Optimised routine flow cytometric enumeration of heterotrophic flagellates using SYBR Green I.

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Heterotrophic flagellates (HF) are the major consumers of bacteria in aquatic ecosystems and dominate heterotrophic nanoplankton in numbers and in biomass. A DNA-staining based flow cytometry (FC) protocol to enumerate HF was described by Zubkov et al. (2007), but has not yet been widely adopted. We tested extensively the method and its limitations using a wide range of sample types and trying several fixation and conservation alternatives. We evaluated simplification of some steps of the method, seeking the best compromise between precision and the quality of distinction of HF from bacteria and phytoplankton in the cytograms. We found that a flow rate of 120-220 μL min^{-1} without using a syringe-pump enhanced machine modification, and running times of 8-10 min allowed enumeration of HF even at values below 102 cells mL^{-1}. SYBR Green I, at final concentrations of 1:10000 and a minimum staining time of 10 min at room temperature in the dark, was adequate for staining and detecting HF. No significant differences were found between cell numbers obtained from freshly analyzed samples and those previously frozen in liquid-N. FC and epifluorescence microscopy (EpiM) were in - agreement and FC yielded lower variability between replicate samples than EpiM. One limitation we encountered was that, in the presence of large bacteria and/or bacterial aggregates, enumeration was difficult. However, in absence of bacterial aggregates samples with Bact/HF ratios > 1000, HF could be well-enumerated.