Supporting Online Material for

Revisiting Carbon Flux Through the Ocean’s Twilight Zone

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Supplemental Materials: Analytical methods, sampling equipment and fecal pellet carbon content

Methods
Sinking flux- NBSTs
Sinking flux was collected using neutrally buoyant sediment traps (NBSTs) (Figs. S1 & S2). Up to 3 separate NBSTs were deployed at a given sampling depth during deployments of 3, 4 and 5 days at 150, 300 and 500 m, respectively. This Lagrangian drifting reduces potential sampling bias due to hydrodynamics associated with horizontal flow over the trap and motions related to the mooring line (e.g. Gardner, 2000). Sample collection tubes go down open, and are closed after a pre-set period and the NBSTs return to the surface and relay position via GPS for NBST location and sample recovery. Immediately after retrieval, samples were gravity filtered through a 350 μm screen to remove large swimmers and wet split into 8 subfractions which were filtered and subsequently dried, chemically preserved or frozen on board. Splitting precision was assessed on a previous cruise to be better than ±1% based upon split solution weights and ± 4% based upon analyses of 234Th in trap particulate materials (± std. deviation of n=4 splits). All sample handling was carried out under trace-metal clean conditions in a clean air bench, and one sampling tube was deployed closed to serve as a processing blank (average PC blank = 50 +/- 10 μg C; PC per sample average = 150 μg ALOHA; 350 μg K2). Formalin (37 mM) and mercuric chloride (180 μM) poisons were used to minimize sample degradation during collection (Lee et al., 1992), and both produced comparable results. Separate experiments of poisoned trap material were used to show an insignificant impact of in trap degradation on particles collected at depth. Both poison treatments were held in a confining brine layer (salinity>70 ppt) formed by freeze-concentrating salt from prefiltered open-ocean seawater. VERTIGO also took significant effort to remove and quantify zooplankton “swimmers” that actively enter the traps and can be a large bias on POC flux collected in an open tube (Karl and Knauer, 1989). On board microscopic analyses of the screens and samples was used to identify and quantify possible sinking material caught on the screen or small zooplankton “swimmers” passing through the
screen, and these corrections were generally minor for POC (<5-20% for ALOHA and <4-10% at K2 for small swimmer C not removed by screen relative to total C flux).

**Geochemical Analyses**

POC is obtained by difference from measurement of total C by CHN and particulate inorganic carbon. PIC was determined by acidification of the sample with phosphoric acid and titration of CO$_2$ by a coulometric method with a UIC coulometric analyzer and acidification module. Biogenic silica was determined using the hot NaOH extraction method as described in Nelson *et al.* (1989). Chlorophyll $a$ is total chlorophyll $a$ pigments (monovinyl plus divinyl chlorophyll $a$) determined by HPLC on sediment trap samples that were immediately LN$_2$ frozen at sea. Mass was determined gravimetrically after filtration onto pre-tared 0.45$\mu$m pore sized Nucleopore filters, rinsing with buffered DI water to remove salts and desiccation to a constant weight.
Supplemental Figure 1. Photograph of Neutrally Buoyant Sediment Trap used in VERTIGO and as described in Valdes and Price (2000).
Supplemental Figure 2. Schematic diagram showing relative difference between NBST and standard surface tethered traps for the direct collection of sinking particles in the ocean. Particles derived from surface ocean biological processes are sinking at velocities of 10 to >100 m/d within a flow field of ocean currents on the order of km/d. The NBST moves at the same relative speed as the water flow ($v \sim 0$) so there is near zero flow across the trap mouth and within the trap ($v=\text{approach velocity}$). For standard traps, the surface tether and float result in flow across the trap mouth ($v = 1 \text{ to } >15 \text{ cm/s are common}$), flow within the trap, possible tilting and vertical motion, and particles entering the trap at non-vertical angles. Any of these hydrodynamic effects can alter the collection characteristics of the trap (e.g. Gardner, 2000).
Supplemental Figure 3. Fecal pellet carbon distribution from 150m Neutrally Buoyant Sediment Traps at ALOHA and K2 normalized to 1000 pellets for each location. Deployments 1 and 2 are combined for each station. The median carbon per pellet for ALOHA at 150m was significantly lower (0.036 μg C, n=421 pellets counted from 3 traps) than K2 (0.170 ugC, n=3068 pellets counted from 4 traps) (Mann-Whitney two-sample test p<0.0005). A χ² test indicated fecal pellet carbon frequency distributions were also significantly different between sites (χ² = 524.4, DF=13, p<0.0005). Carbon content of individual pellets was calculated from pellet volume and applying a conversion factor of 0.08 mgC/mm³ (Silver and Gowing 1991, Carroll et 1998, Urban-Rich et al. 1998, Riser et al. 2001). Pellet volume was calculated from measurements of individual pellets and their shape (e.g. sphere, cylinder, ovoid).
References for supplemental materials:


