan, C. W., D. J. McQueen, E. Demers, S. A. Popiel, A. M. Rocchi, N. D. Yan, A. H. Wong, and K. D. Hughes. 1995. A comparative approach to determining the role of fish predation in structuring limnetic ecosystems. *Archives fur Hydrobiologie* 133:389-416.
- Reinertsen, H., A. Jensen, J. I. Koksvik, A. Langeland, and Y. Olsen. 1990. Effects of fish removal on the limnetic ecosystem of a eutrophic lake. *Canadian Journal of Fisheries And Aquatic Sciences* 47:166-173.
- Reynolds, C. S. 1994. The ecological basis for the successful biomanipulation of aquatic communities. *Archives fur Hydrobiologie* 130:1-33.
- Rigler, F. 1966. Radiobiological analysis of inorganic phosphorus in lakewater. *Internationale Vereinigung Fur Theoretische Und Angewandte Limnologie* 16:465-470.
- Rothhaupt, K. O. 1992. Stimulation of phosphorus-limited phytoplankton by bacterivorous flagellates in laboratory experiments. *Limnology and Oceanography* 37: 750-759.

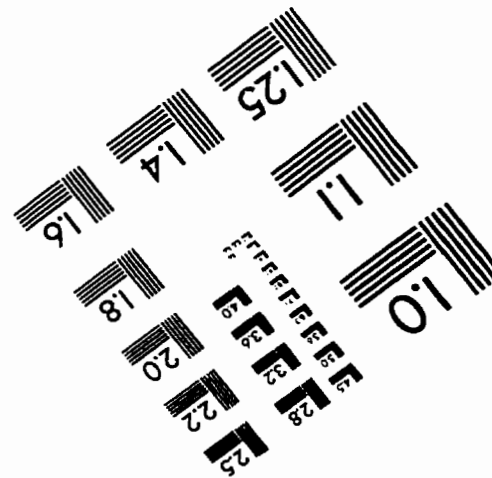
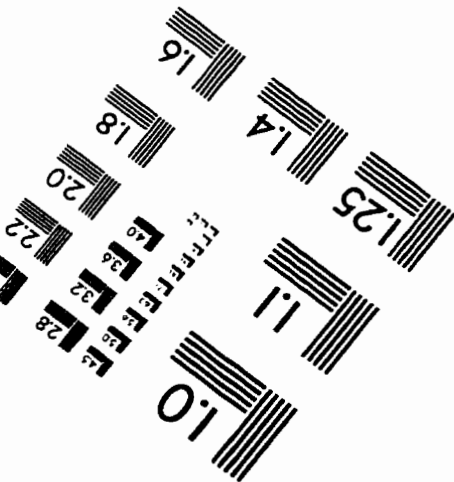
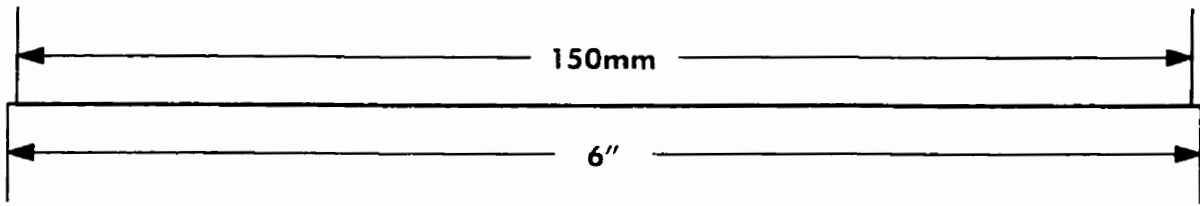
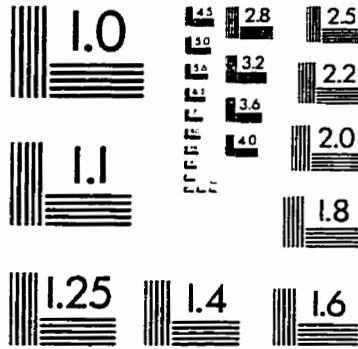
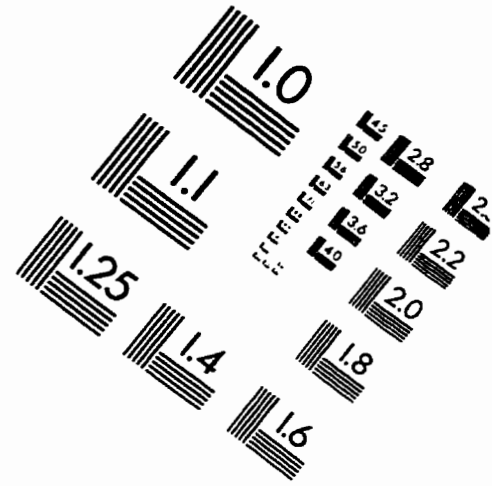
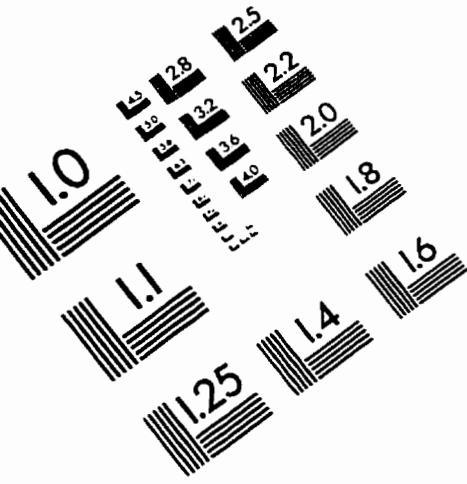
- Sarnelle, O. 1992. Nutrient enrichment and grazer effects on phytoplankton in lakes. *Ecology* 73:551-560.
- Schindler, D. E. 1992. Nutrient regeneration by sockeye salmon (*Oncorhynchus nerka*) fry and subsequent effects on zooplankton and phytoplankton. *Canadian Journal of Fisheries And Aquatic Sciences* 49:2498-2506.
- Schindler, D. E., J. F. Kitchell, X. He, S. R. Carpenter, J. R. Hodgson, and K. L. Cottingham. 1993. Food web structure and phosphorus cycling in lakes. *Transactions of the American Fisheries Society* 122:756-772.
- Schindler, D. W. 1974. Eutrophication and recovery in experimental lakes: implications for lake management. *Science* 184:897-899.
- Schindler, D. W. 1978. Factors regulating phytoplankton production and standing crop in the world's freshwaters. *Limnology and Oceanography* 23:478-486.
- Shapiro, J., V. Lamarra, and M. Lynch. 1975. Biomanipulation: an ecosystem approach to lake restoration. Pages 85-89 in P. L. Brezonik and J. L. Fox, editors. *Proceedings of a symposium on Water Quality Management Through Biological Control.*, 1st Edition. University of Florida Press, Gainesville.
- Shapiro, J., and D. I. Wright. 1984. Lake restoration through biomanipulation: Round Lake, Minnesota the first two years. *Freshwater Biology* 14:371-383.
- Stern, R. W. 1989a. The role of grazers in phytoplankton succession. Pages 107-170 in U. Sommer, editor. *Plankton ecology: succession in plankton communities*, 1st Edition. Springer-Verlag, Berlin.
- Stern, R. W. 1989b. Resource competition during seasonal succession toward dominance by cyanobacteria. *Ecology* 70: 229-245.

- Sterner, R. W., J. J. Elser, T. H. Chrzanowski, J. H. Schampel, and N. B. George. 1996. Biogeochemistry and trophic ecology: a new food web diagram. Pages 72-80 in G. A. Polis and K. O. Winemiller, editors. *Food Webs*, 1st Edition. Chapman and Hall, New York.
- Stockner, J. G., and K. G. Porter. 1988. Microbial food webs in freshwater planktonic ecosystems, p. 69-83. In S. R. Carpenter [ed.], *Complex interactions in lake communities*. Springer.
- Stone, L., and R. S. J. Weisburd. 1992. Positive feedback in aquatic ecosystems. *Trends in Ecology and Evolution* 7:263-267.
- Strong, D. R. 1992. Are trophic cascades all wet? differentiation and donor-control in speciose ecosystems. *Ecology* 73:747-754.
- Suttle, C. A. 1994. The significance of viruses to mortality in aquatic microbial communities. *Microbial Ecology* 28: 237-243.
- Tarapchak, S. J., and L. R. Herche. 1988. Orthophosphate concentrations in lake water: analysis of Rigler's radiobioassay method. *Canadian Journal of Fisheries and Aquatic Science* 45:2230-2237.
- Tarapchak, S. J. 1989. Phosphate uptake by microbial assemblages: model requirements and evaluation of experimental methods. *Journal of Environmental Quality* 18:17-25.
- Taylor, W. D., and D. R. S. Lean. 1981. Radiotracer experiments on phosphorus uptake and release by limnetic microzooplankton. *Canadian Journal of Fisheries and Aquatic Science* 38: 1316-1321

- Taylor, W. D. 1984. Phosphorus flux through epilimnetic zooplankton from Lake Ontario: relationship with body size and significance to phytoplankton. *Canadian Journal of Fisheries And Aquatic Sciences* 11:1702-1712.
- Taylor, W. D., and D. R. S. Lean. 1991. Phosphorus pool sizes and fluxes in the epilimnion of a mesotrophic lake. *Canadian Journal of Fisheries And Aquatic Sciences* 48:1293-1301.
- Threlkeld, S. T. 1987. Experimental evaluation of trophic-cascade and nutrient-mediated effects of planktivorous fish on plankton community structure. Pages 161-173 in W. C. Kerfoot and A. Sih, editors. *Predation: Direct and indirect impacts on aquatic communities*, 1st Edition. University Press of New England, Hanover.
- Tremaine, S. C., and A. L. Mills. 1987. Inadequacy of the eucaryote inhibitor cycloheximide in studies of protozoan grazing on bacteria at the freshwater-sediment interface. *Applied and Environmental Microbiology*. 53: 1969-1972.
- Vadstein, O., Y. Olsen, H. Reinertsen, and A. Jensen. 1993. The role of planktonic bacteria in phosphorus cycling in lakes—sink and link. *Limnology Oceanography* 38: 1539-1544.
- Vanni, M. J. 1987. Effects of nutrients and zooplankton size on the structure of a phytoplankton community. *Ecology* 71:921-937.
- Vanni, M. J., and D. L. Findlay. 1990. Trophic cascades and phytoplankton community structure. *Ecology* 71:921-937.
- Vanni, M. J., and J. Temte. 1990. Seasonal patterns of grazing and nutrient limitation of phytoplankton in a eutrophic lake. *Limnology and Oceanography* 35:697-709.
- Vanni, M. J., C. Luecke, J. F. Kitchell, Y. Allen, J. Temte, and J. J. Magnuson. 1990. Effects on lower trophic levels of massive fish mortality. *Nature* 344:333-335.

- Vanni, M. J. 1996. Nutrient transport and recycling by consumers in lake food webs: implications for algal communities. Pages 81-95 in G. A. Polis and K. O. Winemiller, editors. *Food Webs*, 1st Edition. Chapman and Hall, New York.
- Visman, V., D. J. McQueen, and E. Demers. 1994. Zooplankton spatial patterns in two lakes with contrasting fish community structure. *Hydrobiologia* 284:177-191.
- Vollenweider, R. A. 1968. The scientific basis of lake and stream eutrophication, with particular reference to phosphorus and nitrogen as eutrophication factors. Technical Report of the OECD, DAS/CSI 68:1-182.
- White, E., G. Payne, and F. R. Pick. 1982. Factors influencing orthophosphate turnover times: a comparison of Canadian and New Zealand lakes. *Canadian Journal of Fisheries And Aquatic Sciences* 39:469-474.
- Wilkinson, L., M. A. Hill, J. P. Welna, and G. K. Birkenbeuel. 1992. *SYSTAT for Windows*, 5th Edition. SYSTAT, Evanston.
- Wright, D. I., and J. Shapiro. 1984. Nutrient reduction by biomanipulation: an unexpected phenomenon and its possible cause. *Internationale Vereinigung Fur Theoretische Und Angewandte Limnologie* 22:518-524.

IMAGE EVALUATION TEST TARGET (QA-3)



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