Bacteria Abundance protocols

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Icescape 2010 samples:

Samples were fixed with glutaraldehide (EM grade, 0.5% final concentration), let fix for 30 min in the dark at 4ºC and stored at -80ºC until further processing at Banyuls sur Mer (within 2 months after collection).

Bacterial cells (BA) were counted by flow cytometry using a FACS Calibur (Becton Dickinson, San Jose, CA) equipped with a 488 nm laser and the standard filters setup. SYBR Green-I was added at a final dilution of 1 / 10000 and samples were incubated in the dark for 10 min before analysis. Bacteria were detected on a plot of green fluorescence (FL1, 515–545 nm) versus right angle light scatter (SSC), using the green fluorescence as threshold parameter. High Nucleic Acid (HNA) and low Nucleic Acid (LNA) bacteria were discriminated according to their green fluorescence and counted separately.

Icescape 2011 samples:

Samples were fixed with glutaraldehide (Sigma EM grade, 0.5% final concentration), let fix for 30 min in the dark at 4ºC, and stored at -80ºC until further processing. Bacterial cells (BA) were counted onboard (within 1-2 days after collection) by flow cytometry using a Accuri C6 (Becton Dickinson, San Jose, CA) equipped with a 488 nm laser and the standard filters setup. SYBR Green-I was added at a final dilution of 1 / 10000 and samples were incubated in the dark for 10 min before analysis. Bacteria were detected on a plot of green fluorescence (FL1, 515–545 nm) versus right angle light scatter (SSC), using the green fluorescence as threshold parameter. High Nucleic Acid (HNA) and low Nucleic Acid (LNA) bacteria were discriminated according to their green fluorescence and counted separately.