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Deep-Sea Research I 51 (2004) 1781–1792

DEEP-SEA RESEARCH
PART I

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Instruments and Methods

An instrument for collecting discrete large-volume water samples suitable for ecological studies of microorganisms

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Received 23 June 2003; received in revised form 26 February 2004; accepted 27 May 2004

Available online 26 August 2004

Abstract

Microbiological investigations utilizing molecular genetic approaches to characterize microbial communities can require large volume water samples, tens to hundreds of liters. The requirement for large volume samples can be especially challenging in deep-sea hydrothermal vent environments of the oceanic ridge system. By and large studies of these environments rely on deep submergence vehicles. However collection of large volume (>100 L) water samples adjacent to the benthos is not feasible due to weight considerations. To address the technical difficulty of collecting large volume water samples from hydrothermal diffuse flow environments, a semi-autonomous large-volume water sampler (LVWS) was designed. The LVWS is capable of reliably collecting and bringing to the surface 120 L water samples from diffuse flow environments. Microscopy, molecular genetic and chemical analyses of water samples taken from 9°N East Pacific Rise are shown to demonstrate the utility of the LVWS for studies of near-benthos environments. To our knowledge this is the first report of virioplankton abundance within diffuse-flow waters of a deep-sea hydrothermal vent environment. Because of its simple design and relatively low cost, the LVWS should be applicable to a variety of studies which require large-volume water samples collected immediately adjacent to the benthos.

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Keywords: Sampling method; Microbiology; Hydrothermal vent; Viruses; Bacteria

1. Introduction

Measurements of biological, chemical and physical characteristics of natural waters rely on devices capable of collecting water samples from discrete depths or locations. In general, either Niskin bottles or a simple sampling hose and shipboard pump are employed for accurate sample

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collection. While this approach is quite suitable for sampling the water column, it fails when attempting to collect water samples from specific locations close to the benthos. Collection of near-benthos water samples requires a device able to withdraw and store water from a discrete location. Past investigations of microbial activity and particulate matter within water of the benthic boundary or nepheloid layer required the development of a tethered bottom water sampler (BWS) capable of collecting 15 L of water at four different heights within the first meter above the bottom (Boetius et al., 2000; Ritzrau and Thomsen, 1997; Thomsen et al., 1994).

In deep-sea hydrothermal vent environments, dramatic gradients in chemical and biological characteristics of water occur in close proximity to both diffuse and high flow vent fluids. To accurately characterize these waters requires capture of samples from discrete microenvironments. In instances where in situ analyses of vent fluids from microenvironments using a flow through device (Massoth et al., 1995) are not possible, it is necessary to collect and store water samples for shipboard analyses. An example of such a need was the chemical characterization of diffuse flow vent fluids within the tubes of the polychaetous hydrothermal vent annelid, *Alvinella pompejana* (Di Meo et al., 1999). While some chemical analyses of water samples require only small volumes, ca. 50 ml, analyses for detection of trace elements in seawater can require collection of samples on the order of hundreds of liters followed by concentration of metal species (Bodman et al., 1961; Gerard and Ewing, 1961). In regards to required water sample volume, the biological analogue of trace element quantification in chemical oceanography is molecular genetic characterization of microbial communities. Such analyses necessitate larger volume samples of 1–100 L with less productive, oligotrophic environments, e.g. the deep sea, generally requiring the largest samples. In population genetic studies of viruses, the need for large sample volumes is paramount, as subsequent characterization of viral communities requires viral particle densities of 100–1000 times that of natural abundance (Wommack et al., 1999).

Sampling ephemeral hydrothermal vent fields requires highly discrete techniques because of the steep thermal, chemical, and biological gradients that are present along the tectonically active deep ocean ridges. As a result, studies of the biology and ecology of vent organisms have relied exclusively on DSVs or remotely operated vehicles for the collection of samples and physical/chemical data. To investigate and characterize communities of viruses and bacteria associated with diffuse flow environments of hydrothermal vents, it was necessary to design a sampling device capable of collecting large volumes of diffuse flow water from distinct locations within the vent field. This report describes a simple and relatively inexpensive sampling apparatus, the large-volume water sampler (LVWS), which is operated from a DSV and designed to collect >100 L samples of diffuse flow water. Viral and bacterial abundance data as well as chemical data from samples collected by the LVWS at the 2500 m deep 9°N East Pacific Rise vent site (9°50'N; 104°8'W) are shown to demonstrate the efficacy of this sampling apparatus. The LVWS should be of use in a variety of oceanographic research endeavors where large volumes of discrete water samples are required.

2. Design and operation

The philosophy behind the design and operation of the LVWS was to keep these aspects simple for reliability and durability under the extreme conditions of the deep-sea. The simple design can be summarized by describing the operation of the LVWS: a small battery-operated centrifugal pump draws water through the sample hose and then pushes the water sample into two 60 L Tedlar plastic bags. Tubing is plumbed with a check valve and two over-pressure valves to prevent back-flow of the sample and rupture of the sample bags, respectively. Routing of water flow by a three-way valve and actuation of the pump are controlled simultaneously by a single control arm.

The LVWS is built upon a standard Woods Hole Oceanographic elevator platform (A) shown in Fig. 1. The base of the elevator is made of high-density plastic in an open honeycomb form that

allows for easy attachment of the component parts to the platform. Essentially, each piece of the LVWS is held down by a custom designed aluminum bracket and backing plate (B). Stainless steel bolts with spring washers and locking nuts are used to join the bracket and backing plate with the elevator platform sandwiched in between. Sixty-liter Tedlar plastic bags (C) [part no. 10-050E75-S, MiDan Corp., Chino, CA] are encased in 57 L Nalgene high-density polyethylene rectangular tanks (D) [part no. 14100-0020, Nalge Nunc International], which are attached to the platform with a bracket-backing plate assembly (B). Tedlar bags are held to the lid of the Nalgene box with 3/4" Jaco fittings (F) supplied by the bag manufacturer. Lids of the Nalgene box are strapped down

to the bracket with two standard SCUBA weight belts with expanding clasps (E). The LVWS has two sample bag assemblies, attached to either side of the long axis of the elevator platform, which are plumbed in series using 3/4 in. i.d., 1/8 in. wall silicone tubing (G). Between the two sample bag assemblies is an over pressure relief valve (J, and G on Fig. 2) [part no. 46505K35 McMaster-Carr] with low cracking pressure to prevent rupture of the sample bags. The valve is attached to the mast of the elevator (I) with plastic cable ties.

Between the two sample bag assemblies is mounted, in perpendicular orientation, a battery (H) [part no. SB-12/84, Deep Sea Power and Light, San Diego, CA] which powers the sampling pump. The battery is mounted to the platform

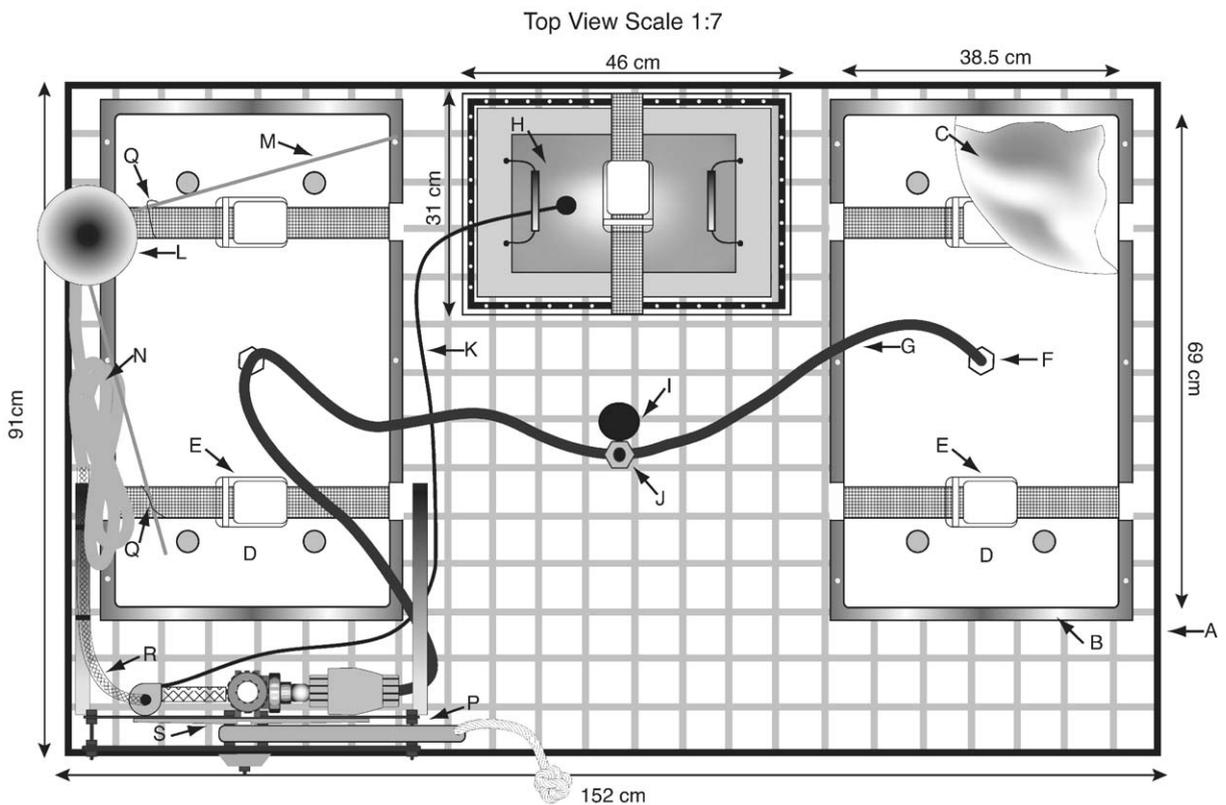


Fig. 1. Overhead view, scale drawing of large-volume water sampler on standard Woods Hole Oceanographic elevator: (A) elevator platform; (B) mounting bracket for Nalgene Box; (C) Tedlar sample collection bag; (D) Nalgene high-density polyethylene box; (E) SCUBA expansion weight belt; (F) through-hull fitting for Tedlar bag; (G) sample hose; (H) Deep Sea Power and Light 12 V battery; (I) yoke post of elevator platform; (J) over-pressure relief valve; (K) electrical cable from battery to pump; (L) sample collection port; (M) legs of sample collection port; (N) sample collection hose; (O) angle bracket for mounting pump panel; (P) pump panel; (R) Tygon tubing; and (S) aluminum runner for control arm.

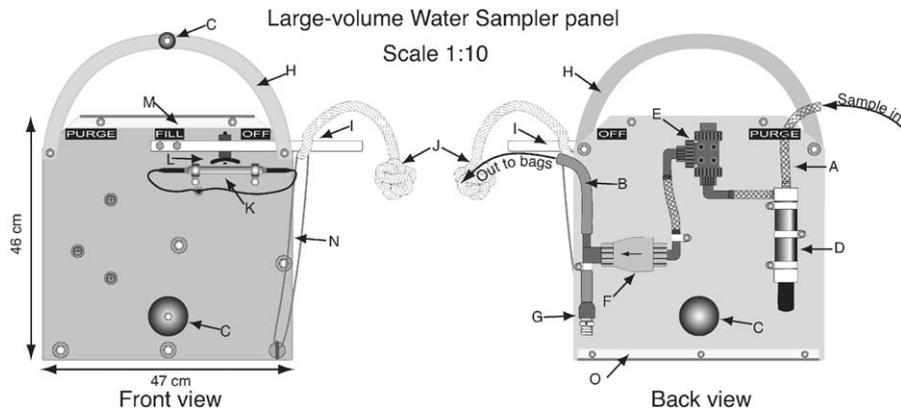


Fig. 2. Scale drawing of large-volume water sampler pump panel: (A) Tygon sample hose; (B) tubing to sample bags; (C) sacrificial zinc; (D) Sea Bird pump; (E) 3-way PVC valve; (F) one way flapper valve; (G) over-pressure relief valve; (H) protective arch for valve handle; (I) valve-pump control arm; (J) retaining rope; (K) magnetic switch; (L) magnet; (M) aluminum runner for control arm; (N) elastic restraining band for retaining rope; and (O) mounting bracket.

with an aluminum bracket-backing plate assembly and a strap similar to that used for the Nalgene boxes. Due to its weight (49 kg, in air) the battery is mounted near the center of the platform. An electrical cord (K) runs directly from the battery to a magnetic switch (K, Fig. 2) on the panel (P), which is mounted to the elevator platform with an angled aluminum brace (O). The water sample is drawn through 3/4 in i.d., 1/4 in. wall, gum rubber tubing (N) which is attached to 3/4 in i.d. PVC pipe at the end of the sample port (L). All materials needed to construct the sample port are available in a typical hardware store. The bowl-shaped end of the sample port (L in Fig. 1 and A in Fig. 3), made from a PVC roof sewer vent cap, is attached to 3/4 in i.d. PVC pipe (B) which is, in turn, attached to the leg assembly (C & D). The stainless steel legs (D) of the leg assembly are welded to a short section of 3/4 in i.d. stainless steel pipe (C). A second section of 3/4 in i.d. PVC pipe is attached to the leg assembly and then the sample tubing (N in Fig. 1 and E in Fig. 3). The gum rubber sample tubing is connected to nylon reinforced Tygon tubing (R in Fig. 1; or A in Fig. 2) which is connected directly to the sample pump. The Tygon tubing is attached to the angled aluminum brace by plastic cable ties. Gum rubber tubing was chosen for the sample hose as this type of tubing resists kinking yet is pliable at the near

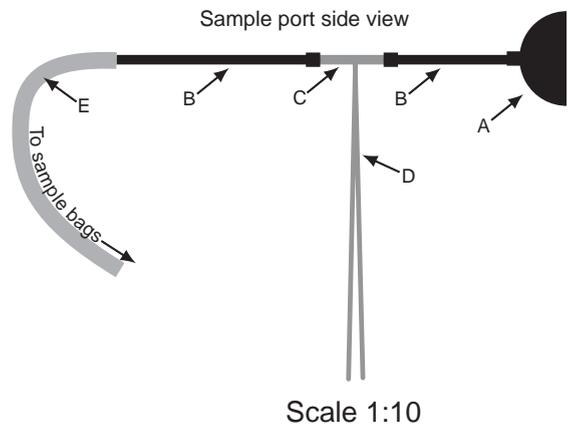


Fig. 3. Scale drawing of large-volume water sampler sample port: (A) bowl-shaped end; (B) 3/4 in i.d. PVC pipe; (C) 3/4 in i.d. stainless steel pipe; (D) stainless steel legs; (E) 3/4 in i.d. gum rubber tubing.

freezing temperatures of the deep sea. To hold the sample port in place during deployment the stainless steel legs (M) of the sample port are loosely attached to the straps of the bag assembly with rubber bands (Q).

One drawback of latex and to a lesser extent, Tygon, is the demonstrated toxicity of these materials to marine phytoplankton and bacteria. For investigations involving rate measurements or cultivation it would be advisable to plumb the

LVWS using silicon tubing which has been shown to be generally non-toxic to marine microorganisms (Price et al., 1986). As the focus of our investigations was cultivation-independent microscopy and molecular genetic approaches, the toxicity of tubing and sample bag materials were not accounted for in the design of the LVWS.

The heart of the LVWS is the panel shown in Fig. 2 which consists of an 1/8 in. aluminum plate to which the Sea Bird sample pump (D) [part no. SEB 5T, Sea-Bird Electronics, Inc], three-way PVC valve (E) [part no. 47125K81, McMaster-Carr Supply Company], and PVC swing-check valve (F) [part no. 45275K42, McMaster-Carr Supply Company] are mounted. Sacrificial zinc anodes (C) are also attached to the panel to prevent galvanic corrosion of panel components. The three-way PVC valve is mounted so that the handle penetrates the panel on the opposite side. Attached to the handle is a high-density polyethylene pump-valve control arm (I). Mounted immediately below the control arm is a magnetic switch (K) [model no. 1543, Pelagic Electronics] which controls current flow from the battery to the Sea Bird pump. The switch is controlled by a magnet (L) attached to the control arm. The switch-valve control arm assembly is configured so that when the magnet is engaged with the switch no current flows to the pump and the three-way valve is in the off position. When the control arm lever is moved to the 180° or 90° positions the magnet is disengaged from the switch and current flows to the pump.

Operation of the LVWS sample pump relies on manipulation of the control arm from a submersible. To prevent accidental activation of the sample pump during deployment, the control arm is restrained in the 0° off position by a polypropylene rope (J) to which a Monkey's Fist is tied in the bitter end and an Eye splice in the running end. It is necessary to use polypropylene rope as this material floats in seawater thus making it easier to grab with the manipulator arm of the submersible. The Eye splice end of the restraining rope is attached to the panel with elastic gum rubber tubing (N) and threaded over the control arm. The Monkey's Fist should be large enough to be easily grasped by the claw of

the manipulator arm (ca. 6–8 cm in. diameter). Once the LVWS is moved into position and the sample port placed within a diffuse flow field, the restraining rope is removed from the control arm and the control lever moved to the purge (180°) position. To facilitate easy movement of the control arm, an arch of 1/4 inch aluminum plating (H) is mounted to the panel outside of the control arm. Essentially, the control arm is protected on the front side (i.e., the side facing the submersible) by the aluminum arch. The sample collection hose of the LVWS is allowed to purge for at least 2 min, then the control lever is moved to the fill (90°) position. To make it easier to locate the 90° position a tapered aluminum runner with a shallow (ca. 1/4 inch deep) notch in the center (M, Fig. 2 and S, Fig. 1) is mounted to the front side of the panel. Maximum width of the runner occurs at the center while each end is tapered. At the 90° position the control arm rests in the notch. At near atmospheric pressure the sample bags fill in ca. 30 min. The approximate time needed to fill the bags at depth is not known, but is likely less than 45 min.

Surface deployment and recovery of the LVWS was performed with the ship's crane. The sequence of events was to deploy the LVWS in the evening by releasing the elevator and float package at a position near the seafloor collection site. Accounting for midwater currents and the sinking velocity of the package, the LVWS generally landed within 100 m of the intended target. On the next day's dive, the LVWS was located and moved into position by the submersible. Once the sample port was positioned in close proximity to the diffuse flow field, the system was purged and then the sample was collected. After the sample bags were full, the pump arm was moved to the off position (0°) and the 137 kg (seawater weight) package of cast iron plates, which were attached to the bottom of the elevator to facilitate sinking, was released from the elevator platform. The now positively buoyant elevator ascended to the surface and was retrieved by the ship. Seawater weight of the LVWS package was 128 kg, which was brought to the surface with 59 kg of positive buoyancy in the form of plastic-encased glass floats attached to the retrieval line (Fig. 4).

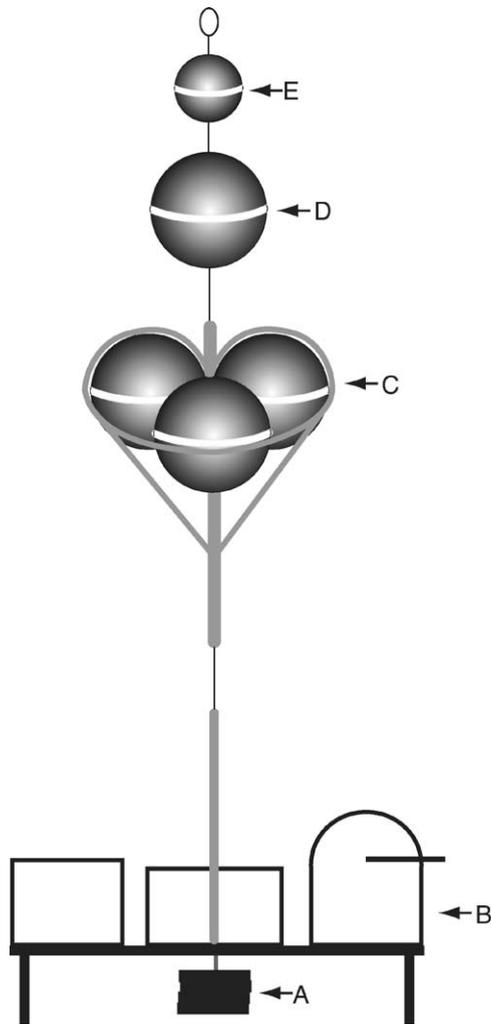


Fig. 4. Schematic of large-volume water sampler and float package (weights are in seawater): (A) four cast iron plates (137 kg); (B) LVWS (128 kg); (C) three 17 in glass float in a frame (–126 kg); (D) single 17 in glass float (–56 kg); (E) SONAR transponder sphere (–5 kg).

3. Sample processing and analyses

Once returned to the surface, 50 ml sub-samples from the LVWS were collected for microscopy and chemical analyses. This was done prior to processing the entire water sample for concentration of viruses and bacteria. Samples for microscopy were immediately fixed with glutaraldehyde (1% final) and stored at 4 °C. Bacteria and virus-like particles

(VLP) in fixed water samples were counted within 2 months of collection according to [Chen et al. \(2001\)](#). Sub-samples for chemical analyses were immediately examined by voltammetric and colorimetric methods. Aliquots of the sample were separated for dissolved Fe(II) and Fe(total) [defined as Fe(total) = dissolved Fe(III) + dissolved Fe(II)] and analyzed by colorimetry with a Spectronic 601 (Milton Roy) following the ferrozine method ([Stookey, 1970](#)).

Electrochemical analyses employed a standard three-electrode cell. The working electrode was a gold amalgam (Au/Hg) electrode of 0.1 mm diameter made in commercially available PEEK (polyethyl ether ketone) tubing sealed with epoxy as described by [Brendel and Luther III \(1995\)](#). Counter (Pt) and reference (Ag/AgCl) electrodes, each of 0.5 mm diameter, were made similarly. For the voltammetric measurements, the voltage range scanned was from –0.1 to –2.0 V. In linear sweep voltammetry (LSV) and cyclic voltammetry (CV) scan rates of 200, 500, or 1000 mV s^{–1} were run, depending on targeted chemical species ([Rozaan et al., 2000](#)). The parameters for square wave voltammetry (SWV) were as follows: pulse height, 24 mV; step increment, 1 mV; frequency, 100 Hz; scan rate, 200 mV s^{–1}. LSV and CV were used to measure oxygen and sulfur species, while SWV was employed for detection of metal redox species ([Theberge and Luther III, 1997](#)). Prior to sample measurements standard curves were produced for O₂, Mn, and sulfur species, as described previously ([Luther et al., 2001](#)).

After collection of 50 ml sub-samples for microscopy and chemical analyses, viruses and bacteria within the remaining ca. 120 L water sample were concentrated by tangential flow filtration (TFF) according to [Suttle et al. \(1991\)](#) with modifications. Briefly, virus particles were concentrated in a three-step filtration process. In the first step, bacteria were concentrated from the large volume water sample with a 0.1 m², 0.22 μm TFF cartridge (Pellicon, Millipore Corp.). The retentate of the Pellicon cartridge became the bacterial concentrate while the permeate was used to feed a second 10 ft² 30 kD ultrafiltration TFF cartridge (Amicon S10Y30, Millipore Corp.). The retentate of the Amicon TFF cartridge was reduced to a 2 L viral

concentrate after four hours of filtration. In the second step, the 2 L viral concentrate was reduced to a volume of 250 ml with a smaller 1 ft², 30 kD TFF cartridge (PrepScale, Millipore Corp.). After the second step, the 250 ml viral concentrate was filtered through a 0.22 µm syringe filter (Sterivex, Millipore Corp.), aliquoted into 50 ml conical centrifuge tubes and frozen for transport to the laboratory. In the final step, viruses within the sample were further concentrated into a final volume of ca. 2 ml with 30 kD spin filters (Centricon, Millipore Corp.). Altogether this three-step procedure achieves a 60,000-fold reduction in water volume. A 500 µl viral concentrate was washed 3X with 5X TBE using Centricon spin filters (30,000 MWCO). 500 µl of buffer was exchanged and the viral concentrate pelleted in a micro-ultracentrifuge (25,000 rpm, 10°C, 12h). The pellet was then resuspended in 20 µl of 5X TBE and heated to 60°C for 40 min. The entire suspension was run on a gel with no purification.

4. Results and discussion

Over the course of three research cruises, the LVWS has been successfully deployed and recovered by the crew of the *R/V Atlantis* twelve times. On the 2001 cruise, four large volume water samples were collected from three vents sites, Bio 9, Q vent and M vent. In addition, a large-volume water sample was collected from an off-vent control site for comparison of diffuse flow water and near-benthos seawater. Bio9 and Q vent were populated with colonies of *A. pompejana* and the giant tubeworm *Riftia*, respectively, while no macrofauna were present at M vent. In most cases, positioning and operation of the LVWS required 45 min to one hour of bottom time. Viruses and bacteria within large volume water samples were concentrated over 100-fold using tangential flow filtration. The efficiency to which viruses and bacteria were concentrated varied widely (from 6% to 69%) with the best efficiencies occurring in samples with higher ambient concentrations of viruses (Table 2). Concentration efficiency was interpreted as the abundance of viruses or bacteria in the final concentrate divided

Table 1
Chemistry data for water collected with LVWS (values in µM except for FeS, given in electrochemical signal current, nA)

Dive	Location	O ₂	H ₂ S	Dissolved Fe (II)	Total dissolved Fe
3714	Bio 9	93.7	21.5	0.4	0.28
3716	Bio 9	142.2	11.7	1.43	1.58
3718	Q vent	39.5	14.3	1.18	1.18
3721	M vent	22	0	3.27	3.63

by the ambient viral or bacterial abundance times the concentration factor. Initial reports of concentration efficiency with TFF were greater than 80% for viroplankton in coastal Gulf of Mexico water samples where viral abundance generally exceeds 10⁷ virus-like particles per ml (Suttle et al., 1991). To our knowledge there has been no such analysis of viral concentration efficiency from oligotrophic environments, which, in general, display lower abundances of viruses and bacteria.

Voltammetric and colorimetric analysis of water samples collected from hydrothermal diffuse flow environments demonstrated that the LVWS successfully collected diffuse flow water. Results reported in Table 1 reveal that these water samples were enriched in sulfur and iron species. Linear sweep voltammograms from ambient seawater revealed the presence of only O₂, while samples collected at Bio 9 and Q vents revealed sulfur and iron species (Fig. 5). While source hydrothermal vent fluid is anoxic, diffuse flow water was microaerophilic to oxidic, as iron and sulfide rich vent waters meet and mix with ambient, O₂ saturated bottom water. Dissolved iron can react with sulfide to form solid and dissolved FeS, distinguishable from free H₂S/HS⁻ and polysulfides by voltammetry (Rickard and Luther III, 1997). It is also likely that some diffusion of atmospheric O₂ occurred during sub-sampling from the large volume water sample. In contrast to the diffuse flow water samples from Bio 9 and Q vent, the M vent sample contained undetectable H₂S but higher amounts of total and dissolved Fe(II). Unlike samples from Bio9 and Q vent, no animals were present within the diffuse flow field of M vent. A lack of available reduced sulfur

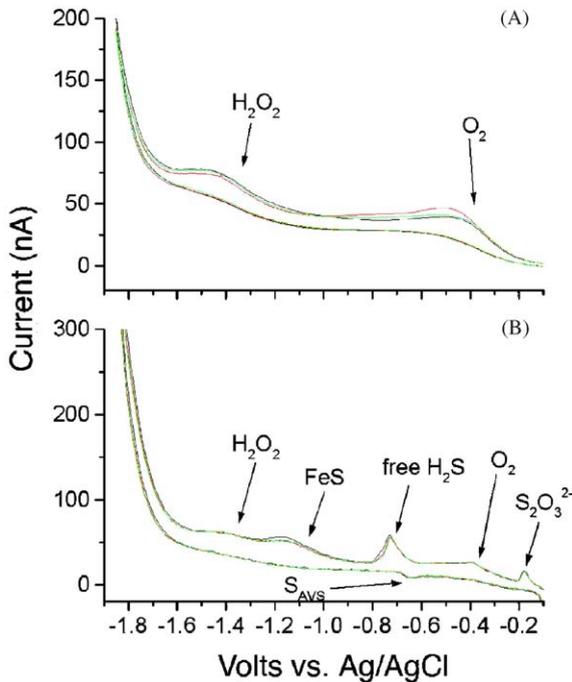


Fig. 5. Linear sweep voltammograms. (A) ambient seawater and (B) diffuse flow vent water collected from Bio9 using the LVWS. The added presence of FeS, free H₂S, S₂O₃²⁻ and acid-volatile S is apparent in the diffuse flow water sample.

species within M vent diffuse flow fluid is a reasonable geochemical explanation for the lack of macrofauna at this site. Previous in situ electrochemical studies have demonstrated a predominance of Fe(II) and FeS and a complete lack of free sulfide within individual *Alvinella* tubes, and more free H₂S/HS⁻ associated with *Riftia* colonies. From fine scale characterization of diffuse flow environments, Luther and co-workers demonstrated that local geochemistry can drive the distribution of vent macrofauna (Luther et al., 2001; Luther III et al., 2001). Despite the possibility that small volumes of ambient seawater were likely entrained with diffuse flow water when collected with the LVWS, geochemical data from these water samples appears to reflect the chemical characteristics of the collection site.

A general feature seen in deep sea hydrothermal environments are enhanced levels of bacterial

activity (Karl et al., 1988) and abundance in portions of the water column directly affected by plumes of vent water (Naganuma and Seki, 1994; Winn et al., 1986). The presence of reduced metals (Mn(II), and Fe(II)) sulfur, and hydrothermally derived H₂ and CH₄ have been shown to contribute to enhanced levels of bacterial production in this otherwise oligotrophic environment (Cowen et al., 1998; Cowen et al., 1986). These effects can be seen from a few centimeters to several kilometers down current from the vent site while high temperature discharge fluids are devoid of living bacteria (Winn et al., 1986). The sole report of viral abundances associated with deep-sea hydrothermal activity were opportunistic water column samples taken of a hydrothermal event plume along the Gorda Ridge (Juniper et al., 1998). Plume waters were identified by temperature anomalies of up to 0.12 °C. As compared to background seawater, bacterial abundance was significantly higher in plume waters, whereas viral abundance was lower for a young plume (ca. 10 days old) and higher for older plumes (ca. 3 weeks old). While exact numbers were not reported, viral and bacterial abundance within event plumes was on the order of 10⁴ cells or VLP per ml. In general, numbers of viruses and bacteria were higher both in the water column and in diffuse flow waters of the 9°N site [on the order of 10⁵ ml⁻¹ (Table 2)] as compared to hydrothermal plumes of the Gorda, Juan de Fuca or Endeavour Ridges (Cowen et al., 1998; Kaye and Baross, 2000; Winn et al., 1986) or the North Fiji Basin (Naganuma and Seki, 1994). There are two likely explanations for the higher numbers seen in this study. First, the diffuse flow water samples examined in this study were collected adjacent to the benthos, whereas previous studies mostly examined seawater obtained from the parts of the water column directly affected by vent plumes. Thus, it is conceivable the diffuse flow samples did indeed contain enhanced levels of bacteria and viruses as seen for other near-benthos environments (Boetius et al., 2000). A second explanation is that the use of SYBR Gold improved our ability to visualize small bacteria and viruses as compared to the DAPI and Yo-Pro stains used previously for counts of bacteria and viruses, respectively. It is

Table 2

Abundance of virus-like particles and bacteria in water column and diffuse-flow water samples

Dive or depth	Location	Water column and diffuse-flow water		VLP: bacteria	Viral concentrate		Bacterial concentrate
		VLP $\times 10^5$ ml ⁻¹	Bacteria $\times 10^5$ ml ⁻¹		VLP $\times 10^7$ ml ⁻¹	Efficiency (%)	
3714	Bio 9	ND ^a	ND	ND	6.95 \pm 1.6		1.35 \pm 0.31
3716	Bio 9	3.13 \pm 1.3	2.64 \pm 0.91	1.19	3.92 \pm 0.98	28	ND
3718	Q vent	3.64 \pm 1.2	0.98 \pm 0.23	3.73	4.54 \pm 0.77	33	ND
3721	M vent	14.8 \pm 4.2	4.3 \pm 1.1	3.45	3.55 \pm 0.44	6	ND
3723	Control ^b	5.93 \pm 1.2	3.48 \pm 1.5	1.71	11.5 \pm 2.4	48	5.41 \pm 0.69
30 m	WC ^c	48.9 \pm 20	18.4 \pm 3.5	2.66	126 \pm 43.2	65	ND
100 m	WC	33 \pm 6.2	5.63 \pm 1.2	5.85	87.2 \pm 19.3	69	ND
500 m	WC	16.1 \pm 1.2	4.85 \pm 1.7	3.33	6.41 \pm 0.7	9	ND
1500 m	WC	25.3 \pm 4.6	5.4 \pm 1	4.65	2.78 \pm 1.13	3	ND

^aNot determined.^bNear benthos site away from vent field.^cWater column.

known that SYBR stains (SYBR Gold and SYBR Green I) can dramatically improve detection and enumeration of viruses as compared to DAPI (Chen et al., 2001; Noble and Fuhrman, 1998).

The only other reports of viral abundance from the deep sea found trends similar to those seen in the 9°N water column (Fuhrman, 2000; Hara et al., 1996). In the western North Pacific, highest viral abundances (38–19 $\times 10^5$ VLP ml⁻¹) occurred at 50 m with counts decreasing to ca. 4 – 0.6 $\times 10^5$ VLP ml⁻¹ at a depth of 5000 m (Hara et al., 1996). At 9°N, highest viral abundances (49–33 $\times 10^5$ VLP ml⁻¹) occurred around the deep chlorophyll maximum, 100 m, with counts steadily decreasing to a nearly constant level of ca. 1.4 $\times 10^5$ VLP ml⁻¹ below 2000 m (data not shown). With the exception of M vent, VLP abundance in diffuse flow and near benthos water samples was not significantly different from abundance in waters below 2000 m ($P > 0.05$). It is interesting to note that viral abundance was significantly higher in the M vent water sample ($P < 0.05$), which also had dramatically different water chemistry (Tables 1 and 2).

With regards to bacterial abundance, near-benthos water samples were similar to ambient seawater up to a depth of 1500 m ($P > 0.05$). In these samples bacterioplankton abundance ranged from 3 to 25 $\times 10^5$ bacteria per ml (Table 2). These deep water and near-benthos values were similar

to a subtropical station sampled in the North Pacific where bacterial abundance was nearly constant at 1 $\times 10^5$ bacteria per ml below 2000 m (Hara et al., 1996). As observed by Hara et al. (1996), both bacterial and viral abundance decreased exponentially and correlated with depth ($P < 0.01$; $r < -0.8$) in 9°N water samples.

The ratio of viral to bacterial abundance (VBR) has been a useful metric for interpreting the relationship between virio- and bacterioplankton populations. In all samples, viral abundance exceeded bacterial abundance (Table 2); but not to the degree seen in more eutrophic environments (Wommack et al., 1992). The range of VBR values seen in 9°N water samples were similar to those reported in other pelagic and deep-sea environments (Fuhrman, 2000; Hara et al., 1996; Juniper et al., 1998), but, unlike deep water samples from the subtropical North Pacific, did not show a tendency to increase below 2000 m. Owing to a lack of empirical or observational data, direct interpretation of the relationship between VBR and the level of viral-mediated bacterial mortality is not currently possible. However, recognizing that propagation of virulent bacteriophages (lytic lifecycle) requires that host–virus contacts are frequent enough to maintain the viral population, it is believed that significant populations of virulent phages can be maintained only in environments exhibiting high viral abundance and VBR

(Wilcox and Fuhrman, 1994). In the case of deep-sea and diffuse flow environments where viral and host abundances are low it is possible that virioplankton consist mainly of temperate bacteriophages (lysogenic lifecycle) produced through lytic induction of lysogenic hosts.

Bacteria and viruses within concentrates prepared from large-volume diffuse flow water samples proved to be sufficiently abundant to provide DNA for molecular genetic analyses. As shown in Fig. 6, DNA isolated from bacterial concentrates was used as a template for PCR amplification of 16S rDNA. Separation of 16S rDNA amplicons by denaturing gradient gel electrophoresis (DGGE) demonstrates a surprising level of similarity between banding patterns in five diffuse-flow water samples. This result indicates the possibility that prokaryotic communities are relatively stable in composition at different sites and over a period of 1 year. Definitive support for

this statement would require genotypic identification of common bands through sequence analysis. Although only small quantities of DNA are required for successful PCR amplification of 16S rDNA, the average amount of bacterial DNA provided from an LVWS bacterial concentrate ($15.6 \pm 3.7 \mu\text{g}$) would be sufficient for hybridization procedures and construction of shotgun fosmid libraries where a great deal more DNA is required (M. Suzuki, pers. comm.). Recombinant DNA approaches, such as fosmid cloning, which are capable of producing libraries with large inserts (ca. 40 kb), have been critical to genomic studies of uncultivated marine prokaryotes (Stein et al., 1996).

In the case of viral communities, where the average DNA content of a virus is 50- to 100-fold lower than that of a typical bacterium (e.g. 40 vs. 2000 kb, respectively), concentration of virus particles from a large volume water sample is prerequisite to most molecular genetic analyses. A critical first step towards application of molecular genetic tools in microbial ecology is purification of nucleic acids. To demonstrate the applicability of large volume water samples to future investigations of viruses within deep-sea hydrothermal vent environments, nucleic acids were isolated from a diffuse flow viral concentrate collected at the Bio 9 site in December 2003 (Fig. 6). A 500 μl viral concentrate was washed 3X with 5X TBE using Centricon spin filters (30,000 MWCO). 500 μl of buffer was exchanged and the viral concentrate pelleted in a micro-ultracentrifuge (25,000 rpm, 10°C , 12h). The pellet was then resuspended in 20 μl of 5X TBE and heated to 60°C for 40 min. The entire suspension was run on a gel with no purification. DNA isolated from the diffuse-flow viral concentrate migrated to a point just above the 23 kb marker band indicating the genomic origin of this DNA. In this preparation, DNA was isolated from 3.3×10^9 virus particles within a viral concentrate. Viral origin of the genomic DNA shown in Fig. 6 is substantiated from previous work demonstrating the effectiveness of TFF for concentration of viruses from large volume water samples (Suttle et al., 1991) and the insignificant contribution of dissolved DNA to total DNA within the typical bacteriophage

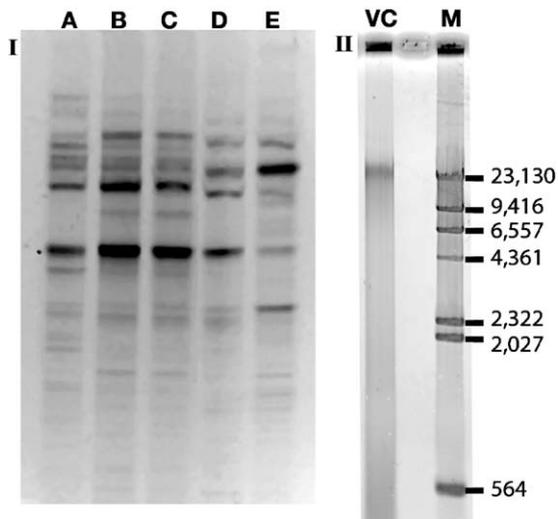


Fig. 6. Molecular genetic data from diffuse flow water samples collected using the large volume water sampler. (I) Denaturing gradient electrophoretic gel of 16S rDNA amplified from diffuse flow bacterial concentrates. Lanes are as follows: (A) Bio 9, October 20, 2001; (B) Bio 9, October 22, 2001; (C) Q vent, October 24, 2001; (D) M vent, October 28, 2002; (E) Microbial mat, October 31, 2002. (II) Agarose electrophoretic gel of virioplankton DNA isolated from diffuse flow viral concentrates. Lanes are as follows: M) λ Hind III molecular weight marker; VC) Virioplankton DNA from large volume water sample collected at Bio9, December 2004. Molecular weights are in base pairs of DNA.

genome size fraction of oligotrophic seawater (DeFlaun et al., 1987; Steward et al., 2000; Wommack et al., 1999). Using phage λ as a guide, this preparation should have contained ca. 150 ng of dsDNA. While absolute quantitation of the virioplankton DNA shown in Fig. 6 was not possible, it appears that the band contains in excess of 50 ng of DNA. With this amount of DNA, PCR-based molecular approaches are easily possible including construction of linker-amplified shotgun libraries for metagenomic exploration of virioplankton genetic diversity (Breitbart et al., 2002). In conclusion, the unique sampling capabilities provided by the LVWS enables investigation of the ecology of microorganisms within a new range of benthic environments.

Acknowledgements

The authors wish to thank the crew of the *R/V Atlantis* and the pilots of the *DSV Alvin* for assistance with deployment, operation and recovery of the LVWS. We are also grateful to Peggy O'Day and George Luther for assistance and discussion regarding chemical analyses. Financial support from the University of Delaware Research Foundation and the NSF (LExEn program (9907666); Biocomplexity in the Environment (0120648); and OCE-0136671) made this work possible.

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