Phosphorus dynamics related to phytoplankton growth with newly sensitive measurement of orthophosphate and growth experiments in north basin of Lake Biwa

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Orthophosphate (PO_4-P) is a common growth-limiting factor for primary production in lakes. Quantification of PO_4-P concentrations in epilimnion of meso- and oligotrophic lakes were rarely reported because the concentrations were under the detection limit (60 nM) using traditional spectrophotometrical method. In this study, we measured PO_4-P concentrations with sensitive method using an ion chromatography with a detection limit of 5 nM, determined seasonal and vertical distributions of PO_4-P during 2018 and 2019 with other environmental factors in north basin of Lake Biwa, to clarify phosphorus (P) dynamics for phytoplankton growth in this mesotrophic lake.

Field investigations were conducted from Apr to Dec in 2018 and Mar to Dec in 2019 at station K4 (50 m deep). P-enrichment experiments were conducted at each sampling occasion, to determine threshold concentration of phosphate for phytoplankton growth. Lake water including phytoplankton assemblage collected from epilimnion (0-20 m) was enriched with 1 μM of PO_4-P (+P) and incubated together with untreated one as control for 2 days under ambient light and temperature conditions. To evaluate effect of potentially nutrient-rich bottom water, the filtered bottom water was combined with untreated epilimnion water including phytoplankton at 1:1 of volume (+B) and incubated at the same conditions as the +P treatment and control. Phytoplankton growth rate (g) was calculated by equation of $g=\ln(C_t/C_0)/2$, where C_0 and C_t are chlorophyll *a* (chl. *a*) concentrations at start and end of the experiment, respectively.

In epilimnion, water temperature showed similar seasonal variation between the two years; Strict thermocline was developed in 10-20 m from Jun to Sep; Annual mean was 17.6 ºC in 2018, and 1.8 ºC higher than that in 2019. Phytoplankton biomass showed bimodal temporal distribution in both years, though annual mean concentrations of chl. *a* were higher in 2018 compared to those in 2019.

In 2018, mean PO_4-P concentrations in epilimnion were <20 nM from Apr to Jun, increased from Jul, and >30 nM from Aug to Dec. In 2019, they were higher than those in 2018, ca. 40 nM from Mar to Jul, but decreased to <20 nM after Aug until Dec. Subsurface maxima of 60-70 nM were observed around 10 m from May to Jun and 10-20 m in Jul 2019, while they were always <50 nM throughout the year in 2018. PO_4-P concentrations increased below 30 m with depth; high concentrations up to 200 nM were observed near the bottom in both years. Concentrations of >150 nM were frequently observed from Sep to Dec 2018, whereas it was just in Aug during 2019. Total P, dissolved organic P and particulate P exhibited similar distributions between the two years. Seasonal and vertical distributions of nitrogen forms were almost the same between the two years; ammonia concentrations increased in 10-20 m from May to Jul, whereas nitrate concentrations decreased in epilimnion after Jun while increased in hypolimnion.

Phytoplankton growth in epilimnion was mostly limited by PO_4-P because the g in control always lower than those in +P and +B treatments. In 2018, the highest g in control was obtained in Sep probably due to high nutrient loading caused by typhoon. Since no obvious perturbations in 2019, the g in both +P and control treatments increased after Jun, reached to the maximum in Aug, and decreased toward winter

depending on seasonally progressing temperature.

Different spatio-temporal distributions in PO_4-P between the two years might be caused by both abiotic and biotic factors, and subsurface maxima indicate possible transportation of nutrients from the littoral slope with internal wave, suggesting horizontal transportation of nutrients from littoral sediment may play an important role as nutrient source for pelagic phytoplankton production. P-enrichment experiments showed that <50 nM of PO_4-P was P-limited conditions for phytoplankton, and 15 nM of PO_4-P might be critical concentration for in situ phytoplankton growth.

Keywords: phosphorus, nutrient dynamics, phytoplankton production, P-limitation, nutritional horizontal transportation, freshwater lake

