

**Protocol for determination of Bacterial Carbon Concentration in
Bioassay Experiments (NAAMES campaign)**

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Samples to estimate particulate organic carbon (POC) were collected at the initiation of a bioassay and during the stationary phase in the bacterioplankton growth curve, determined by bacterioplankton abundance. Each 1 L sample was filtered through a polypropylene inline filter cartridge loaded with two combusted Advantec Grade 25 mm 0.3 μm glass fiber filters (GF75) (James et al., 2017). Two filters were used to increase cell retention (mean $78 \pm 9\%$). At each station of NAAMES 2 and 4, 1 L of either 0.2 μm or 30 kDa tangential flow filtration (TFF) 10 m seawater filtrate was also filtered through a pair of stacked GF75s, which were subsequently used as blanks. These blanks were used to determine a universal blank correction for all POC estimates. Each filter was folded twice, with the sample material on the inside, placed into separate combusted 20 mL borosilicate glass vials and frozen at -20°C . Filters were analyzed on a Costech ECS 4010 CHNS-O elemental analyzer by Bigelow Analytical Services, which has a detection limit of 0.1 $\mu\text{g C}$ (Bigelow Laboratory for Ocean Sciences, Maine).

Bacterioplankton carbon (BC, $\mu\text{mol C L}^{-1}$) refers to the carbon content of a population at any given time. BC at the initial and stationary growth conditions of each bioassay were estimated using empirical carbon conversion factors (CCFs, fg C cell^{-1}). These CCFs were calculated by dividing POC estimates by the corresponding POC filter cell abundance.

References:

James, A. K., Passow, U., Brzezinski, M. A., Parsons, R. J., Trapani, J. N., and Carlson, C. A. (2017). Elevated pCO_2 enhances bacterioplankton removal of organic carbon. *PLoS One* 12, e0173145. doi:10.1371/journal.pone.0173145