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The spatial and temporal dynamics of deep chlorophyll layers in high-mountain lakes: effects of nutrients, grazing and herbivore nutrient recycling as growth determinants

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Deep chlorophyll layers (DCL) are a common feature of oligotrophic lakes, yet the mechanisms that form and maintain them are not understood fully. These phytoplankton populations occur in the metalimnia of lakes where light levels are moderate to low, and where nutrient levels and zooplankton grazing pressure are different than in the epilimnion. To test the importance of nutrients and grazing pressure for algal growth in different lake strata, microcosm experiments and monitoring were conducted in two oligotrophic lakes in the Rocky Mountains of North America that contain DCL. In situ *microcosm experiments with natural phytoplankton communities from three depth strata were conducted with macronutrient additions and with and without the natural zooplankton grazing communities. Alkaline phosphatase assays and the* in situ *microcosm experiments indicated less nutrient limitation in the metalimnia than in the epilimnia of both lakes. Zooplankton grazing in the experiments decreased algal population growth rates by as much as 6% day^{-1}, with impacts shifting to progressively deeper strata over the summer. Zooplankton grazing losses, however, were partially offset by nutrient recycling that increased algal growth rates. Depth-differential nutrient deficiency and zooplankton grazing and recycling interacted to maintain the DCL in these lakes.*

INTRODUCTION

Although many studies have quantified deep chlorophyll layers (DCL) and their productivity, and several hypotheses for DCL formation have been forwarded and discussed (Cullen, 1982), the mechanisms that explain their origin and permit their maintenance remain unclear. The presence of a metalimnetic algal community may result from *in situ* productivity (Fahnenstiel and Glime, 1983), depth-specific zooplankton grazing (Pilati and Wurtsbaugh, 2003), phytoplankton sedimentation (Watson *et al.*, 1975), or a combination of these factors. For *in situ* metalimnetic growth, phytoplankton must be capable of buoyancy regulation to avoid settling loss and must possess adequate light-harvesting pigments for photosynthesis (Camacho *et al.*, 2000). Without these capabilities, sustained *in situ* population growth is not possible, and the more likely explanation for a DCL is passive sedimentation of epilimnetic algae to a depth where cell density equals water density.

DCL have been noted in oligotrophic to mesotrophic freshwater lakes and in the oceans. Nutrients are necessary for fueling *in situ* growth, and some research has found that greater metalimnetic nutrient availability may

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result in elevated subsurface chlorophyll concentrations (Fee, 1976; Moll and Stoermer, 1982; Moll *et al.*, 1984). Therefore, it is reasonable to expect that trophic status will play a role in determining DCL formation and maintenance mechanisms. In oligotrophic systems, available limiting nutrients are in short supply and rapidly lost from the surface waters. A small, continually recycled nutrient pool supports the phytoplankton community that continues to grow at the surface. Phytoplankton may then establish beneath the surface layer where nutrients are higher and light remains adequate for growth. In mesotrophic lakes, DCL are more pronounced than in oligotrophic systems and have also been reported to be less nutrient limited than their epilimnetic counterparts (St. Amand, 1990). Light availability, greater rates of *in situ* production and sedimentation of epilimnetic algal cells into the metalimnion are predominant DCL formation mechanisms in mesotrophic systems. Studies in ocean systems have also found that *in situ* production fueled by nutrients delivered via hydrographic processes (currents and upwelling) results in cell accumulation in a deep stratum in these oligotrophic systems (Cullen, 1982; Furuya, 1990).

Because the presence of an algal population in a natural system is the result of a dynamic balance between production and loss factors, zooplankton grazing may also contribute to DCL formation (Pedros-Alió *et al.*, 1987; Pilati and Wurtsbaugh, 2003). Many studies have demonstrated top-down effects on phytoplankton community dynamics (Carpenter *et al.*, 1987; Elser *et al.*, 1988). Large herbivorous zooplankton, such as *Daphnia* sp., are efficient grazers, and if predation pressure on these zooplankton is decreased, grazing pressure on phytoplankton increases. This may, in turn, decrease phytoplankton biomass and increase water transparency (Lampert *et al.*, 1986). For example, pelagic grazers can consume up to 70% of daily primary production in oligotrophic parts of the ocean (Moll and Stoermer, 1982). Therefore, phytoplankton community composition and biomass may be set by the interaction of grazing pressure (Vanni, 1987) and nutrient dynamics, which depend on supply rates and phytoplankton uptake demands (Kilham and Kilham, 1984).

In addition to grazing impacts, nutrient recycling by consumers contributes directly to nitrogen and phosphorus pools available to algal populations (Peters and Rigler, 1973; Lehman, 1980). Low dissolved nutrient concentrations do not necessarily indicate strong control of phytoplankton by nutrient limitation, since the pools may be rapidly renewed through remineralization of N and P by heterotrophs (Andersen *et al.*, 1991). Where nutrient limitation is relatively severe, the negative effects of grazing mortality can be nearly compensated by the beneficial effects of nutrients recycled by grazers (Carpenter and Kitchell, 1984; Elser and MacKay, 1989). Experiments by Sterner *et al.* (Sterner *et al.*, 1995) showed that recycled nutrients were the dominant nutrient sources for pelagic bacteria and phytoplankton. A model by O'Neil (O'Neil, 1992) predicted that when herbivory recirculates nutrients to the available pool that would otherwise be lost from the system, per capita primary production would often be highest at relatively high levels of grazing consumption. Therefore, a nutrient-poor epilimnion may have high-specific productivity that is dependent upon consumer recycling for limiting nutrients.

Past studies of the oligotrophic Sawtooth Valley lakes, North America, that are the focus of this work have suggested various different mechanisms that may cause the DCL. Gross *et al.* (Gross *et al.*, 1997) and Wurtsbaugh *et al.* (Wurtsbaugh *et al.*, 2001) proposed that plunging inflow stream delivers nutrients to metalimnetic and hypolimnetic water first, thereby driving *in situ* production. Other work found that top-down grazing control of phytoplankton was not as important as nutrient limitation in determining phytoplankton production in the Sawtooth lakes (Gross *et al.*, 1993; Wurtsbaugh *et al.*, 1997). Recent research by Pilati and Wurtsbaugh (Pilati and Wurtsbaugh, 2003) revealed that epilimnetic grazing coupled with sedimentation supports DCL persistence.

Researchers have not simultaneously considered both nutrient and grazing impacts on DCL formation in oligotrophic lakes. Consequently, our study objectives were to quantify the effects of nutrient limitation and inhibition and the effects of grazing mortality and nutrient recycling on phytoplankton communities both in and outside the DCL and temporally through a growing season. Six 4-day factorial microcosm experiments, two specific primary production experiments and regular nutrient deficiency indicator assays were utilized to address these questions.

METHOD

Study site

Yellow Belly Lake and Stanley Lake are located in the granitic Sawtooth Mountains (a section of the Rocky Mountains) in south-central Idaho, USA (44°0′ N, 114°53′ W) at elevations of 2157 and 1985 m, respectively. Both lakes were formed by Pleistocene glaciers, are dimictic and ice-covered from December through May and have one perennial inflow. Respective areas of Yellow Belly and Stanley lakes are 0.80 and 0.73 km^2, and the maximum depth of both lakes is 26 m.

The two study sites are highly oligotrophic (Budy *et al.*, 1995), and their watersheds lie largely in undisturbed wilderness areas where atmospheric deposition of nitrogen is low (~1.3 kg ha^{-1} $year^{-1}$). Historically, anadromous salmon provided some marine-derived nutrients, but their contribution is now negligible (Gross *et al.*, 1998). Streams contribute 72–88% of the N and P loading, and most of this occurs during 2 months of spring snowmelt (Gross *et al.*, 1998). Molar N : P ratios of loading to Sawtooth Valley lakes average 27:1 (Gross *et al.*, 1998), but most of the nitrogen enters as dissolved organic nitrogen rather than as nitrate and thus may be recalcitrant (Wurtsbaugh, unpublished data). Being colder and denser, the inflows tend to plunge when entering the lakes, thereby delivering a portion of the nutrients to subsurface strata and contributing to relative epilimnetic oligotrophication (Steinhart *et al.*, 1994; Wurtsbaugh *et al.*, 2001). Epilimnetic chlorophyll concentrations are closely related to nutrient loading rates (Steinhart *et al.*, 1994).

Previous research has pointed out some differences in aquatic communities between the two study lakes. Steinhart *et al.* (Steinhart *et al.*, 1994) found that Chlorophyta represented >75% of the phytoplankton biovolume in the Yellow Belly Lake DCL during midsummer. Dominant genera were *Chlorella* sp. and *Oocystis* sp. with the smaller *Chlorococcales* spp., *Chlamydomonas* sp. and Desmidiaceae contributing minor amounts. *Dinobryon* sp. were also abundant (23% of biovolume), and larger Cyanophyta contributed <1% of the biovolume (picocyanobacteria were not counted). During this same time, the DCL phytoplankton biovolume in Stanley Lake was comprised of Chlorophyta (~68% of the biovolume: mostly *Chlorella* sp. and *Oocystis* sp.), diatoms (18%; *Synedra*, *Cyclotella* and *Melosira*) and *Dinobryon* sp. (14%). The upper levels of the food web in the two lakes also demonstrate some differences. Fish densities are greater in Stanley Lake than in Yellow Belly Lake because of higher natural recruitment and stocking (Steinhart *et al.*, 1994), resulting in lower densities of smaller zooplankton.

Lake profile monitoring

During the 1999 summer growing season, physical and chemical profile data were collected once every 2 weeks in both lakes. Daily photosynthetically active radiation (PAR) rates (units of μmol photons m^{-2} day^{-1}) for the experimental periods were estimated by measuring PAR as a percentage of total radiation every 30 min using a Kipp and Zonen net radiometer equipped with CM3 and CG3 pyranometers and mounted at the Yellow Belly Lake weather station. Vertical profiles of PAR were measured using a LiCor Model LI-1000 and a 4-Pi sensor at 1-m intervals to lake bottom. Extinction coefficients were calculated as the slope of the regression of the length (% of surface intensity) against depth (Wetzel and Likens, 1991) and then were used to calculate daily PAR rates using the weather station data. Secchi transparencies were determined using a 25-cm disk. Temperature profiles were measured at 3-m intervals before the start of each experiment using a YSI Model 58 thermistor. Water for nutrient analyses was collected at 3-m intervals and filtered through acid-washed and rinsed GF/F filters (nominal pore size of 0.7 μm). Samples were promptly frozen until analysis. Ammonia was analysed colorimetrically using the phenolhypochlorite method of Solórzano (Solórzano, 1969). Nitrate + nitrite was processed using hydrazine reduction followed by colorimetric analyses (APHA, 1995a). Water samples for total nitrogen (TN) and total phosphorus (TP) analyses were simultaneously digested with persulfate (Valderrama, 1981) with the modification that recrystalized potassium peroxodisulfate was used to minimize blanks (Nydahl, 1978). Soluble reactive phosphorus (SRP) and TP were analysed colorimetrically using the molybdate-absorbic acid method (APHA, 1995b). TN was determined using the second derivative spectrophotometric analysis of the nitrate produced from the digestion (Crumpton *et al.*, 1992). The samples were analysed on a Cary 50 UV/Vis spectrophotometer using 5- or 10-cm path length cells to maximize sensitivity. TN : TP ratios were calculated and used as indicators of which macronutrient may have been limiting. A TN : TP molar ratio of <20 was taken to indicate N-deficient growth, a ratio of >50 to indicate P-deficient growth and intermediate ratios to indicate that either nutrient may be deficient (Guildford and Hecky, 2000).

Vertical profiles of chlorophyll and algal physiological parameters were also monitored every 2 weeks in both lakes. Chlorophyll *a* (Chl *a*) concentrations and primary production were measured at 3-m intervals from the surface to 21 m. A 3-m interval is sufficient for sampling the DCL in these lakes, because the layers typically persist in bands up to 10-m wide. Water was collected for Chl *a* measurements at two sites near the middle of each lake and filtered onto 0.45-μm cellulose acetate filters. The filters were frozen and pigments extracted in methanol for 24 h in darkness at room temperature. Following extraction, Chl *a* concentrations were measured fluorometrically with the method of Welschmeyer (Welschmeyer, 1994) using a Turner 10 AU fluorometer. The method provided a detection limit of 0.02 μg L^{-1} and sensitivity of 0.025 μg L^{-1}. *In situ* algal primary production was measured using the ^{14}C method described in Wetzel and Likens (Wetzel and Likens, 1991). Briefly, water from each depth was filled into three 60-mL glass biological oxygen demand (BOD)

bottles. Seventy-five microliters of 20 μCi mL^{-1} of $^{14}CHO_3$ was pipetted into each bottle. To measure dark ^{14}C uptake, one of the three bottles was injected with 500 μL of stock dichloro-phenyl-dimethylurea (DCMU) saturated solution (42 mg L^{-1}) in water (Legendre *et al.*, 1983). The bottles were suspended at their respective depths for a 4-h incubation near midday. Immediately following incubation, water from each bottle was filtered onto 0.45-μm cellulose nitrate filters. Bottles and filtration towers were then rinsed with 0.1 N HCl to solubilize any carbonates that may have formed. Following filtration, filters were placed into 25-mL plastic scintillation vials and allowed to air dry. Each vial received Readysafe cocktail (Beckman Coulter) and was then counted via liquid scintillation spectrometry (Beckman 6500). Production rates were calculated by subtracting ^{14}C uptake in the DCMU controls from uptake by the light bottles. Carbon fixation in the DCMU treatments was normally ~<4.5% of the maximum uptake rates observed in epilimnetic light bottles. Water samples for dissolved inorganic carbon were collected in 10-mL vacutainers, preserved with one drop of chloroform and analysed using a Tekmar-Dohrman Phoenix 8000 UV-Persulfate TOC analyzer.

Nutrient deficiency indicator assays

Two nutrient deficiency indicators, alkaline phosphatase activity (APA) and ammonium enhancement response (AER) assays, were used to measure the vertical and temporal dynamics of phosphorus and nitrogen deficiency of the bacteria picoplankton and phytoplankton during the regular, biweekly sampling periods throughout the growing season (June through September) and once after thermal mixing in November. Separate assays were conducted on water sampled from two sites near the middle of each lake every 3 m to a depth of 21 m except during lake mixing in November when only 3, 12 and 18 m were sampled.

APA is a fluorometric indicator of phosphorus deficiency that detects externally bound enzymes produced by phytoplankton to cleave $PO_{4^{-3}}$ from organophosphates (Vincent, 1981) and indicates a level of P-stress sufficient to induce synthesis of the enzyme (Flynn *et al.*, 1986). Whole-community APA was measured in the laboratory at a temperature of 40°C following the method of Voichich and Lebouton (Voichich and Lebouton, 1994). Activity was expressed with respect to Chl *a* (units of nmol $PO_{4^{-3}}$ μg Chl a^{-1} h^{-1}). Statistical analysis of the APA data consisted of two-way analysis of variance (ANOVA) with depth and date as factors and alpha (∀) set at 0.05 for statistical significance testing.

AER indicates nitrogen deficiency by detecting assimilatory dark carbon fixation associated with the uptake of ammonium (Yentsch *et al.*, 1977). Lake water was incubated *in situ* in 30-mL dark, polyethylene bottles with and without added NH_{4^+}–N in the presence of $NaH^{14}CO_3$ following the method of Voichich and Lebouton (Voichich and Lebouton, 1994). AER was calculated by dividing treatment (NH_{4^+}–N) values by control values to give an index of N limitation. Index values were centered at zero by subtracting one; values greater than zero indicated enhanced N uptake and possible N stress. Unpaired *t* tests were used to compare NH_{4^+}–N treatment values to control values (∀ = 0.05).

Microcosm experimental design

Three microcosm experiments were conducted in late June (27 June–2 July), July (26–31) and August (25–30) 1999 in each lake to examine the effects of nutrients, herbivory and interactions of these factors on phytoplankton growth rates, measured as incremental changes in Chl *a* concentration. The microcosm experiments utilized an *in situ* incubation and factorial design similar to that of Huovinen *et al.* (Huovinen *et al.*, 1999) with the duplicated treatments summarized in Table I. Water was sampled at 3, 12 and 18 m for Yellow Belly and 3, 9 and 12 m for Stanley. The depths for Yellow Belly were chosen based on DCL dynamics observed during 1998. Light-extinction coefficients (Gross *et al.*, 1997) and an exponential model of light decay were used to estimate three depths of similar light intensity for Stanley Lake. Water for the bioassays was collected both during the night and day and pooled by depth so that zooplankton compositions and abundances would represent a daily average. This was important because zooplankton in Yellow Belly Lake are distributed diffusely throughout the epilimnion and metalimnion with some weak migration that changes seasonally, while Stanley Lake zooplankton exhibit strong diel migration possibly to avoid

Table I: In situ microcosm experiment treatments

Treatment name	Symbol	N	P	Zooplankton
Control	C	–	–	–
Nutrients	+N+P	+	+	–
Phosphorus	+P	–	+	–
Nitrogen	+N	+	–	–
Zooplankton	+Z	–	–	+
Nutrients and zooplankton	+N+P+Z	+	+	+
Phosphorus and zooplankton	+P+Z	–	+	+
Nitrogen and zooplankton	+N+Z	+	–	+

–, nutrient was not added or macrozooplankton were removed; +, nutrient was added or zooplankton were present.

predation from the higher fish densities present in the lake (Brindza, 2002).

Macrozooplankton were removed for the C (control), +N+P (nitrogen and phosphorus added), +P (phosphorus amended) and +N (nitrogen amended) treatments by filtering sample water through an 80-μm mesh. This treatment did not remove smaller zooplankton, such as small rotifers, nauplii and protozoans. Testing revealed that this filtration did not remove any chlorophyll. To ensure that all zooplankton treatment containers received approximately equal zooplankton biomasses, unfiltered water containing ambient concentrations of zooplankton for use in the zooplankton treatments was thoroughly mixed before filling the experimental containers. Enclosures were 4-L, translucent polyethylene cubitainers (Hedwin Corporation) that allowed 98% light penetration. Nutrient treatment containers received 16 μM N as NH_4NO_3 (i.e. 8 μM each of NH_{4^+}–N and NO_3–N added concentration) or 0.8 μM P as KH_2PO_4 (added concentration) or a mixture of both nutrients at the above concentrations. The initial Chl *a* concentrations in the 80-μm filtered and unfiltered stock water were measured from each depth.

Treatments were incubated *in situ* at their original depths for 4 days. After the incubation period, Chl *a* concentration in each microcosm was measured as the primary response variable. Zooplankton from each microcosm were also preserved in Lugol's iodine for subsequent enumeration and identification. Zooplankton biomass was estimated using count data and mean weights of zooplankton sampled in the lakes at the same depths and approximately the same times as the bioassay experiments (unpublished data). Mean weights were estimated by applying species-specific length–weight regressions (Downing and Rigler, 1984). Zooplankton mortalities (only noted in the July 3-m and August 3-m treatments in Stanley Lake) were counted by noting the presence of fungus on carapaces.

Microcosm data analysis and statistical design

Algal daily net growth rates (*GR*) were calculated by $GR = \ln(C_t - C_0)/t$, where C_t is the final Chl *a* concentration, C_0 is the initial Chl *a* concentration and t is the duration of the experiment in days. For graphical analysis and presentation, the change in net growth rate (*GR*; units of day^{-1}) was calculated as the difference in average net *GR* in cubitainers that had received a given treatment and cubitainers that had not received the treatment. For example, the change in *GR* due to nitrogen addition was $[(GR_{+N} + GR_{+N+P} + GR_{+N+Z} + GR_{+N+P+Z})/4] - [(GR_C + GR_{+P} + GR_{+Z} + GR_{+P+Z})/4]$ (see Table I for definition of abbreviations). The impacts of nutrients, zooplankton and their interactions were evaluated for each experiment independently using a multifactor, two-way ANOVA for each depth. Factors were nutrients (N = 0,1 and P = 0,1) and zooplankton (Z = 0,1). When zooplankton mortality occurred, only the impacts of nutrients were evaluated using one-way ANOVA. *P* values smaller than the preset ∀-level of 0.05 were used to reject null hypotheses. With this analysis, the N+P treatment was not considered significant unless chlorophyll levels were above (or below) the additive effects of N and P added separately.

Data from the factorial microcosm experiments allowed the estimation of direct zooplankton effects (grazing) and indirect zooplankton effects (algal growth stimulation) for each depth in each experiment (Elser and Goldman, 1991). Macrozooplankton grazing rates (*G*; units of $mL\ L^{-1}\ day^{-1}$) were calculated as the difference in net growth rate between the +N+P and +N+P+Z treatments, assuming that at nutrient-saturated conditions, all phytoplankton death was due to macrozooplankton grazing ($G = GR_{+N+P} - GR_{+N+P+Z}$). A positive *G* value indicated grazing loss. Clearance rates (*CR*; units of $mL\ \mu g^{-1}$ zooplankton day^{-1}) were estimated as the absolute value of the slope of linear regressions of zooplankton biomasses and net algal growth rates in the +N+P and +N+P+Z treatments. Therefore, a negative slope indicated grazing loss. *In situ* or gross phytoplankton growth rates (GR_{is}; units of day^{-1}) were estimated as $GR_{is} = GR_Z + G$, where GR_Z is the growth rate in treatments containing zooplankton but no added nutrients. To assess indirect zooplankton effects (enhancement of algal growth by zooplankton-recycled nutrients), net growth rate in the absence of zooplankton (GR_C) was compared with the net *in situ* growth rate (GR_{is}) in the presence of zooplankton. Indirect effects (*IE*) were calculated as $IE = GR_{is} - GR_C$ (Elser, 1992). Significance of community grazing rate was tested by using contrast statements within the two-factor ANOVA to compare +N+P and +N+P+Z treatments. *CR* were evaluated by regression r^2 and *P* values with ∀ = 0.05. The significance of *IE* was also tested using contrast statements to compare GR_{is} with GR_C.

Specific primary production experimental design and data analysis

The July microcosm experiments in both lakes included two additional treatments to better address the question of whether and where in the water column phytoplankton experienced grazing-induced compensatory growth or 'recycling benefit'. Like *IE*, compensatory growth is production that is possible in spite of grazing losses that can largely be attributed to zooplankton-driven nutrient recycling. The modified experimental design

featured two additional treatments for each depth: a dilution to one-half ambient zooplankton concentrations (½Z) and a concentration to four times ambient zooplankton concentrations (4Z). The additional treatments allowed consideration of four zooplankton levels: 0Z (C), ½Z, 1Z (Z) and 4Z. All treatments were duplicated and incubated *in situ* at their original depths, as previously described. At the end of the incubation period, Chl *a* and primary productivity were measured as previously described from all replicates of the four zooplankton treatments. Zooplankton from each microcosm were preserved in Lugol's iodine for enumeration, identification and biomass estimation, as described previously.

Specific primary production (*PPr*) (carbon fixed per unit of chlorophyll; units of mg C mg Chl a^{-1} h^{-1}) was calculated for each treatment. Data analysis by depth consisted of linear and nonlinear regression for three relationships: Chl *a* and zooplankton biomass, *PPr* and zooplankton biomass and specific *PPr* and zooplankton biomass. Michaelis-Menton equations were used to model specific *PPr* as specific $PPr = (V_s \times \text{zooplankton biomass})/(K_m + \text{zooplankton biomass}) + c$, where V_s is the maximum *PPr* or the asymptote, K_m is the biomass of zooplankton present when specific *PPr* is half of maximum and *c* is a constant defining the y-intercept.

Table II: Secchi transparency and photosynthetically active radiation (PAR) during the three 1999 microcosm experiments in Yellow Belly and Stanley lakes

Experiment	Secchi (m)	3-m PAR/day (μmol m^{-2} day^{-1})	12-m PAR/day (μmol m^{-2} day^{-1})
Yellow Belly Lake			
June	8.0	3.55×10^7	1.99×10^6
July	13.0	3.00×10^7	2.89×10^6
August	16.4	2.87×10^7	3.31×10^6
Stanley Lake			
June	3.8	3.13×10^7	1.22×10^6
July	8.0	2.37×10^7	1.02×10^6
August	9.5	2.19×10^7	1.12×10^6

PAR measurements represent the mean daily irradiance received at 3 and 12 m for the 4-day incubation period.

RESULTS

Lake profile monitoring

Although both Yellow Belly Lake and Stanley Lake develop DCL, the lakes have several important differences. Stanley Lake had lower transparency and a shallower compensation depth (1% light level) than did Yellow Belly Lake (Table II). Thermal stratification developed slowly beginning in late June; with well-developed epilimnia establishing from ~0 to 6 m. Thermal development occurred more slowly in Stanley Lake, because it receives colder inflows than Yellow Belly Lake. A system of severe thunderstorms in early August 1999 caused mixing and decreased Secchi transparency in the weakly stratified Stanley Lake. While nutrient concentrations in both lakes were low (Table III), both total and dissolved nutrient concentrations were slightly higher in Yellow

Table III: Mean nutrient concentrations for Yellow Belly and Stanley lakes during the three 1999 microcosm experiments

Experiment	Mean TN	Mean TP	Mean 3-m NH_3	Mean mid-depth NH_3	Mean low-depth NH_3	Mean 3-m SRP	Mean mid-depth SRP	Mean low-depth SRP
Yellow Belly Lake								
June	89.1 ± 20.7	2.3 ± 0.4	–	–	–	3.0 ± 0.3	2.1 ± 1.5	2.8 ± 2.0
July	66.6 ± 10.6	7.3 ± 2.8	3.4 ± 1.6	3.5 ± 2.1	10.3 ± 8.4	4.3 ± 1.1	3.8 ± 1.5	3.8 ± 3.1
August	71.9 ± 16.4	4.7 ± 2.4	0.8 ± 1.1	1.3 ±1.4	13.2 ± 2.1	1.1 ± 0.1	1.3 ± 0	1.1 ± 0.4
Stanley Lake								
June	81.0 ± 20.0	4.7 ± 1.4	–	–	–	–	–	–
July	47.9 ± 7.8	2.7 ± 0.8	1.3 ± 1.8	0.6 ± 0.8	0.9 ± 0.9	0.9 ± 0.2	0.6 ± 0.3	0.5 ± 0.1
August	50.9 ± 9.4	2.2 ± 0.3	6.8 ± 9.5	1.2 ± 1.7	0.9 ± 1.2	2.5 ± 1.2	1.9 ± 0.3	2.4 ± 0.8

The units for all values are in μg L^{-1}. Concentrations were measured at microcosm incubation depths: 3 m, mid-depth (12 m in Yellow Belly Lake and 9 m in Stanley Lake) and low-depth (18 m in Yellow Belly and 12 m in Stanley Lake). Values given for total nitrogen (TN) and total phosphorus (TP) represent means (±SD) across the three experimental depths. Ammonia (NH_3) and soluble reactive phosphorus (SRP) concentrations represent means (±SD) of two replicates per depth. –, dates for which data is not available.

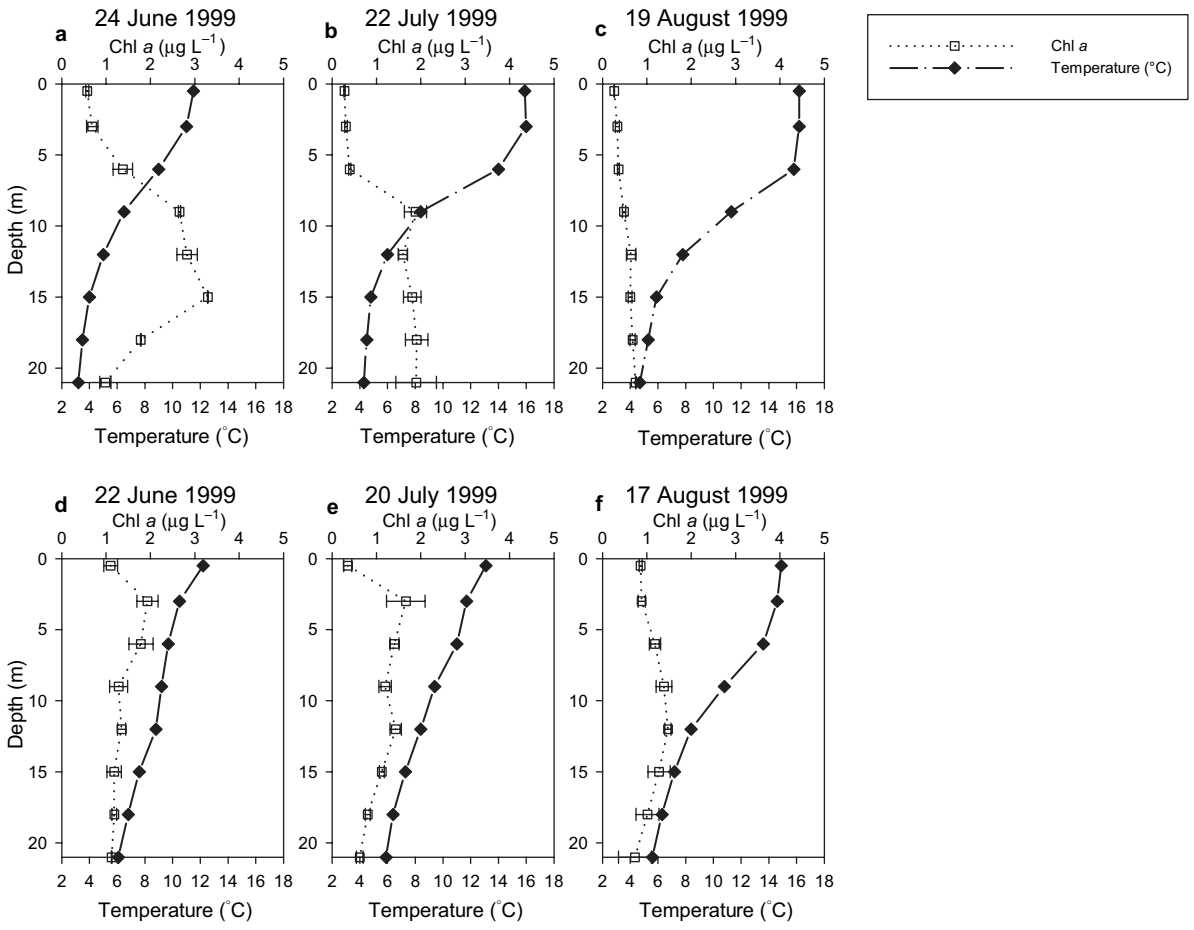

Fig. 1. Vertical profiles of chlorophyll *a* (Chl *a*) and temperature in Yellow Belly Lake (**a**–**c**) and Stanley Lake (**d**–**f**) before the June, July and August microcosm experiments. Error bars represent ±1 SD of duplicates.

Belly Lake, which supported higher Chl *a* concentrations than Stanley Lake until August, when concentrations decreased >2-fold (Fig. 1; Table IV). Primary production rates were similar for the two lakes, but peaks in production were highest near the surface in Stanley Lake and deeper (6–9 m) in Yellow Belly Lake, which sustained higher production rates later into the season (data not shown). Stanley Lake contained lower densities of smaller zooplankton than did Yellow Belly Lake (Table V).

DCL summer dynamics

Early in the summer growing season (June), the DCL was well established in Yellow Belly Lake. Chl *a* concentrations throughout the water column were highest during this time (Fig. 1a), and primary production was greatest in shallow depth strata (mean epilimnetic *PPr* = 1.12 mg C m^{-3} h^{-1} versus 0.65 and 0.10 at 12 and 18 m, respectively), despite Chl *a* peaking at 15 m (3.2 μg L^{-1}). By July, Chl *a* concentrations decreased (Fig. 1b), and primary production peaked in the metalimnion (2.11 mg C m^{-3} h^{-1} at 9 m). In August, Chl *a* levels were at their lowest

Table IV: Chlorophyll a (Chl a) and primary production (PPr) data for Yellow Belly and Stanley lakes during the three 1999 microcosm experiments

Experiment	Initial 12-m Chl *a*	3-m *PPr*	Mid-depth *PPr*	Low-depth *PPr*
Yellow Belly Lake				
June	2.5	1.21	0.65	0.10
July	1.6	0.69	0.75	0.18
August	0.7	1.27	0.52	0.14
Stanley Lake				
June	0.8	1.36	0.11	0.08
July	1.1	1.38	0.50	0.81
August	1.3	1.52	1.02	1.04

Chl *a* concentrations represent those initially present at 12 m prior to experiment start and are given in μg L^{-1}. *PPr* rates for the experimental depths were measured in the lakes, represent the mean of two replicates and are given in mg C m^{-3} h^{-1}. Mid-depth was 12 and 9 m for Yellow Belly Lake and Stanley Lake, respectively; low-depth was 18 m in Yellow Belly Lake and 12 m in Stanley Lake.

Table V: Microcosm zooplankton biomass and composition for Yellow Belly and Stanley lakes during the three 1999 experiments

Experiment	3-m zooplankton biomass (μg L^{-1})	Metalimnetic zooplankton biomass (μg L^{-1})	Hypolimnetic zooplankton biomass (μg L^{-1})	% cladoceran biomass	Mean daphnid weight (μg)
Yellow Belly Lake					
June	110 ± 36	49 ± 10	13 ± 4	25	3.58
July	168 ± 38	116 ± 12	49 ± 12	54	4.96
August	86 ± 9	92 ± 10	60 ± 17	50	5.02
Stanley Lake					
June	19 ± 4	1 ± 1	0.5 ± 0.4	32	3.25
July	–	22 ± 8	14 ± 3	29	2.94
August	–	26 ± 6	19 ± 5	52	2.77

Mean biomass estimations (±SD) are in μg L^{-1} and mean daphnid weight in μg $individual^{-1}$. –, those experiments where zooplankton data is not available due to unexplainable mortality in the 3-m treatments.

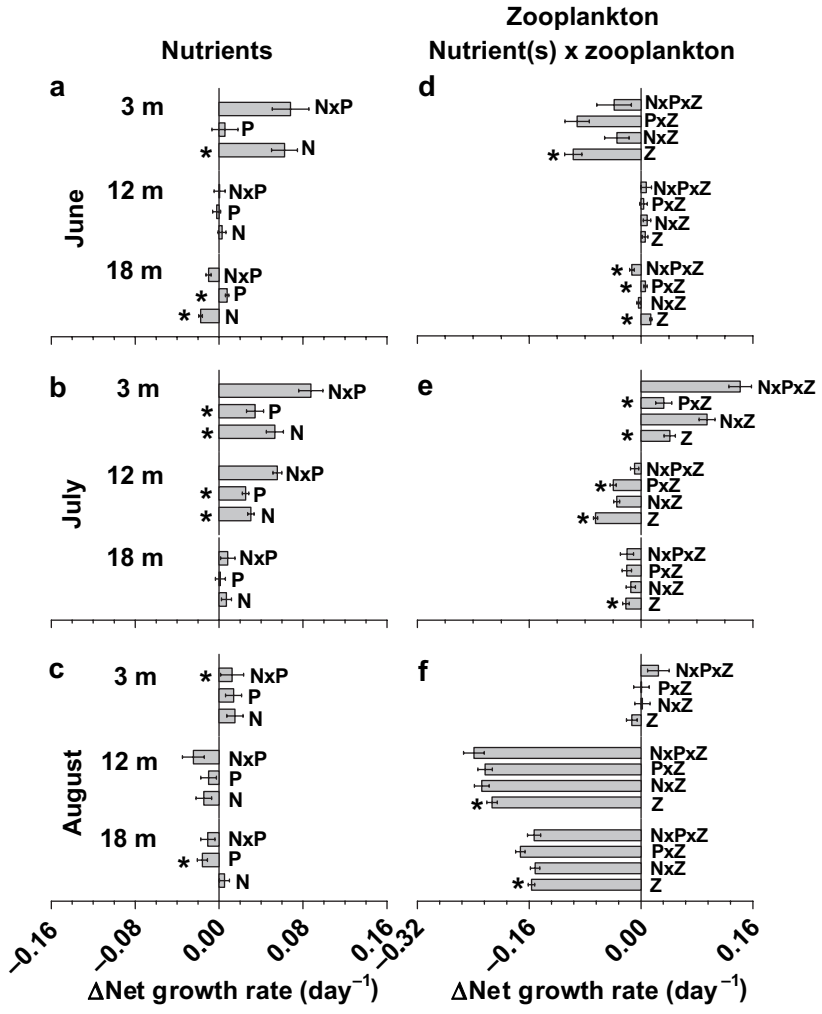

Fig. 2. Changes in Yellow Belly Lake net phytoplankton growth rates as measured by changes in chlorophyll *a* (Chl *a*) (change in net growth rate; units of day^{-1}) caused by nutrient addition (first column) and zooplankton presence and nutrient–zooplankton interactions (second column) in June (panes **a** and **d**), July (panes **b** and **e**) and August (panes **c** and **f**) microcosm experiments. Error bars show ±1 SD associated with the observed change in net growth rate. Asterisks represent significant results ($P < 0.05$) from the multifactorial, two-factor ANOVA. A significant interaction (designated by *) occurs when two treatments interact to yield a different result than would be expected based on the simple addition of the effects of the two treatments alone. Note scale differences between columns.

(<1.0 μg L^{-1}) with little remaining of a DCL (Fig. 1c). The DCL in Stanley Lake was slower to establish and was less pronounced than the Yellow Belly Lake DCL in 1999. It was not apparent until mid-July when epilimnetic Chl *a* levels and turbidity had decreased, allowing deeper light penetration and slightly higher chlorophyll levels in the metalimnion (Fig. 1d–f). Little photoinhibition of production occurred in Stanley, and *PPr* was almost always highest in the surface stratum (data not shown).

Nutrient limitation and inhibition effects

Nutrient limitation and inhibition were significant factors controlling phytoplankton growth in Yellow Belly Lake throughout the summer growing season. Molar TN : TP ratios were greatest early in stratification (102 at 3 m and 77 in the DCL during the June experiment) indicating possible P deficiency; however, in the June experiment, phytoplankton in the epilimnion were N limited. N additions significantly stimulated the algal growth rate by 150% (Fig. 2; Table VI).

Table VI: Statistical information [degrees of freedom (df), type III sums of squares, F values, P values and percent variance explained by each factor] for Yellow Belly Lake microcosm experiments based on two-factor analysis of variances (ANOVAs)

Depth (m)	Factors	*df*	Type III sums of squares	*F* value	*P* value	% variance explained
June experiment						
3	N	1	0.0156	12.76	0.0073	10.9
3	Z	1	0.0375	30.78	0.0005	56.0
3	Error	8	0.0098			15.0
12	Error	8	0.0009			75.0
18	N	1	0.0012	55.38	0.0001	27.3
18	P	1	0.0002	10.36	0.0122	4.5
18	Z	1	0.0008	35.57	0.0003	18.2
18	PxZ	1	0.0006	26.21	0.0009	13.6
18	NxPxZ	1	0.0012	52.28	0.0001	27.3
18	Error	8	0.0002			4.5
July experiment						
3	N	1	0.0113	21.36	0.0017	34.2
3	P	1	0.0047	8.94	0.0173	14.2
3	Z	1	0.0068	12.96	0.0070	20.6
3	PxZ	1	0.0029	5.47	0.0476	8.8
3	Error	8	0.0042			12.7
12	N	1	0.0037	51.19	0.0001	14.6
12	P	1	0.0026	35.67	0.0003	10.2
12	Z	1	0.0169	236.36	0.0001	66.5
12	PxZ	1	0.0014	19.15	0.0024	5.5
12	Error	8	0.0008			3.1
18	Z	1	0.0018	10.18	0.0128	41.9
18	Error	8	0.0015			34.9
August experiment						
3	NxP	1	0.0029	6.04	0.0395	30.2
3	Error	8	0.0038			40.0
12	Z	1	0.1817	410.54	0.0001	96.5
12	Error	8	0.0035			1.9
18	P	1	0.0010	5.62	0.0452	1.0
18	Z	1	0.0981	546.85	0.0001	96.6
18	Error	8	0.0014			1.4

Only factors significant at the $P = 0.05$ are shown in the table. Percent of variance explained is the sum of squares divided by the total corrected sum of squares. Abbreviations for factors are as follows: N, nitrogen addition; P, phosphorus addition; Z, zooplankton present; P×Z, phosphorus and zooplankton interaction; N×P×Z, nitrogen, phosphorus and zooplankton interaction; N×P, nitrogen and phosphorus interaction; error, amount of variance in ANOVA model attributable to error.

Adding N and P together did not provide additional stimulation beyond that of N alone. In June, nutrients did not significantly stimulate nor inhibit phytoplankton in the metalimnion. However, N inhibited and P stimulated chlorophyll production in the hypolimnion. As the summer progressed, TN : TP ratios declined and consistently indicated either N deficiency or conditions where either nutrient may have been limiting. In July, phytoplankton in Yellow Belly Lake showed strong co-limitation (almost equal and statistically significant stimulation by each nutrient) by N and P in the epilimnion and metalimnion (Fig. 2), and the combined additions of N+P markedly increased algal growth rates. This combined stimulation was not, however, significantly greater than expected based on the additive effects of N and P added individually. N and P together explained 48% of the model variance for 3 m and 25% for 12 m (Table VI). In contrast, the hypolimnion was not nutrient limited at this time. In August, N and P slightly stimulated phytoplankton growth in the epilimnion but not significantly (Fig. 2; Table VI). N and P added together at 3-m stimulated growth rates but not to the extent expected based on positive additive effects of N alone and P alone. Nutrient addition inhibited growth in the metalimnion, though not significantly; P additions significantly decreased growth of hypolimnetic phytoplankton.

The phytoplankton communities of Stanley Lake also responded to nutrient addition throughout the summer growing season (Fig. 3). Molar TN : TP ratios were intermediate (ranging from 22 to 50) throughout summer

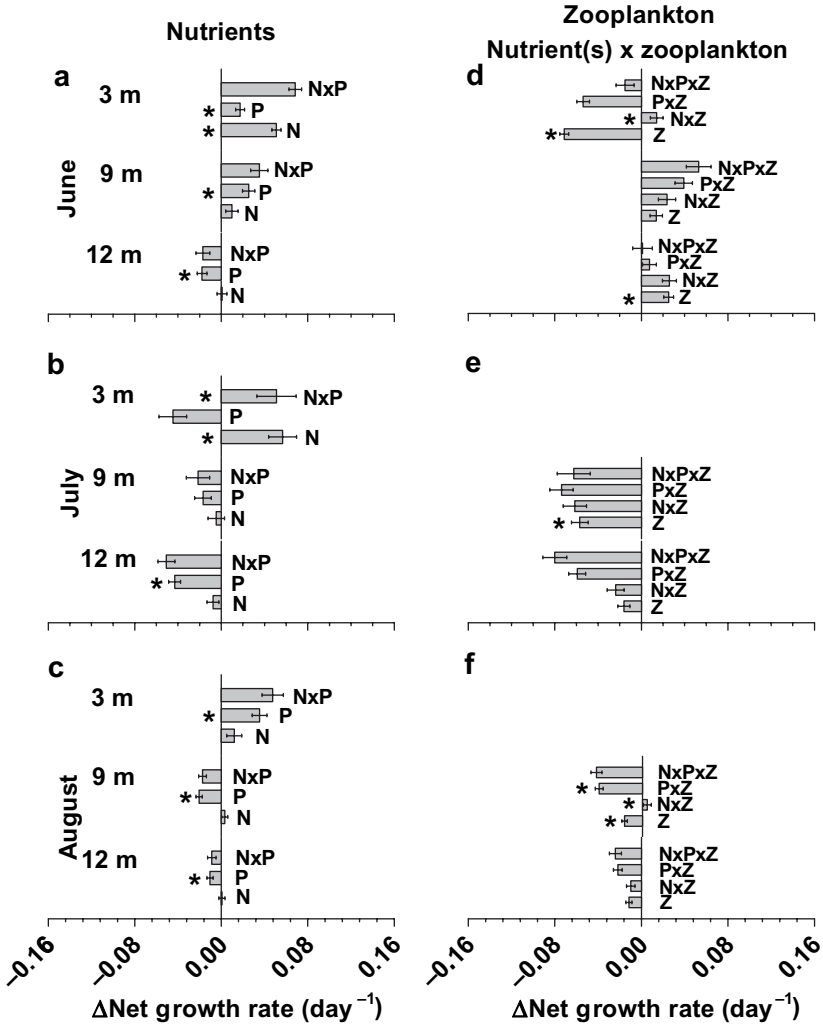

Fig. 3. Changes in Stanley Lake net phytoplankton growth rates (Δnet growth rate; units of day^{-1}) as influenced by nutrient additions (left) and zooplankton (right) in the mesocosm experiments in June (panes **a** and **d**), July (panes **b** and **e**), and August (panes **c** and **f**) microcosm experiments. See Fig. 2 caption for details. Zooplankton and nutrient × zooplankton data are not shown for 3 m July and August experiments due to zooplankton mortality in microcosms.

stratification suggesting that either N or P could have become deficient. In June, phytoplankton growth at 3 m was stimulated by both N and P addition (Table VII). N addition increased growth rates by 142% and P addition by 50%, relative to controls. Phytoplankton at 9 m were P limited, but P caused inhibition at 12 m. In July, phytoplankton growth in the epilimnion was significantly limited by N (Fig. 3), but P addition slightly decreased growth rates. Interestingly, growth rates in N+P-amended microcosms were similar to growth rates in N-amended microcosms, causing the significant N×P interaction term. Nutrients had no effect at 9 m, but once again phosphorus caused significant inhibition at 12 m. In August, P additions significantly stimulated the phytoplankton at 3 m in Stanley Lake but significantly inhibited algae at 9 and 12 m.

In Yellow Belly Lake, APA strongly increased and extended into the DCL as the summer progressed (Fig. 4a). Activity was low throughout the water column during June, but by the end of July, activity had quadrupled in surface waters. As the summer progressed, plankton deeper in the water column showed greater

Table VII: Statistical information [degrees of freedom (df), type III sums of squares, F values, P values and percent variance explained by each factor] for Stanley Lake microcosm experiments based on two-factor analysis of variances (ANOVAs)

Depth (m)	Factors	*df*	Type III sums of squares	*F* value	*P* value	% variance explained
June experiment						
3	N	1	0.0104	75.53	0.0001*	28.5
3	P	1	0.0012	8.89	0.0175*	3.3
3	Z	1	0.0203	147.41	0.0001*	55.6
3	N×Z	1	0.0024	17.79	0.0029*	6.6
3	Error	8	0.0011			3.0
9	P	1	0.0026	9.99	0.0134*	42.6
9	Error	8	0.0021			34.4
12	P	1	0.0013	7.67	0.0243*	23.6
12	Z	1	0.0026	15.51	0.0043*	47.3
12	Error	8	0.0013			23.6
July experiment						
3	N	1	0.0064	9.84	0.0349*	23.5
3	N×P	1	0.0142	21.70	0.0096*	52.1
3	Error	8	0.0026			9.5
9	Z	1	0.0129	27.73	0.0008*	62.3
9	Error	8	0.0037			17.9
12	P	1	0.0074	30.43	0.0006*	58.8
12	Error	8	0.0020			15.9
August experiment						
3	P	1	0.0025	13.10	0.0223*	56.8
3	Error	8	0.0008			18.2
9	P	1	0.0015	28.66	0.0011*	23.4
9	Z	1	0.0010	18.56	0.0035*	15.7
9	N×Z	1	0.0014	27.28	0.0012*	21.9
9	P×Z	1	0.0014	26.60	0.0013*	21.9
9	Error	8	0.0004			6.3
12	P	1	0.0004	6.10	0.0429*	26.7
12	Z	1	0.0005	7.33	0.0303*	33.3
12	Error	8	0.0004			26.7

Only factors significant at the $P = 0.05$ are shown in the table. Due to zooplankton mortality in July and August at 3 m, results reported for those depths and experiments are based on one-factor ANOVAs. Error, amount of variance in ANOVA model attributable to error; N, nitrogen addition; N×P, nitrogen and phosphorus interaction; N×Z, nitrogen and zooplankton interaction; P, phosphorus addition; P×Z, phosphorus and zooplankton interaction; Z, zooplankton present.
*$P < 0.05$.

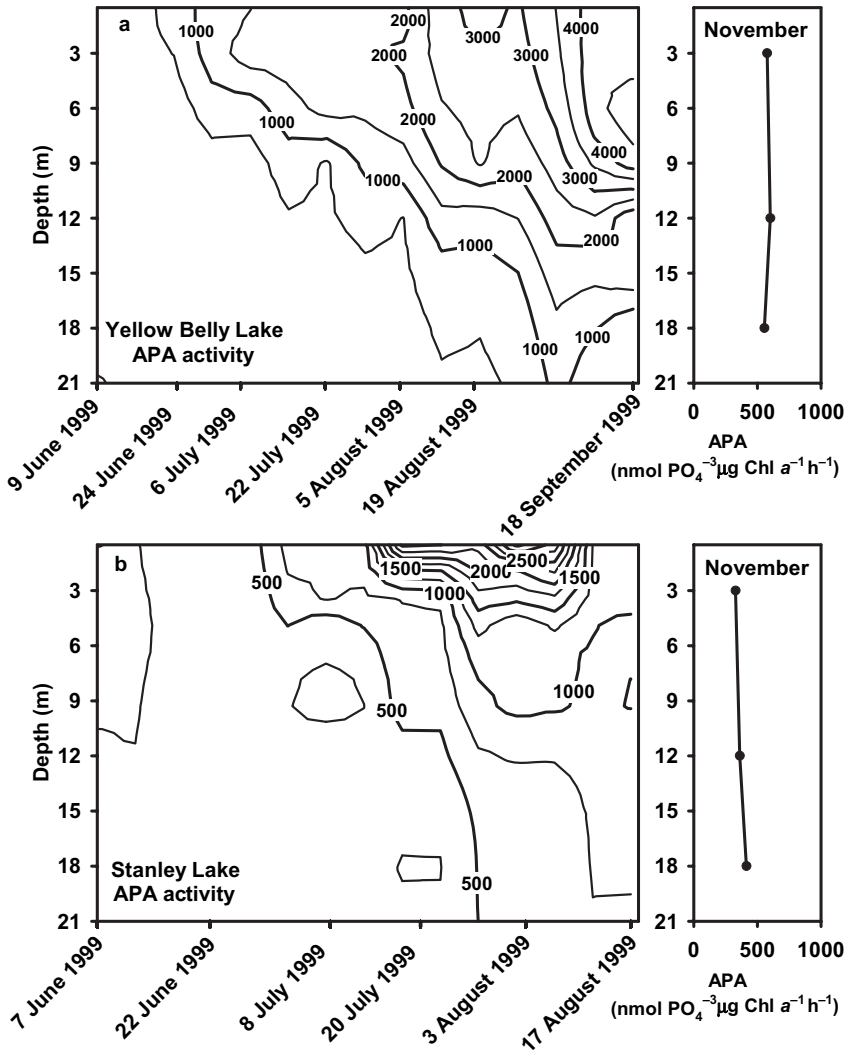

Fig. 4. Spatial and temporal trends in alkaline phosphatase activity (APA) [units of nmol PO_4^{-3} µg chlorophyll *a* (Chl a^{-1}) h^{-1}] in Yellow Belly Lake (**a**) and Stanley Lake (**b**) during the 1999 growing season until mixing in November. Isopleth lines represent an interpolated average of two assayed sites sampled on various dates in June, July, August and September. For Yellow Belly Lake and Stanley Lake data, solid lines between numbered lines represent increments of 500 and 250 nmol PO_4^{-3} µg Chl a^{-1} h^{-1}, respectively. November data are not included in the isopleth graphs but are plotted linearly.

response, although the epilimnetic community always demonstrated greatest P limitation, an effect that may be due in part to warmer epilimnetic temperatures. Despite increasing phosphatase activities, net phytoplankton growth rates did not respond to nutrient additions during the August microcosm experiment. Phosphatase activity returned to early June levels in November when mixing occurred. The two-factor ANOVA confirmed the significance of spatial and temporal patterns. Date, depth and the date–depth interaction were all highly significant ($P < 0.0001$), explaining 94% of the variance in phosphatase activity. For the entire summer, APA was greatest and did not significantly differ among plankton in the mixed layer (0.5, 3 and 6 m). Plankton at 9 m demonstrated intermediate activity, while algal communities of 12, 15, 18 and 21 m had low phosphatase activities and did not significantly differ.

APA in Stanley Lake was lower than in Yellow Belly Lake throughout the summer. Activity began to increase in surface waters down to 6 m in early July (Fig. 4b). Phosphatase activity continued to increase throughout the water column during the summer, with the plankton community exhibiting greatest P limitation in the epilimnion in early August. Epilimnetic APA levels remained elevated in mid-August when microcosm phytoplankton growth rates were also stimulated by P addition. Phosphatase activity was greatest >12 m. Date,

depth and the date–depth interaction were statistically significant factors affecting algal growth.

AERs in Yellow Belly Lake were small during summer stratification. Significant differences ($P \leq 0.05$) between N treatments and controls only occurred on June 24, August 5, September 18 and November 21 (Fig. 5a). Responses measured on June 24 in the epilimnion and on August 5 in the epilimnion and metalimnion agree closely with microcosm results, where N additions significantly increased growth rates in the same strata in the June and July experiments. AERs of Stanley Lake phytoplankton were also low. Significant responses were detected for only four of the eight sampling dates: June 22, August 3, August 17 and September 20 (Fig. 5b). These results suggest that phytoplankton were N stressed in the epilimnion and suggest the onset of N stress deeper in the water column during August. The largest responses were measured on August 17, yet N additions in the August microcosm experiments did not significantly increase net phytoplankton growth rates.

Zooplankton effects

The crustacean zooplankton biomass in Yellow Belly Lake was typically composed of 60–90% calanoid copepods *Aglodiaptomus lintoni* and *Leptodiaptomus tirrelli* and the cladoceran *Daphnia rosea*. The calanoid copepod *Epischura* cf. *nevadensis* and *D. rosea* dominated the zooplankton community of Stanley Lake, contributing 90–100% of zooplankton biomass within microcosms.

In Yellow Belly Lake, the negative effect of zooplankton grazing on algal growth shifted progressively deeper as the summer progressed (Fig. 2). Zooplankton had a

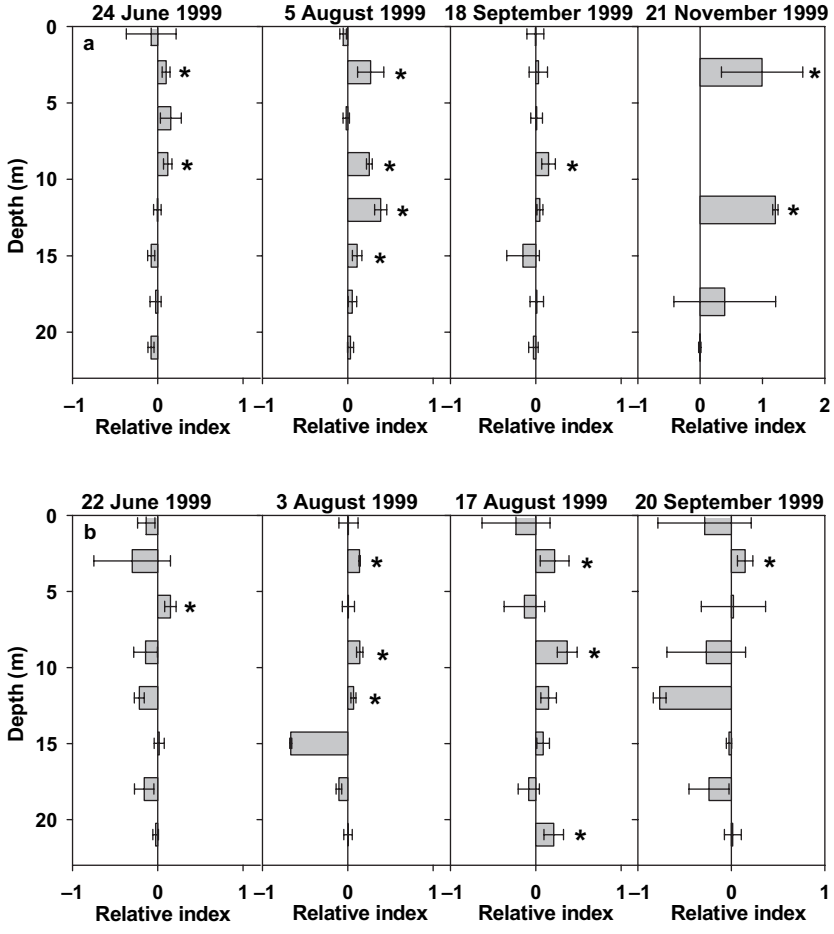

Fig. 5. Spatial and temporal trends in ammonium enhancement response (AER) in Yellow Belly Lake (**a**) and Stanley Lake (**b**) during the 1999 summer. The graph shows only those dates when significant enhancement of $P \leq 0.05$ occurred at one or more depths, although sampling dates for Yellow Belly Lake were June 9 and 24, July 6 and 22, August 5 and 19, September 18 and November 21; Stanley Lake samples were taken on June 7 and 22, July 8 and 20, August 3 and 17, September 20 and November 20. The significant differences are indicated by asterisks with positive relative index values indicating N limitation. Error bars represent ±1 SD of the relative index.

significant negative effect (Fig. 2; Table VI) on epilimnetic phytoplankton net growth rate in June. However, in the hypolimnetic strata where zooplankton biomasses were lower (Table IV), zooplankton significantly enhanced growth rates. In July, zooplankton significantly increased growth rates at 3 m but negatively affected algal growth rates at 12 and 18 m. In August, zooplankton markedly decreased algal growth at 12 and 18 m, and zooplankton biomass explained 97% of the variance in algal growth rate for both depths (Table IV). In Yellow Belly Lake, zooplankton decreased algal growth rates more often than they increased them. In most experiments, the effects of nutrients and zooplankton were additive with nutrient addition ameliorating negative zooplankton grazing effects on growth rate (Fig. 2).

Zooplankton stimulation and grazing effects were important for phytoplankton in Stanley Lake as well (Fig. 3). In June, zooplankton significantly decreased algal growth rates in the surface strata (Fig. 3; Table VII) and stimulated phytoplankton in the hypolimnetic strata. In the July and August experiments, zooplankton decreased phytoplankton net growth rates at 9 and 12 m (Table VII). As in the Yellow Belly Lake experiments, significant negative impacts occurred more frequently than significant stimulatory impacts. In June, nutrients, which were limiting in Stanley Lake surface waters, mitigated negative grazing impacts on algal growth rates (Fig. 3); however, nutrient additions often decreased phytoplankton growth in deep waters, adding to the grazing impacts.

Direct and indirect zooplankton effects

Direct grazing effects, as measured by community grazing rate (community *G*) and clearance (*CR*), were more important in Yellow Belly Lake than in Stanley Lake where zooplankton biomasses were low. In Yellow Belly Lake, community *G* was positive in six of the nine observations, with values between 16 and 243 mL L^{-1} day^{-1}, and four of the six values were highly significant (Table VIII). Additionally, the slope of the regression used to calculate *CR* was negative in six of the nine Yellow Belly observations, and five of the six *CR* values (ranging from 0.6 to 3.2 mL

Table VIII: Parameters estimating direct (grazing) and indirect (algal growth stimulation) zooplankton effects on phytoplankton net growth rate calculated from Yellow Belly Lake and Stanley Lake microcosm experiments

Experiment	Depth (m)	Community *G* (mL L^{-1} day^{-1})	*CR* (mL μg^{-1} day^{-1})	*CR* r^2 value	GR_{is} (day^{-1})	GR_C (day^{-1})	*IE* (day^{-1})
Yellow Belly Lake							
June	3	73.3[a]	0.84[b]	0.89	–0.13	–0.06	–0.07
June	12	–4.6	–0.05	0.04	0.01	0.01	0.01
June	18	15.6[c]	1.05[c]	0.98	–0.01	–0.03	0.03[c]
July	3	–35.7	–0.25	0.42	–0.26	–0.29	0.04
July	12	73.8[c]	0.62[b]	0.95	0.03	0.01	0.02[a]
July	18	23.3	0.63	0.69	–0.06	–0.05	–0.01
August	3	–3.0	–0.04	0.03	–0.09	–0.08	–0.02
August	12	243.0[c]	3.19[c]	0.99	0.08	0.03	0.06[a]
August	18	148.0[c]	1.97[b]	0.94	0.09	0.08	0.01
Stanley Lake							
June	3	53.5[c]	2.41	0.66	0.02	0.08	–0.06[c]
June	9	–8.2	–11.02	0.70	–0.02	–0.04	0.02
June	12	–14.5	–22.44	0.09	0.03	0.02	0.01
July	9	42.3	2.74	0.42	0.00	0.02	–0.02[b]
July	12	6.3	0.39	0.00	–0.12	–0.06	–0.06[a]
August	9	17.9[a]	0.67	0.57	0.04	0.05	–0.01
August	12	10.7	0.67	0.52	0.07	0.06	0.01

No results are reported for the 3-m depth in Stanley Lake in August due to unexplainable zooplankton mortality in experimental units. *CR*, clearance rate; *G*, macrozooplankton grazing rate; GR_C, net algal growth rate in the absence of zooplankton; GR_{is}, *in situ* phytoplankton growth rate; *IE*, indirect effect.
[a]Those significant at $P \leq 0.10$.
[b]*G*, *CR* and *IE* values significant at $P \leq 0.05$.
[c]Those significant at $P \leq 0.01$.

μg^{-1} day^{-1}) were statistically significant (Table VIII). Negative values of *G* and positive values of *CR* were never statistically significant. In Stanley Lake, *G* was positive (ranging from 6 to 54 mL L^{-1} day^{-1}), and *CR*s ranged from 0.4 to 2.7 mL μg^{-1} day^{-1} in five of the seven experiments (Table VIII); however, only two positive *G* values were significant. Negative *CR* values, negative *G* values and positive *CR* values were never significant. In general, grazing and community *CR* measured from Stanley Lake were lower than those estimated for Yellow Belly Lake.

The importance of direct zooplankton effect varied among depths and with season for both Yellow Belly and Stanley lakes. Early during summer stratification in Yellow Belly Lake, direct grazing effects were more important in the epilimnion than in deeper depth strata due to higher zooplankton concentrations in the epilimnion (Table V). As epilimnetic temperatures warmed and surface Chl *a* levels decreased, zooplankton biomasses increased, and the biomass peak shifted deeper in the water column (Table V), thereby increasing grazing effects at 12 and 18 m (Table VIII). Community grazing rates generally increased during the summer in Yellow Belly Lake, reaching peak levels in August at 12 m (community $G = 243$ mL L^{-1} day^{-1}). In Stanley Lake, grazing effects also became more important for phytoplankton of deeper depths later in summer stratification (Table VIII). However, no information on epilimnetic grazing was available in July and August due to zooplankton mortalities in the treatments. The cause(s) of the observed mortality are not apparent. Grazing and community *CR* remained low throughout the summer in Stanley Lake.

In the microcosm experiments, *IE* of zooplankton (algal growth stimulation due to nutrient recycling) generally slightly increased net algal growth rates in Yellow Belly Lake but never significantly stimulated growth rates in Stanley Lake experiments (Table VIII). In Yellow Belly Lake, GR_{is} was slightly greater than GR_C in four of six tests, but in only one of these cases did zooplankton nutrient recycling significantly stimulate phytoplankton growth rates (Table VIII).

In the July specific *PPr* microcosm experiments, which included several levels of zooplankton, three general trends were noted for Yellow Belly Lake: (i) Chl *a* levels declined significantly with increased zooplankton biomass (Fig. 6); (ii) primary production slightly decreased or remained about the same with increased zooplankton biomass and (iii) specific primary production followed a nonlinear curve approaching an asymptote with increased zooplankton density (Fig. 7). The algal benefit response, as measured by specific *PPr*, saturated at high biomasses of zooplankton (400 μg L^{-1} for 3 m, 162 μg L^{-1} for 12 m and 80 μg L^{-1} for 18 m). Interestingly, ambient levels of zooplankton for each depth lay on steep portions of the nonlinear functions, where specific *PPr* increased maximally per unit of zooplankton biomass. While *PPr* was highest in the metalimnion, epilimnetic and metalimnetic phytoplankton benefited about equally from recycling. At maximal zooplankton densities, grazing-induced compensatory growth doubled specific primary production of shallow and mid-depth plankton in Yellow Belly Lake. However, in Stanley Lake, maximal zooplankton biomasses (in the 4Z treatments) were only ~13% of maximal levels in Yellow Belly Lake, and neither Chl *a*, primary production nor specific primary production showed any relationship to increasing zooplankton biomass (data not shown).

Depth, nutrient and zooplankton interaction effects

Significant nutrient and zooplankton interactions were infrequent occurrences in Yellow Belly Lake, since nutrient and zooplankton effects were additive in most cases (Fig. 2). In this lake, significant interactions (three P×Z and one N×P×Z) occurred when nutrient additions stimulated algal growth rates more strongly in the absence of zooplankton than in their presence (Fig. 2, panes a and d and b and e). The three significant P×Z interactions indicated that phytoplankton were less P limited when zooplankton were present than when zooplankton had been removed.

Significant nutrient and zooplankton interactions were less frequent in Stanley Lake than in Yellow Belly Lake (Fig. 3). Significant N×Z interactions in Stanley Lake were the result of greater phytoplankton stimulation by N in the presence of zooplankton than in their absence (Fig. 3, panes a and d and c and f). Phytoplankton grew significantly faster with N addition when zooplankton were present than when zooplankton had been removed. The P×Z interaction was only significant in one of the seven cases, and in this case phytoplankton were more P inhibited when zooplankton were present than when they were removed (Fig. 3, panes c and f).

DISCUSSION

Chemical factors affecting algal growth in the DCL

Macronutrient availability is known to limit primary production in aquatic ecosystems (Elser *et al.*, 1990), and limitation is a balance between supply and demand. Nutrient limitation was an important factor restricting deep chlorophyll production in Yellow Belly and Stanley lakes, but metalimnetic phytoplankton from both lakes consistently exhibited less nutrient limitation than phytoplankton in the epilimnion (Figs 2–5). The lower nutrient demand in the metalimnion may have been due to (i) light limitation of phytoplankton in the deeper

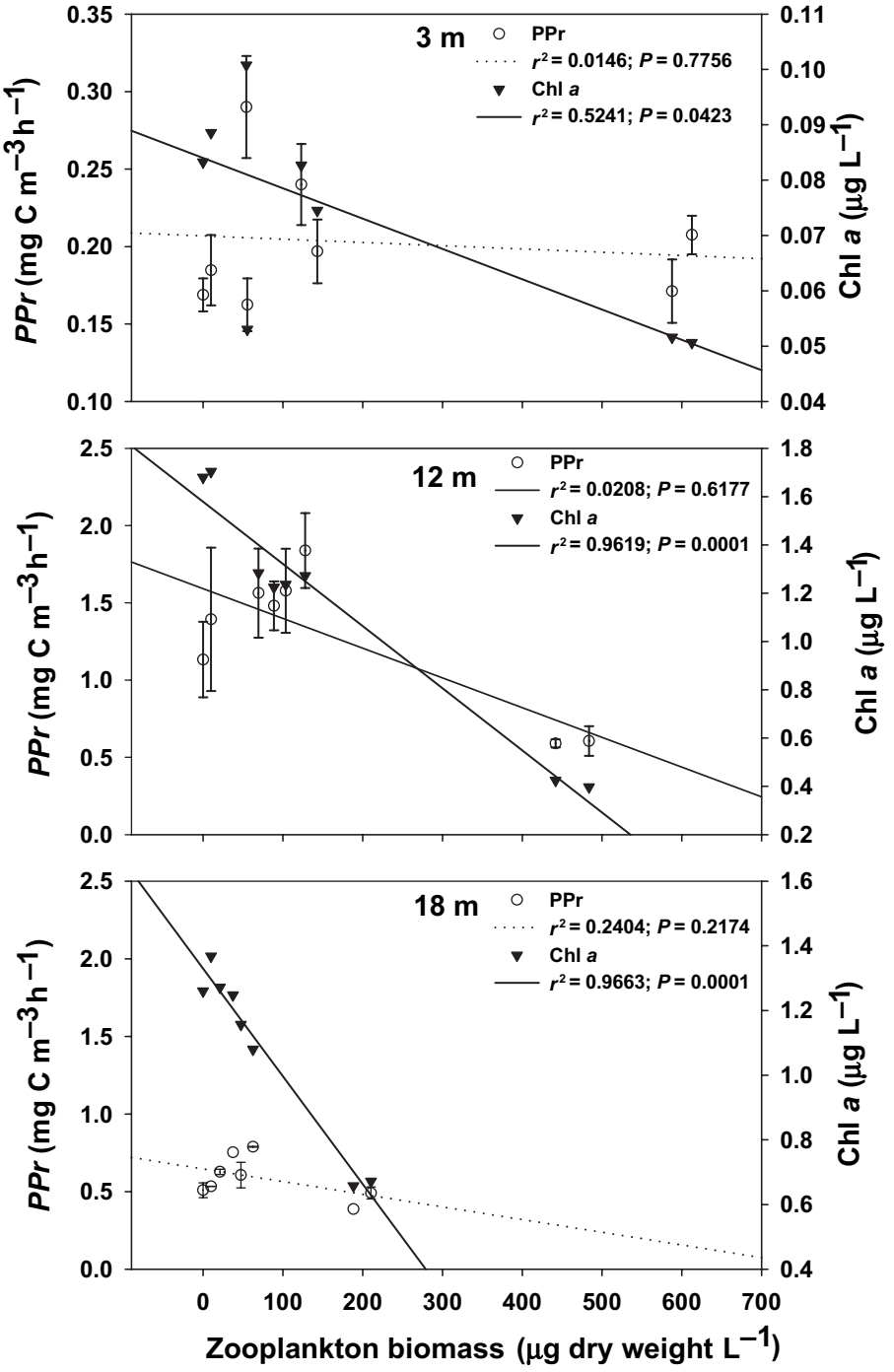

Fig. 6. Responses of primary production (*PPr*) and chlorophyll *a* (Chl *a*) to zooplankton biomass (measured in μg dry weight L^{-1}) in the specific primary production experiment in Yellow Belly Lake. *PPr* error bars represent the average of duplicates ±1 SD. Regressions significant at the $P < 0.05$ level are represented by solid lines, while nonsignificant trends are represented by dotted lines. Note differing scales for *PPr* and Chl *a* for 3-, 12- and 18-m depths.

strata (Camacho *et al.*, 2000); (ii) greater nutrient availability supplied by plunging stream inflow inputs (Vincent *et al.*, 1991; Gross *et al.*, 1998) and/or (iii) sedimentation of zooplankton excrement and decaying organisms from the surface stratum (Wetzel *et al.*, 1972; Pilati and Wurtsbaugh, 2003). Despite these additional nutrient sources, the bioassays usually indicated that the deep phytoplankton were nutrient limited. Small-scale bioassays have indicated that at times, phytoplankton in these layers can be as limited or more limited than their

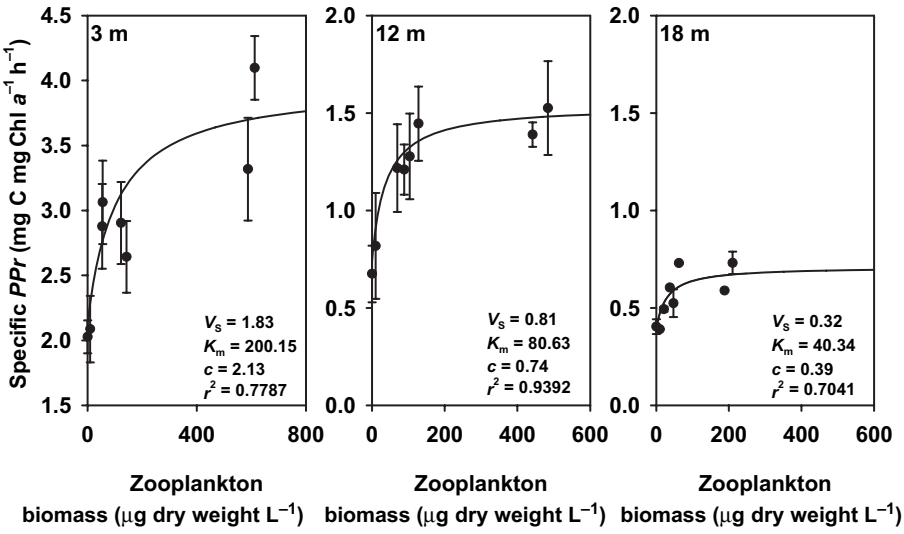

Fig. 7. Specific primary productivity (*PPr*) response to zooplankton biomass (measured in μg dry weight L^{-1}) in the specific primary production experiment in Yellow Belly Lake. The error bars are ±1 SD of the mean of duplicates. The solid line represents the Michaelis-Menton model used to fit the data [specific $PPr = (V_s \times \text{zooplankton biomass})/(K_m + \text{zooplankton biomass}) + c$]. Parameter values are shown in each frame. The r^2 values represent the square of the residuals versus predicted. Note differing scales for x- and y-axes.

epilimnetic counterparts (Wurtsbaugh *et al.*, 1997). Wurtsbaugh *et al.* (Wurtsbaugh *et al.*, 2001) also found that N+P additions to the metalimnia of mesocosms in another Sawtooth Mountain lake significantly stimulated algal growth, so it appears that phytoplankton in the DCL of some lakes in this region are nutrient limited, at least for portions of the summer growing season.

P addition significantly inhibited algal production at certain times of the season in both study lakes. P inhibition occurred only once in Yellow Belly Lake (in the hypolimnion) and four times in Stanley Lake, decreasing growth rates by as much as 5% per day. This peculiar result was possibly due to competition for phosphorus between phytoplankton and bacteria, which are present in higher numbers in Stanley Lake than in Yellow Belly Lake (unpublished data). Bacteria may have uptake advantages compared with phytoplankton because of smaller surface area to volume ratios (Fuhs *et al.*, 1972). Stimulation of bacteria by P may have allowed the bacteria to out compete algae for a secondary nutrient such as N or micronutrients, which were previously reported to secondarily limit algal growth in the lakes (Wurtsbaugh *et al.*, 1997). Rhee (Rhee, 1972) found that algal growth can be severely restricted in the presence of bacteria when P is limiting. APA data confirm that the planktonic communities in the lakes consistently produced phosphatases in order to compete in a P-limited environment, but this assay does not differentiate between algae and bacteria. A particularly interesting nutrient inhibition result occurred in the July Stanley Lake 3-m treatments when P alone inhibited algal growth, but the N+P treatments exhibited growth rates almost equal to those observed in the N-amended treatments (Fig. 3). This result suggests that heterotrophic bacteria may have been P limited, assuming that they were not carbon limited. When P was added exclusively, bacteria may have ceased to be P limited and with their superior N-uptake efficiency could have out competed phytoplankton in the N-deficient environment. When both nutrients were added together, the N-limited phytoplankton did not have to compete with the P-limited bacteria and grew almost as rapidly as in the N-amended treatments.

Yellow Belly Lake and Stanley Lake responded differently to macronutrient addition during the summer growing season. In Yellow Belly Lake, epilimnetic phytoplankton were primarily limited by N, although significant P stimulation often occurred concurrently (Fig. 2). Nutrient limitation increased from June to July but not from July to August, suggesting that some other factor(s) was controlling phytoplankton growth during the late summer. Possibilities include (i) micronutrient limitation (Wurtsbaugh *et al.*, 1997); (ii) grazing mortality, which increased dramatically between July and August; (iii) strongly nutrient-limited heterotrophic bacteria may have been primarily P limited but secondarily N limited and consequently have been out-competing phytoplankton for both nutrients and (iv) extremely low initial August concentrations of chlorophyll (<1 μg L^{-1} throughout the water column and ~0.2 μg L^{-1} near the surface) may not have allowed for a measurable response during the 4-day

incubation period. Even though phytoplankton growth rates did not respond to nutrient additions in August, AER and particularly APA activity results indicated that N and P demand remained high during August and September. To provide perspective, APA levels in the Stanley lakes were ~10 times those reported by St. Amand (St. Amand, 1990) for two mesotrophic lakes (150 nmol PO_{4-3} μg^{-1} Chl *a* h^{-1}). The August AER and APA data and TN : TP ratios suggest that the phytoplankton may have been primarily N limited (Fig. 5) and most of the observed APA may have been due to heterotrophic bacteria. In Stanley Lake, a shift from N- to P-limitation occurred between the July and late August experiments, underscoring the case for co-limitation (Fig. 3). However, during this same period, APA activity decreased by two-thirds (Fig. 4b) probably due to strong thunderstorms which caused mixing in the weakly stratified water column (Fig. 1) and brought nutrients into the lake. Nutrient addition stimulated epilimnetic algae in Stanley about equally throughout the summer, but phytoplankton in the DCL actually demonstrated less N- and P-limitation in the nutrient addition bioassays as the summer progressed, despite increased light penetration into the metalimnion (Table V).

The magnitude of limitation responses exhibited by phytoplankton in Yellow Belly Lake and Stanley Lake also differed. In the microcosm experiments, Stanley Lake phytoplankton did not respond to nutrient addition as strongly as did Yellow Belly Lake phytoplankton. Nutrients stimulated growth rates up to 9% per day in Yellow Belly Lake and 7% per day in Stanley Lake. Additionally, Stanley Lake plankton never demonstrated the degree of APA activity or AER demonstrated by Yellow Belly Lake algae. This is consistent with the lower transparency of Stanley Lake, which may have resulted in light limitation of algal growth early in the summer. Stanley Lake also has a higher watershed to lake area ratio (49:1) than does Yellow Belly Lake (42:1), meaning that larger amounts of nutrients in runoff are concentrated into a unit of lake surface area. Yellow Belly Lake also has two large lakes above it in the watershed, and these can trap nutrients (Wurtsbaugh *et al.*, 2005). Autumnal mixing decreased APA levels to baseline (~500 nmol PO_{4-3} μg Chl a^{-1} h^{-1}) in both lakes, but Yellow Belly Lake phytoplankton continued to respond to NH_{4+} addition (Fig. 5a), suggestive of a strongly N-limited system. These results contrast with those of Wurtsbaugh *et al.* (Wurtsbaugh *et al.*, 1997), who found that Stanley Lake phytoplankton were more nutrient limited than those in Yellow Belly Lake during 1992. A possible reason for this difference may be due to a massive landslide that occurred between 1992 and 1998 in the Stanley Lake watershed resulting in decreased average summer Secchi transparencies from 9.1 m in 1992 to 7.1 m in 1999. This possibly increased nutrient loading and light limitation, thereby decreasing both nutrient limitation and demand.

Zooplankton control of algal growth in the DCL

Crustacean zooplankton grazing strongly impacted phytoplankton growth in both Yellow Belly and Stanley lakes. Community grazing rate estimates showed that zooplankton could filter up to 24% of lake water per day or 96% of the volume contained in a microcosm during the course of the 4-day experiment. In general, early in stratification zooplankton grazing limited epilimnetic production in both lakes (Figs 2 and 3). Later in stratification, when herbivores moved deeper in the water column, grazing limited metalimnetic growth rates, while being less important for the epilimnion (Table VI). This shift in phytoplankton response may have been due to one or more of the following factors: (i) decreased grazing in the epilimnion when food became inadequate there and (ii) increased diel vertical migration and consequent feeding in the metalimnion. Although food quality in the Stanley lakes is lower in the metalimnion (Cole *et al.*, 2002), feeding in both zones may have provided complimentary nutrient resources (DeMott, 1998) for the zooplankton; and (iii) loss of trophic coupling (i.e. strength of predatory effect) in the dilute (<1 μg L^{-1} Chl *a*) epilimnion (Elser and Goldman, 1991). Additionally, community composition in both lakes shifted toward more efficient grazers (i.e. Daphnids) (Haney, 1973; Cyr and Pace, 1992), inducing greater grazing pressure later in the summer.

Yellow Belly Lake phytoplankton were more heavily impacted by direct zooplankton grazing than were Stanley Lake phytoplankton. Herbivory decreased algal growth rates by up to 25% per day in Yellow Belly Lake as compared with a maximum of 7% per day in Stanley Lake. Additionally, Chl *a* concentration was inversely related to zooplankton biomass in Yellow Belly Lake (Fig. 6), but these parameters showed no relationship in Stanley Lake. Presumably, direct effects were more important in Yellow Belly Lake because of more abundant and larger zooplankton and a greater proportion of *Daphnia* than in Stanley Lake.

Zooplankton also indirectly influenced phytoplankton growth, particularly in Yellow Belly Lake. Zooplankton stimulated algal growth slightly in the hypolimnion during the June experiment and strongly in the epilimnion during the July experiment when nutrient additions also stimulated algal growth (Fig. 2), suggesting that zooplankton may have supplied a limiting nutrient. Significant nutrient–zooplankton interactions, which

occurred when nutrient additions stimulated algal growth rates more strongly in the absence of zooplankton than in their presence, also suggest that zooplankton were regenerating nutrients and partially relieving algal nutrient limitation. Most significant nutrient–zooplankton interactions occurred in July in Yellow Belly Lake (Fig. 2) when nutrient demand and grazing pressure were high. In both study lakes, the significant interactions indicated that zooplankton were recycling P and/or releasing a low ratio of N : P. Such patterns are characteristic of lakes where copepods contribute a significant portion of the community biomass (Sterner *et al.*, 1992; Elser *et al.*, 1996). Copepods were an important component of the zooplankton community in both Yellow Belly and Stanley lakes, contributing as much as 75% of the total biomass in cubitainers.

Calculation of zooplankton *IE* and results from the specific primary production (*PPr*) experiment also suggest that nutrient recycling affects phytoplankton growth in Yellow Belly Lake. *IE* calculated from microcosm experiment data were generally positive though small for the individual treatments at each depth (Table VIII). Zooplankton in the microcosm treatments though were prevented from migrating to other depth strata. Specific *PPr* increased with zooplankton biomass in July (Fig. 7) and zooplankton stimulated epilimnetic phytoplankton growth in July (Fig. 2). Taken together, these results suggest that zooplankton do transport some nutrients from deep layers to the highly dilute epilimnion. The mechanism whereby zooplankton stimulate production particularly in the epilimnion may be upward nutrient transport rather than grazing and recycling in the same stratum.

In interpreting the zooplankton grazing data, it is important to recognize that microzooplankton <80 μm were present in all microcosms, and our experiments did not address their impacts on algal growth. Elser and Frees (Elser and Frees, 1995) showed that microconsumers are an important part of the food web, especially in oligotrophic systems. Because large zooplankton can negatively affect microzooplankton density and grazing rate (e.g. Scavia and Fahnenstiel, 1988), microzooplankton grazing may have been greater in treatments lacking macrozooplankton, thereby causing an underestimation of macrozooplankton grazing effects.

DCL dynamics—formation and maintenance mechanisms

Phytoplankton densities in Yellow Belly Lake were the result of a dynamic balance between production and loss rates. Early in the summer, zooplankton effectively depleted phytoplankton in the epilimnion (Figs 2 and 3), but macrozooplankton biomasses in the meta- and hypolimnion were likely insufficient to affect phytoplankton abundance. Our results are consistent with those of Pilati and Wurtsbaugh (Pilati and Wurtsbaugh, 2003) who conducted a mesocosm experiment in Yellow Belly Lake and found that zooplankton grazing in the epilimnion decreased phytoplankton abundance and increased nutrient transport to the metalimnion. Lampert and Grey (Lampert and Grey, 2003) also found that Daphnia preferentially graze in the warm epilimnion, even though food resources may be lower than in the metalimnion. By July, epilimnetic food quantity had decreased, and low Chl *a* (< 0.5 μg L^{-1}) and particulate organic carbon levels (8–12 μg C L^{-1}) in the epilimnion (Cole *et al.*, 2002) likely could not support zooplankton growth. Lampert (Lampert, 1977), e.g., showed that threshold concentrations of POC permitting *Daphnia pulex* growth ranged from 40 to 120 μg C L^{-1}, considerably greater than levels found in the epilimnion of the study lakes by midsummer. Zooplankton community composition shifted toward higher percentages of efficient grazers, and zooplankton began to heavily graze the DCL (Figs 2 and 3), the zone of greatest standing algal biomass. This argument is consistent with the results of Williamson *et al.* (Williamson *et al.*, 1996) who found that metalimnetic food supported greater zooplankton growth and reproduction than did food collected from the epilimnion. By August in Yellow Belly Lake, zooplankton grazing had little effect on epilimnetic algal growth, and rates of algal production were primarily controlled by nutrient availability. Production was limited by nutrients in the epilimnion and was fueled to some extent by zooplankton nutrient regeneration that compensated for consumption losses. Taken together, the results indicate that the spatiotemporal shifts in zooplankton grazing played an important role in Yellow Belly Lake DCL formation and persistence.

Mechanisms for DCL formation in the two lakes seem different. Based on the data, net *in situ* production fueled by adequate light penetration and a low but continually recycled nutrient pool resulted in the Yellow Belly Lake DCL. As the summer progressed, phytoplankton in Yellow Belly Lake experienced heightened nutrient demand (Fig. 4a) and grazing losses (Fig. 2; Table VIII), factors that may have led to the eventual collapse of the DCL (Fig. 1). In contrast, the Stanley Lake metalimnion was not the ideal stratum for production until August after light penetration had increased. It is likely that DCL establishment in August was the result of greater light and nutrient availability both for cells actively growing there and for cells that passively settled from earlier epilimnetic growth. Nutrient stress and zooplankton grazing pressure were lower in Stanley Lake than in Yellow Belly Lake, and

consequently water column chlorophyll concentrations did not decline during summer stratification.

Our results support those of Elser and Goldman (Elser and Goldman, 1991), who proposed that the degree of coupling (i.e. the strength of predatory impacts) between trophic groups varies among lakes of differing productivities. In Yellow Belly Lake and Stanley Lake, productivity varied with depth and season. The epilimnetic strata of the lakes later in the season (August) were similar in trophic state to Lake Tahoe with Chl *a* concentrations <1 μg L^{-1} (Elser and Goldman, 1991). In these dilute zones, trophic groups were weakly coupled due to grazing-induced compensatory growth and food quantity limitation. This lack of coupling has been called a 'trophic bottleneck' (Neill, 1988; Elser and Goldman, 1991), because the predator fails to deplete its prey. Conversely, the metalimnetic strata in this study were similar in trophic state to Castle Lake, which was also studied by Elser and Goldman (Elser and Goldman, 1991) with Chl *a* levels 1–4 μg L^{-1}. In the metalimnia where nutrient demand was lower, phytoplankton were plentiful, so tight coupling between the zooplankton and phytoplankton occurred. Algae in the DCL also benefited from internal nutrient cycling. Predator–prey coupling may have been strong enough to diminish DCL Chl *a* levels to <1 μg L^{-1} in late season (Fig. 1).

In conclusion, our results suggest that depth-differential nutrient demand and herbivore grazing and nutrient cycling processes interact to determine phytoplankton distribution in Yellow Belly Lake and Stanley Lake. These depth-differential processes, which ultimately control net production, provide a unique phytoplankton habitat in the metalimnion.

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