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Instruments and Methods

A suspended-particle rosette multi-sampler for discrete biogeochemical sampling in low-particle-density waters

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ABSTRACT

To enable detailed investigations of early stage hydrothermal plume formation and abiotic and biotic plume processes we developed a new oceanographic tool. The Suspended Particulate Rosette sampling system has been designed to collect geochemical and microbial samples from the rising portion of deep-sea hydrothermal plumes. It can be deployed on a remotely operated vehicle for sampling rising plumes, on a wire-deployed water rosette for spatially discrete sampling of non-buoyant hydrothermal plumes, or on a fixed mooring in a hydrothermal vent field for time series sampling. It has performed successfully during both its first mooring deployment at the East Pacific Rise and its first remotely-operated vehicle deployments along the Mid-Atlantic Ridge. It is currently capable of rapidly filtering 24 discrete large-water-volume samples (30–100 L per sample) for suspended particles during a single deployment (e.g. >90 L per sample at 4–7 L per minute through 1 μm pore diameter polycarbonate filters). The Suspended Particulate Rosette sampler has been designed with a long-term goal of seafloor observatory deployments, where it can be used to collect samples in response to tectonic or other events. It is compatible with *in situ* optical sensors, such as laser Raman or visible reflectance spectroscopy systems, enabling *in situ* particle analysis immediately after sample collection and before the particles alter or degrade.

1. Introduction

Suspended particulate material is ubiquitous throughout the hydrosphere. In its broadest definition it is composed of living and non-living material and spans a range of particle sizes, compositions, and concentrations. The formation, transport, dissolution, and burial of particulate material are fundamental to biogeochemical cycles (Anderson et al., 2003). The distribution and transport of the biotic components of suspended particles – larvae, plankton, microbes, and viruses – are equally important in understanding aquatic ecosystems. Niskin bottles, *in situ* filtering pelagic pumps, and a variety of specialized apparatus have served most suspended particle sampling needs. However, in deep-sea hydrothermal plumes, we are undertaking new geochemical and microbial research in environments that require spatially and temporally precise sampling.

To accomplish this, we have developed a novel Suspended Particulate Rosette (SUPR) multi-sampler that can be deployed either on a mooring for unattended time series sample collection or on a remotely operated vehicle (ROV) to enable sampling tasks such as vertical-profiling of rising hydrothermal plumes. The SUPR sampler is

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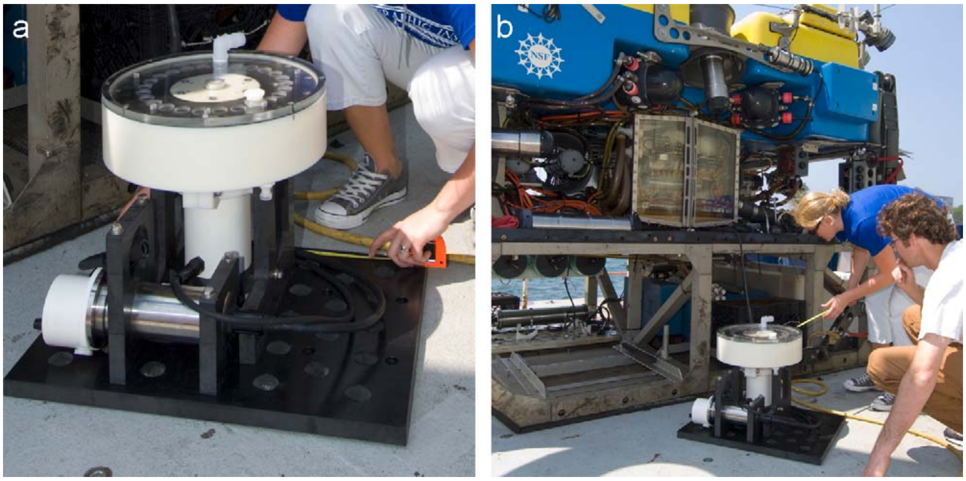

Fig. 1. The SUPR sampler is a WHOI designed and built, optical-sensor compatible, multi-sample filtering head interfaced to a McLane Research Laboratories high-flowrate pumping system. When configured for ROV deployments the SUPR system (a) is compact enough to fit into any science payload position on the ROV *Jason* (b).

currently capable of rapidly filtering 24 discrete large-water-volume samples (30–100 L per sample at 4–7 L min^{-1} through 25–47 mm diameter filters) for suspended particles during a single deployment. The ROV version weighs 7 kg in water and occupies a volume $<0.1\,\text{m}^3$ (Fig. 1). It is designed to host *in situ* optical sensors, such as a dedicated laser Raman spectroscopy system, to enable *in situ* particle analysis. In this paper, we discuss the scientific need for the SUPR-sampler, the sampler design, and results from the first two at-sea deployments: a) moored on the East Pacific Rise (EPR) in 2007, and b) ROV-deployed on the Mid-Atlantic Ridge (MAR) in 2008.

2. Scientific background

Processes in hydrothermal plumes alter the gross chemical fluxes from hydrothermal vents to the oceans. Two general processes have been identified: *Process I*) co-precipitation of Fe and other chalcophile elements to form polymetallic sulfide phases immediately when vent fluids enter the ocean (i.e. a "quenching" effect); and *Process II*) co-precipitation of trace elements with, and sorption of dissolved metals onto, freshly-formed Fe oxyhydroxide phases as the reduced vent fluids mix with more oxidizing ambient ocean waters (Feely et al., 1987; Lilley et al., 1995; German and Von Damn, 2003). While much of the chemical flux from *Process I* is deposited on the seafloor as metaliferous sediments, *Process II* results in the generation of a low-density particle floc that can be dispersed many kilometers through the water column. Also during *Process II*, seawater nutrients and trace elements (e.g. P, As, Cr, and V) are scavenged by the highly reactive Fe- and Mn-rich plume particles (Mottl and Mcconachy, 1990; Metz and Trefry, 2000). It may take less than 10,000 years for the entire ocean to pass through the plumes of Earth's deep-sea vents (Feely et al., 1991; Kadko, 1993; Elderfield and Schultz, 1996). This is rapid in geologic terms; therefore, the processes that occur within hydrothermal plumes may have a direct effect on global seawater chemistry (Kadko et al., 1995).

In addition to geochemical transformations, Fe and Mn oxidation reactions are thought to be important energy sources for chemosynthetic microbial communities at and below the seafloor (Edwards et al., 2003; Bach et al., 2006). Microbial processes may also be important in hydrothermal plumes (e.g. Cowen and German, 2003), affecting oxidation rates and the ultimate fate of hydrothermal plume particles. Estimates of the chemical energy available within hydrothermal plumes show that a variety of chemosynthetic metabolic pathways are possible (McCollom and Shock, 1997). Deming and Baross (1993) reported elevated particulate DNA and cell concentrations in rising vent fluids. Several studies have shown evidence of microbial activity within neutrally buoyant hydrothermal plumes based on Mn and CH_4 oxidation and biomass production (Cowen et al., 1986; De Angelis et al., 1993; O'Brien et al., 1998; Dick et al., 2006). It is unknown whether such microbial communities are opportunistic or endemic, and if endemic how they persist in such a physically dynamic setting.

It should be stressed that most of our understanding of hydrothermal plume processes has come from sampling fluids near vent orifices and particles from neutrally buoyant plumes, typically 100–200 m above the seafloor. Because of the technical challenges, comparatively few samples have been collected from the buoyant (rising) portion of hydrothermal plumes, where most particles initially form and are most reactive. To develop a better understanding of biotic and abiotic plume processes, we have developed a new tool that can systematically sample particles from discrete locations within these rising and laterally spreading hydrothermal plumes.

2.1. Sampling needs

To systematically sample rising hydrothermal plumes, sample collection must be rapid enough that an adequate sampling plan can be carried out within a single ROV dive. Samples must be collected over a wide range of suspended particle concentrations; because hydrothermal fluids are diluted by a factor of 10^4 by the time the vent fluid/seawater mixture reaches the level of a dispersing plume (Lupton, 1995). Samples must also be collected across a range of particle sizes. Excluding plankton, the largest hydrothermal particles are on the order of 10 s of microns, while the smallest particles are at the nano-scale (although the currently accepted, operationally-defined limit is 0.2 μm). For microbial analysis, cells as small as 0.2 μm are significant and must be collected. For geochemical analysis, collecting ≥1 μm sized particles and larger is currently the most pragmatic option because it allows sufficient material for a variety of analyses. Evidence suggests that in hydrothermal plumes most of the particle mass is associated with larger particles (Bennett, *pers. comm*., unpublished data), though a better quantification of this for rising plumes is an excellent application for our SUPR-sampler.

In addition to the sampling requirements listed above, the sampler design must be compatible with both trace-metal and microbial cleanliness practices and standards. The sampler should be depth rated to at least 5500 m, allowing access to all currently known deep-sea hydrothermal vents, such as the recently discovered Ula Nui vent field on the abyssal flanks of Loihi seamount at 5000 m (Davis et al., 2007). When operated on an ROV, it should be small and compact, and when deployed autonomously, it should have sufficient battery capacity to filter as much as 2400 L total for up to a 12-month period. For quantitative data reduction, it must record the water volumes filtered for each sample. Finally, since our long-term goal is to perform *in situ* particle analysis using optical techniques, such as laser Raman and visible reflectance spectroscopies (Breier et al., 2009), we require an optically compatible particulate sampler.

2.2. *Existing particulate samplers*

The time-tested particulate multi-sampler is a set of Niskin or GO-FLO® bottles combined with surface filtration. Some examples of this type of sampling include the following. On the Endeavor Segment of the Juan de Fuca Ridge, Straube et al. (1990) collected 25 whole-water samples from the rising buoyant plume using 1.5- and 5-L GO-FLO® bottles deployed on the Alvin submersible. On the Cleft Segment of the Juan de Fuca Ridge during Alvin dives on January 1990, Cowen et al. (1990) collected whole-water samples (1–10 m above vents) using 1.7- and 10-L Niskin bottles in the lower portion of the plumes. The same sampling approach was also used at TAG on the MAR (Edmond et al., 1990; Rudnicki and Elderfield, 1993). There have also been instances where the upper portions of buoyant plumes were sampled by vertical CTD-rosette and wire-deployed, stand-alone pump casts (e.g., Bennett et al., 2008; Edmonds and German, 2004; G. Wheat and J. Resing, *pers. comm.*), but this method is highly dependent on surface and subsurface conditions and has been neither systematic nor reliable. There are three major drawbacks to the bottle approach: i) sample size is limited by bottle volume, ii) sample payload is limited by collecting whole-water samples and iii) samples can be biased by particle adherence to the bottle walls. Mitra et al. (1994) describes the bias that can occur when filtering particles from water bottles.

Sampling by *in situ* filtration is a good solution to the disadvantages of whole-water samplers. However, there is a tradeoff; whole-water samplers collect a sample almost instantaneously, whereas *in situ* filtration may take several minutes to collect a sample from a comparable water volume. The time-scales of the processes involved and science needs will dictate whether a water sampling or *in situ* filtration approach to sample collection is more appropriate. For our applications, the advantages of *in situ* filtration out weigh the tradeoff in sampling speed. Several *in situ* filtration approaches to particle and fluid multi-sampling have been developed for specific scientific applications. We considered four existing samplers during the SUPR design process: the Continuous Plankton Recorder (CPR), the Butterfield et al. (2004) Hydrothermal Fluid and Particulate Sampler, the McLane Water Transfer System (WTS) series multi-samplers, and the Sholkovitz et al. (2001) Buoy-Mounted Aerosol Sampler.

The CPR is a towed device that has been used to sample plankton in the surface ocean for decades (Hardy, 1939). It collects plankton onto a spool of mesh that is continuously wound through the device by a propeller driven mechanism. While a pumped, discrete-sample adaptation of the CPR design could be a very compact device, ensuring sample integrity would require a relatively complex design. In addition, opportunities for *in situ* optical analysis would be limited to one instance immediately after collection.

The Hydrothermal Fluid and Particulate Sampler (HFPS) was developed by Butterfield et al. (2004) for controlled sampling of diffuse hydrothermal fluid at the seafloor by ROV. It can be configured to collect a combination of fluid (filtered or unfiltered), particulate, and gas-tight samples. It continuously measures temperature, just within the inlet and further along the intake tube, to ensure that samples are actually diffuse hydrothermal discharge. It uses a McLane dual multi-port valve to draw water through different sampling ports. Filtered samples are collected on individual 47 mm diameter filters. Fluid samples are limited to 800 mL, and filtration can take 10 to 15 min to complete (Huber et al., 2003).

Like the HFPS, the McLane WTS series samplers also use a similar dual multi-port valve to draw water (<10 L) through individual, typically 47 mm diameter, filters (e.g. Rendigs and Bothner, 2004). This general design has been adapted to a variety of applications with similar sampling needs, including seafloor microbial sampling at hydrothermal vents (Taylor et al., 2006). Both the HFPS and the McLane WTS systems are intended for relatively small volume samples where minimizing the collection time is not critical. The design of the valve head limits the diameter of the flow path and consequently, the sampling flow rate. In addition, the filter holders do not allow for optical access to a collected sample and the fact that the holders are separate makes interfacing them to an optical instrument problematic. Other than the filtering apparatus, several of the McLane components found in these systems (e.g. the stepper motor, controller, and pump) met our requirements with only minor modifications and were used in the SUPR sampler prototype.

The general arrangement of our multi-sample filtering head is based, primarily, on the WHOI Buoy-Mounted Aerosol Sampler, which collects 24 aerosol samples onto filters arranged in a circle (a filter rosette) (Sholkovitz et al., 2001). In this design, one position is for sample collection, and filters are rotated into place as necessary. The flow path is straight through the filter. Another position is available for a sensor for analyzing the Fe content of the aerosols (e.g., visible reflectance or XRF). This arrangement maximizes the cross-sectional area of the flow path, and the circular pattern of the filter rosette simplifies interfacing with an analysis system. Since the aerosol sampler is not wetted, the filters can be contained in a single, common enclosure and still ensure sample integrity. In this arrangement leaving the samples uncovered provides the optical access necessary for the Fe sensor (also in the same enclosure). However, in submerged applications, samples collected in a single, flooded, enclosure would resuspend, cross contaminate, and compromise sample integrity. However, enclosing each sample individually makes optical access more difficult. Meeting these two design requirements – ensuring sample integrity and allowing optical access to the samples – resulted in the SUPR filtering head design discussed in the following sections. In addition to meeting the requirements discussed above, the SUPR design permits: i) collection of sequential or simultaneous replicate particulate sample pairs for complementary geochemical and microbial analysis, and ii) multi-stage filtering to collect separate particle size-classes simultaneously.

3. The suspended particulate rosette (SUPR) sampler

3.1. *Description*

The SUPR sampler consists of a custom filtering head capable of collecting 24 discrete samples (Fig. 2, Table 1). The design is novel in that it collects multiple samples in a way that is easy to process upon recovery, allows multi-stage and replicate filtering, and is compatible with *in situ* optical analysis (patent pending). It also allows a large diameter flow path so that the filters are the limiting factor determining flow rates. The filtering head consists of a housing and filter rosette (Fig. 3).

For ROV deployments, a hose extension and PVC sampling wand are attached to the inlet for positioning with the ROV manipulator. The housing has two outlets. When samples are collected sequentially, only one outlet is used and the other is plugged. When true simultaneous replicate samples are collected, the two outlets are joined at a common union. The prototype housing was fabricated from nylon, with an acrylic cover, and Viton® rubber gaskets. The housing has planned locations for an optical instrument port and a dosing port for introducing stains and reagents, as required for future joint microbial/geochemical investigations.

The filter rosette consists of a sequence of plates that create separate containments and flow paths for each sample (Fig. 3). For each of the 24 sample locations (plus the purge port) in the filter rosette there is an inlet, a simple closure, a lateral offset, a stack of two filter stages,

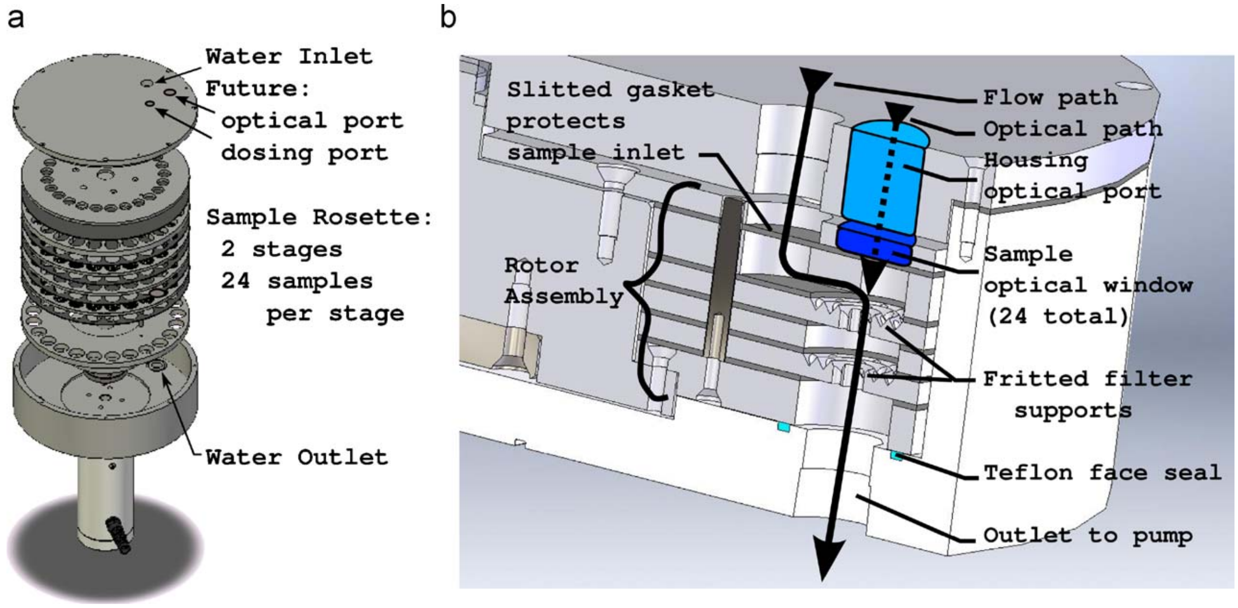

Fig. 2. (a) The SUPR sampling head contains a filter rosette, with 24 filter sample locations, driven by a stepper motor. The water inlet and outlets, and future optical sensor and dosing ports, are stationary. A face seal between the water outlet and the active filter seals the flowpath. (b) A cross section of the SUPR sampler head shows the offset flow path, which provides optical access to the filtered samples. The current prototype has a clear acrylic housing cover and a clear polycarbonate rosette top plate, which allows collection to be monitored in real time via video-link during ROV operations.

Table 1
SUPR sampler specifications.

Functional and physical	
Samples	up to 24
Sequential filtration	up to 2 levels
Filter pore size	0.2 μm and greater
Filter diameter	25–47 mm
Depth rating	5000 m (autonomous) 5500 m (ROV)
Weights (autonomous)	60 kg in water 100 kg in air
Weights (ROV)	15 kg in water 40 kg in air
Materials	
Sample rosette	Polypropylene, polycarbonate, nylon, viton rubber, titanium
Rosette housing	Nylon, Viton® rubber, teflon, nickel alloy (i.e. Hastelloy®), titanium
Pumping system	316 stainless steel, polycarbonate
Pressure housing	Aluminum
Mooring frame	316 stainless steel
ROV frame	Acetal copolymer
Electrical	
Disposable battery	30 Ahr alkaline pack
Rechargeable battery	10 Ahr NiMH pack
ROV power	36–28 V, 1 A max.
Communication	RS-232
Controller	Onset Computer Corp. TattleTale 8

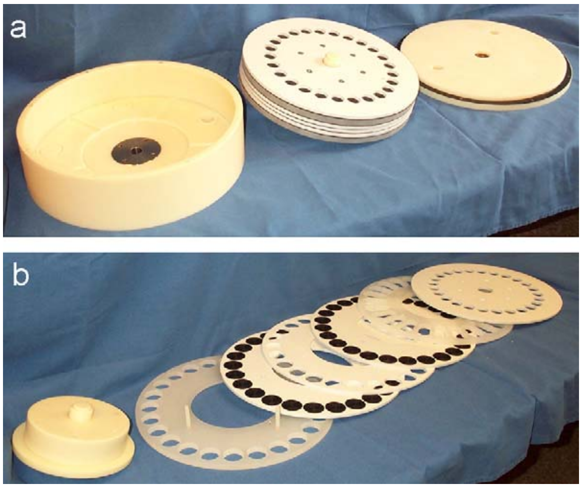

Fig. 3. (a) The SUPR sampling head consists of a filter rosette contained in a housing. (b) The filter rosette itself consists of a sequence of plates that support the filters and create the fluid flow paths and sample chambers; gaskets provide sealing between the plates, which are clamped together and connected to a central shaft with titanium fasteners.

and an outlet (Fig. 2). The closure at each inlet is created by a Viton® membrane, with slits, that acts as a simple check valve to positively retain the sample. Clamped between the inlet plate and the filter support plate is a baffle and gasket combination that serves as a mask, and creates 24 sample containments and lateral offsets. The lateral offsets provide for the optical windows above the samples set at a fixed radius from the filter rosette center (Fig. 2b). Two filter stages are stacked together allowing the option of sub-sampling by size class. The filter rosette was fabricated from polypropylene and polycarbonate with Viton® gaskets and titanium shaft and fasteners.

The entire filter rosette is supported on 5 Teflon® face seals seated in the housing. Two of these face seals also serve to seal between the two housing outlets and the active sample flow paths. Sealing pressure is provided by a single titanium spring pressing down on the rotor shaft. To obtain a sample, water is drawn through each flow path sequentially by the pump, which is located downstream of the filtering head. The filtering head is interfaced with McLane Water Transfer System components: a 25 position actuator (24 sample ports and 1 dedicated purging port), a mechanical flow-meter, 5000 m (5500 m ROV version) pressure housing, a micro-controller (Onset Computer Corporation TT8™), and an adaptation of McLane control software (Morrison et al., 2000). The software uses active feedback to reduce pumping speeds as the filters become loaded with sample and will stop collecting a sample when pump revolutions per minute drop below an operator-controlled threshold (pre-set for autonomous deployments but real-time for ROV deployments).

When configured for ROV operations the sampler is powered through the vehicle (28–36 V at 1 A max), is controlled in real time by serial communication (RS-232), and weighs 15 kg in water and 40 kg in air. When it is configured for autonomous operations, the SUPR sampler uses interchangeable 10 Ah NiMH rechargeable or 30 Ah alkaline battery packs, and, with its dedicated mooring frame, weighs 60 kg in water and 100 kg in air. During ROV deployments individual sample volumes can be recorded with precision by simply monitoring the mechanical flow meter with one of the vehicle's video cameras. During autonomous deployments, a water volume-calibrated digital pump revolution counter is currently used to estimate individual sample volumes.

Based on laboratory pumping tests the current mechanical flow meter is both accurate and precise to within 10% of the actual volumes within the range of 10–100 L. The accuracy of the digital pump revolution counter is comparable until the filters become heavily loaded with particulates at which point pump revolutions begin to underestimate the sample volumes. Therefore, for autonomous deployments the summed individual digital-counter estimates are compared with the total filtered volume per deployment as recorded by the mechanical flow meter. Individual sample-volume estimates are then adjusted by distributing the difference proportionally. A digital flow meter separate from the pump would solve this issue for autonomous deployments and we are testing options now. But our current focus is on ROV applications for which the mechanical flow meter is adequate.

3.2. Future optical sensor integration

The SUPR sampler is designed to host *in situ* optical analysis instruments in the future. The lateral offset in the flow path before the filter allows for this future application. Optical access to the sample is permitted above every sample port, allowing multiple techniques to be applied to the same sample, by moving the sample to specific locations, and to do so repeatedly after the sample has been collected. Techniques such as visible reflectance spectroscopy and image analysis using fluorescent stains are possible, but for hydrothermal mineral analysis we have focused on laser Raman spectroscopy (Brewer et al., 2004; White et al., 2005, 2006). For example, in pursuit of these goals, we have already developed methods and a database of Raman spectral characteristics that can be used to identify individual minerals in hydrothermal particle mixtures *in situ* and quantify the relative mineralogical composition of those mixtures (Breier et al., 2009).

4. Methods

4.1. Sample collection

The SUPR sampler was designed to systematically sample rising hydrothermal plumes during ROV deployments. The sampler can also be used for neutrally buoyant plume sampling either on a CTD water rosette to conduct transects of neutrally buoyant plumes or on fixed moorings for time-series sampling. Time series of up to 1 year are possible.

For rising plume sampling during a dedicated ROV dive, target vents are approached along the seafloor from off-site using the standard ROV navigation techniques (e.g., ultra-short baseline and long baseline navigation). The ROV then rises up through the water column adjacent to, rather than within, a rising buoyant plume. Samples are drawn from the buoyant plume into the SUPR sampler at increasing heights off bottom using an inlet “wand” held forward of the ROV. It is our current practice to collect sequential replicate samples above a focused vent at multiple elevations up through the neutrally buoyant plume. The first replicate pairs are collected just above the vent and subsequent samples are spaced at increasing intervals up into the uppermost region of the non-buoyant plume. The natural variability in particle concentration, due to turbulence, is reduced both by integration during the 10–30 min required to collect each SUPR sample and through the collection of replicate samples that allow a first-order evaluation of temporal as well as spatial variability within these dynamic particle-laden environments. In the future, near-bottom and above plume background particulate samples will be included in the sampling plan and sample collection will be integrated with *in situ* sensor data: a light scattering sensor, Eh, dissolved O_2, T, particle fluorescence and dissolved Fe and Mn. These measurements will allow us to develop three-dimensional geochemical maps of the plume environment to better guide sampling and interpretation of the microbial community data.

For the samples collected during the trial deployments discussed in this paper, both polycarbonate membranes (GE Osmonics) and woven polyethersulfone (SUPOR®) filters were used. The SUPOR® filters do not clog as rapidly and can filter more material but it is more difficult to remove that material from the filter for some subsequent microscopic analyses; in that regard the smooth polycarbonate membranes are superior. Upon recovery the sample rosette was brought into the lab and sheltered from air/dust with polyethylene sheeting. All samples were rinsed with distilled/deionized water, to remove sea salts and frozen until analysis. In the future, all sample handling will be done in a nitrogen purged glove box. Particulate samples will be rinsed upon recovery with filter-sterilized, neutral-pH buffered distilled/deionized water, to remove sea salts. Samples will be vacuum-sealed in nitrogen purged Ted Pella® containers and stored frozen until analysis. Filtered samples to be archived for microbial analysis will be handled in an aseptic manner and preserved consistent with standard protocols.

4.2. Sample analysis

We are using the EPR samples to investigate the fine-scale (μm- and nm-range) mineralogical and biogeochemical composition of non-buoyant hydrothermal plume particles using i) synchrotron-based X-ray absorption spectromicroscopy now and ii) traditional thin-film XRD and elemental analysis in the future (Breier et al., 2008). The Scanning Transmission X-ray Microscopy (STXM) measurements presented here were performed at the Advanced Light Source beamline 11.0.2, Lawrence Berkeley National Laboratory, Berkeley, CA (Kilcoyne et al., 2003). This microscope uses a Fresnel zone plate lens to focus a monochromatic X-ray beam onto a sample to record transmission images. Material was resuspended from the polycarbonate filters and approximately 1 μL of plume particle suspension was deposited onto a silicon nitride window (Silson Ltd.) for analysis. X-ray images were collected using a scintillator-photomultiplier detector assembly. Images recorded at energies below and at the Fe 2p and C 1s absorption edges were converted into optical density images and used to derive elemental maps (optical density is equal to $\ln(I_0/I)$, where I_0 is the incident X-ray intensity and I is the transmitted intensity through the sample). All measurements were performed at ambient temperature and ≤ 1 atm He. The theoretical spatial and spectral resolutions of the beamline were 40 nm and ±0.1 eV, respectively. The main $\mathrm{Fe2p}_{3/2}$ resonance of the reference mineral ferrihydrite at 709.5 eV was used for relative energy calibration at the Fe 2p edges. The energy was calibrated at the C 1s edge using the 3p Rydberg transitions of gaseous CO_2 at 292.74 and 294.96 eV. All STXM data processing was carried out using the IDL (ITT Visual Information Solutions) software package aXis2000.

5. First field deployment: East Pacific Rise, non-buoyant hydrothermal plume

The SUPR sampler was successfully field tested during the LADDER 2007 cruise, led by L. Mullineaux, on a 3-day moored deployment in the non-buoyant hydrothermal plume above Tica vent at 9°50′N EPR in November 2007 (Fig. 4). The SUPR sampler was loaded with 37 mm

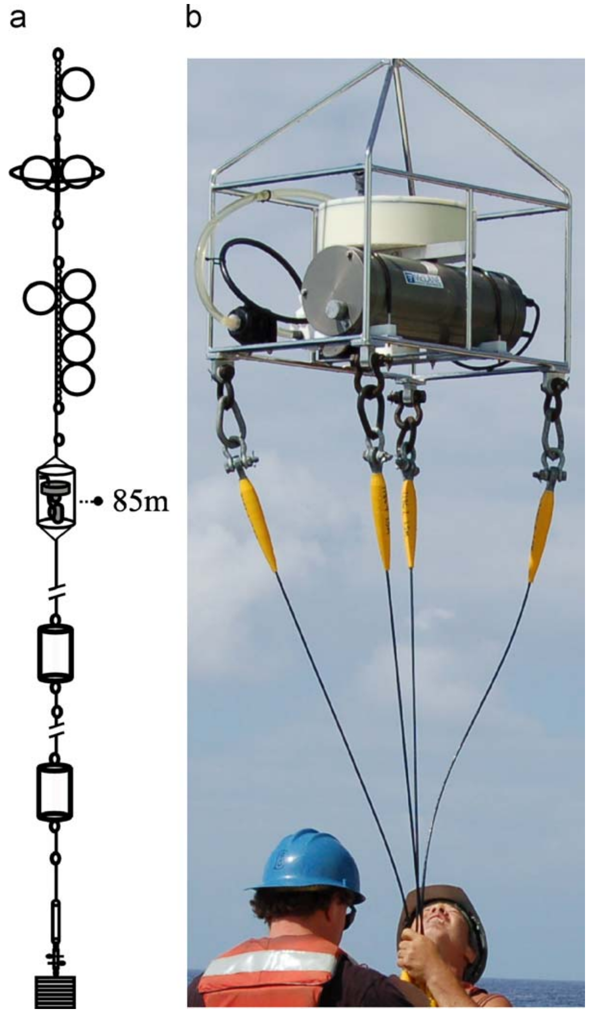

Fig. 4. (a) The SUPR sampler was first field tested during a 3-day mooring deployment in the neutrally buoyant hydrothermal plume 85 m above Tica Vent at 9°50′N East Pacific Rise in November 2007. (b) When configured for autonomous operations the SUPR sampler is powered with either 30 Ah alkaline or 10 Ah NiMH battery packs.

diameter, 1 μm pore size polycarbonate (GE Osmonics) filters and programmed to collect 24 samples. The sampler was positioned at 85 m above bottom, in the upper portion of the non-buoyant plume, on a mooring intended for larval sampling. The mooring was lowered from the surface with a transponder and released above the target site using long baseline navigation and the *Alvin* transponder network. The mooring was then positioned precisely by *Alvin*.

The sampler completed the deployment successfully, filtering up to 97 L per sample (Fig. 5, Table 2). During the 72-h deployment, the sampler was programmed to collect 24 evenly distributed time-series samples with the first starting at 0600 on 23 Nov. 2007 and the last starting at 0600 on 26 Nov. 2007. The sampler also implemented the adaptive sampling routine designed to: i) maximize sample volume during each sampling period, but ii) stop sampling before a filter ruptured. No filters ruptured, but there was large variability in the sample volumes (Table 2). This variability is at least partly due to temporal changes in particle concentration. Changes in particle size distribution or particle quality may also play a role, since the most densely loaded sample was also the one that filtered the highest volume (Fig. 5). However, the variability is also partly due to the sensitivity of the controller (as set by the coefficients of the proportional, integrative, and derivative feedback algorithm). Measurements of particle size distribution and concentrations from these natural samples will allow us to develop test solutions that can be used to more accurately adjust the control algorithm.

We recently completed a particle-by-particle, synchrotron-based X-ray absorption spectromicroscopic analysis of these samples. These measurements yield essential information about the elemental composition, mineralogy, and chemical speciation of major (e.g. Fe and C) and trace elements in the plume particles. These results indicate that a large proportion of plume particles are composed of Fe-bearing nanoparticles aggregated by a pervasive C-rich matrix (Fig. 6). This supports recent findings for descending plume particles collected in Tica vent EPR sediment traps (Toner et al., 2009). We have also used these samples to further test the limits of the *in situ* laser Raman spectroscopy techniques we have recently developed (Breier et al., 2009). These samples are now undergoing traditional thin-film XRD and elemental analysis.

6. First ROV deployments: Mid-Atlantic Ridge, rising hydrothermal plumes

Following the successful mooring deployment at 9°50′N EPR, the SUPR prototype was modified and optimized for use on an ROV. The ROV-version of the SUPR sampler weighs 7 kg in water and is compact enough to fit in either the swing-arm or rear-payload-bay positions of the ROV. The SUPR sampler was successfully deployed during multiple ROV *Jason* dives as part of the MAR July 2008 cruise led by A. Reysenbach. Deployments were completed at: 1) the ultramafic-hosted Rainbow vent site (36°13′N, 33°54′W), and 2) the basalt-hosted, Lucky Strike vent site (37°17′N, 32°16′W). All deployments were successful, with the exception of one dive when a roll pin in the stepper drive mechanism broke; the pin was replaced immediately following that dive and successful operations continued. The deployment at F10 vent (Rainbow) was technically the most challenging because of a strong bottom current that caused the plume to rise at a ~45° angle. Despite this, *Jason* was able to hold lateral position while rising through the water column collecting six replicate pairs of samples at elevations up to 20 m above bottom (i.e. 1, 1, 2, 5, 10, and 20 m) (Fig. 7). The particle-rich ascending plume could be visually identified directly from *Jason's* video feed at all elevations. Three replicate sample-pairs were also collected at 1 m above the orifice of the X3 vent at Rainbow. Similar deployments were conducted above Lucky Strike vents. Replicate samples were collected at 1, 1, 2, 5, 10, and 20 m above

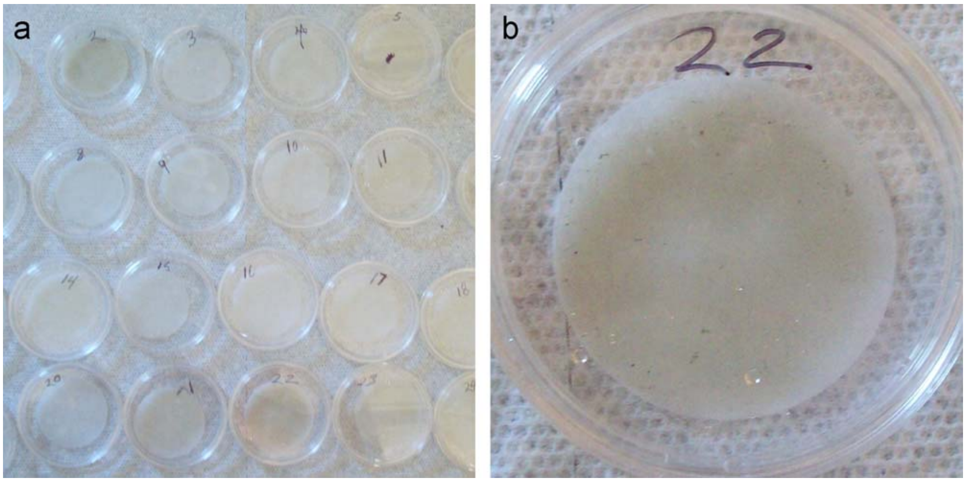

Fig. 5. (a) During the EPR mooring deployment the sampler successfully collected a 24-sample time series. (b) The maximum volume filtered was 97 L (in this case using 37 mm diam., 1 μm polycarbonate filters), achieving high particulate loading at the same time.

Table 2
9°50′N East Pacific Rise neutrally buoyant plume particulate sample time series.

104°17.5′W, 9°50.4′N (25 m NNW of Tica Vent)
85 m above bottom (2540 m)

Collection time		Filters (pore size)		
Start	23 Nov 07	1 μm	0.2 μm	Volume (L)
	0600	x		4.6
	0907	x		90.1
	1215	x	x	0.2
	1523	x		23.9
	1831	x		42.9
	2139	x		18.7
	0046	x		4.6
	0354	x		2.2
	0702	x		7.2
	1010	x		2.3
	1318	x		22.8
	1625	x	x	0.2
	1933	x		63.9
	2241	x		55.6
	0149	x		3.3
	0457	x		2.8
	0805	x		0.6
	1112	x		3.4
	1420	x		3.8
	1728	x	x	0.2
	2036	x		55.0
	2344	x		97.0
	0251	x		44.1
	0600	x		11.7
End	26 Nov 07			

the orifice at the 2608 vent, and three replicate sample-pairs were collected at 1 m above the orifice at Marker 4 vent.

For all dives, the SUPR sampler was loaded with 47 mm diameter, SUPOR® polyethersulfone membranes, in an alternating sequence of 0.8 and 0.2 μm pore-size filters, which were collected as sequential duplicates. The 0.8-μm filter samples collected closest to the vents were heavily loaded with particulates; samples collected from higher elevations were progressively less heavily loaded. Because of dive time constraints, sample volumes were typically limited to 5 L. The 0.8-μm filter sample volumes were limited only by time. The 0.2-μm filters clogged rapidly after filtration of between 300 and 500 mL. The 0.8-μm filters will undergo the same geochemical analysis as the EPR particles. The 0.2-μm filters have been archived for future microbial studies.

7. Future directions

Now that the basic sampler design and ROV sampling strategy have both completed sea trials successfully, we are planning a novel campaign of dedicated rising hydrothermal plume studies. To obtain a comprehensive suite of geochemical and microbial samples, including duplicate samples for a variety of analyses, we plan to expand the sampling capacity of the system in two ways. First we will add a second slaved SUPR sampling head so that 48 filters (24 replicate pairs of 47 mm diameter filters) can be collected per deployment. This will also provide an increased level of operational redundancy. Second, to increase filter volumes and obtain larger samples at the 0.2 μm level, we will add a rack of 16 auxiliary 142 mm diameter filter holders that can be alternately connected to the SUPR pumping system using a manual valve actuated by the ROV manipulator.

In addition, because many particle characteristics are short lived, the SUPR sampler has been designed to be compatible with *in situ* optical techniques, such as laser Raman spectroscopy. Funding of the Ocean Observing Initiative (OOI) project is clear evidence that the future direction of oceanography lies in the development of sensors and sensor networks for observatories. The SUPR sampler is a prototype system for hosting *in situ* optical sensors to track compositional changes in suspended

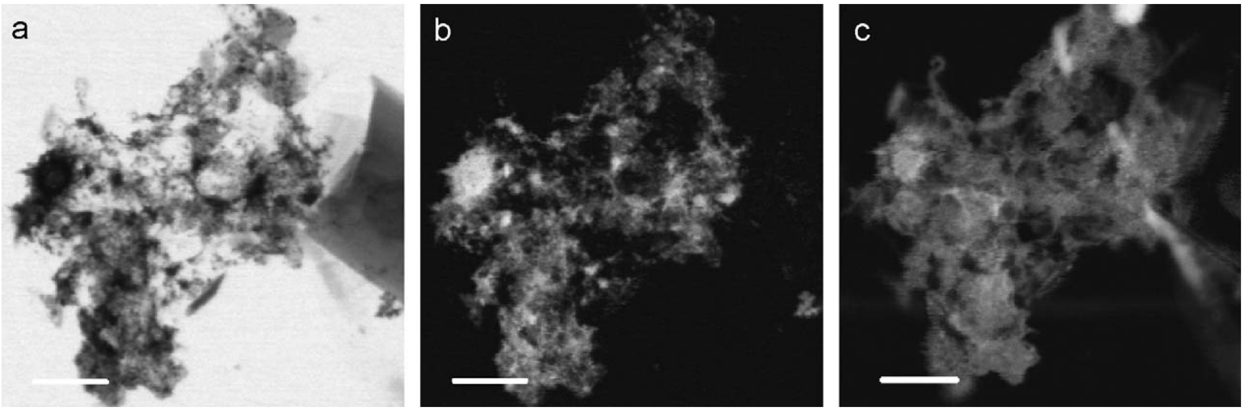

Fig. 6. Scanning Transmission X-ray Microscopy (STXM) images and elemental maps of an EPR Tica vent non-buoyant plume aggregate showing Fe-C association. (a) STXM image collected at the Fe L_3-edge (709.5 eV). (b) Iron distribution map. (c) Carbon distribution map. Scale bar is 2 μm. Iron and carbon maps are in optical density units with values from 0 to 1.66 and 0 to 1.74 respectively.

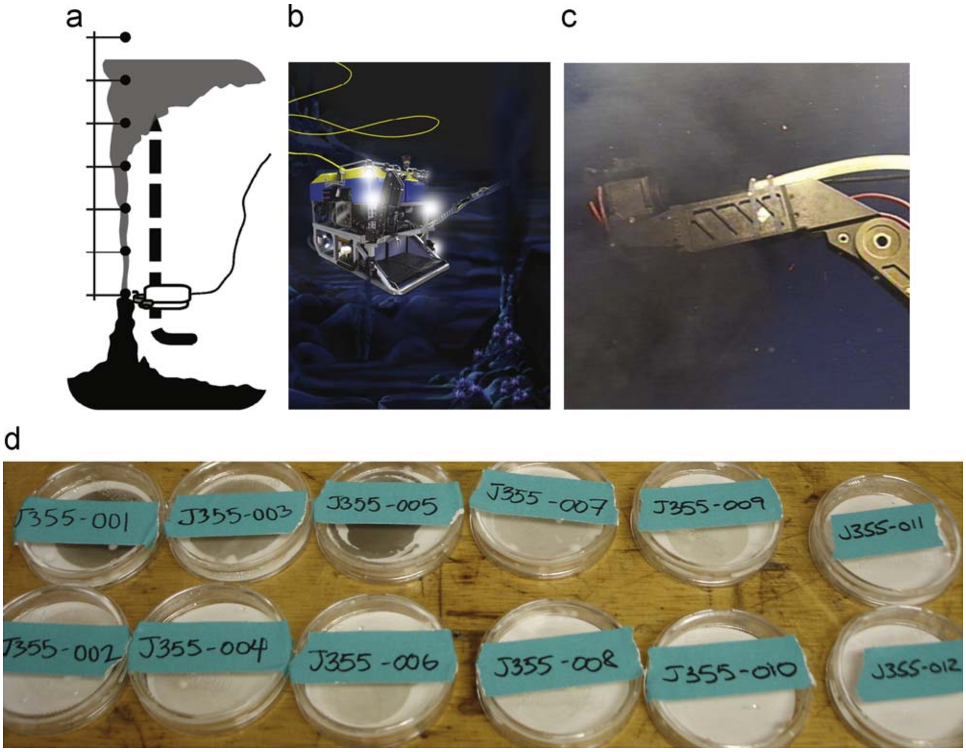

Fig. 7. (a) The SUPR sampler was successfully tested during deployments on ROV *Jason* in July 2008 proving our rising plume sampling strategy in the vent fields of the Mid-Atlantic Ridge. (b) Rising hydrothermal plumes were systematically sampled at increasing heights (a) by rising up through the water column adjacent to, rather than within, the rising buoyant plumes (b) (illustration by Oberlander) and, (c) using an inlet "wand" held forward of the ROV. (d) These 0.8 μm (top row) and 0.2 μm (bottom row) sets of paired samples were collected from the rising plume issuing from F10 vent at Rainbow at increasing plume elevations left to right (1, 1, 2, 5, 10, 20 m).

particles. It can be deployed as a part of an ocean observatory to monitor long-term trends or capture changes in response to episodic (e.g., tectonic and volcanic) events. We are also working to develop a more comprehensive version of this system, that can perform both particulate and water sampling, specifically for autonomous underwater vehicle based studies of coupled physical and biogeochemical processes on the continental shelf. We see an increasing demand for this type of sampling capability – enabling both spatially resolved and temporally resolved sampling of suspended particles, including microbes, from a range of dynamic biogeochemical environments, in addition to hydrothermal systems.

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