Physiological responses in phytoplankton under nutrient-limited epilimnion in north basin of Lake Biwa

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In north basin of Lake Biwa, phytoplankton production has been shown to suffer from phosphorus limitation throughout the year, especially in the stagnation period every year. In this study, we determined physiological responses in phytoplankton under such P-limited environment using dilution-nutrients-enrichment experiments.
The experiments were conducted at 5 times from 14 June to 4 December 2013 at St. 3 (60 m depth) in north basin of Lake Biwa. Lake waters for the experiments were collected from 5-m depth with a Schindler trap and then filtered with a 200-μm-mesh net for eliminating meso-zooplankton. Another waters for measuring chlorophyll a (chl. a), nutrients and sestonic C, N, P were collected from 8 depth layers between 0 and 50 m. Simultaneously, vertical profiles of water temperature and photon flux density were determined with a CTD profiler. A part of the water collected was filtered with a capsule filter set (pore size, 0.45 μm), and then made 5 diluted lake waters with mixing to remaining non-filtered lake water. Three treatments for the experiments, adding NH_4Cl (20 μM) as +N treatment, KH_2PO_4 (2μM) as +P treatment and both as +NP treatment were prepared with control at all 5 dilution levels. All of the experimental bottles were incubated at in situ temperature and light conditions for 48 hours with a shaking incubator. Apparent growth rates (μ_{net}) were calculated using an initial and final concentrations of chl. *a* (chl_0, chl_t) at each experimental bottle with the following equation, $\mu_{net} = \ln (chl_t/chl_0)/48$. Grazing coefficient (g) was calculated using the μ_{net} for +NP treatment from the following equation, $\mu_{net} = \mu_{mean} - g\ x$, where μ_{mean} is potential growth rate without grazing by micro-zooplankton at each dilution, and x is dilution rate. Then, μ_{mean} for +N and +P treatments was calculated from the g obtained. Assuming that phytoplankton could use three nutrient sources, μ_{mean} could be calculated from the following equation, $\mu_{mean}(x) = \ln (1 + Kr\ x + Ki + Ke/x)/48$, where Kr, Ki, and Ke were growth coefficients for recycling nutrients, internal nutrient stock and external dissolved nutrients, respectively. Each growth coefficients were estimated with a stepwise multiple regression analysis. Lake water was strongly stratified from June to September, started vertically mixing at October, and then the mixing layer was deepened after that. NO_3-N concentrations were depressed from July to September, while recovered at October. PO_4-P conc. were always below the detection limit in the epilimnion. Seasonal changes of sestonic C:P and N:P ratios implied that phosphorus limitation for phytoplankton growth was likely in June to July, but relaxed after September. Dilution-nutrient-addition experiments showed that phytoplankton was exposed phosphorus limitation for its growth throughout the study period. Multiple regression analysis indicated that phytoplankton used internal and recycling phosphorus for growth in June to September, and that it used just internal one in October and December. Whereas, for nitrogen, it used recycling one for growth in June to September with lower ratios comparing to those of phosphorus, and internal one in October and December. These results suggested that recycling phosphorus from micro-zooplankton grazing supported phytoplankton growth under phosphorus-limited epilimnion in Lake Biwa. Phytoplankton used internal sources in both phosphorus and nitrogen for its growth in October and December. During this period, phytoplankton could stock nutrients recovering from the deeper layer due to deepening the mixing layer and then grow using the stock one.

Keywords: phosphorus limitation, phytoplankton production, recycling nutrients, micro-zooplankton grazing, Lake Biwa

