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Use of Flow Cytometry and Stable Isotope Analysis to Determine Phytoplankton Uptake of Wastewater Derived Ammonium in a Nutrient-rich River

Calla Schmidt
University of San Francisco, cischmidt@usfca.edu

Tamara E. C. Kraus

Megan B. Young

Carol Kendall

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Calla M. Schmidt[1], Tamara E. C. Kraus[2], Megan B. Young[3], Carol Kendall[3]

[1]University of San Francisco, 2130 Fulton St, San Francisco, CA, 94117, USA
[2]USGS California Water Science Center, 6000 J Street, Placer Hall, Sacramento CA 95819, USA
[3] USGS National Research Program, Menlo Park, CA, USA

Correspondence to: Calla M Schmidt (cischmidt@usfca.edu)

Abstract. Anthropogenic alteration of the form and concentration of nitrogen (N) in aquatic ecosystems is widespread. Understanding availability and uptake of different N sources at the base of aquatic food webs is critical to establishment of effective nutrient management programs. Stable isotopes of N (^{14}N,^{15}N) are often used to trace the sources of N fuelling aquatic primary production, but effective use of this approach requires obtaining a reliable isotopic ratio for phytoplankton. In this study, we tested the use of flow cytometry to isolate phytoplankton from bulk particulate organic matter (POM) in a portion of the Sacramento River, California, during river-scale nutrient manipulation experiments that involved halting wastewater discharges high in ammonium (NH_4^+). Field samples were collected using a Lagrangian approach, allowing us to measure changes in phytoplankton N source in the presence and absence of wastewater derived NH_4^+. Comparison of $\delta^{15}N$-POM and $\delta^{15}N$-phytoplankton ($\delta^{15}N$-PHY) revealed that their $\delta^{15}N$ values followed broadly similar trends. However, after 3 days of downstream travel in the presence of wastewater treatment plant (WWTP) effluent, $\delta^{15}N$-POM and $\delta^{15}N$-PHY in the Sacramento River differed by as much as 7‰. Using a stable isotope mixing model approach, we estimated that in the presence of effluent between 40 and 90% of phytoplankton-N was derived from NH_4^+ after 3 days of downstream transport. An apparent gradual increase over time in the proportion of NH_4^+ in the phytoplankton N pool suggests that either very low phytoplankton growth rates resulted in an N turnover time that exceeded the travel time sampled during this study, or a portion of the phytoplankton community continued to access nitrate even in the presence of elevated NH_4^+ concentrations.