A large volume particulate and water multi-sampler with *in situ* preservation for microbial and biogeochemical studies.

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Running Title: Large Volume Microbial Sampler

34 *Abstract*

35 A new tool was developed for large volume sampling to facilitate marine microbiology and
36 biogeochemical studies. It was developed for remotely operated vehicle and hydrocast
37 deployments, and allows for rapid collection of multiple sample types from the water column
38 and dynamic, variable environments such as rising hydrothermal plumes. It was used
39 successfully during a cruise to the hydrothermal vent systems of the Mid-Cayman Rise. The
40 Suspended Particulate Rosette V2 large volume multi-sampling system allows for the collection
41 of 14 sample sets per deployment. Each sample set can include filtered material, whole
42 (unfiltered) water, and filtrate. Suspended particulate can be collected on filters up to 142 mm in
43 diameter and pore sizes down to 0.2 μm . Filtration is typically at flowrates of 2 L min^{-1} . For
44 particulate material, filtered volume is constrained only by sampling time and filter capacity,
45 with all sample volumes recorded by digital flowmeter. The suspended particulate filter holders
46 can be filled with preservative and sealed immediately after sample collection. Up to 2 L of
47 whole water, filtrate, or a combination of the two, can be collected as part of each sample set.
48 The system is constructed of plastics with titanium fasteners and nickel alloy spring loaded seals.
49 There are no ferrous alloys in the sampling system. Individual sample lines are prefilled with
50 filtered, deionized water prior to deployment and remain sealed unless a sample is actively being
51 collected. This system is intended to facilitate studies concerning the relationship between
52 marine microbiology and ocean biogeochemistry.

1. Introduction

Life processes and ocean chemistry are coupled. Ocean chemistry places constraints on the nature and extent of marine metabolic processes, while life processes alter the speciation, chemical associations, and water-column residence time of organic and inorganic seawater constituents. This relationship exists throughout the oceans, even in sub-environments such as deep-sea hydrothermal plumes where fluid chemistry differs significantly from background seawater (see review by German and Seyfried, 2014). In deep-sea hydrothermal plumes, evidence suggests that biotic processes produce enrichments in organic matter that interact with inorganic vent derived elements through aggregation, complexation, and cellular uptake (e.g., Cowen et al., 1986; de Angelis et al., 1993; Lam et al., 2004; Lam et al., 2008; Mandernack and Tebo 1993; Bennett et al., 2008; Toner et al., 2009; Breier et al., 2012; Sylvan et al., 2012; Li et al., 2014). These relationships are important because they have implications for material transport and thus for understanding chemical exchange between the lithosphere and the oceans (e.g., for Fe: Bennett et al., 2008; Tagliabue et al., 2010), as well as the availability of chemical energy within the ocean interior (McCollom 2000). The instrument developed here is not only suitable for studying hydrothermal systems, but also has important capabilities for any dynamic and rapidly changing marine environment where microbial-biogeochemical coupling remains to be investigated across steep biogeochemical gradients – in coastal, benthic (including other seafloor fluid flow settings, such as at cold seeps) and upper ocean/twilight zone investigations.

Microorganisms play a central role in biogeochemistry due to their abundance, pervasiveness, and metabolic diversity (Arrigo, 2004). The processes involved are complex; consequently, our understanding is incomplete. More studies are required to fully elucidate this

relationship, particularly studies that quantify and relate marine microbial communities with the biogeochemical parameters that influence their growth and mortality. Such studies can involve measurements of microbial community DNA and RNA, as well as environmental concentrations of electron donors and acceptors, major and minor dissolved nutrients, essential biomolecules, toxins, and metabolites (e.g., Anantharaman et al., 2012; Lesniewski et al., 2012; Li et al., 2014). These studies can involve rather comprehensive sample collection. Moreover, some of the most interesting places to study are those where environmental geochemical gradients are high or subject to temporal and spatial variability. These types of environments are logistically difficult to sample systematically e.g., deep-sea hydrothermal plumes (Figure 1).

To facilitate studies of these processes, and enable broader investigation of the relationship between marine microorganisms and their environment, we have developed a novel, large volume sample collection system designed to collect a suite of sample material rapidly and efficiently, and preserve the time-sensitive genetic components (i.e., RNA) immediately after collection. This system is capable of collecting whole water, filtrate, and filtered material, with up to 14 sample sets per deployment (Figure 2). It does so using *in situ* filtration and preservation, thereby overcoming the limitations of Niskin bottle collection and shipboard filtration, specifically: limited volume, post collection changes in RNA expression, and biases related to particle processes (Mitra et al., 1994). Though it differs in design and capability, this system builds upon the goals for our previous Suspended Particulate Rosette Sampler (SUPR) (Breier et al., 2009b); and thus we have kept the name and refer to the new sampler as the SUPR-V2 system. Here we describe this system and present results from a recent field study of Mid-Cayman Rise hydrothermal plumes to illustrate its utility.

2. Background

A variety of dedicated sampling and *in situ* analytical systems have been designed for vehicle-based or vehicle-deployed sampling, particularly for remotely operated vehicle (ROV) deployments at seafloor chemosynthetic environments including hydrothermal vents. These samplers include the Hydrothermal Fluid and Particulate Sampler, the McLane WTS time series samplers, the Autonomous Microbial Sampler, the GeoMicrobe and Mobile Pumping System Instruments, the Monterey Bay Aquarium Research Institute GULPER, and the Environmental Sample Processor (Huber et al., 2003; Butterfield et al., 2004; Rendigs and Bothner, 2004; Taylor et al., 2006; Bird et al., 2007; Scholin et al., 2009; Cowen et al., 2011; Preston et al., 2011; Ottesen et al., 2011; Ussler et al., 2013). Of these, all but the GULPER collect samples by active pumping. The GULPER is used for surface ocean sampling and collects samples by syringe action, which limits sample volumes to ~2L (Bird et al., 2007). The others use a similar valve design, with a relatively small flow path diameter, that does not permit rapid sample collection. While these flowrates are appropriate for the intended use of these systems near the seafloor where analyte concentrations can be high or for long-term time-series sample collection, they become prohibitive when larger sample volumes become necessary and sampling time is limited. In our new design, we overcome these limitations.

3. The SUPR-V2 Sampling System

The SUPR-V1 design was described and discussed in Breier et al., (2009b). The SUPR-V1 design collects only particulate samples and only on filters <47 mm in diameter, a design intended to facilitate *in situ* optical analyses (Breier al. 2009a). While the SUPR-V2 retains this particle sampling capability, the new design is different and allows for a greater variety of

sample types and configurations, water-tight seals at the inlets and outlets of each sampling loop, and *in situ* preservation of samples.

The SUPR-V2 was designed with the goal of facilitating hydrothermal plume studies specifically and open ocean studies generally. In the case of hydrothermal plumes, samples must be collected over wide gradients of suspended particle and dissolved phase concentrations, which at their limit reach ambient ocean concentrations. Thus to fully characterize a hydrothermal plume, background open ocean samples are required, both for comparative scientific analysis and to gather sufficient information to identify background entrainment in plume samples. Consequently, for geochemical and microbial particulate analyses, between 5 and 250 liters of seawater per sample are necessary. Moreover, as endmember vent fluid flux can vary considerably between vents and with time, the sampling rate must be variable in order to control entrainment of background seawater while sampling plume fluid. Sample collection must be compatible with both trace metal and microbial cleanliness practices and standards while the sampler must be capable of both ROV and hydrocast deployments and, therefore, must be small and compact. Importantly, sample collection must be rapid, and sampling capacity must be sufficient to carry out an adequate sampling plan within a single dedicated ROV dive; for our research this has equated to a capacity of 14 sample sets per deployment. Water bottles and stand-alone *in situ* pumps have been used on ROVs in the past; however, their size alone makes achieving these sampling needs difficult if not impossible.

The most important component of the SUPR-V2 system is the sample control valve. As noted in Section 2, the valve design commonly used in several other sampling systems limits the flow channel diameter and pumping speed. To overcome this we designed a custom sample control valve for the SUPR-V2. The SUPR-V2 system uses a double-throw, 14 port valve,

meaning the valve has two sets of 14 ports: one set to open and close the inlets of the sample containers and one set to open and close the outlets of the sample containers (Figure 3). There is a sample line bypass position for each sample port that allows intake preflushing. The inlet ports are connected to a single common master inlet in the center of one valve face, and the outlet ports are likewise connected to a single common master outlet in the center of the opposing valve face. The master outlet is connected to the pump. The master inlet is led to the point of sample collection or, when needed, to a stepper driven 3-way inlet control valve that can alternately connect the master inlet to the sampling environment or a bladder of preservative. Each fixed port is sealed by a spring loaded (Hastalloy – nickel alloy) polytetrafluoroethylene (PTFE) sealing surface, which the ported smooth polycarbonate valve selector plate is free to slide past. The two sets of 14 ports are locked together and selected in tandem by the sample control valve stepper motor. This motor is offset from the valve rotational axis through a spur gear in order to produce sufficient torque to actuate the valve. Valve position is determined by step counting and a PTFE coated magnet (i.e., stir bar) embedded in the rotating portion of the valve, and a magnetic pickup on the valve body.

It is the port sealing design and drive train arrangement that permits the relatively large cross-sectional diameter of the valve flow channels and selection ports. The flow path diameter of the SUPR-V2 is 9.525 mm. Scaling this design to control both the number of ports and the port diameter is greatly simplified by the fact that these parameters are only influenced by radial dimensions, which in this face seal valve design have no influence on sealing performance. The critical dimensions with regard to sealing performance in this valve only concern the axial components. It is the stackup of these axial dimensions that control the compression of the spring-loaded PTFE seals. The valve is designed so that thin sheets of acetal copolymer can be

inserted permanently into the valve so these axial dimensions can be adjusted to reach the optimal sealing force. This provides a means of ensuring sealing integrity while minimizing the tolerances to which the valve must be machined.

The rest of the SUPR-V2 sampling system currently consists of (i) a pressure compensated oil filled electronics housing that contains a pressure tolerant microcontroller, stepper motor drivers, and a brushless DC motor driver, (ii) a digital flowmeter (Seametrics Model SPX) and a physical volumetric totalizing meter, (iii) a McLane Research Laboratories Inc. 8 L min⁻¹ deep sea pump, and (iv) a modular suite of filter holders and water bottles (Figure 4). For our preliminary hydrothermal studies, the SUPR-V2 systems were used to collect a combination of whole water, filtrate, and filtered particulate samples in water bottles and filter holders of several sizes. Custom 142 mm filter holders are used due to the need to seal the inlet and space constraints, these filter holders share several design features (e.g., materials, particle distribution control) with the MULVS style filter holders (Bishop et al., 2012). Essentially any sample container that can be made with an inlet and outlet can be placed in one of the sample loops of a SUPR-V2 and used for sample collection. Suspended particulate samples are collected by *in situ* filtration thereby overcoming (i) limitations of Niskin bottle sample volume and (ii) the artifacts associated with post-collection precipitation, scavenging and particle adherence to Niskin bottle walls (Figure 5). Each SUPR-V2 sampler has the capacity to sequentially collect 14 sample sets per deployment, filtering at the same rates as the stand-alone McLane LVP pumps used routinely to-date by the US GEOTRACES program among others, (e.g., 2 LPM for 142 mm diameter 0.2 µm polyethersulfone filters). Whole water and filtrate samples are collected as part of the SUPR filtering process; these water bottles are in-line in each sampling loop (in the order whole water bottle, filter holder, and filtrate bottle). The SUPR systems are constructed of

189 plastics (perfluoroalkoxy, PTFE, low-density polyethylene, polycarbonate, and acetal
190 copolymer) with titanium fasteners and nickel alloy spring loaded PTFE seals; there are no
191 ferrous alloys in the sampling system. Concerning material choices and sampler cleanliness, we
192 follow the principles and choices used for the MITESS system for trace metal clean samples
193 (Bell et al., 2002).

194 The SUPR-V2 is either powered through the ROV (20-36 V at 5A max) or by deep-sea
195 24 V batteries when deployed on a CTD hydrocast. System weight varies by configuration but
196 ranges between 22 to 28 kg in water and 67 to 130 kg in air (Table 1). Several independent low-
197 level microcontrollers are used to control the pump, the valves, and to measure flowrate. Each of
198 these is controlled from the surface by serial communication through the ROV or the CTD-
199 rosette sea cable. Real-time control is manual and on-demand, with the scientist using a software
200 interface program on a laptop computer to select the sample channel and control pumping, while
201 the ROV pilot (during ROV deployments) positions the sample intake wand.

202 4. Methods

203 4.1. Instrument Testing

204 Prior to deployment, dye tests were performed to determine the purging volumes needed
205 to flush fluid from the 2 L bottles and the 142 mm diameter filter housings. These sample
206 containers were filled with concentrated solutions of Alura Red food coloring and then flushed,
207 using the SUPR-V2 system, with undyed artificial seawater. This was repeated with varying
208 flush volumes. Cases using both seawater and freshwater for the initial dyed fluid were
209 performed with the 2 L bottle; for the filter housings only seawater was used for the initial dyed
210 fluid. Dye concentration was quantified spectrophotometrically based on a calibration curve

made from controlled dilutions. Dye concentrations of aliquots of flushed fluid and the original dye solution were compared on this basis.

4.2. Sample Collection

For hydrothermal plume sampling at Mid-Cayman Rise vents, the following ROV-based sampling operations were conducted. Operational blanks were taken for each analytic method. First, a set of near bottom background samples was taken in the vicinity of the vents but outside of the plumes (> 3 m distance). Next, the manipulator was used to position the SUPR sampler intake directly within the rising plume starting at a height of ~0.5 m above the vent orifice, a position from which the first set of plume samples was collected. This was repeated at progressive heights within the detectable rising plume. Background samples above the plume were collected in the mid-water column during ROV ascent. SUPR-V2 systems were also deployed on the CTD -rosette and used to sample the upper portion of the rising plume, the non-buoyant plume, and the background water column between ROV dives. A limited number of CTD -rosette Niskin bottle samples were also collected and filtered shipboard for comparison with the SUPR-V2 collected microbial samples. In addition, high temperature hydrothermal end-member fluids were also collected as part of these profiles using titanium isobaric gas tight fluid samplers (Seewald et al., 2002); see Reeves et al., (2014) for end-member chemistry results.

Three different SUPR-V2 sampler configurations were used. Filter media selection follows that of previous studies (Bennett et al. 2011; Breier et al. 2012; Anantharaman et al. 2013). First, for microbial community genomic analyses a SUPR-V2 was configured to collect a set of 14, acid cleaned (in 10% HCl), 142 mm diameter, 0.2 µm poresize, polyethersulfone (PES; SUPOR; Pall, Port Washington, NY, USA) filter samples and preserve them *in situ* with RNAlater (Qiagen, Valencia, CA, USA); this is referred to as the Micro configuration. Second,

for particulate and dissolved inorganic chemistry, nutrient chemistry, and X-ray micro-spectroscopy a SUPR-V2 was configured to collect a set of 14, acid cleaned (in 10% HCl), 142 mm diameter, 0.2 μ m poresize, polycarbonate (Isopore; Millipore, Billerica, MA, USA) filter samples with post-filter, in-line 2 L perfluoroalkoxy (PFA; Savillex) water bottles; this is referred to as the Iron configuration. Third, for organic carbon analyses, a SUPR-V2 was configured to collect a set of 14, 47 mm diameter, pre-combusted glass fiber filter (GF/F; Whatman, Pittsburgh, PA, USA) samples with both pre- and post-filter, in-line 250 mL water bottles); this is referred to as the Carbon configuration. Typically, two differently configured SUPR-V2 samplers were deployed and operated synoptically. At the Von Damm site (characterized by low Fe fluid), the Micro and Carbon configurations were used. At the Piccard site (characterized by high Fe fluid), the Micro and Iron configurations were used.

All sampling components were thoroughly cleaned prior to the cruise by a sequence of 5% ethanol and 5% hydrochloric (HCl) acid baths. All plasticware was rinsed with MQ water and leached with 10% HCl at pH 2. Water sample bottles for inorganic analysis were washed with detergent (Detergent 8; Alconox, White Plains, NY, USA) and acid leached prior to the cruise. All pre-deployment filter handling was performed within a shipboard fabricated polyethylene enclosure with positive-pressure provided by a HEPA filter box, and all oxidation sensitive post-deployment shipboard and laboratory filter handling was performed in an argon-purged glovebox. Upon recovery, the particulate samples for organic carbon analysis were enclosed in combusted aluminum foil and frozen until analysis. The particulate samples for trace metal and mineral analyses were rinsed with distilled/deionized water (pH buffered with trace metal grade ammonia) to remove sea salts, transferred to a glovebox, sealed in mylar bags, and frozen until analysis. All acids were Fisher (Pittsburgh, PA, USA) Optima grade.

4.3. Sample Analysis

The detailed microbiology and biogeochemistry of these samples will be described elsewhere (Sheik et al., in prep.; Breier et al., in prep.; Bennett et al., in prep.). Here we describe only the essential measurements necessary to illustrate the efficacy of the SUPR-V2 sampling approach.

For microbial analyses, 142 mm filters stored at -80°C were thawed on ice, split into quarter portions, and total community DNA and RNA was extracted from separate quarter filters. Total DNA was extracted using a combination of chemical and physical lysis (Dick and Tebo, 2010) and quantified with Picogreen (Invitrogen, Carlsbad, CA, USA). Total RNA was extracted using the *mirVana* miRNA kit (Ambion, Grand Island, NY, USA), treated with DNAase I, concentrated with the RNeasy MinElute kit (Qiagen) and finally quantified with Ribogreen (Invitrogen). Methods used to amplify microbial community DNA are described in depth by Anatharaman et al. (2013). Briefly, using polymerase chain reaction (PCR) we amplified the 16S rRNA ribosomal gene with primers that amplify both bacteria and archaea (V4 region 515F/806R). PCR was performed in triplicate for each DNA sample. Triplicate reactions were then combined and cleaned with a MoBio UltraClean PCR clean-up kit. Individual PCR-DNA was then combined into a single sample at equivalent concentrations and amplicons were sequenced using 454 Titanium chemistry in the laboratory of Dr. Vincent Young at the University of Michigan. Amplicon sequences were quality screened, truncated by length, and clustered into operational taxonomic units (OTUs) at 97% similarity, which is a proxy for a theoretical species, with UPARSE (Edgar, 2013). OTUs were taxonomically classified to the Silva taxonomy using MOTHUR (Schloss et al., 2009). For determination of cell counts, 30-ml of whole water from niskin bottles was filtered under low vacuum pressure onto 0.2 μm 25 mm

white polycarbonate membrane filters (Isopore; Millipore). Cells were fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS; pH 7.2), washed with PBS and water, air dried in the dark, stored in sterile petri dishes at -20°C, and counted using epifluorescence microscopy with 4',6-diamidino-2-phenylindole (DAPI).

For bulk elemental analysis, particulate filter sample splits were completely digested in 30 mL acid cleaned perfluoroalkoxy vials (Savillex) using the following procedure based on Bowie et al., (2010). Aliquots of sample digest, at a 1:10 dilution, were analyzed for Fe, Cu, and Zn by optical emission spectroscopy (OES) on a Varian 730-ES axial spectrometer. External standards were used for instrument calibration. Digestion and analysis were both monitored by processing portions of basalt geostandard BHVO-2 and an internal laboratory chalcopyrite reference material with these samples (Raczek et al., 2001; Breier et al., 2009a). OES analyses were performed at Activation Laboratories. Sample and geostandard processing and preparation were performed at Woods Hole Oceanographic Institution (WHOI). Filtrate sample aliquots (100 mL) were stored frozen in acid-cleaned polyethylene bottles and analyzed for dissolved silicate on a Lachat Instruments QuickChem 8000 four-channel continuous flow injection system at the WHOI nutrient analysis facility. Filtrate sample aliquots (500 mL) were acidified to a pH of 1.7 with concentrated nitric acid and stored for 6 months at room temperature prior to analysis of dissolved Fe by the Ferrozine spectrophotometric method (Stookey et al., 1970).

For particulate organic carbon analysis, GF/F filters were oven dried (60°C) and treated with concentrated HCl fumes under vacuum for 24 hours to remove the inorganic carbonates. Filters were redried (60°C), quartered, and packaged in tin cups ready for analysis. Organic carbon concentrations were measured using a Costech Analytical Technologies, Inc. Elemental Analyzer, fitted with a Zero Blank Autosampler and interfaced with a Thermo Scientific MAT

253 stable isotope ratio mass spectrometer in continuous flow mode. Sulfanilamide and acetanilide were used throughout as calibration standards, having been verified against certified reference materials, and were run as part of each sample set.

The fine-scale particle morphology and carbon distribution were measured using scanning transmission X-ray microscopy (STXM) at beamline 5.3.2.2, Advanced Light Source, Lawrence Berkeley National Laboratory, USA (Kilcoyne et al., 2003). Samples for analysis were prepared shipboard by rinsing particles from SUPR-V2 generated polycarbonate membrane filters with 1 mL of purified water. A 1 μ L aliquot of the resulting suspension was deposited on a Si₃N₄ membrane (Silson Ltd, Northampton, UK), and the liquid water was allowed to evaporate. Samples were packed under N₂ gas, sealed in heavy mylar bags (7.5 mil; IMPAK Corp.) with an oxygen scrubber (AnaeroPack, Mitsubishi Co., Inc.), and frozen for shipping and storage.

At the beamline, the samples were transferred from sealed mylar bags to the microscope with ~ 2-5 minutes of exposure to ambient atmosphere. The microscope chamber was operated with helium at just under 1 atm pressure. X-ray images were acquired in transmission mode using a scintillator-photomultiplier detector assembly. Carbon distribution maps were measured by recording images at energies below (280 eV) and above (305 eV) the carbon 1s absorption edge, and converting to optical density (OD) maps; where $OD = \ln(I_0/I)$, I_0 is the incident X-ray intensity, and I is the transmitted intensity through the sample. The photon energy was calibrated at the carbon 1s edge using the Rydberg transitions of gaseous CO₂ at 292.74 eV and 294.96 eV. All data processing was carried out using aXis2000 software.

5. Flushing Tests

The flushing tests showed that the dye concentration in the 2 L water bottles was reduced to <0.5% of the original concentration after being flushed with 500% of their internal volume (Figure 6). Whether the bottles were initially filled with either freshwater or saltwater made little difference. Based on this, it is our practice to use deionized, ultrapure water for the initial bottle fill and to purge each 2 L bottle with 10 L of sample fluid prior to sample collection. For the 142 mm filter holders, the tests showed that the dye concentration was reduced to $\leq 1\%$ of the original concentration after being flushed with 5 times their internal volume (100 mL per filter holder). Based on this, the filter holders should be flushed with at least 0.5 L of RNAlater after sample collection to ensure the residual fluid in the sample holder has an RNAlater concentration >99%. Therefore, the preservation of 14 samples requires 7 L of RNAlater. During this cruise we deployed the system with 10 L of RNAlater per deployment; however, due to dive priorities, we were typically limited to collecting ≤ 4 samples for plume microbiology. Consequently, it was our practice to be conservative with regards to sample preservation and use a ≥ 2 L RNAlater flush. This approach to preservation has the advantage of being reliable to implement in a wide range of sampler configurations. The disadvantage is the volume of preservative required. Future work will examine ways to minimize the preservative needed by minimizing the internal volume of the filter holders and improving their flushing efficiency.

6. First Field Deployment: Mid-Cayman Rise Hydrothermal Fields

The SUPR-V2 system was used to sample hydrothermal plumes during RV Atlantis Cruise AT18-16 in January 2012 (German et al., 2012). The two hydrothermal fields studied,

Piccard and Von Damm, are within 21 kilometers of each other, but separated by >2500 m water depth and are distinctly different in terms of their geology and geochemistry. Von Damm is a hydrothermal mound with high temperature fluid primarily emanating from two orifices at its top: one emitting pure vent fluid and the other a mixture of vent fluid and seawater. It is situated atop an oceanic core complex on the western slope of the Mid-Cayman Rise valley at a depth of 2300 m (Connelly et al., 2012; Cheadle et al., 2012). Piccard comprises a suite of active and extinct massive sulfide chimneys hosting an array of high temperature chimneys and diffuse flow all hosted in neovolcanic pillow basalts on the deep Mid-Cayman Rise rift-valley floor at depths to 4987 m (Kinsey & German, 2013). Von Damm and Piccard hydrothermal fluids are both enriched in H₂, H₂S, and CH₄ (in the case of Von Damm by an order of magnitude more) but where Piccard fluids are high temperature (max 398°C) and metal rich, the Von Damm fluids are lower in temperature (max 232°C) and metal poor (McDermott et al., in prep).

Numerous deployments of the SUPR-V2 system using the ROV *Jason* and a CTD-rosette were conducted. Samples were collected from the rising plumes emanating from the top of the Von Damm hydrothermal mound and the ≤ 400°C Beebe Vents complex within the Piccard hydrothermal field. Samples for microbiology were also collected from remote background seawater (similar depths but > 20 km distant from the vents). Samples were collected for microbiology (n = 37), particulate and dissolved inorganic geochemistry (n = 29), spatially resolved particulate chemistry (n = 29), and particulate and dissolved organic chemistry (n = 20).

The sampling system performed as intended. Due to the reconfigurable nature of the system there were numerous ways to prepare and use the components, as a result, there remains continuing scope for further, future method optimization. Even within the duration of the 2012 cruise, the efficiency of our methods concerning pre- and post-deployment instrument setup and

sample processing improved significantly. A key focus of post-cruise instrument and method evaluation has been quantifying background contamination for the microbial samples. While background contamination can be minimized, it is always anticipated. Accordingly, we take the approach of quantifying microbial contamination through the use of operational blanks, which were included in every deployment. For bulk chemical measurements, these operational blanks can simply be quantified and subtracted from the sample measurements. For microbiology, several approaches to quantifying and evaluating contamination have been utilized. First, analogous to the approach for chemical measurements, total DNA and RNA extracted from samples and operational blanks was quantified and compared. DNA concentrations from operational blank filters were 15-20% of the values retrieved from plumes and backgrounds, on average (Table 2). Second, attempts to count cells directly from filters were done by taking subsamples from filters used for DNA extraction and preserving with a 4% paraformaldehyde solution (German et al., 2010). However, the filters used to collect large quantities of material for DNA extraction were not suitable for cell counting. Instead, 30 ml of water was collected from hydrocast collected water samples, filtered, preserved and counted according to methods in (German et al., 2010). Third, the composition of the microbial community in samples and operational blanks was determined by high-throughput DNA sequencing of 16S rRNA gene amplicons. Operational blanks were dominated by OTUs with high sequence similarity (>98%) to *Cupriavidus metallidurans* (Burkholderiaceae), *Psuedomonadales* spp., and *Sphingomonad* spp. (Figure 7a). *Cupriavidus metallidurans* and related species are commonly found in strictly cleaned environments such as the surfaces of spacecraft and spacecraft assembly rooms and in hospitals and ultrapure water systems (Mijnendonckx et al., 2013 and references therein). This organism also forms biofilms on plastics (Mijnendonckx et al., 2013). We infer that the

occurrence of *Cupriavidus metallidurans* on SUPR sampler operational blanks reflects contamination of either the ultrapure (Millipore® Milli-Q™) water used to flush the SUPR sampler or from Tygon® tubing. A variety of lower-abundance proteobacteria were also recovered from operational blanks (Figure 7a). While we did recover microbial communities from the operational blanks, we observed variability in the level of contamination (Figure 7b). The observed decrease in contamination between traditional hydrocast bottle collection (CTD.1.07) and SUPR-V2 sampling (all other samples) is notable (Figure 7b). However, contamination was not uniform for all SUPR-V2 collected samples. Rather we observed high variability within and between casts (Figure 7b, see CTD.06.47, CTD.08.50, J2.614.09 and J2.615.24). We believe that variation stems from two potential factors; first, low microbial biomass associated with Mid-Cayman Rise plumes ($\sim 2.0 \times 10^3$ cells/ml in off-axis neutrally buoyant plumes, German et al., 2010) and second, shipboard sample handling.

Reassuringly, the true deep-sea organisms identified in samples were not present at significant abundance in the operational blanks, confirming that cross-contamination between filters was not an issue. This provides a further indication that the valve performs as designed and, specifically, that dead volume (volume within the sampling system that cannot be flushed) is minimal. We estimate the dead volume to be $<100 \mu\text{l}$ per sample and restricted to the interstitial area within the PTFE seals at the sample line inlets, equivalent to 0.001% of a 10 L sample. Even this volume is not truly “dead” but it is less efficiently flushed. Continuing method development will focus on further reducing these background contaminants, both by evaluating the potential for contamination from shipboard ultrapure water supplies, and by minimizing the potential for contamination from within the sampling system itself, particularly from the tubing that connects the components. For these deployments, Tygon® was used for this tubing, and it

was initially cleaned, after installation on the system, by filling the system with successive solutions of 5% ethanol and 5% HCl acid. Substitution of semi-rigid plastic tubing for the Tygon® may allow for more effective cleaning.

At Von Damm, concentrations of carbon in the plume ranged from 29 to 579 nmol/kg, with highest concentrations measured in samples collected with the ROV within the warm fluids (18 to 77° C) suggesting a sub-seafloor source of carbon (Table 3). In agreement with the bulk carbon observations, particle-specific STXM observations showed aggregated particle flocs rich in carbon (Figure 8,a-b). Mean microbial cell counts were $\sim 2 \times 10^4$ cells/ml seawater with maximum counts of $\sim 4 \times 10^4$ cells/ml seawater. *Epsilonproteobacteria*, which are commonly abundant members of seafloor and hydrothermal vent animal-associated microbial communities, were the most abundant microbial group identified in the Von Damm rising plume (Figure 9). Other microbial groups included Marine Group I Archaea, SUP05 *Gammaproteobacteria*, and SAR324 *Deltaproteobacteria*, which have been observed in deep-sea hydrothermal plumes previously (Baker et al., 2012; Anantharaman et al., 2013; Sheik et al., 2014). Particulate metal concentrations were not measurable at these filtered volumes, consistent with the metal-poor nature of the vent fluid.

For Piccard, results show a plume dominated by Fe minerals (Table 4). Notably, there is no measureable particulate Al in these samples indicating the contribution of background marine particulate material is minor. At the lowest plume elevation, 1 m above the vent, 55% of the Fe was in the form of particles $>0.2 \mu\text{m}$ in diameter. The relative fraction of particulate Fe decreased to 13% by an elevation of 157 m above the vent. This is consistent with Fe sulfide particle dissolution or settling. Interestingly, the ratio of Fe to Cu, the next most abundant metal, is initially 10 but decreases to 4 by an elevation of 126 m above the vent. Assuming similar

initial rates of particle formation, this suggests different rates of particle dissolution or settling for these two sulfide-forming metals. This illustrates the influence of either mineralogy or particle size, and most likely both, on the relative transport properties of hydrothermal metals. Perhaps related to this, STXM observations revealed that particles from the plume at the Beebe Vents site have a dense granular texture indicative of aggregated mineral particles containing carbon (Figure 8, c-d).

Piccard plume communities showed similarity in membership to those present at Von Damm, with the notable shifts in abundance for Archaea, Marine Group A (MGA)/SAR406 and *Epsilonproteobacteria* (Figure 9). Within the Archaea, the Marine Group I and DHVEG-6 were observed at greater abundance at Piccard than Von Damm, while the putative heterotrophic groups Marine Group II & III were present at lesser abundance. MGA/SAR406 are markedly more abundant at Piccard (Figure 9), consistent with this group being linked to elevated sulfur concentrations at Piccard, as this group has been linked to sulfur cycling (Wright et al., 2014). Most notable is the decrease in *Epsilonproteobacteria* from Von Damm to Piccard. Mean microbial cell counts were $\sim 2 \times 10^4$ cells/ml seawater with maximum counts of $\sim 4.7 \times 10^4$ cells/ml seawater.

Overall the results illustrate two different types of hydrothermal plumes. Like all hydrothermal plumes, they are notable for the mixing of two distinct fluids: seawater and reducing vent fluid. In this case, differences in metal concentrations between the two vent fluids result in first order differences in plume biogeochemistry; however, variations in mixing, entrainment, and other chemical constituents, as well as depth and pressure, may also play important roles in constraining plume microbial community composition.

7. Summary

We have developed and successfully field-tested a new oceanographic sampling tool. The SUPR-V2 system has been designed to facilitate marine microbiology and biogeochemical studies and developed for both ROV operations and CTD hydrocast deployments. It was used successfully in both modes during a recent cruise to the hydrothermal vent systems of the Mid-Cayman Rise. The Suspended Particulate Rosette V2 large volume multi-sampling system allows for the collection of 14 sample sets per deployment. Each sample set can include filtered material, whole water, and filtrate. The suspended particulate filter holders can be filled with preservative and sealed immediately after sample collection. Up to 2 L of whole water, filtrate, or a combination of the two, can be collected as part of each sample set. This system performed successfully in multiple modes during its first ROV and CTD-rosette deployments at the Mid-Cayman Rise collecting a suite of novel sample types. These and future investigations will greatly facilitate both our understanding of the biotic and abiotic processes at work in hydrothermal plumes, and the relationship between marine microbiology and ocean biogeochemistry in a range of other dynamic, high-gradient oceanographic settings.

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Table 1. SUPR V2 Sampler Specifications.

Functional	
Depth rating	5500 m
Samples per dive	14 sample sets
	Each sample set may consist of multiple types of subsamples.
Sample types	whole water ≤ 2 L per sample
	filtered water ≤ 2 L per sample
	filtered material on 47 to 142 mm diameter filters
	<i>in situ</i> sample preservation by dosing with reagents
Pumping system	McLane Research Laboratories, Inc. 8 L per minute deep-sea pump. Actual sampling speed is dependent on filtering media. Volume filtered is limited only by filter media and filtering time.
Weight	Varies by configuration: 22 to 28 kg in water, 67 to 130 in air
Materials	
SUPR System	acetal copolymer, polycarbonate, high density polyethylene, perfluoroalkoxy, polytetrafluoroethylene, titanium, nickel alloys
Electronics housing	oil filled high density polyethylene housing
Electrical	
ROV Power	20-36 V, 5 A maximum
Communication	RS-232 with cross-platform console and graphical user interfaces

Table 2.

Table 2

Table 2. Mid-Cayman Rise hydrothermal plume particle chemistry^{a,b}

Field	Deployment	Sample		Depth	Elev.	Vol.	T	Dissolved	Particulate			
								SiO ₂	DNA	RNA	Cells	
Von Damm	CTD	10	SUPR 58	APB ^c	1479	812	26	4	10800	28	NA ^d	NA
Von Damm	CTD	10	SUPR 57	Plume	2254	37	22	4	11200	27	NA	NA
Von Damm	CTD	10	SUPR 56	Plume	2290	1	24	4	12800	34	NA	NA
Von Damm	ROV	J-614	SUPR 9	Plume	2288	8	6	18	128000	320	NA	NA
Von Damm	ROV	J-614	SUPR 4	Plume	2290	1	5	64	398000	220	110	NA
Von Damm	ROV	J-614	SUPR 1	Plume	2296	1	5	77	317000	1100	NA	NA
Von Damm	ROV	J-614	SUPR 7	NBB ^e	2296	1	8	4	NA	110	57	NA
Von Damm	CTD	3	SUPR33	NBB	2312	-21	18	4	12300	83	NA	1.2
Von Damm	CTD	3	CTD03-4	Plume	2207	84	10	4	NA	39	NA	1.4
Von Damm	CTD	3	CTD03-8	Plume	2164	127	10	4	NA	38	NA	0.9
Von Damm	CTD	3	CTD03-9	NBB	2312	-21	10	4	NA	32	NA	1.2
Von Damm	CTD	3	CTD03-14	APB	1797	494	10	4	NA	30	NA	1.0
Von Damm	CTD	4	SUPR37	Plume	2204	87	6	4	NA	59	79	1.6
Von Damm	CTD	6	SUPR45	Plume	2042	249	36	4	NA	34	39	2.3
Von Damm	CTD	6	SUPR46	Plume	2041	250	32	4	NA	34	18	3.8
Von Damm	CTD	6	SUPR47	Plume	2238	53	29	4	NA	30	66	3.1
Von Damm	CTD	6	SUPR48	Plume	2256	35	13	4	NA	53	100	2.0
Piccard	ROV	J-615	SUPR 16	APB	2896	APB	11	4	15300	86	NA	NA
Piccard	ROV	J-615	SUPR 30	Plume	4906	51	21	4	30300	28	NA	NA
Piccard	ROV	J-615	SUPR 28	Plume	4926	31	15	4	22500	45	NA	NA
Piccard	CTD	8	SUPR 49	Plume	4316	641	52	4	NA	23	8	NA
Piccard	CTD	5	CTD05-8	Plume	4849	108	10	4	NA	51	NA	1.5
Piccard	CTD	8	SUPR 50	Plume	4869	88	44	4	NA	23	40	NA
Piccard	CTD	5	CTD05-3	Plume	4882	75	10	4	NA	60	NA	1.9
Piccard	CTD	5	CTD05-5	Plume	4945	12	10	4	NA	49	NA	4.7
Piccard	CTD	8	SUPR 51	Plume	4946	11	42	4	NA	27	32	NA
Piccard	ROV	J-615	SUPR 26	Plume	4941	16	18	6	23000	34	NA	NA
Piccard	ROV	J-615	SUPR 24	Plume	4950	7	19	8	50500	21	NA	NA
Piccard	ROV	J-615	SUPR 22	Plume	4956	1	10	98	166000	27	NA	NA
Piccard	ROV	J-615	SUPR 19	NBB	4956	1	10	4	23300	200	NA	NA
Background	CTD	1	CTD01-7	OB ^f	2400		10	4	NA	67	NA	NA
Background	CTD	1	CTD01-15	OB	2200		10	4	NA	30	NA	NA
Background	CTD	11	SUPR59	OB	2202		34	4	NA	33	23	NA
Background	CTD	11	SUPR60	OB	2327		34	4	NA	20	13	NA
Background	CTD	11	SUPR61	OB	4850		29	4	NA	23	20	NA
Background	CTD	11	SUPR62	OB	4950		28	4	NA	15	16	NA
Precision:							5%		1%	<10% ^g	<10% ^g	10-40% ^{g,h}

^aAll filters 0.2 um poresize, 142 mm diameter^bSiO₂ in nmol per kg seawater; DNA and RNA in ng per kg seawater; cell counts in x10⁴ cells per ml.^cabove plume background^dnot available^enear bottom background^focean background, plume elevation not relevant^gBased on propagation of both analytic and volumetric uncertainty.^hEstimate based on microscope field count variability on individual filters.

Table 3

Table 3. Mid-Cayman Rise hydrothermal plume particle organic carbon concentrations^{a,b}

Table 3. Von Damm CTD and ROV hydrothermal plume particle organic carbon concentrations									Dissolved	Particulate
Field	Deployment	Sample	Type	Depth (m)	Elev. (m)	Vol. (L)	T (°C)		SiO ₂	POC
Von Damm	CTD	3	SUPR36	APB ^c	1797	494	18	4	19900	44
Von Damm	CTD	3	SUPR35	Plume	2003	288	18	4	20500	33
Von Damm	CTD	3	SUPR31b	Plume	2094	197	19	4	20400	62
Von Damm	CTD	3	SUPR34	Plume	2105	186	17	4	16000	54
Von Damm	CTD	3	SUPR32	Plume	2164	127	17	4	13000	80
Von Damm	ROV	J-614	SUPR 10	Plume	2288	8	6	18	128000	220
Von Damm	ROV	J-614	SUPR 6	Plume	2290	1	5	64	398000	130
Von Damm	ROV	J-614	SUPR 2	Plume	2296	1	5	77	317000	570
Von Damm	ROV	J-614	SUPR 8	NBB ^d	2296	1	8	4	NA ^e	90
Von Damm	CTD	3	SUPR33	NBB	2312	-21	18	4	12300	26
Precision:							5%		1%	<7% ^f

^aAll filters precombusted GF/F, 47 mm diameter^bAll concentrations in nmol per kg seawater^cAbove plume background; taken during ascent for ROV deployments^dNear bottom background^eNot available^fBased on propagation of both analytic and volumetric uncertainty.

Table 4

Table 4. Mid-Cayman Rise hydrothermal plume particle chemistry^{a,b}

Table 1. Von Damm ROV J-614 and Piccard ROV J-615 hydrothermal plume particle chemistry												
Field	Deployment		Sample		Depth (m)	Elev. (m)	Vol. (L)	T (°C)	Dissolved		Particulate	
									SiO ₂	Fe	Fe	Cu
Von Damm	CTD	10	SUPR 58	APB ^c	1479	812	26	4	10800	- ^d	64	-
Von Damm	CTD	10	SUPR 57	Plume	2254	37	22	4	11200	311	32	-
Von Damm	CTD	10	SUPR 56	Plume	2290	1	24	4	12800	-	320	3.0
Von Damm	ROV	J-614	SUPR 11	Plume	2288	8	6	18	128000	-	180	67
Von Damm	ROV	J-614	SUPR 5	Plume	2290	1	5	64	398000	-	170	260
Von Damm	ROV	J-614	SUPR 3	Plume	2296	1	5	77	317000	-	120	150
Piccard	ROV	J-615	SUPR 17	APB	<4000	>1000	11	4	15300	-	-	-
Piccard	CTD	5	SUPR 42	Plume	4761	196	11	4	13000	-	-	-
Piccard	CTD	5	SUPR 43	Plume	4661	296	15	4	2410	-	7.9	-
Piccard	CTD	5	SUPR 41	Plume	4831	126	12	4	13700	-	20	5.2
Piccard	CTD	5	SUPR 38	Plume	4800	157	10	4	13300	536	83	19
Piccard	CTD	5	SUPR 39	Plume	4860	97	10	4	12500	-	110	17
Piccard	CTD	5	SUPR 40	Plume	4849	108	11	4	11500	311	130	25
Piccard	ROV	J-615	SUPR 30	Plume	4906	51	21	4	30300	2250	740	120
Piccard	ROV	J-615	SUPR 28	Plume	4926	31	15	4	22500	-	730	120
Piccard	ROV	J-615	SUPR 26	Plume	4941	16	18	6	23000	2820	3800	590
Piccard	ROV	J-615	SUPR 24	Plume	4950	7	19	8	50500	5820	5400	850
Piccard	ROV	J-615	SUPR 22	Plume	4956	1	10	98	166000	591000	720000	67100
Piccard	ROV	J-615	SUPR 19	NBB ^e	4956	1	10	4	23300	865	24	8.3
Precision:							5%		1%	5%	<10% ^f	<10% ^f

^aAll filters 0.2 um poresize, 142 mm diameter^bAll concentrations in nmol per kg seawater^cAbove plume background; taken during ascent for ROV deployments^dBelow detection limit^eNear bottom background^fBased on propagation of both analytic and volumetric uncertainty.

1 Figure Legends

2

3 Figure 1. SUPR-V2 system, deployed on *Jason*, preparing to collect samples from the
 4 rising hydrothermal plume emanating from the Beebe Vents chimney complex in the
 5 Mid-Cayman Rise. The (a) inlet control wand is held by the ROV manipulator and is
 6 connected by (b) intake lines to two SUPR-V2 units on the ROV science basket. Video of
 7 plume sampling is available in an online supplement. *Jason* imagery and video courtesy
 8 of the National Science Foundation, Woods Hole Oceanographic Institution, and C. R.
 9 German. (*Note to editor: the image of this photo is the first frame of the supplementary*
 10 *video, which can be linked to this figure; color web only. Two column figure.*)

11

12 Figure 2. SUPR-V2 samplers mounted on ROV *Jason* during a hydrothermal research
 13 cruise to the Mid-Cayman Rise. (a) Two SUPR samplers being deployed simultaneously
 14 in separate configurations for synoptic collection of particulate organic carbon samples
 15 and microbial community samples preserved *in situ*. (b) A third SUPR sampler, used on a
 16 different dive, configured with 2L PFA bottles particulate and dissolved metal samples
 17 along with a whole water and filtrate sample set. (*Note to editor: One column figure.*)

18

19 Figure 3. (a) The SUPR-V2 sampler uses a 14 port, double-throw, distribution valve to
 20 pump sample through any 1 of 14 sample loops. Each sample loop can contain various
 21 combinations of (b) filter housings and water bottles. The (c) entire system consists of the
 22 distribution valve (Dist. Valve), sample containers, electronics, pump, flow meters and a
 23 two-port selector valve used for *in situ* sample preservation. For *in situ* preservation, after

each sample is collected, the two-port selector valve is used to connect the distribution valve inlet to a bladder filled with preservative. Electrical power and control lines shown in black; sample lines shown in dotted green; preservative supply line shown in dashed red. Dimensions in cm. *(Note to editor: color web only. Two column figure.)*

Figure 4. SUPR-V2 system components: (a) the base system consisting of the distribution valve, oil-filled electronics housing and pump (not visible); (b) the preservation valve and preservative bladder; (c) the digital and mechanical flow meters; (d) fourteen 142 mm diameter filter housings; (e) fourteen sets of 47 mm filter housings with whole water and filtrate collection bottles; and (f) fourteen 2 L PFA water bottles. *(Note to editor: color web only. Two column figure.)*

Figure 5. Samples collected 20 cm above the orifice of a high temperature black smoker on the Mid-Cayman Rise: (a) particulate material on a 142 mm diameter, 0.2 μm polycarbonate filter enables a variety of bulk geochemical analyses while (b) particle-laden, whole water samples enable supplementary analyses related to particle morphology and aggregation. *(Note to editor: One column figure.)*

Figure 6. Laboratory purging experiments show relative residual concentration of Allura Red dye remaining in sample containers after variable amounts of flushing with salt water. Volume flushed reported as percentage of sample container volume. Experimental cases were the flushing of 2 L bottles originally containing fresh water, 2 L bottles originally containing salt water, and 142 mm filter holders originally containing salt

1 water. Also shown, are theoretical curves for cases of pure displacement of original fluid
2 by flushing fluid (i.e., no mixing) and complete, continuous mixing. (*Note to editor: One*
3 *column figure.*)

4
5 Figure 7. Identification of microbial contaminants on SUPR-V2 operational blank filters,
6 SUPR-V2 sample filters, and a Niskin collected sample: a) dominant phylotypes
7 (“theoretical species”) and their associated abundances, and b) abundance of contaminant
8 phylotypes relative to the total recovered microbial community. See table 2 for sample
9 location. (*Note to editor: color web and print. Two column figure.*)

10
11 Figure 8. Transmission images and carbon maps for Beebe and Von Damm plume
12 particles. Carbon 1s STXM for 1 m plume-elevation within the main Von Damm vent
13 (low temperature, high flux). (a) Image at 288.2 eV for sample Von Damm J2-614-
14 SUPR05-1m. (b) Distribution of carbon in particle aggregate (288.2 eV normalized by
15 280 eV). Optical density is 1.98. (c) Image at 280 eV for sample J2-615-SUPR30-50m.
16 (d) Distribution of carbon in particle aggregate (288.6 eV normalized by 280 eV).
17 Optical density is 2.17. All scale bars are 2 μm . (*Note to editor: One column figure.*)

18
19 Figure 9. Composition of microbial communities, post contaminant removal, from two
20 representative buoyant plume samples at Mid-Cayman Rise. (*Note to editor: color web*
21 *and print. One column figure*)

Figure 1

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