WATER COLUMN TIME SERIES PROTOCOL
Sample Processing for Total Organic Carbon (TOC)
Moorea Coral Reef LTER

1. Collect unfiltered TOC samples as described in the Protocol for water column field collection.
2. Place samples in a -80° C freezer immediately on return to the Gump lab
3. Return samples to Santa Barbara on dry ice and store in a -20° C freezer until analysis.
4. TOC samples are analyzed via high temperature combustion using a Shimadzu TOC-V in shore based laboratory at the University of California, Santa Barbara. The operating conditions of the Shimadzu TOC-V have been slightly modified from the manufacturer's model system. The condensation coil has been removed and the headspace of an internal water trap was reduced to minimize the system's dead space. The combustion tube contains 0.5 cm Pt pillows placed on top of Pt alumina beads to improve peak shape and to reduce alteration of combustion matrix throughout the run. CO_2 free carrier gas is produced with a Whatman® gas generator (Carlson et al. 2004). Sample are drawn into 5 ml injection syringe and acidified with 2M HCL (1.5%) and sparged for 1.5 minutes with CO_2 free gas. Three to five replicate 100 µl of sample are injected into combustion tube heated to 680° C. The resulting gas stream is passed though several water and halide traps, including an added magnesium perchlorate trap. The CO_2 in the carrier gas is analyzed with a non-dispersive infrared detector and the resulting peak area is integrated with Shimadzu chromatographic software. Injections continued until the at least three injection meet the specified range of a SD of 0.1 area counts, CV ≤2% or best 3 of 5 injections.
5. Extensive conditioning of the combustion tube with repeated injections of low carbon water (LCW) and deep seawater is essential to minimize the machine blanks. After conditioning, the system blank is assessed with UV oxidized low carbon water. The system response is standardized daily with a four-point calibration curve of potassium hydrogen phthalate solution in LCW. All samples are systematically referenced against low carbon water and deep Sargasso Sea reference waters (2600 m) and surface Sargasso Sea water every 6 – 8 analyses (Hansell and Carlson 1998). The standard deviation of the deep and surface references analyzed throughout a run generally have a coefficient of variation ranging between 1-3% over the 3-7 independent analyses (number of references depends on size of the run).
6. Daily reference waters were calibrated with DOC CRM provided by D. Hansell (University of Miami; Hansell 2005).
7. TOC calculation µMC = (average sample area – average machine blank area) / (slope of std curve)

Carlson, C.A., S.J. Giovannoni, D.A. Hansell, S.J. Goldberg, R. Parsons, and K. Vergin. 2004. Interactions between DOC, microbial processes, and community structure in the mesopelagic zone of the northwestern Sargasso Sea. *Limnology and Oceanography* 49: 1073-1083.

Hansell, D.A. and C.A. Carlson 1998. Deep ocean gradients in the concentration of dissolved organic carbon. *Nature,* 395: 263-266.

Hansell, D.A. 2005 Dissolved Organic Carbon Reference Material Program. EOS, 35:318-319.