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# Temporal and vertical dynamics of phytoplankton net growth in Castle Lake, California

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**Abstract.** The impact of nutrient additions, zooplankton grazing and light intensity on phytoplankton net growth with depth and season was studied with five microcosm experiments in meso-oligotrophic, subalpine Castle Lake, California, during the period of summer stratification in June–September 1994. The incubations (4 day) were performed at 5 m intervals from the surface to the bottom using natural phytoplankton and zooplankton assemblages, with enrichments of phosphorus and nitrogen. The phytoplankton community was only limited by nutrients in the upper 5 m (epilimnion), as indicated by change in chlorophyll concentration. Nutrient enrichments had the greatest effect on the phytoplankton net growth in June and July. High light inhibited the phytoplankton net growth at the surface. Low light intensities limited phytoplankton at 20 m and below, and at the end of the growing season already around 10–15 m. A deep chlorophyll maximum in the hypolimnion in June–August was not limited by either light or nutrients. The results showed variation in grazers' impact on phytoplankton. These results suggest the importance of nutrient limitation only in the epilimnion with light inhibition at the surface, light limitation in the hypolimnion, and varying impact of zooplankton grazing in influencing the development of the phytoplankton in Castle Lake.

## Introduction

Phytoplankton dynamics in freshwater ecosystems are affected by complex interactions between phytoplankton, zooplankton and other processes in the food web, as well as numerous physical and chemical factors. The impact of zooplankton grazing on phytoplankton can be either negative (reduction of algal biomass) or positive (stimulation of growth through nutrient recycling) (Bergquist and Carpenter, 1986; Sterner, 1986). The zooplankton–phytoplankton interactions are species specific (Lehman and Sandgren, 1985; Bergquist and Carpenter, 1986; Elser *et al*., 1987) and are affected by lake trophic status, with the strongest coupling between phytoplankton and zooplankton seen in lakes with intermediate productivity (Elser and Goldman, 1991). The ability of phytoplankton to grow is dependent on adequate light intensity and the availability of nutrients, which again depends on external loading of nutrients (Goldman, 1981) and internal processes within the lake (Elser *et al*., 1988). Temporal (interannual and intra-annual) and spatial variations in environmental conditions complicate the understanding and interpretation of these interactions, and patterns controlling phytoplankton growth.

Algal production has been measured in meso-oligotrophic, subalpine Castle Lake, California, for 39 years (Goldman and De Amezaga, 1984). Based on these

long-term data, interannual variation in primary productivity has been associated with climatic conditions (Goldman *et al*., 1989), as well as with cascading trophic interactions and direct physical effects (Jassby *et al*., 1990). Extensive research has been conducted on the role of grazers and nutrients on phytoplankton in the epilimnion and mixed layer of Castle Lake (e.g. Redfield, 1980; Elser and Goldman, 1990, 1991; Elser, 1992; Brett *et al*., 1994; Elser *et al*., 1995). Although deep chlorophyll and productivity maxima in Castle Lake are well documented (Priscu and Goldman, 1983; Jassby *et al*., 1990), less attention has been paid to factors controlling phytoplankton growth below the epilimnion.

In the present study, the dynamics of phytoplankton net growth (as chlorophyll *a*) in the whole water column at different times of the growing season were studied in Castle Lake, California. The main objective was to explain the vertical and temporal *in situ* dynamics of phytoplankton net growth, i.e. where in the water column and when during the growing season the phytoplankton are susceptible to nutrient limitation, zooplankton grazing, and light limitation or inhibition. These questions were studied with five 4-day microcosm experiments, performed at 5 m intervals from the surface to the bottom. Ambient phytoplankton and zooplankton assemblages were used, with enrichments of phosphorus and nitrogen.

## Method

### *Study site and experiments*

Castle Lake is a small (0.20 $km^2$) subalpine lake with a maximum depth of 35 m and a mean depth of 11.4 m. It is located in the Siskiyou mountains of northern California, USA (41°13′N, 122°22′W), at an elevation of 1657 m. According to earlier studies, phytoplankton growth in meso-oligotrophic Castle Lake is often limited by nutrients (Goldman and De Amezaga, 1984).

During the summer of 1994, five microcosm experiments designed to examine vertical effects of nutrients, zooplankton and light intensity on phytoplankton net growth were performed in Castle Lake at a central deep-water sampling station with a total depth of ~35 m. In order to describe the time scale of these events, the experiments covered most of the growing season: 21–25 June (experiment 1), 11–15 July (experiment 2), 11–15 August (experiment 3), 27–31 August (experiment 4) and 14–18 September (experiment 5). Lake water was collected in 10 l polyethylene containers using a Van Dorn sampler at depths of 0, 5, 10, 15, 20, 25 and 30 m, with the exception of the first experiment in which the depths 15 and 25 m were not sampled. Owing to strong diel vertical migration of zooplankton in Castle Lake (Redfield and Goldman, 1978), water samples were taken both at night and in the morning so that zooplankton abundance and species composition would be representative of a daily average. The samples taken at night preceding the experiment were kept *in situ* until pooled with the samples taken in the morning from the same depth.

Phytoplankton net growth, using chlorophyll *a* concentration as an estimate of biomass, was studied using four different treatments: control (C; zooplankton removed and no nutrients added); zooplankton (Z; no zooplankton removed and no nutrients added); nutrients (N; zooplankton removed and nutrients added);

zooplankton and nutrients (ZN; nutrients added and no zooplankton removed). The vertical effects of light intensity (L) on phytoplankton net growth were studied through treatments similar to controls except for incubation depths. Each treatment was executed in duplicate.

Phosphorus (as $K_2HPO_4$) and nitrogen (as $NH_4NO_3$) were added to nutrient-enriched treatments (N, ZN) at concentrations of 50 µg P $l^{-1}$ (1.6 µM) and 300 µg N $l^{-1}$ (21.4 µM), which are based on previous studies in Castle Lake (Elser *et al.*, 1995). Crustacean zooplankton were removed by filtering the water through 83 µm mesh. Removal of large phytoplankton while removing zooplankton was determined by measuring initial chlorophyll concentrations both from filtered and unfiltered lake water. In two-thirds of the initial samples, filtering removed ≤10% of phytoplankton, and in less than one-third 11–30% of phytoplankton were removed. In two samples, removal of phytoplankton was higher (41% in experiment 5 at 30 m, 53% in experiment 4 at 25 m). This removal of phytoplankton was corrected by comparing filtered initial chlorophyll concentrations with filtered final concentrations (treatments C, N, L) when calculating net growth rates. Accordingly, unfiltered initial chlorophyll concentrations were used when calculating net growth rates for treatments (Z, ZN) with no removal of zooplankton.

To characterize phytoplankton and crustacean zooplankton abundance and species composition, samples for enumeration were taken at the beginning of each experiment. Phytoplankton samples were preserved with Lugol's solution for later identification and enumeration using inverted microscopy (Utermöhl, 1958). Volumes used in estimating phytoplankton biomass were based on measured dimensions of cells and colonies, and approximations of their geometric shape, as well as on values previously used for Lake Tahoe (D.Hunter, unpublished data) and Castle Lake (P.Arneson, unpublished data) phytoplankton. Zooplankton samples were taken by pouring 10 l of pooled sample water through an 83 µm mesh screen, and preserved with Lugol's solution and sucrose. Juveniles and adults were enumerated under a dissecting microscope and biomass calculated using dry masses determined previously for Castle Lake zooplankton (Redfield, 1979).

Samples (treatments C, N, Z, ZN) were incubated at their original depths *in situ* for ~4 days in 1 l acid-washed transparent polyethylene bottles. In the light treatment (L), the samples from different depths were displaced and incubated at 5 m in a separate rack close by. Based on the routine monitoring data collected by the Castle Lake Research Group at times (30 June, 14 July, 12 August, 25 August, 15 September) representing the experimental incubations, intensities of photosynthetically active radiation measured with a submersible Li-Cor quantum sensor at 5 m were 486, 408, 245, 229 and 128 µE $m^{-2}$ $s^{-1}$ (24, 28, 17, 17 and 12% of the surface light), and corresponding Secchi disc transparencies were 10.3, 8.9, 6.4, 6.1 and 4.0 m. After incubation, a 100 ml subsample from each bottle was filtered onto a Whatman GF/C filter. Filters were kept frozen until chlorophyll *a* was extracted with methanol for 24 h at 4°C in the dark (Marker *et al.*, 1980). Chlorophyll *a* was measured using the fluorometric method with acid correction for phaeophytin (Strickland and Parsons, 1972).

*Physical and chemical characteristics of the lake water*

The routine monitoring data collected by the Castle Lake Research Group at a central deep-water sampling station were used to describe the physical and chemical characteristics of the lake water. The data from dates around the times of each experiment were considered representative of the experimental conditions. The first experiment (21–25 June) was conducted nearest to the monitoring of 30 June, and the fourth experiment (27–31 August) nearest to the monitoring of 25 August. The monitoring samplings of 14 July, 12 August and 15 September coincided with the second (11–15 July), the third (11–15 August) and the fifth (14–18 September) experiment.

Vertical temperature profiles were measured with a YSI temperature–oxygen meter. Samples for nutrient analysis were pre-filtered through a Whatman GF/C filter. Soluble reactive phosphorus (SRP) was analyzed using the acid molybdate technique (American Public Health Association, 1992), nitrate ($NO_3$-N) concentration with the hydrazine reduction method (Kamphake *et al*., 1967) and ammonium ($NH_4$-N) concentration with the phenol hypochlorite method (Solórzano, 1969).

*Data analysis*

Daily rates of net growth ($day^{-1}$) were calculated from $r = \ln(C_t/C_0)/t$, where $C_t$ is the final chlorophyll *a* concentration, $C_0$ is the initial chlorophyll *a* concentration (from filtered or unfiltered samples, depending on treatment) and $t$ is the duration of the experiment (in days). The net growth rates are expressed relative to the controls.

The significance of the effects of depth, nutrients, zooplankton, and their interactions, was assessed individually for each experiment using a three-factor analysis of variance (ANOVA). Statistical significance was set to $P < 0.05$. Phytoplankton net growth calculated as chlorophyll *a* was used as the main response variable. Since an overall ANOVA summarizes the data from all the depths in each experiment, it does not show where in the water column the effects occurred. Therefore, graphical interpretation was used to outline the vertical variation in treatment effects, and to locate where the effects were the most pronounced. In order to examine the relationships between some variables, linear regressions were calculated. The results of light intensity experiments were not tested statistically.

## Results

*Plankton dynamics*

The lake was thermally stratified throughout the study period (Figure 1). In the epilimnion, chlorophyll concentrations increased towards the end of the summer (Figure 2). A deep chlorophyll maximum had already developed by the time of the first experiment in June. It persisted around 15–20 m in July and August, and disappeared by the last experiment in September. In the deep basin, chlorophyll concentrations remained low throughout the summer (Figure 2).

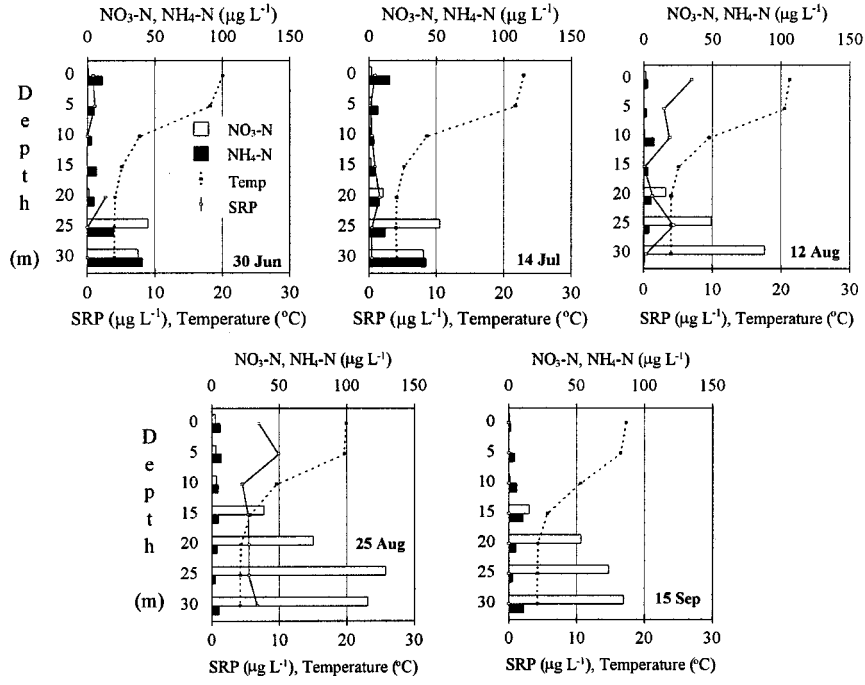


**Fig. 1.** Vertical profiles of temperature, nitrate ($NO_3$-N), ammonium ($NH_4$-N) and soluble reactive phosphorus (SRP) in Castle Lake during summer 1994 (routine monitoring data collected by the Castle Lake Research Group).

Initial phytoplankton biomass followed the vertical profile of chlorophyll *a* ($r^2 = 0.72$, $P < 0.001$, $n = 33$), and maximum algal biomasses were also found at 15–20 m in July and August (Figure 2). This maximum was generally formed by diatoms, large dinoflagellates, cryptomonads and chrysophytes. The epilimnetic phytoplankton community was dominated by green algae and chrysophytes in June, and chrysophytes together with centric diatoms and colonial blue-green algae in July. In August and September, the epilimnion was characterized by colonial blue-green algae (e.g. *Aphanocapsa* spp., *Chroococcus* spp.). In June and July, the hypolimnion was dominated by cryptomonads, large dinoflagellates and diatoms and, later in the season, the deep basin was characterized mainly by diatoms (Figure 2).

In early summer, the most abundant zooplankton taxon in the epilimnion was the filter-feeding cladoceran *Holopedium* sp., which decreased in numbers in August, and the raptorial cyclopoid copepod *Diacyclops* sp. provided a major part of the biomass in the deeper water column. In August and September, the zooplankton community was mainly dominated by the filter-feeding cladoceran *Daphnia* sp. The numbers of the cladoceran *Diaphanosoma* sp. increased in the epilimnion at the end of the summer. The filter-feeding and raptorial calanoid copepod *Diaptomus* sp. accounted for a small portion of the biomass throughout the summer (Figure 2).

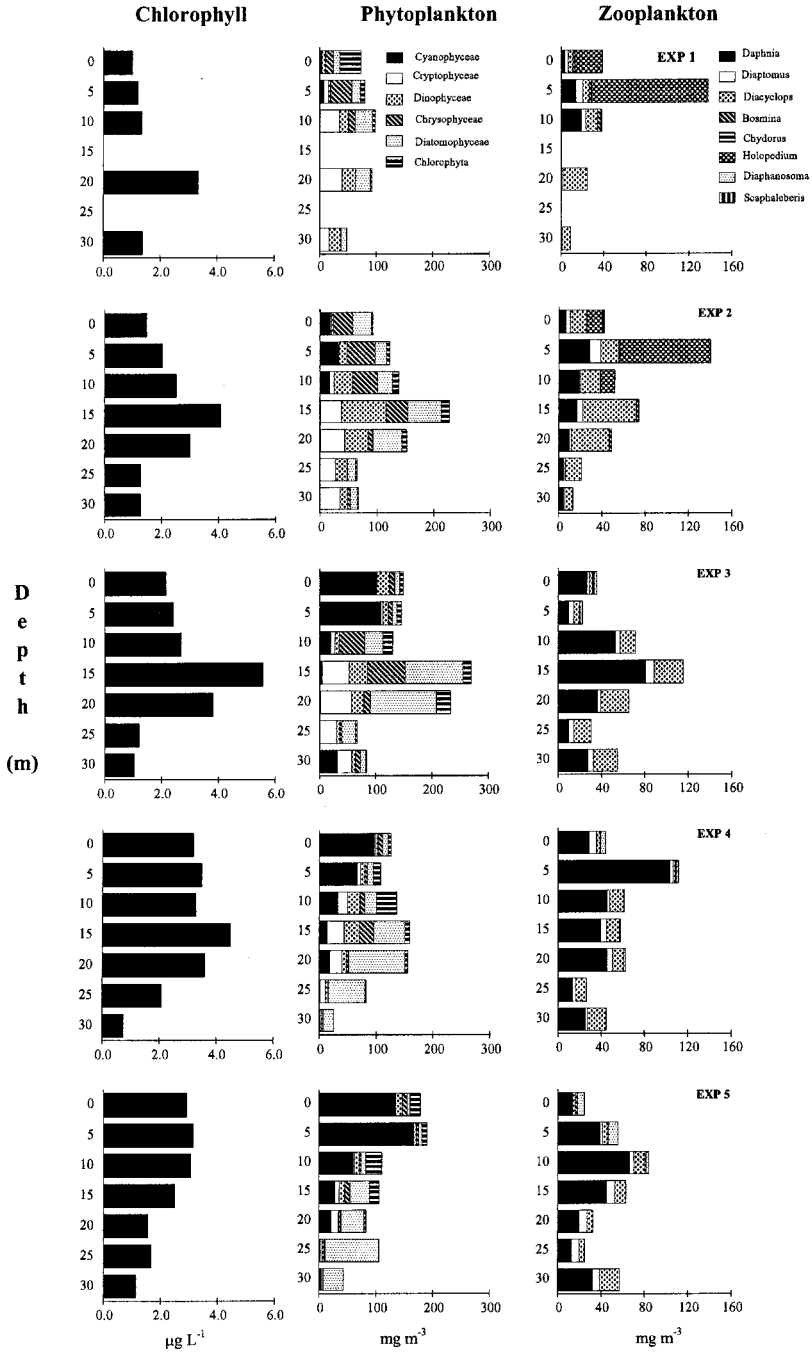


**Fig. 2.** Vertical profiles of initial chlorophyll *a*, phytoplankton biomass and zooplankton biomass in Castle Lake for five microcosm experiments on 21 June (EXP 1), 11 July (EXP 2), 11 August (EXP 3), 27 August (EXP 4) and 14 September (EXP 5) 1994. In the first experiment, the 15 and 25 m depths were not studied.

*Nutrient limitation*

A three-factor ANOVA for an overall treatment effect showed that the effect of nutrient enrichments on phytoplankton net growth was statistically significant from June to August during the first four experiments ($P < 0.05$; Table I). While nutrient enrichments explained approximately one-fifth of the variance in the first experiment in June, the importance of nutrients as a growth-limiting factor decreased towards the end of the summer, and in late August, nutrient treatments explained only 1.6% of the variance. In September, the effect of nutrient enrichments on phytoplankton net growth was not significant (Table I).

**Table I.** The results of an ANOVA of Castle Lake phytoplankton net growth (as chlorophyll *a*) responses to depth, nutrients, zooplankton, and their interactions, in five microcosm experiments in summer 1994. The percent variance explained is the sum of squares divided by the total sum of squares

| Experiment/ date | Treatment | d.f. | Sum of squares | *F*-test | *P* value | % variance explained |
|---|---|---|---|---|---|---|
| 1 | Depth (D) | 4 | 0.1611 | 67.42 | 0.0001 | 40.5 |
| 21–25 June | Nutrients (N) | 1 | 0.0756 | 126.62 | 0.0001 | 19.0 |
| | DN | 4 | 0.1473 | 61.66 | 0.0001 | 37.0 |
| | Zooplankton (Z) | 1 | 0.0000 | 0.00 | 0.9869 | 0.0 |
| | DZ | 4 | 0.0010 | 0.41 | 0.7972 | 0.3 |
| | NZ | 1 | 0.0000 | 0.06 | 0.8129 | 0.0 |
| | DNZ | 4 | 0.0007 | 0.28 | 0.8880 | 0.2 |
| | Error | 20 | 0.0120 | | | 3.0 |
| 2 | Depth (D) | 6 | 0.0385 | 14.33 | 0.0001 | 15.1 |
| 11–15 July | Nutrients (N) | 1 | 0.0396 | 88.50 | 0.0001 | 15.6 |
| | DN | 6 | 0.0942 | 35.11 | 0.0001 | 37.1 |
| | Zooplankton (Z) | 1 | 0.0134 | 29.96 | 0.0001 | 5.3 |
| | DZ | 6 | 0.0408 | 15.20 | 0.0001 | 16.1 |
| | NZ | 1 | 0.0021 | 4.66 | 0.0396 | 0.8 |
| | DNZ | 6 | 0.0131 | 4.88 | 0.0016 | 5.2 |
| | Error | 28 | 0.0125 | | | 4.9 |
| 3 | Depth (D) | 6 | 0.0194 | 6.01 | 0.0004 | 23.6 |
| 11–15 August | Nutrients (N) | 1 | 0.0051 | 9.40 | 0.0048 | 6.2 |
| | DN | 6 | 0.0195 | 6.06 | 0.0004 | 23.8 |
| | Zooplankton (Z) | 1 | 0.0108 | 20.08 | 0.0001 | 13.2 |
| | DZ | 6 | 0.0050 | 1.54 | 0.2010 | 6.1 |
| | NZ | 1 | 0.0005 | 1.00 | 0.3352 | 0.6 |
| | DNZ | 6 | 0.0068 | 2.11 | 0.0839 | 8.3 |
| | Error | 28 | 0.0150 | | | 18.3 |
| 4 | Depth (D) | 6 | 0.0128 | 23.87 | 0.0001 | 28.8 |
| 27–31 August | Nutrients (N) | 1 | 0.0007 | 8.06 | 0.0083 | 1.6 |
| | DN | 6 | 0.0127 | 23.56 | 0.0001 | 28.5 |
| | Zooplankton (Z) | 1 | 0.0075 | 83.48 | 0.0001 | 16.9 |
| | DZ | 6 | 0.0068 | 12.58 | 0.0001 | 15.3 |
| | NZ | 1 | 0.0000 | 0.36 | 0.5557 | 0.0 |
| | DNZ | 6 | 0.0015 | 2.73 | 0.0325 | 3.4 |
| | Error | 28 | 0.0025 | | | 5.6 |
| 5 | Depth (D) | 6 | 0.0069 | 2.87 | 0.0264 | 15.8 |
| 14–18 September | Nutrients (N) | 1 | 0.0001 | 0.25 | 0.6178 | 0.2 |
| | DN | 6 | 0.0133 | 5.53 | 0.0007 | 30.5 |
| | Zooplankton (Z) | 1 | 0.0001 | 0.35 | 0.5577 | 0.2 |
| | DZ | 6 | 0.0105 | 4.39 | 0.0030 | 24.1 |
| | NZ | 1 | 0.0001 | 0.19 | 0.6654 | 0.2 |
| | DNZ | 6 | 0.0014 | 0.58 | 0.7428 | 3.2 |
| | Error | 28 | 0.0112 | | | 25.7 |

According to graphical interpretation, the strongest effects of nutrient enrichments on phytoplankton net growth were seen in the epilimnion, especially in June and July (Figure 3). Although the epilimnetic nutrients were almost depleted in September (Figure 1), nutrient additions did not have a strong effect

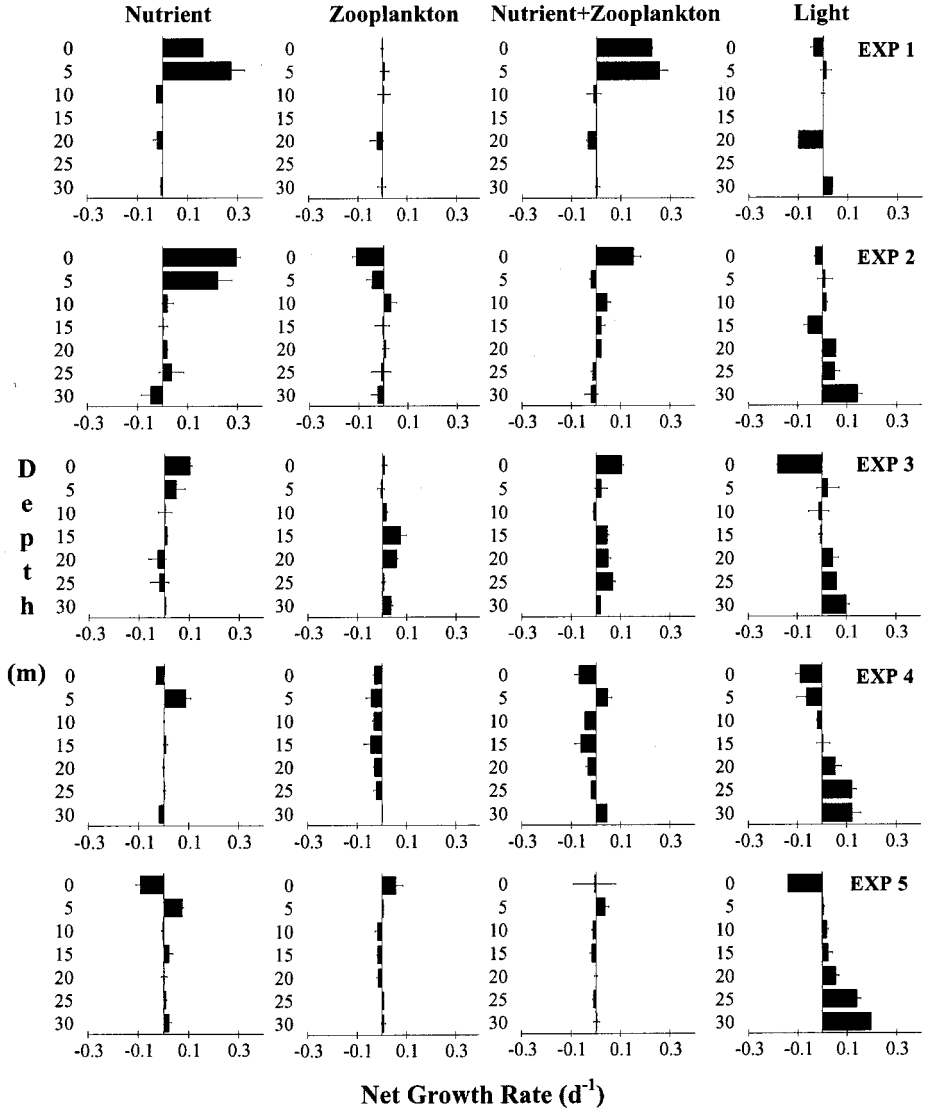


**Fig. 3.** The response of Castle Lake phytoplankton net growth rate (as chlorophyll *a*, relative to the control) to different treatments in microcosm experiments on 21–25 June (EXP 1), 11–15 July (EXP 2), 11–15 August (EXP 3), 27–31 August (EXP 4) and 14–18 September (EXP 5) 1994. In the light treatments, the net growth rates at 0 m are presented as negative to demonstrate inhibition. Net growth rates represent means (± SD) of duplicates (only one result in the following cases: nutrient 0 m and light 30 m in experiment 1; light 20 m in experiment 2; zooplankton 25 m in experiment 5). In the first experiment, the 15 and 25 m depths were not studied.

on phytoplankton net growth (Table I, Figure 3). Phytoplankton net growth was less affected by nutrient additions throughout the research period in the hypolimnion (Figure 3), where concentrations of $NO_3$-N were highest (Figure 1). However, no significant regressions between phytoplankton net growth rates in the nutrient enrichment treatments and nitrogen concentrations of lake water were found in any of the experiments.

*Zooplankton grazing*

The effect of zooplankton on phytoplankton net growth was significant ($P < 0.05$) in the three experiments of July and August, explaining 5.3–16.9% of the variance (Table I). In June and September, the variance in the experiments could not be explained by zooplankton (Table I). The response of phytoplankton net growth to crustacean zooplankton varied from experiment to experiment and no clear pattern in growth stimulation or grazing loss was shown (Figure 3).

*Interactions of depth, nutrients and zooplankton*

Depth, nutrients, zooplankton, and their interactions, explained most of the variance in the phytoplankton net growth rate (74.3–97.0%; Table I). Depth had a statistically significant ($P < 0.05$) effect on phytoplankton net growth rates in all the experiments, and it explained up to 40.5% of the variance. In all the experiments, depth or the interactions of depth and nutrients explained most of the variance. Statistically significant interactions between nutrients and zooplankton were observed only in the second experiment in July, although explaining only 0.8% of the variance in that experiment (Table I).

In the June and July experiments, nutrients had a stronger effect on epilimnetic phytoplankton net growth (Figure 3), explaining more of the variance than zooplankton (Table I). In contrast, in August, zooplankton explained more of the variance than nutrients (Table I), although nutrients showed a stronger effect in the epilimnion, except for the surface water in the fourth experiment (Figure 3). In September, neither nutrients nor zooplankton alone, nor their interactions, explained the variance of phytoplankton net growth (Table I).

*Light inhibition and limitation*

Incubation of samples from different depths at 5 m showed that phytoplankton net growth was inhibited by high light intensity at the surface in all the experiments, especially in August and September (Figure 3). In the first experiment in June, light limitation was only observed in the deepest samples. In July and August, low light inhibited phytoplankton net growth at 20 m and below, and in September already around 10–15 m, since the displaced samples had higher net growth rates than those measured *in situ*. The light limitation increased with depth (Figure 3). The highest phytoplankton net growth was seen in the deepest samples (Figure 3) where chlorophyll concentrations were, with the exception of the first experiment, at their lowest (Figure 2), indicating that the phytoplankton in the deep basin were light limited throughout the summer.

## Discussion

In Castle Lake, both phosphorus and nitrogen are potential factors limiting phytoplankton growth (Elser *et al*., 1995). It has been reported that, in enrichment bioassays, additions of both nitrogen and phosphorus together enhance phytoplankton growth more substantially than either nutrient singly (Elser *et al*., 1990). Thus, no attempt was made to distinguish experimentally between the importance of phosphorus and nitrogen in the present study.

Low epilimnetic chlorophyll concentrations were limited by nutrients at the early part of the growing season. Later in the season, epilimnetic chlorophyll concentrations increased, but still phytoplankton were nutrient limited, until in September nutrient limitation was no longer significant in controlling the phytoplankton net growth. Then, although nutrients were almost depleted from the epilimnion, nutrient additions did not increase the phytoplankton net growth significantly. Also, earlier studies in Castle Lake have reported epilimnetic nutrient limitation of phytoplankton growth, occurring soon after ice-out, and exhibiting both considerable interannual variation resulting mainly from processes that produce differences in spring nutrient concentrations, and intra-annual variation, which appears to be associated with interactions with zooplankton (Elser *et al*., 1995). Because of minimal external inputs of nitrogen in Castle Lake during the growing season, zooplankton excretion and microbial mineralization in the regeneration of nitrogen are considered critical for phytoplankton growth in the epilimnion (Axler *et al*., 1981). Also a potential factor contributing to this is periphyton outcompeting phytoplankton for epilimnetic nutrients during spring (Axler and Reuter, 1996).

Deeper in the water column, a deep chlorophyll maximum had already developed by the first experiment in June, and it persisted around 15–20 m until the end of August. This is typical of Castle Lake, where a deep maximum usually develops soon after ice thaw, and stays in the deep basin until fall overturn, with generally more than half of the total chlorophyll stock existing below 15 m (Priscu and Goldman, 1983). The results of the present study showed that, during the growing season, nutrient additions did not enhance phytoplankton net growth below the epilimnion, suggesting that most of the Castle Lake phytoplankton were not limited by nutrients.

At the surface, the phytoplankton net growth remained depressed by high light intensities, even after 4 day incubations. In July and August, the deep chlorophyll maximum at 15 m was not light limited, but below that low light started to limit the phytoplankton net growth. In September, light limitation of the phytoplankton net growth already began at around 10–15 m, and the deep chlorophyll maximum ceased to exist. At 5 m, the light treatment depth was the same as the control depth. Therefore, the net growth values at this depth represent the differences between the control and light treatment racks used in the incubations. However, since these racks were situated close to each other and had similar incubation conditions, these differences can rather be explained by variation between the samples. This variation was generally small, except for the fourth experiment in which, however, the overall vertical trend typical of other experiments can also

be seen. The phytoplankton net growth increased with depth when exposed to light. The ability of the deep-water phytoplankton, with reduced rates of photosynthesis, to photosynthesize upon exposure to higher light has also been reported earlier at Castle Lake (Priscu and Goldman, 1983) as well as at Lake Tahoe (Tilzer *et al*., 1977).

The incubation depth in the light treatments was kept the same (5 m) throughout the study period. The turbidity in the lake increased towards the end of the summer as the amount of small colonial blue-green algae increased, decreasing light intensities in the water column, which complicates the seasonal interpretation of the data. The incubation of the samples from 10 m at 5 m did not, however, enhance the phytoplankton net growth markedly, which supports the interpretation that phytoplankton net growth was unlikely to be limited by low light at the incubation depth during this period. In addition to altered light climate, the phytoplankton in the light treatments were also displaced into a different temperature, which may also have contributed to the phytoplankton net growth rate results. However, the importance of the temperature may not be estimated easily, since optimum temperatures of photosynthesis are found to be physiologically different in the epilimnetic and hypolimnetic phytoplankton communities of Castle Lake (Priscu and Goldman, 1984). In all, the results of the present study indicated that low light, rather than nutrients, limited the phytoplankton net growth in deep waters. This is in accordance with the study of Elser and Frees (1995) concluding that the Castle Lake deep-water phytoplankton grow at nutrient-saturated rates, and are more likely limited by low light.

In accordance with earlier studies in Castle Lake (Elser, 1992), the effects of zooplankton varied from experiment to experiment, showing variation in the phytoplankton's responses to grazers through time. Both grazing loss and stimulation of the phytoplankton net growth, varying also with depth, were observed. Previously, the effect of zooplankton on phytoplankton productivity has been reported to shift from positive to negative over the summer in the epilimnion of Castle Lake (Redfield, 1980), and both strong direct and indirect impacts of zooplankton on the nutrient-limited phytoplankton assemblages have been revealed (Elser and Goldman, 1991; Brett *et al*., 1994). Although zooplankton had significant effects on phytoplankton net growth in the three experiments of July and August, the pattern of these effects remained unclear. Changes in the phytoplankton and zooplankton species composition over the course of the research period, however, are likely to have contributed to this variation, since grazing effects differ significantly among zooplankton (Brett *et al*., 1994) as well as among phytoplankton species (Lehman and Sandgren, 1985; Elser and Goldman, 1990; Elser, 1992).

In conclusion, the results of the present study indicated both vertical and temporal variation in the phytoplankton's responses to the factors controlling the net growth rates. The phytoplankton net growth could be explained by different factors depending on the depth and the stage of the growing season. The phytoplankton were only limited by nutrients in the upper 5 m (epilimnion), as indicated by change in chlorophyll concentrations, and nutrient enrichments had the greatest effect on phytoplankton at the early part of the growing season. High

light inhibited the phytoplankton net growth near the surface. Low light limited the phytoplankton net growth at the depth of ~20 m, and at the end of the growing season already around 10–15 m. The deep chlorophyll maximum present in June–August was not limited by either light or nutrients. The results showed variation in the phytoplankton's responses to grazers through time and depth, along with changing zooplankton and phytoplankton species composition. The impact of zooplankton grazing on phytoplankton net growth was significant in July and August. These results emphasize the importance of nutrient limitation only in the epilimnion with light inhibition at the surface, light limitation in the deeper water column, and seasonally and vertically varying impact of zooplankton grazing in influencing the development of the phytoplankton community in meso-oligotrophic subalpine Castle Lake.

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