Improved methodology to measure taxon-specific phosphate uptake in live and unfiltered samples.

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Microorganisms play a major role in the marine phosphate biogeochemical cycle but the relative contribution of picoplanktonic groups is not well understood. Previous studies have shown that combining uptake measurements of radiolabeled dissolved inorganic phosphate (P_i) substrate with cell sorting by flow cytometry is a powerful tool for the assessment of P_i fluxes at the cell-specific level. Nevertheless, using ^{33}P to trace P_i uptake, we show that treatments involving fixation and filtration of the sorted groups (i.e., heterotrophic prokaryotes, *Synechococcus* and piconanophytoeukaryotes) induce leakage of radioactive P_i (up to 50% of the signal), resulting in a sizeable underestimation of the taxon-specific P_i uptake. We suggest an alternative protocol, which significantly reduces this bias. Using this optimized protocol, the samples were treated with an excess of nonradioactive Pi to stop the incubation and sorted fractions were directly collected in microtubes for radioactivity counting, avoiding signal loss due to filtration. Sorted groups were strongly and differently impacted by fixation (0.5% PFA), with the exception of *Synechococcus* cells, which showed once a 10% lower signal in samples treated with the previously used protocols compared with samples treated with ours. Based on the integrity of the live sorted cells, our improved protocol provides reproducible and accurate estimations of the taxon-specific P_i uptake ($\leq$ 11% variation on cellular uptake rates, sd/average, n = 69). It was successfully applied to P-depleted oligotrophic seawater samples from the Mediterranean and will allow a comparison of taxon-specific uptake rates between sites.