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UNDERSTANDING THE ROLE OF THE BIOLOGICAL PUMP IN THE GLOBAL CARBON CYCLE

An Imperative for Ocean Science

BY SUSUMU HONJO, TIMOTHY I. EGLINTON, CRAIG D. TAYLOR, KEVIN M. ULMER, STEFAN M. SIEVERT, ASTRID BRACHER, CHRISTOPHER R. GERMAN, VIRGINIA EDGCOMB, ROGER FRANCOIS, M. DEBORA IGLESIAS-RODRIGUEZ, BENJAMIN VAN MOOY, AND DANIEL J. REPETA

Anthropogenically driven climate change will rapidly become Earth's dominant transformative influence in the coming decades. The oceanic biological pump—the complex suite of processes that results in the transfer of particulate and dissolved organic carbon from the surface to the deep ocean—constitutes the main mechanism for removing CO_2 from the atmosphere and sequestering carbon at depth on submillennium time scales. Variations in the efficacy of the biological pump and the strength of the deep ocean carbon sink, which is larger than all other bioactive carbon reservoirs, regulate Earth's climate and have been implicated in past glacial-interglacial cycles. The numerous biological, chemical, and physical processes involved in the biological pump are inextricably linked and heterogeneous over a wide range of spatial and temporal scales, and they influence virtually the entire ocean ecosystem. Thus, the functioning of the oceanic biological pump is not only relevant to the modulation of Earth's climate but also constitutes the basis for marine biodiversity and key food resources that support the human population. Our understanding of the biological pump is far from complete. Moreover, how the biological pump and the deep ocean carbon sink will respond to the rapid and ongoing anthropogenic changes to our planet—including warming, acidification, and deoxygenation of ocean waters—remains highly uncertain. To understand and quantify present-day and future changes in biological pump processes requires sustained global observations coupled with extensive modeling studies supported by international scientific coordination and funding.

BACKGROUND

The pelagic and coastal oceans, together with the Great Lakes, contain over 90% of Earth's bioactive carbon (bio-C) and exert a major influence on the global environment by modulating fluxes and transformations between various carbon reservoirs. In particular, the ocean's bathypelagic zone (including abyssopelagic and hadalpelagic zones) is by far the single largest inventory of bio-C on Earth. It contains 3,150 Pmol (Pmol = 10^{15} mole; Figure 1), more than 50 times greater than the amount of CO_2-C in the atmosphere, currently estimated to be about 62.5 Pmol (pre-industrial levels are estimated to have been about 48.3 Pmol; IPCC, 2007), and more than an order of magnitude greater than all the bio-C held in terrestrial vegetation, soils, and microbes combined. The sink-strength (or "feedback efficiency"; Falkowski et al., 2000) of this reservoir is critical in buffering Earth's atmosphere from a rapid CO_2 increase. Operating in parallel, the inorganic gas-exchange pump (which includes the carbonate/bicarbonate buffer-driven solubility pump) is estimated to account for only ~ 10% of the total transfer of dissolved inorganic carbon (DIC) from surface to deep waters in the modern ocean (e.g., Sarmiento and Gruber, 2006). In this article, we exclusively focus on the biological pump.

The biological pump starts in the

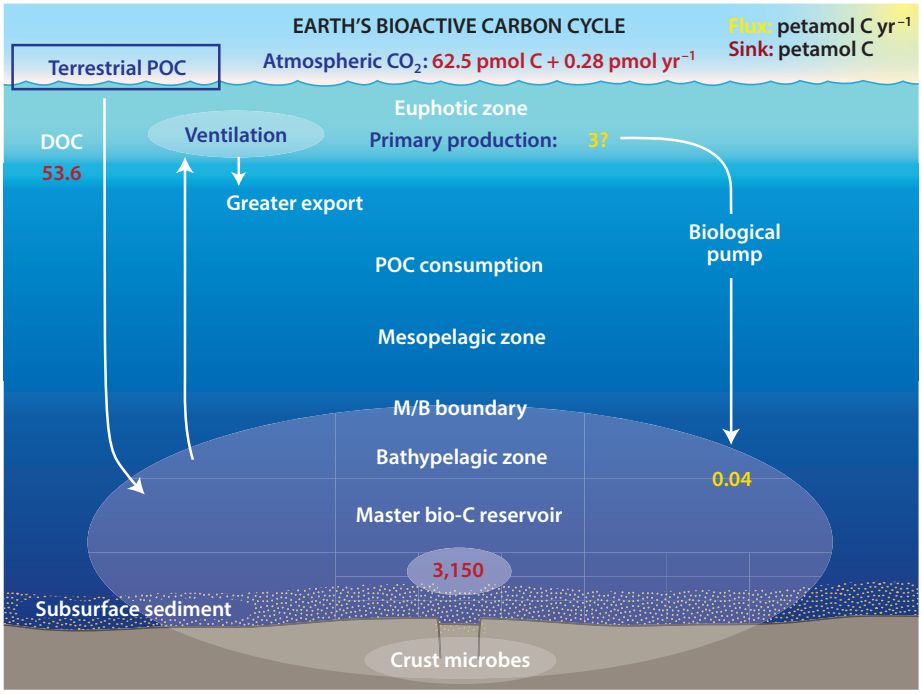

Figure 1. A simplified conceptual diagram of Earth's bioactive carbon cycle with the size (petamol C) of the atmospheric reservoir as CO_2 (CO_2-C). The deep ocean sink is shown in red, and key fluxes (petamol C yr^{-1}) are in yellow. The current CO_2-C inventory in Earth's atmosphere (62.5 petamol C) is increasing at the rate of 0.28 petamol C yr^{-1}. POC (particulate organic carbon) exported to the bathypelagic zone by the biological pump is estimated at 0.04 petamol C yr^{-1}. This zone, containing 3,150 petamol C, represents Earth's master reservoir of bioactive C. For clarity, the solubility pump, which is estimated to account for ~ 10% of the total transfer of DOC (dissolved organic carbon) from surface to deep waters in the modern ocean (Sarmiento and Gruber, 2006), is not included. M/B = mesopelagic/bathypelagic.

euphotic zone with the photosynthetic fixation of inorganic carbon into phytoplankton biomass. Current estimates of global oceanic primary production (G-PP) are between 3 and 4 Pmol C yr^{-1} (e.g., Berger, 1989; Antoine, 1996; Behrenfeld and Falkowski, 1997; Chavez et al., 2011). Research undertaken during the US Joint Global Ocean Flux Study (US JGOFS, ca. 1987–2005) and subsequent programs clarified that a fraction of this bio-C is rapidly removed from surface waters and exported to the ocean's interior in the form of particulate organic matter (POM) through a complex interplay of biological processes combined with gravity (eco-dynamic transport; e.g., Honjo et al., 2008; Online Supplement, Section 1). Chemoautotrophic processes in the meso- and bathypelagic realms may also play important roles in modulating deep ocean carbon inventories (e.g., Arístegui et al., 2009; Swan et al., 2011; Online Supplement, Section 2).

Prior studies suggest that the annual flux of bio-C to the bathypelagic ocean by direct transport of POC is ~ 0.04 Pmol yr^{-1} (Figure 1; Honjo et al., 2008). Notably, this flux represents only 14% of the current annual increase of carbon as atmospheric CO_2, highlighting the importance of understanding how the biological pump will respond to increasing atmospheric CO_2 concentrations, and whether the bathypelagic carbon reservoir can remain a sink for this anthropogenic carbon.

There are serious deficiencies in our ability to place these processes in a quantitative context, to determine their dynamics, and to assess how the ocean will respond to, or exacerbate, climate change, pollution, and over-exploitation of marine resources. For example, our recognition of the large stock of prokaryotic biomass throughout the ocean and in subsurface and subseafloor environments (e.g., Whitman, et al., 1998; Arístegui, et al., 2009; Lauro and Bartlett, 2008; Kallmeyer et al., 2012) and of dissolved organic carbon residing in ocean waters (Hansell and Carlson, 2013) sharply contrasts with our limited knowledge of their roles in biogeochemical processes. A complete mechanistic and quantitative understanding of the biological pump is essential for determining its importance in modulating atmospheric CO_2 and predicting its future behavior. Programs such as the Global Carbon Project (http://www.globalcarbonproject.org) as well as other global carbon flux modeling efforts are in need of far more extensive and comprehensive ocean data to further refine their predictive capabilities. Input from this community will be critical in guiding the prioritization for measurements needed to address current deficiencies in our models.

ADDRESSING KNOWLEDGE GAPS: THE GRAND CHALLENGE

Current global flux estimates of bio-C generally stem from data acquired from highly diverse and often asynchronous observations. There is considerable uncertainty in these estimates due to sparse and heterogeneous data coverage that may fail to capture seasonal

variability or incorporate geographical biases. For example, although US JGOFS provided a wealth of new insights, derivation of global-scale carbon fluxes from this and other programs is fraught with uncertainty because discontinuous observations spanned > 10 years and various parameters were not measured simultaneously. These deficiencies reflect both a lack of technology and limited opportunities for ocean observations of the type and scope required to develop precise constraints on the biological pump on temporal and spatial scales suitable for assessing links and sensitivity to global change.

SPATIAL AND TEMPORAL VARIATIONS IN BIO-C CYCLING

In the euphotic zone, or "phytoplankton domain," accurate constraints on marine primary production must be established in terms of absolute flux, photoautotrophic community structure, and biomineral (ballast) production and removal rates. Satellite-based surface ocean color observations have yielded the most spatially comprehensive view of G-PP (e.g., Behrenfeld and Falkowski, 1997) and will be indispensable in future ocean observing efforts. However, these measurements probe only the surface layers of the euphotic zone and presently deliver only restricted information on the diversity of primary producers (e.g., Alvain et al. 2005; Bracher et al., 2009) and on the fate of this photosynthetically derived carbon. While new constraints on organic carbon export are being realized through coupling of satellite observations with food web models (Siegel et al., 2014), high-resolution time-series measurements (e.g., Taylor and Howes, 1994) would provide greatly improved assessment of carbon and biomineral production throughout the euphotic zone and of autotrophic processes at all ocean depths (Figure 2).

In the mesopelagic zone, or "prokaryote/zooplankton domain," both prokaryotic and eukaryotic organisms are understood to strongly influence biogeochemical processes. However, their impacts on the net flux and composition of settling particulate organic carbon (POC), and of dissolved organic carbon (DOC), remains poorly constrained (e.g., Steinberg et al., 2002; Buesseler et al., 2007). In particular, the diel vertical shuttling of zooplankton through the mesopelagic zone (e.g., Angel and Baker, 1982) involves complex ecodynamic transport and transformation of POC (Figure 2), imposing serious challenges to the characterization and parameterization of this important but elusive component of the biological pump. Microbes occur abundantly in mesozooplankton guts (e.g., Gowing and Wishner, 1998), free settling fecal pellets (Honjo, 1997) and marine snow (e.g., Alldredge and Cox, 1982; Alldredge and Silver, 1988). Quantitative research on these microbes is greatly needed for understanding the transport of bio-C throughout the water column (Online Supplement, Section 3).

The bathypelagic zone or "prokaryotic domain" comprising Earth's bio-C master reservoir (Figures 1 and 2) is crucial in the context of the oceanic carbon cycle, yet it remains grossly undersampled. The metabolic activity of prokaryotic/eukaryotic communities largely controls in situ organic matter remineralization to ΣCO_2-aq in the bathypelagic water column and underlying sediment because of the scarcity of zooplankton in this zone. Globally, the amount of prokaryote biomass in subsurface ocean sediments remains a topic of debate. The standing crop of bio-C in subsurface sediment is estimated to be 25 Pmol C (40% of the amount of current atmospheric CO_2-C) and includes diverse assemblages of microorganisms (Whitman et al., 1998; Kallmeyer et al., 2012; Figure 1). Further research is necessary to elucidate and quantify rates of carbon transformation in bathypelagic waters and underlying sediments.

The dynamics of ocean margin ecosystems and associated bio-C are even more complex than pelagic ocean dynamics. The margins are regions of large ecological diversity (Levin and Sibuet, 2012) and of high carbon productivity, export, and burial (Tsunogai

Susumu Honjo *(shonjo@whoi.edu) is Scientist Emeritus, Wood Hole Oceanographic Institution (WHOI), Woods Hole, MA, USA.* **Timothy I. Eglinton** *(timothy.eglinton@erdw.ethz.ch) is Professor, Eidgenössische Technische Hochschule, Zurich, Switzerland, and Adjunct Scientist, WHOI, Woods Hole, MA, USA.* **Craig D. Taylor** *is Associate Scientist, WHOI, Woods Hole, MA, USA.* **Kevin M. Ulmer** *is Executive Director, Seaquester, Inc., East Sandwich, MA, and Guest Investigator, WHOI, Woods Hole, MA, USA.* **Stefan M. Sievert** *is Associate Scientist, WHOI, Woods Hole, MA, USA.* **Astrid Bracher** *is Professor, Alfred Wegener Institute for Polar and Marine Research, Bremerhaven, Germany.* **Christopher R. German** *is Senior Scientist, WHOI, Woods Hole, MA, USA.* **Virginia Edgcomb** *is Associate Scientist, WHOI, Woods Hole, MA, USA.* **Roger Francois** *is Professor, University of British Columbia, Vancouver, Canada.* **M. Debora Iglesias-Rodriguez** *is Professor, University of California, Santa Barbara, CA, USA.* **Benjamin Van Mooy** *is Associate Scientist, WHOI, Woods Hole, MA, USA.* **Daniel J. Repeta** *is Senior Scientist, WHOI, Woods Hole, MA, USA.*

et al., 1999; Thunell et al., 2000; Muller-Karger et al., 2010; Montes et al., 2012). Characterizing processes on the continental margins and their influence on deep ocean bio-C inventories is therefore a prerequisite for developing a complete understanding of the global carbon cycle, yet ocean margins remain woefully underrepresented in global carbon databases and models (e.g., Thunell et al., 2007).

THE GLOBAL BIOGEOCHEMICAL FLUX OBSERVATORY CONCEPT

The rapid pace of atmospheric carbon accumulation is likely to increase as a result of positive feedback mechanisms: ocean warming, deoxygenation, and acidification are proceeding at measurable rates and on a global scale. Assessment of the impacts of these and other perturbations related to global climate change on ocean biogeochemical processes can only be addressed via sustained observations (e.g., Wunsch et al., 2013). Linking changes in the physical/chemical environment with biological and biogeochemical properties and processes and accurate modeling and prediction of the effects of global change (e.g., Siegel, et al., 2014) requires scientists across multiple ocean research disciplines to develop and build upon technological innovations toward cost-effective implementation of reliable systems. It is also important to instill in society appreciation of the ocean as a vital global resource, understanding of its role in maintaining the habitability of our fragile planet, and recognition of the need for multidecadal observations of ocean processes.

The Global Biogeochemical Flux Observatory (GBF-O) concept offers a framework for implementing a sustained observation and sampling program that complements elements of the US Ocean Observatory Initiative (OOI) and other ocean observatory programs (e.g., http://www.oceansites.org, http://www.ioc-goos.org), as well as other observational approaches, such as satellite-based global investigations of marine primary productivity (Behrenfeld and Falkowski, 1997), shipboard time-series programs (Church et al., 2013), and widespread dissemination of floats and gliders equipped with sensors for constraining ocean biogeochemical processes (Johnson et al., 2009). The GBF-O concept is based on a combination of established technologies and advanced autonomous instrumentation operated synchronously. Among the key facets of the GBF-O that distinguish it from the OOI are an emphasis on long-term sample acquisition, preservation of the samples for subsequent retrieval of maximum biogeochemical (e.g., genomic) information, and return of the samples for detailed laboratory-based analyses (Online Supplement, Section 3). These elements are vital for extracting the greatest level of information and for developing a sample legacy that will be invaluable for future research as new analytical technologies emerge.

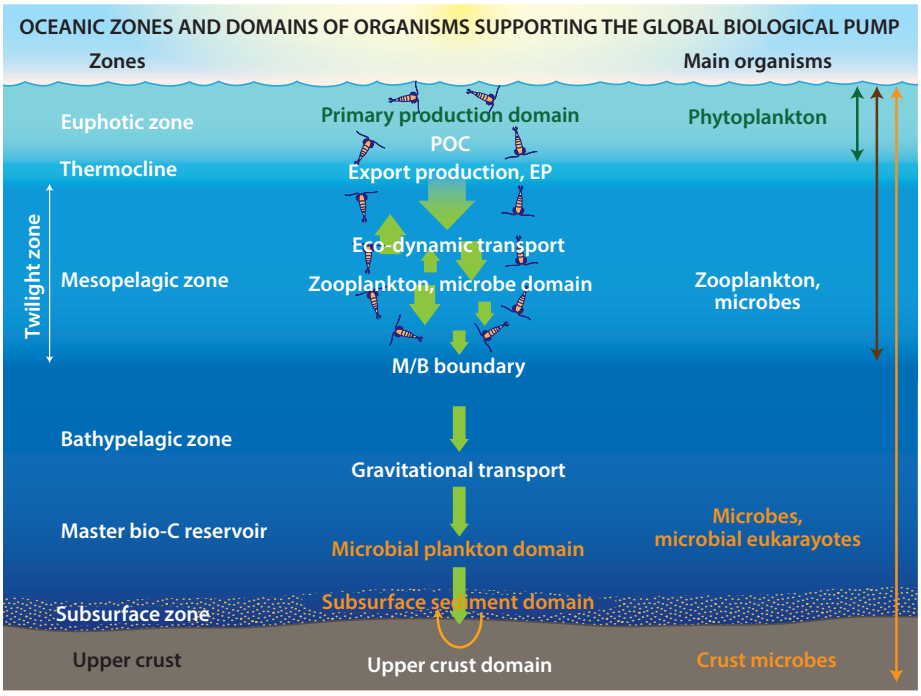

Figure 2. Schematic illustration of major oceanic zones and biological domains between the air-sea interface and the deep ocean floor, including the subsurface zone. Below the mesopelagic/bathypelagic (M/B) boundary, there is little zooplankton activity, so, hypothetically, the large population of prokaryotes near the bottom of the water column is supported by gravitational transport of biomineral-ballasted particles that descend from surface waters.

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Methodology

Key methodological elements of the GBF-O concept are:

1. Observations from the air-sea interface through the euphotic, mesopelagic, and bathypelagic zones to the seafloor
2. Sustained, synchronized time-series observational modes to monitor the seasonal and interannual rhythms of the biological pump
3. Ecosystem characterization encompassing a broad spectrum of organisms from pelagic to benthic communities, and from prokaryotes to zooplankton
4. Implementation and maintenance of centralized laboratories for accurate and precise determination of core biogeochemical flux parameters
5. Incorporation of profiling and fixed-depth contextual instrumentation
6. Construction of a long-term archive that acquires and preserves samples for future in-depth "omics" and related studies associated with biogeochemical and paleoceanographic proxy research (Online Supplement, Section 2)

Technical Readiness

The challenges of implementing the GBF-O approach are formidable, but they must be met in order to fully understand the workings of the biological pump and associated processes in the context of global change. Autonomous observation of ocean properties represents a major new emphasis within the ocean science community (e.g., Johnson et al., 2009; Bishop, 2009), and remote observation capabilities are continuously being developed. Mooring systems that support full ocean depth biogeochemical experiments also have advanced during US JGOFS and related programs. As for any observatory, it is essential that all of the associated instruments and supporting materials be designed and manufactured to produce consistent results. Mass production of instruments and mooring platforms is crucial to ensure broad availability of serviceable, cost-effective systems that meet rigorous specifications.

Orchestration of GBF-O Arrays

Synchronization of instruments and sensors within and between observatory arrays is critical for understanding the rhythms of global ocean biogeochemical processes. The majority of POC (often 70% to 90% of annual export) and other biogenic particulates are produced during episodes that usually occur only once or a few times a year in response to seasonal phytoplankton blooms (e.g., Wefer et al., 1988). The resulting sharp export pulses gradually diminish in amplitude with depth (reviewed in Honjo et al., 2008). Defining the annual pattern and evolution of this curve throughout the water column represents an important aspect of constraining the functioning of the biological pump and its impact on ocean-atmosphere carbon balances (Kwon et al., 2009).

Preliminary Vision for GBF-O Implementation

Figure 3 presents one vision of a stand-alone GBF-O instrument. Although dependent upon local bathymetric conditions, the moorings within the array would typically be set from several to 12 nm apart (to allow for unobstructed deployment). Each mooring would be kept in vertical alignment by a single syntactic-foam sphere with the appropriate buoyancy. In this example of a GBF-O array, samplers are deployed at specific intervals along each mooring to cover different water column domains. Such an array could host more than 25 major time-series devices as well as many contextual sensors and "guest" instruments. Further details of the GBF-O array and instruments are in the Online Supplement, Section 4.

A single array of this type, equipped with the instrumentation capabilities depicted in Figure 3, would yield a wealth of new information. Deployment of multiple arrays throughout the major ocean basins would form the basis for a GBF-O. Selection of specific locations for array deployments would be based on multidisciplinary perspectives and consensus in order to maximize our level of understanding and predictive capability regarding biological pump processes. Criteria for determining array locations would, for example, involve assessments of primary production based on ocean color (e.g., Behrenfeld and Falkowski, 1997), ocean biogeochemical provinces (e.g., Longhurst et al., 1995), observations from prior studies (e.g., Honjo et al., 2008), bathymetric variations, and maritime logistics.

CONCLUSION

Our ability to model the workings of the oceanic biological pump comprehensively and accurately is a critical component of global efforts to forecast the trajectory and effects of anthropogenic climate change. We have begun to understand the major features of the biological pump and its key role in the sequestration of carbon in the ocean, but we are still blind to many of its characteristics and far from developing comprehensive mechanistic and quantitative constraints on its myriad processes. Assessment of the impact of climate change on ocean biogeochemical processes and ecosystems, and vice versa, can only be addressed via global, standardized, sustained,

synchronous observations over coming decades. Indeed, we hope to galvanize the oceanographic community to champion the need for a century of ocean observation—deploying a truly global array of state-of-the-art sensors and other instrumentation that will be necessary for understanding not only carbon flow in the ocean but also all of the ocean's intimately related inhabitants.

Recent rapid progress in underwater technologies, particularly ocean robotics and novel in situ sensors, experimentation platforms, and discrete samplers, has made it feasible to develop high-endurance sentry instruments capable of operating in diverse ocean environments to provide these essential data. However, the magnitude of the undertaking will require international scientific coordination and funding. We must strive as a community to integrate all emerging ocean observatories to forge the best possible global planetary observation network and elevate its priority above that which already exists for other bodies in our solar system and far beyond. The scientific and societal imperatives are clear—and the clock is ticking.

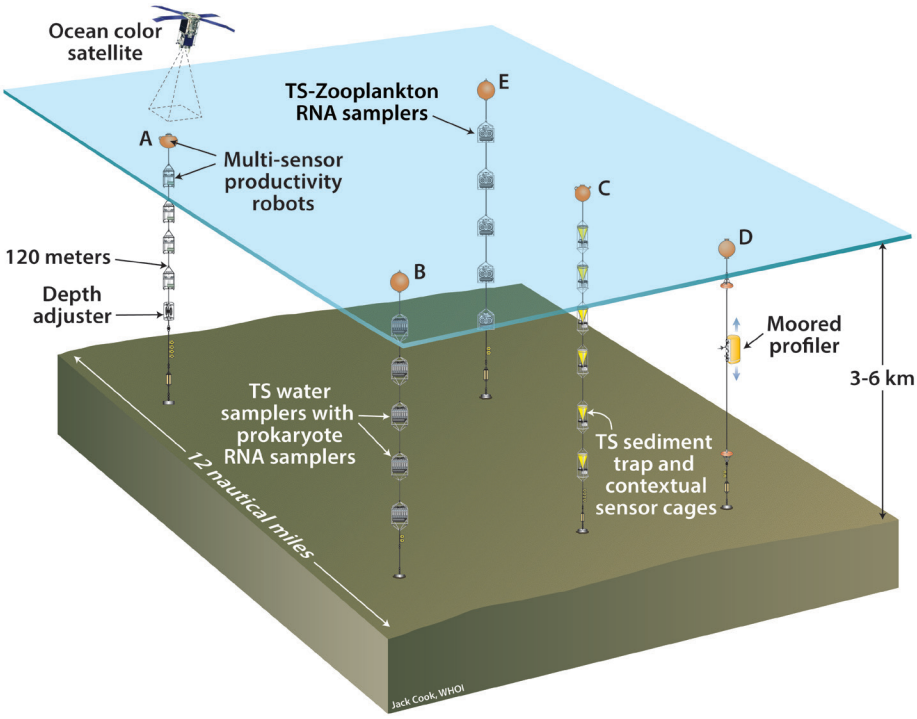

Figure 3. A preliminary vision of a Global Biogeochemical Flux Observatory (GBF-O) instrumented mooring array for sustained deployment at a given ocean location. The array consists of five moorings that include (A) primary production measurements, (B) in situ time-series (TS) water samplers and quantitative prokaryote collectors that preserve RNA, (C) TS sediment traps to capture settling POC and other particulate matter, (D) a moored profiler for seamless recording of conductivity-temperature-depth, current, and acoustic ecosystem imaging data, and (E) quantitative zooplankton samplers with autonomous RNA fixing processors. Further details are in the Online Supplement, Section 4.

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ONLINE SUPPLEMENT

The Online Supplement can be accessed at http://www.tos.org/oceanography/archive/27-3_honjo.html.

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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

by S. Honjo, T.I. Eglinton, C.D. Taylor, K.M. Ulmer, S.M. Sievert, A. Bracher, C.R. German, V. Edgcomb, R. Francois, M.D. Iglesias-Rodriguez, B. van Mooy, and D.J. Repeta

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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 ($\leq$ 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.

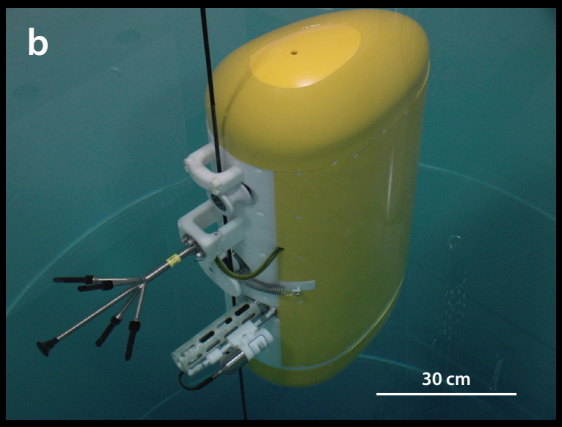
SECTION 4. GLOBAL BIOGEOCHEMICAL FLUX OBSERVATORY COMPONENTS

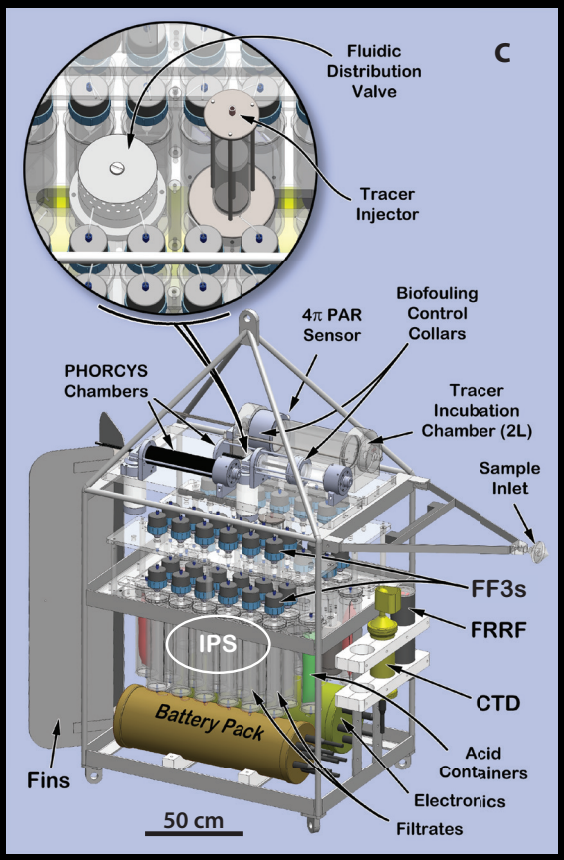
In order to track and assess the transport and transformation of bioactive carbon (bio-C) and to properly sample oceanic particles and microbes from all oceanographic zones and domains in all seasons, we must sample and examine at ecological, metabolic, and genetic levels all of the life forms (eukaryotes,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

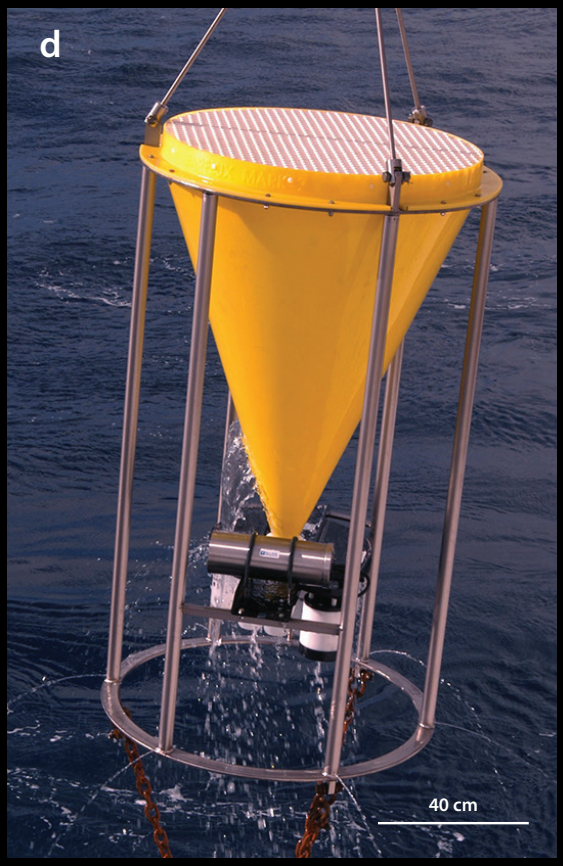
Mooring designs and the instruments intended for incorporation in the Global Biogeochemical Flux Observatory (GBF-O) are described below. Many of the sensors and samplers have been in active use for various oceanographic objectives and have endured deployment for up to a year or more. However, some are 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.

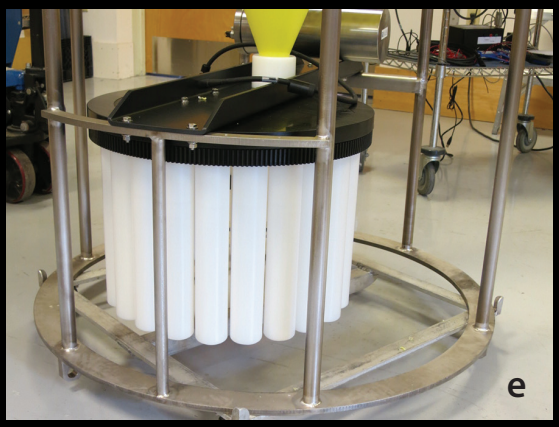
Mooring A: Primary Production Array

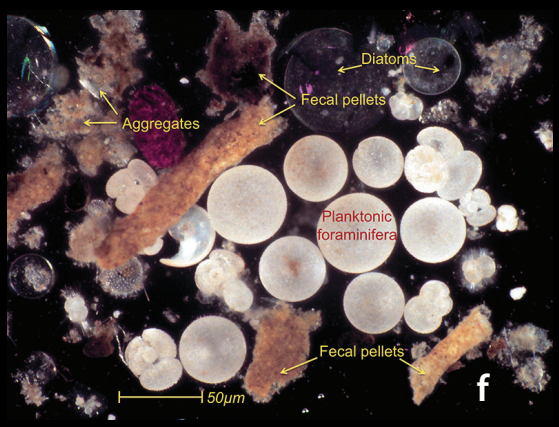
Mooring A (Figure 3 of the main text) is a fully submerged, bottom-tethered array. It consists of three main types of instruments. (1) Five sets of in situ robotic incubators for non-radioactive C and N isotopic tracer research (Incubation, Productivity with Samplers [IPSs]) that are based on earlier 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.

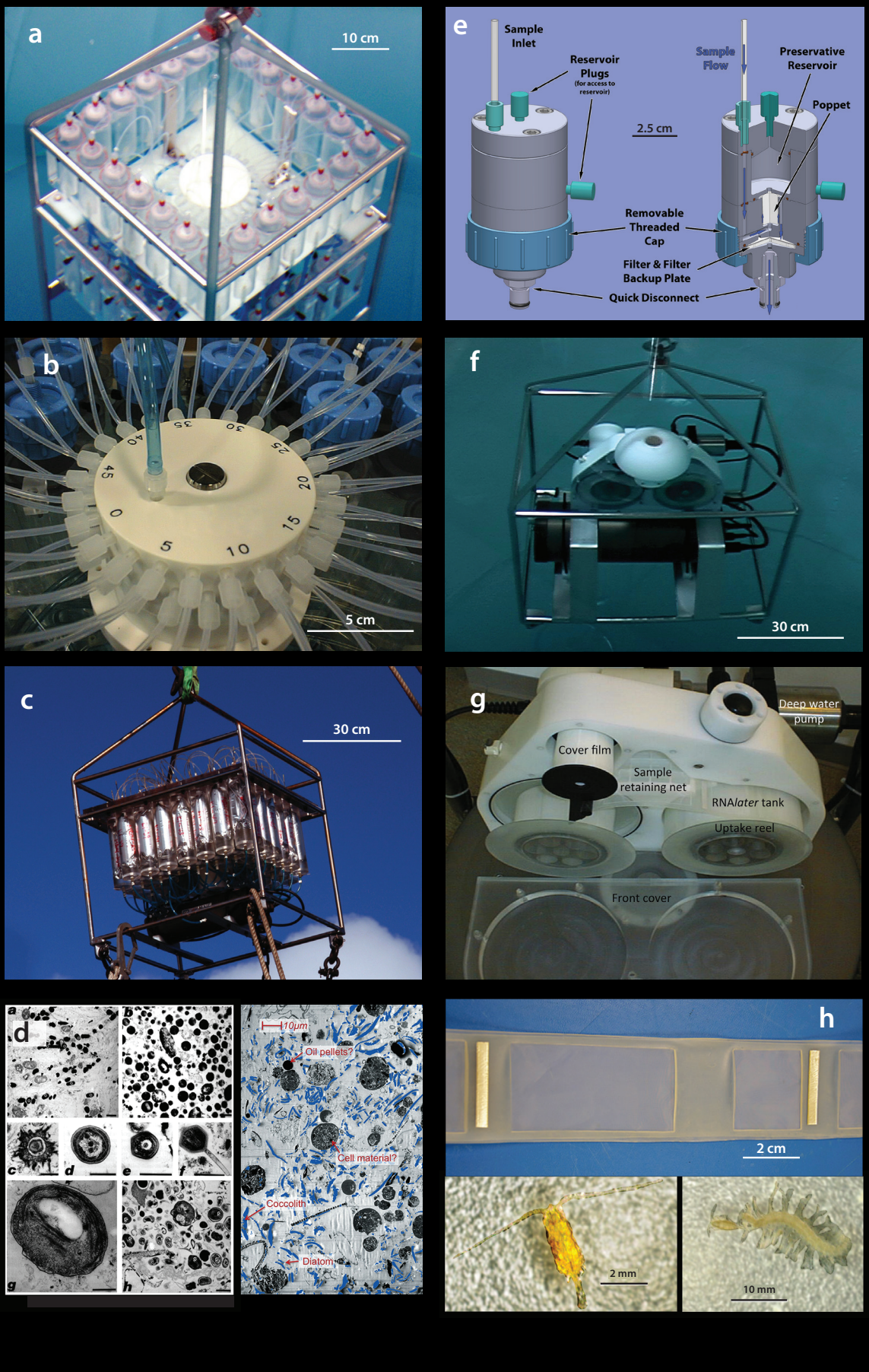
An array of independent sensors can be deployed along a TS-trap mooring to measure contextual ocean properties. A TS-trap is supported by six titanium rods, each 2 m long (Supplement Figure 1d,e), that provide ideal platforms for at least a dozen additional miniaturized, independent sensors. In instances 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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