THE OFFICIAL MAGAZINE OF THE OCEANOGRAPHY SOCIETY

Oceanography

Supporting Online Material for

Understanding the Role of the Biological Pump in the Global Carbon Cycle: An Imperative for Ocean Science

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2014, *Oceanography* 27(3):10–16, http://dx.doi.org/10.5670/oceanog.2014.78

This article has been published in *Oceanography*, Volume 27, Number 3, a quarterly journal of The Oceanography Society.

SECTION 1. VERTICAL SETTLING OF PARTICULATE ORGANIC CARBON BY ZOOPLANKTON: ECO-DYNAMIC TRANSPORT

Knowledge of biomineral production and fate is important for assessing controls on the global biological pump as well as on the carbonate chemistry of the ocean. Zooplankton remove newly formed particulate organic carbon (POC) from the euphotic zone as they excrete waste pellets. This fecal material, ballasted with biomineral particles such as coccoliths ($CaCO_3$) and diatom frustules (biogenic opal SiO_2), can transport POC through the mesopelagic to the bathypelagic zone at a speed of a few hundred meters a day (e.g., Honjo, 1997; Honjo et al., 2008; Berelson, 2002; Francois et al., 2002). Marine snow (aggregated POC associated with mineral ballast particles; e.g., Alldredge and Silver, 1988) is also entrained in this vertical flux as are microorganisms ingested by zooplankton.

Agglomerated settling particles constitute an essential food source for organisms residing in or passing through greater depths where no photosynthesis takes place. Therefore, residual organic materials are likely repeatedly consumed and repackaged by zooplankton during diel vertical migration up and down through mesopelagic and euphotic waters. Such zooplankton behavior may at least partially explain apparent inconsistencies in POC flux as determined by sediment traps (e.g., Harbison and Gilmer, 1986). In addition, based on deep tows and wide-band sonar surveys, it is estimated that 15–50% of zooplankton biomass above 500 m water depth migrates vertically into shallow layers at night (e.g., Wiebe et al., 1979; Angel and Baker, 1982; Kikuchi and Omori, 1985; Angel, 1989; Steinberg et al., 2002; Madin et al., 2006). Angel and Baker (1982) indicated that, during diel migration, zooplankton are potentially capable of removing one to two orders of magnitude more POC to the deeper layers than non-diel migrating animals of a similar standing crop. Beyond the mesopelagic, where the ocean's bathypelagic zone and Earth's master bioactive carbon (bio-C) reservoir begin, the export of POC may hypothetically depend on gravity's pull on the ballast particles ("terminal gravitational transport"; Honjo, et al., 2008). Mooring E (see Section 4 below) is designed to clarify eco-dynamic transport by mesozooplankton.

SECTION 2. ASSESSING FUNCTIONAL DIVERSITY OF OCEANIC PROKARYOTES: THE ROLES OF PROKARYOTES AND PROTISTS IN THE BIOLOGICAL PUMP

While the majority of vertically transported POC is known to be remineralized into ΣCO_2-aq by the combined activities of prokaryotes and protists in the ocean's dark bathypelagic realm (e.g., Arístegui et al., 2009), critical questions remain: On what forms of carbon (settling or suspended POC, or DOC [dissolved organic carbon]) do they act and how do they influence exchange of carbon between these pools? Which communities at different depths are responsible? How and at what rates does remineralization proceed? To what extent are these processes responsible for maintaining the master bio-C reservoir? The recent realization of a potential widespread sink for inorganic carbon (≤ 50% of heterotrophic production) in the meso- and bathypelagic zones now begs the question of identifying the reductant required to support this vast chemoautotrophy, highlighting the complexity and deficiency of our knowledge of microbially mediated processes in the deep ocean (e.g., Herndl et al., 2005; Hügler and Sievert, 2011). This sink is both poorly constrained and inadequately represented in current global carbon models (Arístigui et al., 2009). Detailed, quantitative understanding of the role of microbial processes in the biological pump requires a holistic approach, coupling depth profiles of microbial species abundance, metabolic activities, and rates with corresponding measurements of vertical particle flux, and characterization of contributing sources and the compositions of POM and DOM (particulate and dissolved organic matter).

SECTION 3. ASSESSING FUNCTIONAL DIVERSITY OF OCEANIC PROKARYOTES: GENOMIC AND TRANSCRIPTOMIC ANALYSES

Knowledge of the composition and functional properties of populations and communities of the oceanic prokaryotes has increased exponentially over the last decade through major advances in genomic technologies and in the bioinformatic power to interpret the vast amount of data generated (e.g., DeLong et al., 2006). The application of genomic and transcriptomic tools to oceanographic questions can aid in the determination of gene diversity and activity, the extent to which gene expression is controlled by environmental conditions, and the reconstruction of genomes to infer community structure and metabolism (Tyson et al., 2004). Ongoing developments include efforts to establish adequate sampling protocols for prospecting microorganisms and genes that may be overlooked with conventional sampling approaches.

When coupled with emerging methods for exquisite preservation of labile biomolecules such as nucleic acids, proteins, and intact polar lipids (under development by author Taylor; Supplement Figure 1), in situ time-series preservation of genomic, proteomic, and lipidomic information becomes feasible. An array of devices with these capabilities would enable gathering information at many levels, including those of prokaryotes, protists, and small eukaryotes, from molecular (e.g., DNA, RNA, lipids) to bulk biogeochemical constituents (e.g., N, C). Use of genomes of sentinel species representing important biogeochemical functions will be key to this endeavor. These approaches can also aid in the discovery of novel organisms and compounds, and of the mechanisms driving biogeochemical processes of the biological pump.

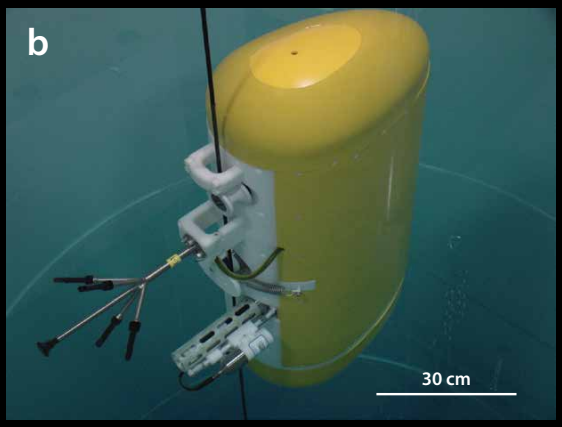
SECTION 4. GLOBAL BIOGEOCHEMICAL FLUX OBSERVATORY COMPONENTS

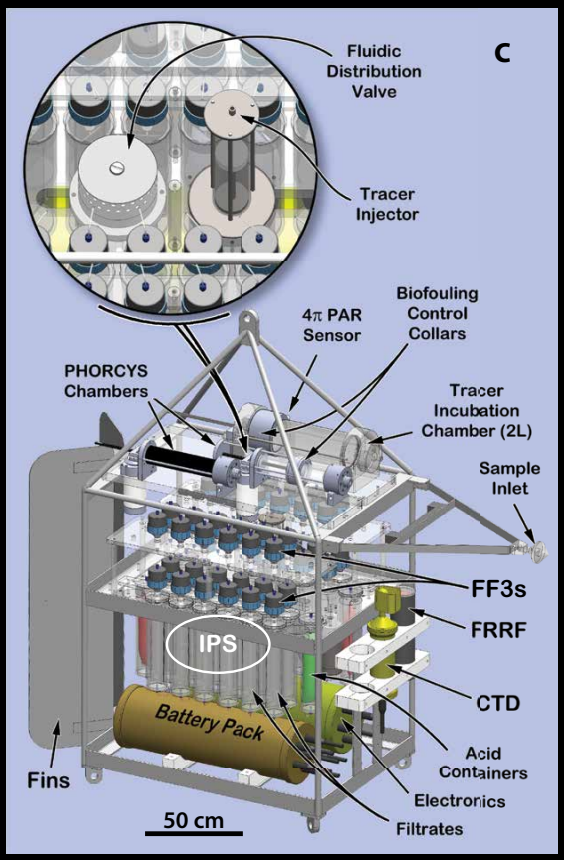
In order to track and assess the transport and transformation of bioactive carbon (bio-C) and to properly sample oceanic particles and microbes from all oceanographic zones and domains in all seasons, we must sample and examine at ecological, metabolic, and genetic levels all of the life forms (eukaryotes, prokaryotes, and viruses) involved in the biological pump. The timing of measurements and sample collection must be coordinated under a uniform time-series schedule.

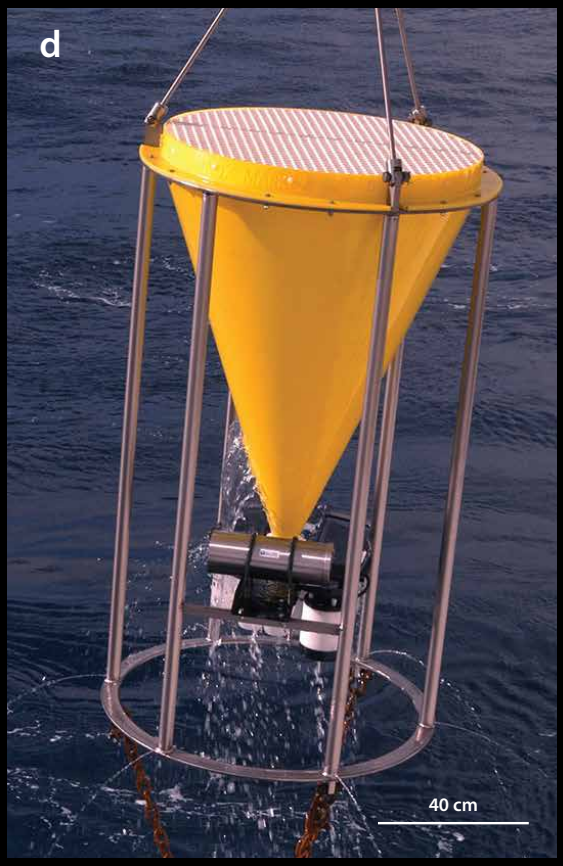
Mooring designs and the instruments intended for incorporation in the Global Biogeochemical Flux Observatory (GBF-O) are described below. Many of the sensors and samplers have been in active use for various oceanographic objectives and have endured deployment for up to a year or more. However, some are still in various stages of development and testing, and instruments other than those described here may also be adapted for GBF-O use. International collaboration will be indispensable for developing more appropriate and reliable robotic instruments to better understand the biological pump and bioactive carbon in the world ocean.

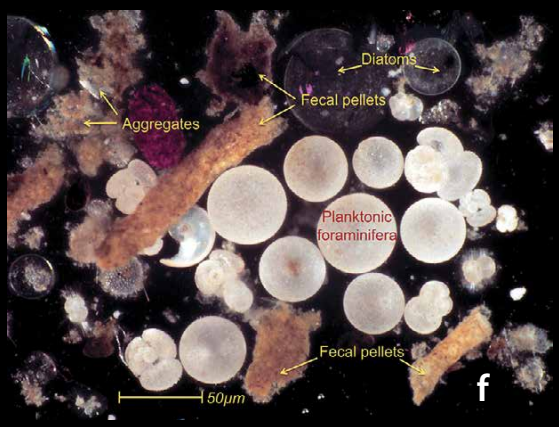
Mooring A: Primary Production Array

Mooring A (Figure 3 of the main text) is a fully submerged, bottom-tethered array. It consists of three main types of instruments. (1) Five sets of in situ robotic incubators for non-radioactive C and N isotopic tracer research (Incubation, Productivity with Samplers [IPSs]) that are based on earlier articles (e.g., Taylor and Doherty, 1990). Other tracers for biogenic $CaCO_3$ (coccoliths) and opal (diatom frustules) primary production (PP) could be added to this robotic incubator. (2) PHOtosynthesis, Respiration, and Carbon-balance Yielding Systems (PHORCYS) being developed by author van Mooy and Rick Keil, University of Washington, employ two optodes to monitor the dissolved oxygen under light and dark incubation. A prototype PHORCYS has been extensively tested in the field. (3) Prototype Fast Repetition Rate Fluorometers (FRRFs) are extensively deployed (Kolber et al., 1998; Cheah et al., 2011). The FRRF provides seamless fluorometric data that can be incorporated into the primary production assessment package (Supplement Figure 1c). Another potential method would be long-term deployment of the imaging FlowCytobot (not shown), which is designed to reveal the ebb and flow of a diverse range of microscopic plankton (Olson and Sosik, 2007; Sosik and Olson, 2007). The shallowest instrument cluster on a type A mooring









Supplement Figure 1

(a) A single syntactic-foam flotation sphere supports each mooring.

(b) A moored profiler is shown in a testing well. A three-dimensional current meter, a conductivity-temperature-depth (CTD) instrument, and a dissolved O_2 sensor are mounted on this particular model.

(c) Primary production sensor package made up of a combination of three independent instruments with separate modes of operation: (1) Incubation Productivity System (IPS; Taylor and Doherty, 1990; Taylor et al., 1993; Taylor and Howes, 1994). (2) A Photosynthesis, Respiration and Carbon-balance Yielding System (PHORCYS; recent work of author van Mooy and Rick Keil, University Washington). (3) In situ Rapid Repetition Rate Fluorometers (FRRFs; Kolber et al., 1998). See text and Supplement Figure 2e regarding FF3s (bacterioplankton/protist sampling devices).

(d) Time-series sediment trap whose titanium frame can support many independent physical and biogeochemical sensors (Honjo and Doherty, 1988).

(e) Each sampling bottle in this array collects two weeks of vertical flux of particles over a total of one year. Each bottle is filled with a pH-buffered preservative solution.

(f) Micrograph examples of settling particles collected in a 1,000 m trap in the Arabian Sea.

will be maintained at 15 m (a half-wave depth) within the main syntactic-foam float, allowing the incubators to be exposed to sunlight, and measurements will be closely compared with satellite-based ocean color observations. An Automated "Depth Adjuster" (ADA) is currently under development to be located at 150 m depth (tentative) on Mooring A to control the depth of the instrument string above the ADA and allow the depth of the uppermost IPS to be maintained at 15 m, hopefully within ± 2 m (or smaller error range) while other instruments are deployed at specific depths within the euphotic zone. This new technology will allow a depth-sensitive string of PP instruments to be deployed closer to the sea surface, irrespective of ocean bottom depth and potential issues associated with stretching of the mooring cable.

Mooring B: Discrete Water Sampler Array

The objective of this mooring design is to deploy five sets of discrete water samplers (Remote Access Samplers [RASs]), primarily for time-series DOC and DON collection at five depths; the water samplers should be integrated and synchronized with bacterioplankton/protist sampling devices (FF3). The RASs (Supplement Figure 2a,b,c) collect 48 water samples of 500 ml each at depth, drawing the water into multilayered gas-impermeable sample bags that may be unfiltered or filtered through 1.0-, 0.6-, or 0.4-µmØ-diameter nominal pores.

The FF3 device, designed to collect bacteria-sized microorganisms in situ through 1.0- and 0.2 µmØ-diameter filters while preserving RNA, DNA, and protein (Supplement Figure 2e), is a recent development that is being vigorously tested by author Taylor and collaborators. An outstanding feature of the FF3 is that each microfilter is continuously bathed in a saturated RNA*later*® (Life Technologies™) solution during filtration to preserve it for genomic, transcriptomic, and proteomic analyses following recovery. FF3s can also be installed on a robotic primary production incubator (Supplement Figure 1c).

Mooring C: Deep Ocean Biogeochemical Mass-Flux and Contextual-Sensor Array

The mooring C design builds on the traditional TS (time-series) sediment trap array that has successfully served international Joint Global Ocean Flux Study (JGOFS) and other field programs for over 30 years (Supplement Figure 1d,e); reviewed in Honjo et al., 2008). For each pelagic C-type mooring, we propose to deploy seven quasi-equally spaced TS-traps below the euphotic zone (e.g., three traps in the mesopelagic; three traps in the bathypelagic master bio-C reservoir zone, including the benthic layers; and one trap at 2,000 m) each collecting settling particles for 24 equally spaced periods over a 12-month deployment. The mooring is intended to be turned around and redeployed immediately. The open-close cycles of all TS-traps will be synchronized in order to estimate the bulk settling speed of particles.

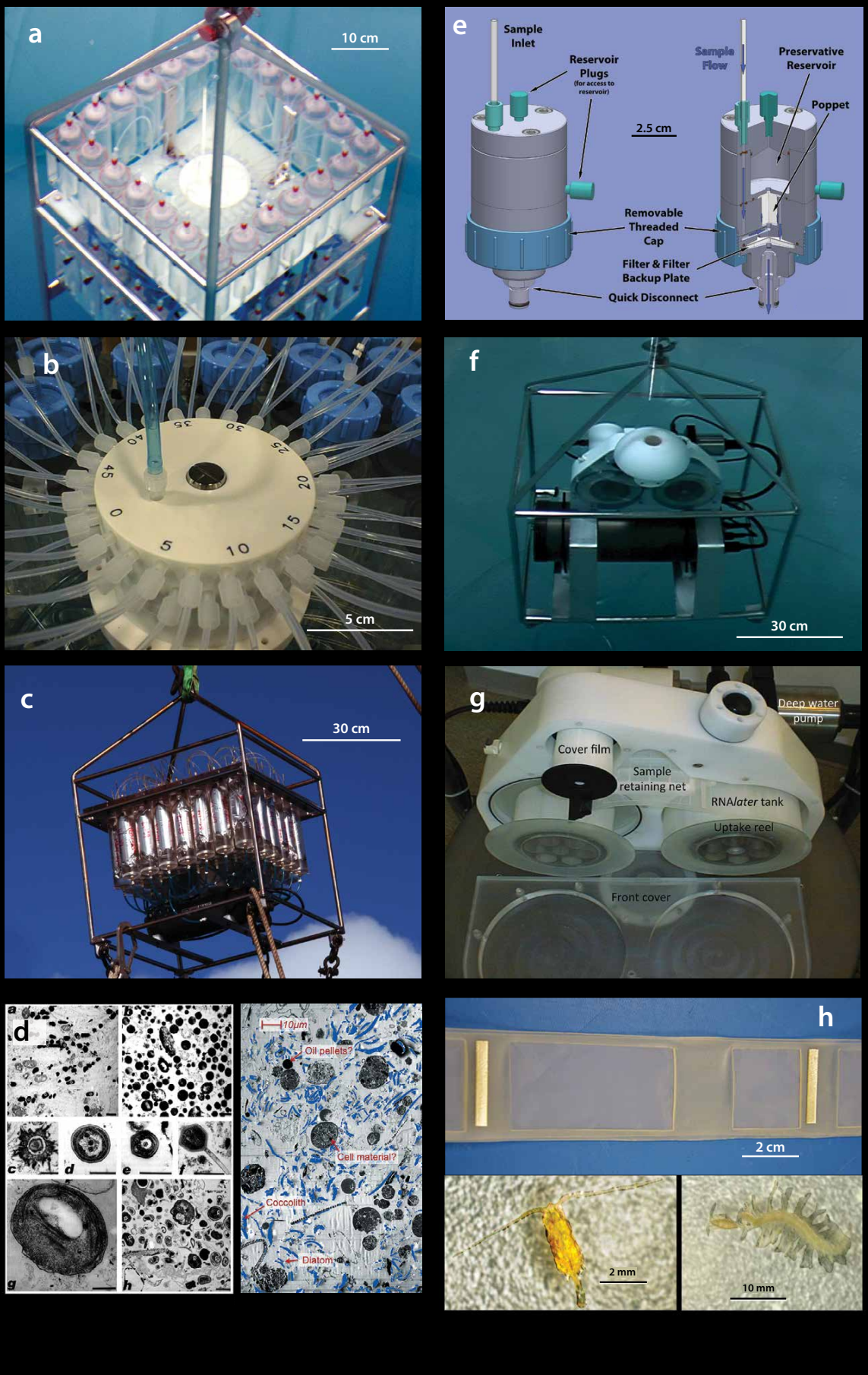
An array of independent sensors can be deployed along a TS-trap mooring to measure contextual ocean properties. A TS-trap is supported by six titanium rods, each 2 m long (Supplement Figure 1d,e), that provide ideal platforms for at least a dozen additional miniaturized, independent sensors. In instances where eight TS-traps are deployed within Mooring C, it would therefore be possible to accommodate 80 to 100 sensors at seven depths (conductivity-temperature-depth [CTD], pCO_2, nutrient sensors, dissolved oxygen optodes, transmissometer, and other ocean optics and acoustic transmitters, to name but a few). In this context, a C-type mooring should be able to serve the Ocean Observatory Initiative (OOI) as well as numerous independent experiments from diverse research groups.

Mooring D: Full Ocean Depth Moored Profiler (MMP)

Mooring D comprises a wire-crawling profiling instrument package (Supplement Figure 1b) designed to serve as a bridge between the OOI and the GBF-O programs by accommodating seamless observation of the entire water column using CTD sensors, three-dimensional current vectors, and dissolved-O_2 probes. In order to better understand the diel vertical migration of the zooplankton community, mini acoustic transponders could be mounted on an MMP (this concept is being tested). In the future, a holographic zooplankton imager (Benfield et al., 2007) could be mounted on an MMP.

Mooring E: Zooplankton Sampler Array

Mooring E consists of five robotic, quantitative zooplankton samplers (ZPS; Supplement Figure 2f,g,h) with in situ RNA*later*® fixation capacity. The ZPS draws meso-zooplankton into a mesh sampler through a sample inlet engineered to minimize "escape response" loss of organisms. It is possible to collect 50 samples that are synchronized with other sensors and samplers. Mesozooplankton are captured between two mesh sheets located ~1 mm apart to avoid crushing the organisms (Supplement Figure 2h); they are preserved in a container with concentrated RNA*later*® to facilitate subsequent molecular/genomic analysis. A ZPS can operate under a variety of sampling modes that may include synchronization with a TS-trap or rapid collection



Supplement Figure 2

(a) A time-series Remote Access Sampler (RAS) collects phytoplankton, suspended particles, and water samples (500 ml).

(b) The central valve system of an RAS. An array of filter holders for phytoplankton and suspended particle collection can be seen in the background.

(c) A side view of (a). All water bags (Al-foil/Teflon laminated) are filled here with collected water, providing one year of time-series sampling.

(d) Transmission electron micrographs of (left) a copepod's gut (Gowing and Wishner, 1998) and (right) a fecal pellet containing coccoliths and diatom frustules (Honjo, 1997).

(e) Bacterioplankton/protist sampling device (FF3) filter holder. Organisms, particularly microbes, that collect on the filter are fixed by a nucleic acid preserving solution (such as RNA*later*®) during filtering and are then immersed in the same solution for long-term storage and preservation. The FF3 filter holders can be used with RASs (a) or other meso-fluidic micro-pumps.

(f, g, h) RNA-preserving, time-series zooplankton sampler (ZPS) systems. Zooplankton are sucked from an intake located on the top of the pump system (f) and introduced into a sample retainer (3 x 5 cm x 0.5 mm) made of a strip of plankton net. The sample retainer is synchronously covered with another plain strip of net so that the collected zooplankton are confined within a few mm space between a pair of plankton nets. The sample retainer then rolls into a tank containing preservative such as RNA*later*®, where the sample is stored. The ZPS is designed to collect 50 time-series samples during a year's deployment.

(i.e., many times a day). As a standard mode of operation, a ZPS is programmed to pass 500 L of water through each sampling cage, repeating this operation 50 times for a total of 25,000 L during a deployment. At this time, ZPS technology has already been applied to quantitative collection of zooplankton during CTD lowerings. Improvement is needed to prevent leakage of preservative from the retainer tank during long-term operations.

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