Resource Summary Report

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Mixotrophy - Quantification of the percent of phytoplankton cells with preys (e.g. fluorescent microspheres or fluorescently labeled bacteria)

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Protocol Information

URL: https://dx.doi.org/10.17504/protocols.io.be2vjge6

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Summary: This methodology describes a protocol modified from Sherr and Sherr (1993) to determine phytoplankton prey uptake using flow cytometry and yellow-green fluorescent polystyrene-based microspheres (YG-beads; 0.5 µm in diameter, Fluoresbrite, Polysciences, Inc., Warrington, PA, USA) or fluorescently labelled bacteria (FLBs) as food proxies. FLBs were prepared according to the protocol of Sherr et al. (1987) using the bacteria Brevundimonas diminuta (strain CECT313, also named Pseudomonas diminuta), obtained from the Spanish Type Culture Collection (CECT, Valencia, Spain). The percent of cells with prey were measured in the laboratory in four Micromonas polaris strains and one mixotrophic strain (Ochromonas triangulata) used as a positive control. The percent of cells with beads was determined in samples fixed with Lugol's iodine solution (protocol explained in detail here) and enumerated with a flow cytometer (Guava easyCyte, Luminex Corporation, USA) where cells that contained chlorophyll as well as green fluorescence (same signal as the prey added, YG-beads or FLBs) were considered to be cells containing prey (quantification protocol explain in detail here). To determine the uptake of prey over time the percent of cells with preys was quantified in each experimental flask by first adding preys and then sub-sampling and fixing after an incubation of 0 (T0), 20 (T20) and 40 (T40) minutes. The T0 sample accounts for the physical attachment of preys to the cell and therefore the percent of cells ingesting prey corresponds to the percent of cells with prey at T20 or T40, minus the percent of cells with prey at T0. References:Sherr, EB. & Sherr, BF. (1993). Protistan grazing rates via uptake of fluorescently labeled prey. In Kemp, P., Sherr, B., Sherr, E. & Cole, J. (eds.) Handbook

of Methods in Aquatic Microbial Ecology, 695–701 (Lewis Publishers: Boca Raton, USA).Sherr, EB., Sherr, BF. & Fallon, RD. (1987). Use of monodispersed, fluorescently labeled bacteria to estimate in situ protozoan bacterivory. Appl Environ Microbiol.

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