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Instruments and Methods

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

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ABSTRACT

A new tool was developed for large volume sampling to facilitate marine microbiology and biogeochemical studies. It was developed for remotely operated vehicle and hydrocast deployments, and allows for rapid collection of multiple sample types from the water column and dynamic, variable environments such as rising hydrothermal plumes. It was used successfully during a 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 (unfiltered) water, and filtrate. Suspended particulate can be collected on filters up to 142 mm in diameter and pore sizes down to 0.2 μm. Filtration is typically at flowrates of 2 L min^{-1}. For particulate material, filtered volume is constrained only by sampling time and filter capacity, with all sample volumes recorded by digital flowmeter. 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. The system is constructed of plastics with titanium fasteners and nickel alloy spring loaded seals. There are no ferrous alloys in the sampling system. Individual sample lines are prefilled with filtered, deionized water prior to deployment and remain sealed unless a sample is actively being collected. This system is intended to facilitate studies concerning the relationship between 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., Lam et al., 2004; Bennett et al., 2008; 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. 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, 2005). The processes involved are complex; consequently, our understanding is incomplete. More studies are required to fully elucidate this relationship, particularly studies that quantify and

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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., 2013; 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 (Fig. 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 (Fig. 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 ~2 L (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.

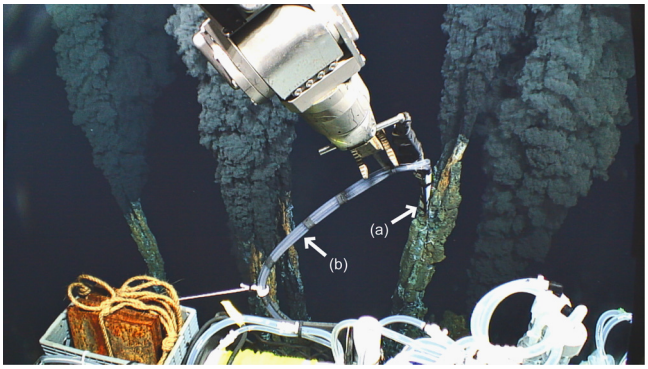

Fig. 1. SUPR-V2 system, deployed on *Jason*, preparing to collect samples from the rising hydrothermal plume emanating from the Beebe Vents chimney complex in the Mid-Cayman Rise: (a) inlet control wand is held by the ROV manipulator and is connected by (b) intake lines to two SUPR-V2 units on the ROV science basket. Video of plume sampling is available in an online supplement. *Jason* imagery and video courtesy of the National Science Foundation, Woods Hole Oceanographic Institution, and C.R. German.

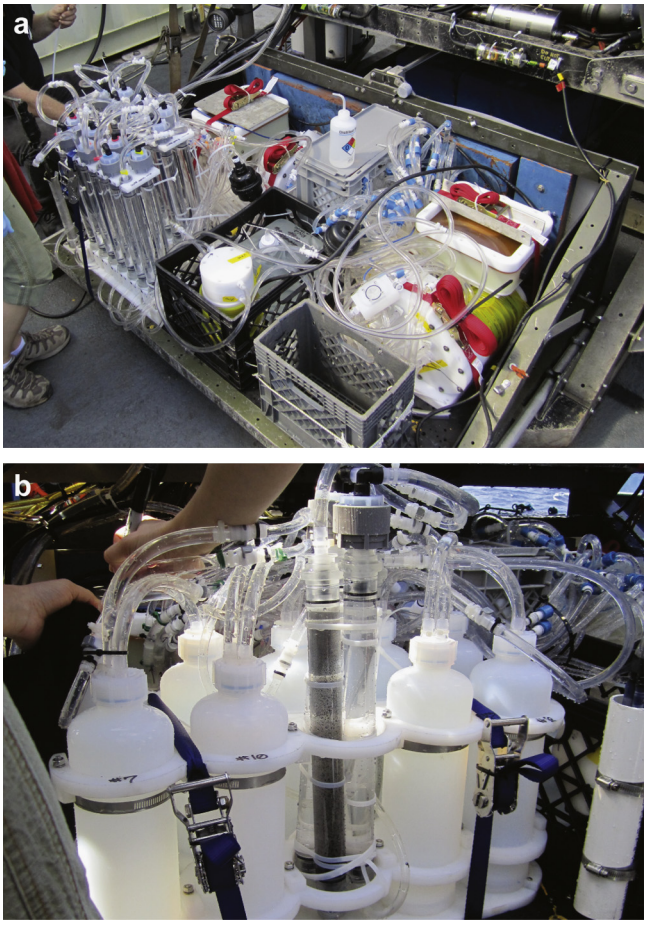

Fig. 2. SUPR-V2 samplers mounted on ROV *Jason* during a hydrothermal research cruise to the Mid-Cayman Rise: (a) two SUPR samplers being deployed simultaneously in separate configurations for synoptic collection of particulate organic carbon samples and microbial community samples preserved *in situ*. (b) A third SUPR sampler, used on a different dive, configured with 2L perfluoroalkoxy (PFA; Savillex) bottles for collection of particulate and dissolved metal samples along with a whole water and filtrate sample set.

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 et 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.