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Instruments and Methods

Advanced instrument system for real-time and time-series microbial geochemical sampling of the deep (basaltic) crustal biosphere

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ABSTRACT

Integrated Ocean Drilling Program borehole CORK (Circulation Obviation Retrofit Kit) observatories provide long-term access to hydrothermal fluids circulating within the basaltic crust (basement), providing invaluable opportunities to study the deep biosphere. We describe the design and application parameters of the GeoMICROBE instrumented sled, an autonomous sensor and fluid sampling system. The GeoMICROBE system couples with CORK fluid delivery lines to draw large volumes of fluids from crustal aquifers to the seafloor. These fluids pass a series of in-line sensors and an in situ filtration and collection system. GeoMICROBE's major components include a primary valve manifold system, a positive displacement primary pump, sensors (e.g., fluid flow rate, temperature, dissolved O_2, electrochemistry-voltammetry analyzer), a 48-port in situ filtration and fluid collection system, computerized controller, seven 24 V–40 A batteries and wet-mateable (ODI) communications with submersibles. This constantly evolving system has been successfully connected to IODP Hole 1301A on the eastern flank of the Juan de Fuca Ridge. Also described here is a mobile pumping system (MPS), which possesses many of the same components as the GeoMICROBE (e.g., pump, sensors, controller), but is directly powered and controlled in real time via submersible operations; the MPS has been employed repeatedly to collect pristine basement fluids for a variety of geochemical and microbial studies.

1. Introduction

Low temperature hydrothermal fluids (<100 °C), vigorously circulate within the fractured and permeable volcanic rocks of the upper ocean crust (basement) (Fisher and Von Herzen, 2005; Fisher et al., 2008), providing temperature and chemical gradients that form habitats for a variety of microbial communities. Indeed, there is growing evidence that a diverse subseafloor biosphere extends throughout the immense volume of aging crust underlying the global system of mid-ocean ridge (MOR) axes, flanks and ocean basins (e.g., Gold, 1992; Baross et al., 1994; Fisk et al., 1998; Torsvik et al., 1998; Furnes et al., 2001; Thorseth et al., 2001; Cowen et al., 2003; Cowen, 2004; Amend and Teske, 2005; Orcutt et al., 2010). The continued development of Circulation Obviation Retrofit Kit (CORK) observatories (Fig. 1) (e.g., Davis et al., 1992; Becker and Davis, 2005; Wheat et al., 2011) installed at Ocean Drilling Program (ODP) and Integrated Ocean Drilling Program (IODP) boreholes (Davis and Becker, 1999) offer unprecedented opportunities to study biogeochemical properties and microbial diversity in deep, sediment-buried crustal environments.

In a preliminary study of the crustal biosphere, fluids escaping from the top of the over-pressured CORK observatory at ODP Hole 1026B (ODP Leg 168) (Davis et al., 1997; Shipboard Scientific Party, 1997), on the flanks of the Juan de Fuca Ridge (JFR) in the Northeast Pacific, were shown to be derived from the oceanic basaltic crust and that the chemical characteristics and temperature (64 °C) of these fluids are conducive to microbial growth (Cowen et al., 2003). However, major questions regarding microbial community structure, key metabolic pathways and rates, as well as the dynamic inorganic and organic geochemistry, of the crustal fluids remain. Originally, 1026B was equipped with an early style CORK observatory (Fig. 1b), in which the entire borehole was sheathed by a steel casing, isolating fluids within the boreholes from direct exchange with sediment pore waters or ocean bottom waters. Seafloor access to fluids ascending within the CORK casing was possible via a sampling port spigot and short

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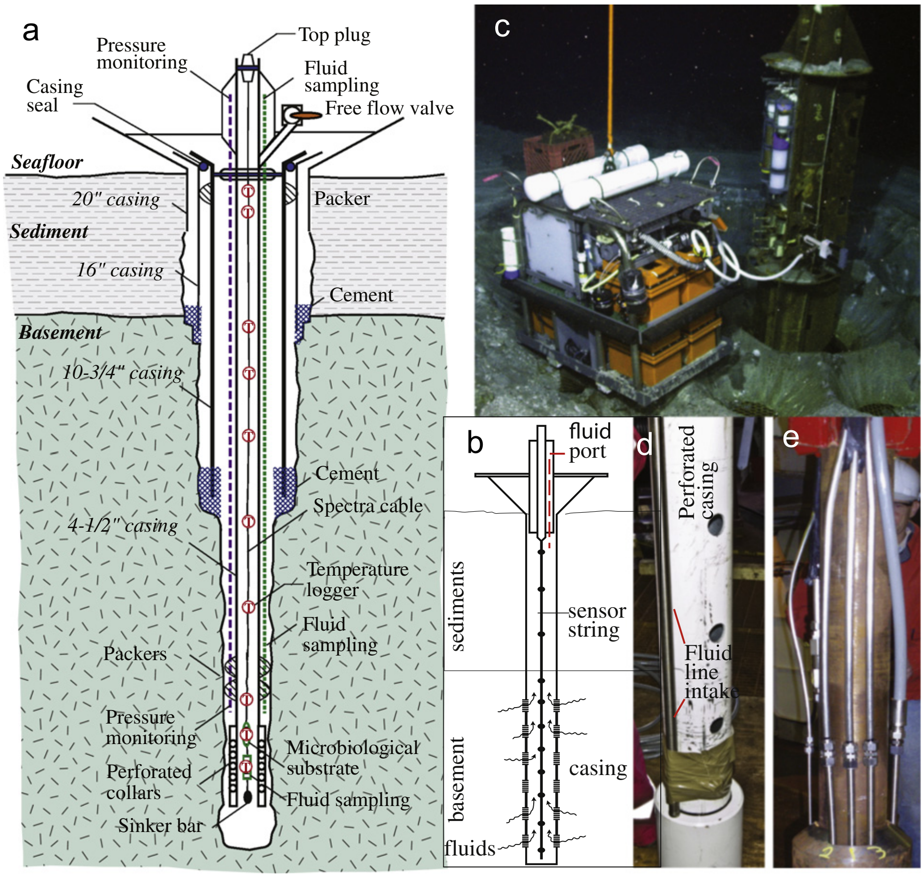

Fig. 1. Schematic drawings of modified CORK-II (a) and original CORK designs (b), the former showing continuous fluid delivery lines (FDL) extending from basement to seafloor platform (dashed green lines) (from Wheat et al., 2011). Photographs of (c) GeoMICROBE instrument sled connected to CORK-II installation at IODP Hole 1301A, (d) FDL intake screen at exterior of base of epoxy-coated CORK casing and (e) 4 stainless steel and one Tefzel (on right) FDLs attached to exterior of CORK casing in preparation for installation.

(2 m), 0.5 in. inner diameter (ID) titanium pipe. The large diameter (10–3/4 in.) of the CORK's casing presented little drag relative to even the modest formation overpressure of the borehole (18 kPa), resulting in substantial unassisted flow out of the sampling port (Davis and Becker, 1999; Cowen et al., 2003). Consequently, a series of simple passive microbiological and geochemical samplers, or 'BioColumns', were designed to take advantage of this artesian flow of crustal fluids from the 1026B CORK (Cowen et al., 2003). The BioColumn device channeled crustal fluids discharged at the CORK spigot past temperature and flow sensors, through particle filters (> 0.4 μm pore size), and an organic compound-scavenging resin column to collect samples for fluid composition, biomass, ribosomal RNA sequencing and lipid analysis (Cowen et al., 2003; Kenig et al., 2003, 2005). The duration of BioColumn deployments varied from days to 1-year, with remarkably constant, unassisted flow rates of ~4.4–5.4 l per minute (L min^{-1}) (Cowen et al., 2003) and estimated crustal (formation) fluid temperature of ~64 °C (Fisher et al., 1997; Davis et al., 1997; Davis and Becker, 2002).

The greatest disadvantages of this early generation sampling arrangement was the potential for chemical and microbial contamination (Cowen et al., 2003; Wheat et al., 2004) and the BioColumns integrated collected materials losing temporal resolution. The fluids collected from the top of the borehole had to flow through 295 m of reactive (low alloy) steel casing. The extent to which the fluids were altered microbially by interaction with casing cement, or the low alloy steel casing is unknown, but the reactive inner surfaces of such iron casings likely support a biofilm community that could contribute to the cellular biomass and molecular diversity observed in the 1026B fluid and BioColumn samples (Cowen et al., 2003). Extensive biofilms have been observed on scrapings recovered from CORK steel components recovered from borehole 1026B CORK in 2004 (Nakagawa et al., 2006). Concerns for the potential impact of the extended exposure of the crustal fluids to the steel casing on the chemical (e.g., iron leaching; hydrolysis reactions leading to H_2 production; loss of environmental H_2S to FeS via abiotic reaction with iron) and microbial composition of the fluids (Cowen et al., 2003) have led to significant improvements in the design of new CORK observatories (Becker and Davis, 2005; Fisher et al., 2011b; Wheat et al., 2011), as well as the development of new generations of seafloor sampling systems designed to both minimize contamination, collect discrete fluids, and meet the new challenges created by key new CORK upgrades.

The historic improvements in CORKs as geochemical and microbiological observatories have recently been described in detail by Wheat et al. (2011) (Fig. 1a, d, e). Key improvements with respect to microbiosphere studies include the use of packers to vertically isolate depth horizons within the upper crust for discreet sampling, the installation of fluid delivery lines (FDLs) that run exterior to the CORK casing as continuous 1/8 in. to 1/2 inch ID tubing, and the use of greatly improved materials for FDLs, intake gratings, and casings adjacent to FDL intake gratings and downhole experiments. Initially, the FDLs consisted of narrow bore 1/4 inch (0.636 cm) OD (outside diameter) stainless steel tubing with internal diameters of ~0.125 (0.318 cm); a 0.5 in. (1.3 cm) ID Tefzel (commercial product of ethylene tetraflurоethylene, ETFE) fluid line was also installed with the CORK at

IODP Hole 1301B, eastern flank of JFR, but this line has failed to produce a significant flow, even with substantial pumping, and is assumed to have sustained damage during installation, since the depth of its intake is coincident with a section of crust with significant hydrologic connectivity (A. Fisher, personal communication). CORK installations at IODP Hole 1362A and 1362B (IODP Exp 327), eastern flank of JFR, are each equipped with multiple 1.3 cm stainless steel chemistry sampling lines and a single 1.3 cm Tefzel microbiology (MBIO)-chemistry sampling line (Fisher et al., 2011b); the new CORK installations (October/November 2011) at North Pond sites (U1382A and U1382B; IODP Exp 336, western flank of the Mid-Atlantic Ridge) include up to three 1.3 cm ID Tefzel MBIO sampling lines servicing different depth horizons for each CORK (Fig. 1e). The stainless steel lines are a great improvement over the low alloy steel with respect to trace element contamination and contributions to microbial (redox) metabolism. However, the Tefzel (ETFE) is essentially inert, providing no chemical contamination or potential energy sources.

This paper focuses on the development of seafloor sampling systems designed to exploit new CORK FDLs to produce pristine crustal fluids for analytical and experimental procedures requiring relatively fresh or large fluid volumes. Volume requirements for analyses of the low biomass basement fluids range from several millliliters (e.g., amino acids, low molecular weight organic acids) to tens of liters (e.g., particulate organic carbon/particulate nitrogen, some molecular biology, ATP, metabolic rate experiments, some organic carbon (lipid) biomarkers) to 100's of liters (e.g., particulate organic carbon biomarkers and viral and environmental genomics). Any sampler must be compatible with the physical and chemical characteristics of crustal fluids: basically altered seawater with warm temperatures to $>64\,^{\circ}C$, oxic to anoxic, depleted in Mg^{++}, alkalinity, total CO_2 and dissolved organic carbon, and mildly enriched in trace metals (e.g., Fe^{++} and Mn^{++}), Si, ammonia and possibly methane and hydrogen (Wheat et al., 2000; Cowen et al., 2003; Lin et al., submitted to). The primary focus of this instrument development effort has been the GeoMICROBE sled (Fig. 1c), intended for time-series, year-long autonomous deployments connected to CORK FDLs. An outgrowth of the GeoMICROBE sled is the Mobile Pumping System (MPS), which incorporates many of the essential GeoMICROBE components but is powered and operated solely from the working basket of a human operated (HOV) or remotely operated vehicle (ROV). Both systems are designed to meet delivery challenges imposed by the FDLs while eliminating contamination. The more complex GeoMICROBE sleds are described in detail below, followed by a brief discussion of the MPS. Finally, preliminary results are presented from a 12-hour (July 2010) and a year-long (August 2010 to July 2011) deployment of the GeoMICROBE sled at the 1301A CORK and from 2011 MPS deployments at the 1301A, 1362A, and 1362B CORKs.

2. GeoMICROBE technical description

The main components, fluid flow, communications, and power distribution of the GeoMICROBE instrument sled are depicted in Fig. 2 (see also Supplementary Fig. 1). Basically, the GeoMICROBE's programmable central computer controller directs one of three primary valves to open and the primary pump to start pumping crustal fluid up one of up to three CORK fluid delivery lines (FDL) through the open valve and the primary pump itself,

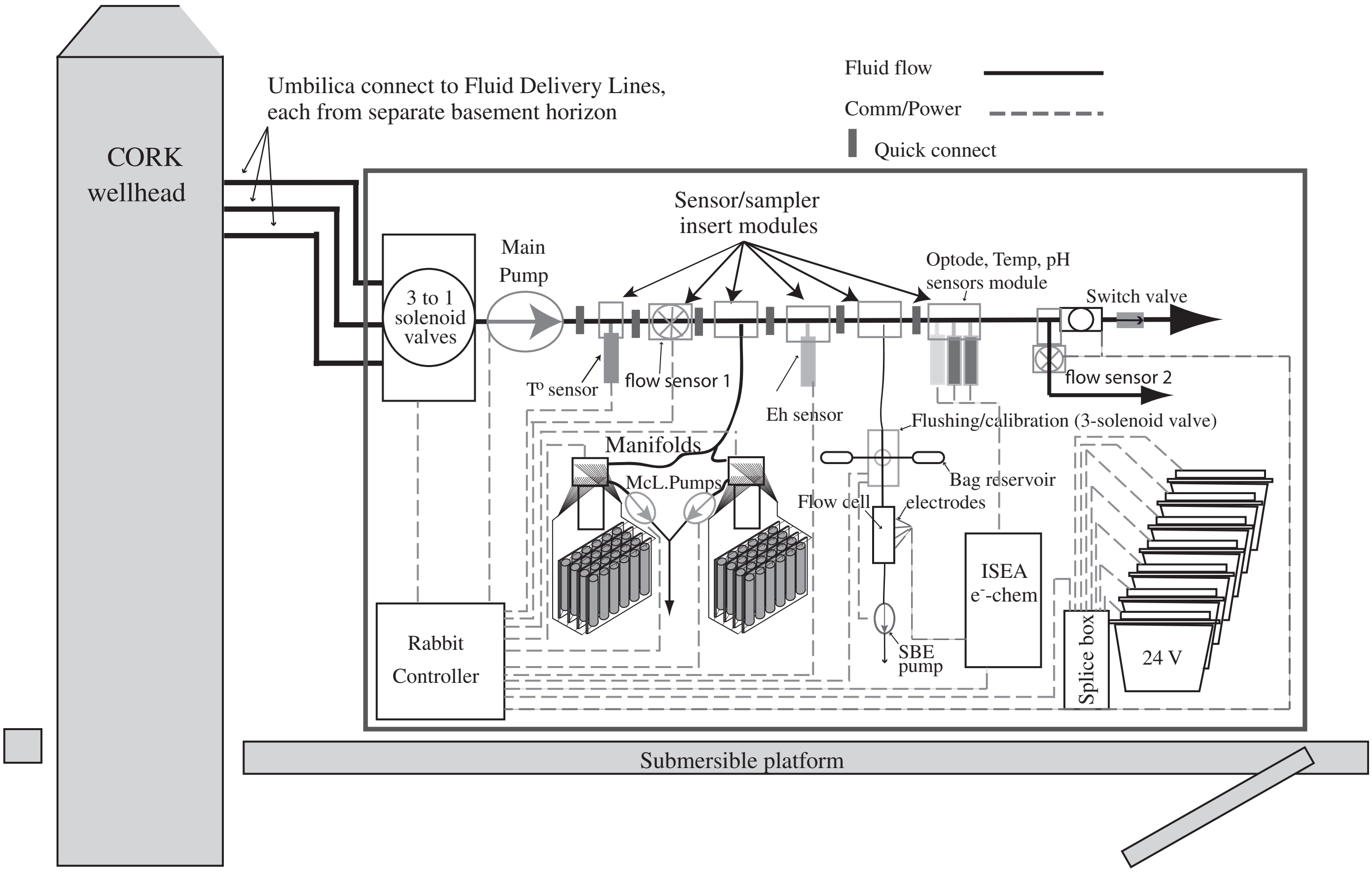

Fig. 2. Cartoon illustrating the fluid flow and communication/power pathways of the newest generation GeoMICROBE sled. Solid black lines represent fluid flow pathways and dashed red lines the power and communications cabling.

and then past a series of sensors and chemical analyzers. Along this path, secondary pumps pull a portion of the main fluid flow through one of two 25-port manifolds to a fluid collection or filtration device or to an in situ electrochemical analyzer. All of the components are controlled to some extent by the RCM3100 microcontroller by Rabbit Semiconductor that distributes power, synchronizes component functions, and records system activity and data (Supplementary Fig. 1). Since the requirement for a robust mechanism to draw fluids up several hundred meters from upper crust to the seafloor through narrow bore tubing is arguably the most dramatic challenge of the overall system, the technical description starts with the pumping system, followed by discussions of the primary valve system, fluid sampling and processing modules, sensors, overall plumbing, computer controller, power and power management, and software. Full mechanical and electronic shop drawings are included in the Supplemental Material (Supplementary Figs. 1–8).

2.1. Primary pumping system

The 9.5 in. inside diameters of the original CORKs offered little drag at modest fluid flow rates, hence the unassisted flow rates of >5 l per minute (L min^{-1}) realized with the BioColumn at borehole 1026B in 1998 (Cowen et al., 2003). However, the use of superior (i.e., stainless steel or inert fluoropolymers) materials for fluid transport from depth necessitated a transition from the large bore low-alloy steel casings to small bore tubing that is attached to and ascends the exterior of the casing (Fig. 1d,e). CORKs installed on the flanks of the JFR in 2004 (IODP Exp 301: 1026B, 1301A, 1301B) are equipped with 0.125″ (0.32 cm) I.D. tubing. This represents a tremendous increase in drag for similar flows. According to the Hazen-Williams equation for pressure drop (P_D, in 'psi/ft'), or drag (Brater et al., 1996):

$$P_D = 4.52 * f^{1.85}/(r^{1.85} * d^{4.86}), \tag{1}$$

at relatively low temperatures (15–20 °C) and under constant flow rate (f) and surface roughness (r), a decrease in diameter (d) from a ~9.5″ ID casing to a 0.5″ ID tubing results in an increase in drag of over 10^6 times, or over 10^9 times for a 0.125″ ID tubing (Supplementary Fig. 9). This relationship is fairly accurate at low temperatures ($<\sim20$ °C), as observed for FDL exit temperatures at 1301A and expected for all new boreholes at North Pond. At higher temperatures the pressure drop will still be an inverse function of the tubing diameter, but somewhat less extreme. Regardless, the small bore FDL will still express passive flow for overpressured boreholes, but at greatly reduced rates relative to that through open casings. The smallest bore fluid lines offer some advantages for OsmoSamplers (Jannasch et al., 2004; Wheat et al., 2011), which require only very slow flow rates for very small daily collection volumes (<1 ml d^{-1}). However, many chemical and microbiological parameters and experiments require substantially larger volumes of fluids retrievable over minutes to hours, rather than months to years. Consequently, positive displacement pumps have been incorporated to overcome the drag imposed by the narrow tubing and to draw fluids from crust depths at rates sufficient to deliver tens of liters of fluid within one to several hours. Ultimately, a compromise must be reached between fluid line diameter, flow rate and power consumption. A change in FDL diameter from 0.375″ to 0.125″ ID results in a ~208 time increase in power consumption (see Supplementary Document 1).

Pumping fluid with a viscosity similar to that of seawater at a flow rate of ~5 L min^{-1} through 400 m of tubing generates a pressure head of about 50 psi. To engage a pump at such rates for ten's of hours over a one-year autonomous deployment with limited battery capacity requires efficient pumping. An off-the-shelf full ocean depth pump, capable of efficiently pumping at 5 L min^{-1} with a pressure drop of 50 psi and inert (i.e., PTFE, PVDF, or Ti) wetted parts does not exist. Consequently, a Micropump positive displacement pump head (Model IDEX #GL–H21.FFT.E), custom made with PTFE and Ti wetted parts, was magnetically joined to a Pittman (Model 119007) brushless DC motor with ultra high power density (1 hp peak in a 2.5″ diameter package) (Supplementary Fig. 2 and 10a,b). A custom pressure balanced oil filled housing and motor to pump head linkage were designed. While the electric motor is housed in an oil-compensated housing, the magnetic coupling eliminates the need for a shaft seal and the inefficiency due to shaft seal friction and isolates the flow path from contamination from both motor components and oil. The result is a powerful, compact, efficient, DC powered, inert flow path, full ocean depth pump.

2.2. Valve system

The GeoMICROBE's front-end manifold valve system (3-to-1 valve) is designed to connect from one to three CORK fluid delivery lines to the instrument sled's single primary pump and primary flow path (Fig. 2; Supplementary Fig. 3 and 10a). The GeoMICROBE sled currently deployed at 1362A does not require such a front-end valve system because the JFR flank CORKs (e.g., 1301A, 1362A, and 1362B) have only a single microbiological fluid delivery line. However, this sled does operate a 3-to-1 valve system of identical design for managing the calibration and rinsing procedures on the GeoMICROBE's ISEA electro-chemistry module (see 2.4. 'Sensors' and Supplementary Document 2). Third generation primary 3-to-1 valve systems are installed on two of three new GeoMICROBE sleds fabricated for deployment at the North Pond site, with modifications to further improve flow rate and system compactness (Supplementary Figs. 3 and 10a,c).

2.3. Fluid sampling

The GeoMICROBE's primary fluid sampling and processing system builds upon a modified commercially-available multisampler system manufactured by McLane Research Laboratories. The original GeoMICROBE employs a single 25-port (including one flushing port) manifold (McLane 25P), either a 125 ml min^{-1} or 250 ml min^{-1} pump (McLane MP125 or MP 250) and a custom modified controller stack consisting of an Onset TT8 CPU, McLane auxiliary stepper controller and a 3-phase controller, and AWT-1-00 firmware. The new GeoMICROBEs achieve 50-port capacity by using 2 parallel manifolds and 2 pumps, supported by two controller stacks (see Supplementary Document 3). Each McLane manifold consists of a dual multi-port valve that directs the fluid through 25 user replaceable filters (including one flushing port), or whole/filtrate fluid collectors, for operation in a multipurpose (different filter types; exchange resins; filter treatments), a time-series or combination mode. Our custom software systems allow independent programming of the initial and minimum flow rates, total volume, maximum pumping time and pumping start times for each of the 24 filter positions. The secondary McLane positive displacement pumps are placed downstream from the filters to prevent sample contamination (Fig. 2).

The McLane manifold and pump are plumbed to a custom sample holder array consisting of 4 removable trays (Supplementary Fig. 4 and 11), each tray is capable of holding up to 6 (JFR sled) or 12 (new NP sleds) 'samplers'. The trays are designed both for ease of loading/unloading and for versatility in 'samplers'. 'Samplers' can include any combination of various in-line filters and whole or filtrate fluid collectors. The latter employ the McLane 'RAS' acrylic containers which collect up to 500 ml of fluid into acid-washed plastic (foil-lined for gases) bags; the bags fill with fluids when the manifold sequences to direct fluid flow to a particular bag sampler while simultaneously sequencing to direct the secondary McLane pump to evacuate the fluids surrounding the bag inside the acrylic cylinder. The negative pressure created by the removal of the water around the bag pulls on the bag to open it, sucking in fluids through the bag's intake. The bags themselves are fitted with 2-way stopcock valves and luer-lock fittings, providing both quick connections and a means of closing the bags for ship-board removal. Bags can also be pre-charged with a tracer, preservative, lysis buffer or other solution.

The sample trays also can accommodate a variety of 'pancake' style or cartridge filters. Typically a combination of Steryx or other cartridge filters and 25 mm and 47 mm 'pancake' filter holders are used. McLane sells a 'pancake' style filter assembly that can be used with or without their simple preservation system (Supplementary Document 4; Supplementary Figs. 12 and 13); likewise this filter assembly can be used in series with the bag sampler to both filter and collect the resulting filtrate of up to 500 ml. A series of calibration experiments were conducted to evaluate the limits of sample filtration volume with which the in situ preservation systems can be confidently employed (see Supplementary Document 4).

The fluid line to the sampling manifold and secondary pump interfaces with the main fluid flow plumbing via a simple 'Tee' module (Fig. 2). The effect of the main flow from the primary pump on the measured flow rate through the sampling system (manifold and secondary pump) was measured as a function of increasing primary pump rate (Supplementary Fig. 14). Up to about 2.2 L min^{-1}, the flow rate through the secondary pump is equal to the programmed rate; at >2.2 L min^{-1} the flow through the secondary pump starts to creep higher than the programmed rate. Furthermore, with the secondary pump turned off, there is still positive flow through the sampling system at the higher primary pump flow rates. In practice, this is a non-issue since the primary pump is programmed to pump most vigorously only during the flushing phase and is throttled down to less than 1 L min^{-1} during the sampling phase in order to save power; this is well below the rate that impacts flow through the sampling line.

2.4. Sensors

A number of inline sensors are inserted within the main fluid flow line, down stream of the pump. Typically on the GeoMICROBE, the sensors include fluid flow, temperature, O_2, pH and voltammetric electrodes. By virtue of the GeoMICROBE's open architecture, additional sensors are possible. In each case a simple interface is custom made for each sensor to insure seal integrity and that the active portion of the sensor is placed appropriately within the fluid flow.

2.4.1. Fluid flow sensor

The measured rate of fluid flow provides a key input to calculate proper FDL flushing times prior to sampling and to monitor flow rates during sampling. The GeoMICROBEs employ a high flow rate sensor (Seametrics SPT–50) with a dynamic range of 0.38–37.9 L min^{-1}, where pump rates are anticipated to be greater than 1 L min^{-1} due to large bore (0.5″) FDL and low flow resistance (Supplemental Fig. 15; see also Figs. 2 and 9 and Suppl. Figs. 1 and 5). At lower expected flow rates (e.g., <1 L min^{-1}), a Gem Sensors FT-210 turbine flow sensor is used; it has a dynamic range of $\sim$0.1 to >2.5 L min^{-1}. Both sensors use dependable 'Hall–effect' sensors (Ramsden, 2006), tested to 10,000 psi. A custom pressure-balanced oil-filled housing was designed to protect the hall effect sensor and transition the delicate hall effect conductors to robust off-the-shelf neoprene jacketed cabling (Supplemental Fig. 15). The sensor output pulses interface with the electronics by opening and closing a time sampling window for counting on a 4 MHZ clock with a LS7366R clock counter (Suppl. Fig. 6i). The count during the window time is inversely proportional to flow rate. Thus, even at slow flow rates, the resolution of the actual flow rate is accurate to several decimal places. This circuitry is part of a custom controller within the main controller housing (Suppl. Fig. 6).

2.4.2. Temperature

The GeoMICROBEs employ the SeaBird Electronics temperature probe, model SBE 38, a stable, robust and accurate analog to digital acquisition thermistor. Its measurement range is -5 to $>+35$ °C, ideal for the temperatures of fluids exiting the fluid delivery lines (<45 °C), although inadequate for measuring downhole fluid temperatures at some of the JFR flank CORKs (e.g., $\sim$65 °C); they will also be ideal for the upcoming deployments at North Pond where upper crustal temperatures are expected to be less than 20 °C. The absolute accuracy of the SBE 38 is better than 0.001 °C, its resolution $\sim$0.00025 °C and its response time about 500 milliseconds. The probe end of the titanium pressure casing (rated for 10,500 m) is conveniently threaded, facilitating the designing of a water tight, non-contaminating interface for the SBE 38 that positions the Ti-probe directly within the main fluid flow line. An additional temperature sensor is integrated with the oxygen optrode on the ISEA subsystem.

2.4.3. In situ electrochemistry: voltammetry and pH measurements are made using an in situ electrochemical analyzer (ISEA, AIS, Inc.)

The AIS model ISEA-IV is similar to the ISEA-III (e.g., Glazer and Rouxel 2009), but has some important custom advancements and modifications for deployment on GeoMICROBE sleds. In addition to containing a potentiostat for running voltammetry, it has the updated capability for acquiring pH and optical oxygen sensor data (see optode description below). Details for in situ solid-state Au/Hg voltammetric electrodes can be found elsewhere; briefly, a 100-micron Au wire is sealed in PEEK tubing using epoxy, polished, electroplated with an Hg film, and calibrated, allowing for simultaneous measurement of a variety of analytes (O_2, Mn^{2+}, Fe^{2+}, H_2S/HS^-, $S_2O_3^{2-}$, $S_4O_6^{2-}$, S^0, and aqueous FeS and Fe(III) clusters, e.g., Luther et al., 2008 for recent review). pH is measured using a commercially-available pressure-compensated pH electrode (AIS, Inc.) For programming on-deck, (or direct connection to cabled observatories) the ISEA–IV communicates via Ethernet connection. Preprogrammed sequences running voltammetric methods (linear sweep, cyclic, and square wave) within the ISEA are coordinated by the GeoMICROBE's RCM3100 microcontroller via RS–232 communication. A key advancement in the ISEA-IV is the capability to power completely off between sampling time points, thus conserving GeoMICROBE system power during longer-term deployments. Sample fluids, deionized rinse water, or Mn^{2+} standard are delivered to the voltammetric flowcell via a Tee'd connection on the primary plumbing line and a 3-to-1 solenoid valve system and minipump (Seabird SBE–5T) controlled by the RCM3100 microcontroller (see Supplemental Document 2 for more details). Optimization of techniques for extending the longevity of individual solid-state voltammetric electrodes are on-going. Frequent rinsing and storage in deionized water between measurements allows electrodes to last for months in laboratory trials, hence the incorporation of the 3-to-1 solenoid valve system to allow for electrode rinsing between sampling and standardization time points. Using current electrode construction techniques, electrode lifetime seems to be constrained to within $\sim$10,000 scans, and ISEA measurement intervals are configured depending upon targeted length of deployment (Supplemental Table 1).

2.4.4. Oxygen optode sensor

Oxygen Optodes 4330F (Aanderaa Data Instruments) provide temperature compensated absolute O_2 concentration with a measurement range of 0–500 μM (0–150% saturation), resolution of <1 μM ($<0.4\%$), and accuracy of <8 μM ($<5\%$). Serial RS–232 output from the Optode is logged by the ISEA (above) internal memory. The Optrode interfaces with the GeoMICROBE's primary fluid flow via a custom flowcell.

2.5. Plumbing

The GeoMICROBE plumbing (Fig. 2) is designed to be modular, versatile and adaptable to the addition or replacement of sensors and sampling units. Other design criteria include robust components, careful attention to materials used for wetted surfaces, minimization of restrictions to fluid flow, ease of maintenance and leak-proof, high integrity fittings and seals. The GeoMICROBE is connected to the CORK with one to three ~3- to 4-m-long umbilicals (1/2 in. SuperFlex tubing with PVDF inner tubing armored with stainless steel followed by a tough plastic exterior sheathing), fitted with an appropriate connector. The modified 'Aeroquip-like' (Wheat et al., 2011) connectors used on CORKs installed prior to IODP Exp 327 can ultimately form strong, high integrity connections, but are prone to operational difficulties in the field. Starting with IODP Exp 327 (Juan de Fuca Ridge flanks, boreholes U1362A and U1362B), a new connector was developed to minimize connection problems (Wheat et al., 2011); their advantages include greater ease of connection and positive visual confirmation that the connector is properly locked in place. Custom designed robust, high integrity 1/2 inch-bore titanium swivels add a second degree of freedom (rotation) and thus help greatly to mitigate the effect of the SuperFlex's relative stiffness on submersible pilots' efforts to connect the sleds to the CORK.

The FDLs consist of nearly continuous ETFE tubing from upper crust to wellhead, with only a few permanent tight titanium compression-fitting connections. The use of 'clean' Teflon/titanium pump heads magnetically coupled to their motor drives allows the pump to draw fluids up the CORK's fluid lines, then push through or past inline sensors and samplers downstream of the pump. Thus any unlikely small leaks downstream of the pump could result in the loss of (overpressured) fluids, but unlikely lead to contamination. The main pump then directs flow past system sensors and secondary fluid sampling ports. The overall main flow path is kept as linear and unobstructed as possible to minimize drag. The sample flow path materials are comprised of PVDF, ETFE and titanium with short PFA components, all microbiologically and chemically essentially inert. Gas permeability of the composite plumbing pathway is low but finite; for example, permeation of O_2 into anoxic crustal fluids transiting a 300 m Tefzel (ETFE) FDL and GeoMICROBE plumbing (predominantly PVDF) should amount to less than ~1% deep water O_2 concentrations of ~80 μM) (see Suppl. Docs). However, quantitative gas measurements rely on the use of stainless steel FDL and the PVDF plumbing of the MPS-fluid-trap (see Section 3. Mobile System).

Each sensor generally necessitates fabrication of a custom adapter (i.e., insert module) that properly positions each sensor in the flow while securely sealing the assembly against fluid leaks. The secondary pumps associated with the fluid sampling and electrochemical modules must have a composite pumping rate less than that of the main pump at all times, or alternate in sampling times. Where possible individual modules within the flow path are connected with quick connectors to facilitate installation and servicing.

2.6. Computer controller

The central controller electronics provides integrated communication control and power distribution over all GeoMICROBE components (Fig. 2; Supplemental Fig. 6). In addition to the science instruments, it controls all of the system platform fluid paths, valves, and pumps. It synchronizes the timing of all instruments, and controls, records, and maintains the performance status of the platform (Supplemental Fig. 1). It also manages the power usage as constrained by its autonomous battery operation.

2.6.1. Microcontroller

The heart of the system is the RCM3100 microcontroller by Rabbit Semiconductor. Software is developed in C programming language, Dynamic C version 8.10 by ZWorld, Inc., which is specifically designed for interfacing to instrumentation. System diagnostic data, flow rate, temperature, and McLane sampler data are stored in the microcontroller's flash memory. All commands and sequences are synchronized by the RCM3100 microcontroller and every command and all status data are logged with a date and time status. The controller can be pre-programmed to operate autonomously as a deployed platform, or operated interactively, command by command by an operator.

2.6.2. Flash memory

For the 2010 and 2011 deployments, the RCM3100 microcontroller was interfaced to 64 Mbytes of flash memory. For future deployments the RCM3100 microcontroller will be interfaced to two sets of 64 Mbytes of flash memory (primary and redundancy). This memory can store all necessary data and permanently maintains the data while the system is powered off.

2.6.3. Timer controller (power consumption)

Power consumption is a crucial issue for the system. In a long-term deployment with large amounts of inactive time, reducing power consumption and extending battery life is essential during the "off" time. The DS1501 timer-controller, by Dallas Semiconductor, performs this function. It powers down the entire system including the RCM3100 microcontroller, effectively reducing the power consumption during that time to less than 1 microwatt, including the DS1501 itself. During the operational periods of the system, it is used to control the logic that switches on full power, up to hundreds of watts.

2.6.4. Timer controller (timing)

Timing and synchronization of instrumentation is also critical, as data sampling must be measured in tandem with required pumping and fluid flow and sampling. The DS1501 uses an external crystal (ABS25-3.768KHz-T by Abracom Corporation). It is rated better than ± 20 ppm or 1 part in 50,000. For a year of 31,536,000 s that is a possible error of ± 630.72 s in one year, or 10.512 min of possible error in one year. Since all instruments and the RCM3100 microcontroller are synchronized to the DS1501 internal clock, relative timing/ synchronization is not an issue.

2.6.5. Communication

The RCM3100 microcontroller communicates with isolated RS422 communication to submersibles and with non-isolated RS232 communication to two McLane water samplers and the ISEA. Circuitry for the isolated RS422 was specifically designed to have total ground isolation from the submersibles.

2.6.6. Valves

The valves are operated by solenoids and generally are power-consuming devices. Specialized circuitry was designed to reduce the power consumed by about 60%. This is done with a PWM (pulse width modulation) circuit that provides the full power necessary to engage the solenoid, then reduce the power to a minimal level to maintain the solenoid until turned off. There are up to 6 of these circuits, three for the primary 3-to-1 valve system associated with the primary pump and multiple fluid lines and three for the 3-to-1 valve system associated with the ISEA.

2.6.7. Flow rates, fluid volume, and pump speed (instantaneous)

Measuring the flow rate of the fluid is essential to evaluate the performance of the system during FDL flushing and sampling. Also, the speed (revolutions per minute or RPM) of the fluid pump-motor is measured to correlate the pump speed with the flow rate of the fluid through the system (Supplemental Document 5).

2.6.8. Pump speed control

The primary pump speed is controlled by a 0 to 5 VDC (volts direct current) input to the Ametek motor controller. The RCM3100 microcontroller controls a digital-to-analog converter that produces this voltage. The voltage is adjusted up or down to maintain the desired rate by either one of two feed back control methods. The primary method measures the fluid flow rate through the flow sensor using the circuit described above and then adjusts the drive voltage to the motor controller until the pump-motor speed produces the desired flow rate in the flow sensor. The second, less desirable, method is to measure the pump RPM's (as described in Supplemental Document 5) and then adjust the drive voltage to the motor controller until the desired pump-motor speed is achieved. The latter method is less accurate in determining actual fluid flow rates as it cannot take into account factors affecting flow rate, especially the back-pressure (i.e., drag).

2.6.9. System operation status

The status of the system is monitored and recorded primarily through engineering data. These data are under the control of the RCM3100 microcontroller through a 12 bit analog-to-digital converter. Status of battery voltages, pressure, temperature, ISEA, and McLane component voltages, primary pump voltage and current, pressure gage and several temperature sensors (monitoring the Ametec motor controller, the Vicor heat mounting plate and the internal temperature of the controller housing are continually logged and recorded). Also, digital input and output lines are used to turn on/off instruments and devices.

2.6.10. Filtered power

Filtered power is supplied to the two McLane manifolds and to the ISEA electrochemical system.

2.6.11. System power and power distribution

The autonomous GeoMICROBE system is powered by Deep Sea Power and Light (DSPL) 24 V, 40 Amp batteries. The major power draws are the primary pump and the ISEA electrochemistry system, each roughly a third of the total power requirement over a typical long-term deployment. The number of batteries is adjustable as necessary for over all power requirements and time of deployment. However, not all of the instruments, or the primary pump motor, operate on 24 V, so various power supplies are needed for power distribution. The ISEA operates on 12 V. The pump motor can operate over a range of power voltages (24–60 VDC), although its efficiency improves at higher voltages. A 24-volt DC/DC converter has been used in series with the 24 V battery voltage to step the motor voltage to 48 V; up to 400 W can be provided to the pump motor. The RCM3100 micro controller controls all the voltages and switches ON/OFF all instruments and the primary pump motor. For the RCM3100 microcontroller and other logic, the power supplies are 5.0 VDC and 3.3VDC; the analog circuitry operates on ± 12 VDC.

2.6.12. Electric current

The system monitors the pump motor current using a hall sensor (F.W. Bell: NT–15). This is critical in order to determine the optimum efficiency for pumping fluids and extending operational time and battery life. By monitoring the voltage and current to the pump motor, and correlating that with the flow rate of the fluid through the flow sensor, optimization of power consumption can be performed. This is a nonlinear function of a number of factors (e.g., back pressure, tubing diameter), but as additional empirical data are accumulated, algorithms will be developed to automatically optimize the system.

2.6.13. Software

The software consist of two basic parts: (1) embedded software in the GeoMICROBE's RCM3100 micro controller and (2) user interface command/control and data monitoring software on a Microsoft Windows laptop or desktop computer. Both are written in C code. The RCM has a development environment specifically designed to program it, and to monitor and debug the code, Dynamic C8.10 by ZWorld Inc. The GUI (graphic user interface) user software is Borland C^{++} Builder Professional, version 6.0, by Borland Software Corporation.

The operator uses only the GUI software, which sends all commands and receives status and data back from the RCM. The only communication path is over a single RS-422 serial port at 57K baud. All commands to the RCM are text based. For example "VALVE 4 1" which would turn valve #4 on, or "VALVE 3 0" which would turn valve #3 off. At this time, 89 various commands can be sent to the RCM. These are subdivided into 22 specific to the McLane commands, 24 specific to the ISEA commands, and 43 specific RCM commands. Along with the capability to send commands to or through the RCM, for autonomous deployments the RCM can be programmed to process a batch of user commands in script files. These are time based, interval paced, commands designed to execute in coordinated sequences for the 2 McLanes, the ISEA, the valves, pumps, etc. In the non-autonomous mode, the GUI display shows a real time status of the system of valves activated, pump speed, flow rates, and volume pumped, and includes the McLane and ISEA operational status.

3. Mobile pumping system

The Mobile Pumping System (MPS) is a modified version of the GeoMICROBE sled, intended for transport directly on the working basket of the remotely (ROV) or human (HOV) operated vehicle for real-time, short-duration sampling (Fig. 3a,b; Supplemental Fig. 1). The MPS consists of the primary pump, GeoMICROBE electronics including the RCM3100 microcontroller, flow sensor (Gem Sensor FT-210) and other selected sensors (e.g., temperature, O_2, and ISEA) as described above for the GeoMICROBE (Fig. 93a). The MPS has been particularly effective at testing the deliverability of new CORK fluid delivery lines (FDL), for flushing working FDLs and providing a source of large volumes of pristine upper crustal fluids. In contrast to the internal battery supplies of the GeoMICROBE, the MPS controller converts the 'unlimited' power from the host submersible into the appropriate voltages. The MPS is ultimately controlled in real time from an operator's laptop within the HOV or ROV's control van using RS-422

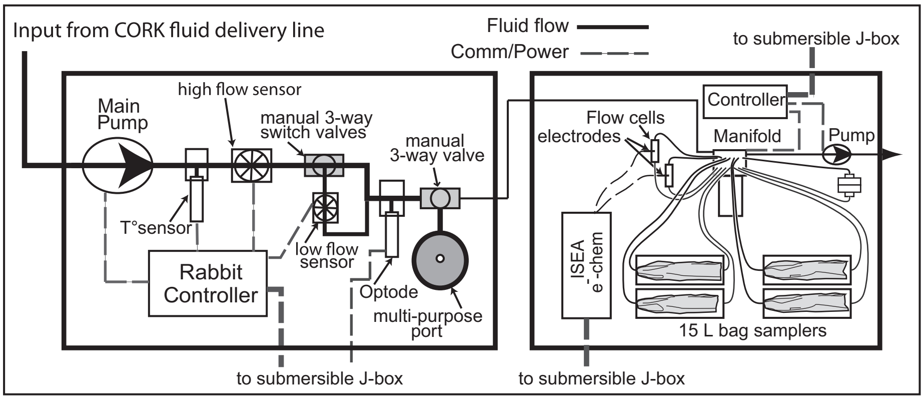

Fig. 3. Flow diagram of Mobile Pump System (MPS) coupled to a Medium Volume Bag Sample system (see text). MPS can also be coupled to multiple Large Volume Bag Samplers or other samplers via the multi-purpose port.

communications. The MPS components reside within a milk-crate, typically secured to the starboard side of the ROV/HOV forward science basket, with a footprint of $\sim$0.6 m $\times$ 0.4 m. The MPS, Superflex umbilical and connector have a combined wet-weight of $\sim$36 lbs (16.4 kg) and air weight of 83 lbs (37.7 kg).

The MPS can direct its fluid flow into a common port that can accommodate a high-aspect ratio funnel-shaped sampling port extension or a fluid-trap that accumulates several liters of constantly refreshed crustal fluids. Both the funnel port and fluid-trap allow the use of a large variety of conventional seafloor samplers (e.g., Ti-majors, Gas-Tights, syringe samplers). The fluid-trap is used for samplers with fluid intake rates that could be greater than the pump rate (e.g., Gas-Tight samplers).

Alternatively, the entire fluid flow from the MPS can be directed into expanding acid-clean bags of 50 l Large Volume Bag Samplers (LVBS) (modified after Wommack et al. (2004)). Each LVBS is capable of holding up to 60 l, a single LVBS can be carried on the submersible or ROV basket, with the MPS or several LVBS can be connected together on an elevator (Fig. 3c). Each LVBS weighs only 6 lbs (2.7 kg) in water, but has an air weight of 136 lbs (62 kg) when full of seawater.

A newer MVBS (Medium Volume Bag Sampler) system integrates the MPS with a McLane 24-port manifold/pump/controller system that distributes fluid to six independent 15-L bag samplers, multiple in-line filters and electrochemical flow cells (Fig. 3b). The McLane system draws fluid from the MPS and directs it sequentially to one of several 15-L MVBS samplers or filters for in situ filtration. PVDF (polyvinyl difluoride) bags (Sampling Bag Technologies, Inc.) with PVDF 3-way valves are used for most chemical and molecular biology parameters with either the LVBS or MVBS; Al-foil, HDPE- (high density polyethylene) lined bags (Jensen Inert Products) are used for gas or redox-sensitive sample collections.

Crustal fluid collected for metabolic or culture experiments should use the inert Tefzel (ETFE) bio-FDLs coupled to the MPS-McLane-MVBS system to collect samples into the foil-lined bags (see Supplemental Document 6). However, for absolute gas concentration measurements, the MPS should be coupled to a CORK stainless steel FDL and the fluid samples collected with a Gas-Tight sampler via the 'fluid-trap' inserted into the MPS's common port.

4. Pre-deployment system cleaning

Pre-deployment cleaning procedures are similar for both the GeoMICROBE and the MPS. Once system components are assembled the entire plumbing path is subjected to a sequence of flushing and overnight soaks with soapy (Alconox) water, tap water, 5% hydrochloric acid in deionized water and deionized, doubly filtered (0.2 um) Milli-Q water. Individual bag or filter holders are cleaned according to investigator (parameter)-specific requirements. Generally, our bags are acid-cleaned and well flushed with Milli-Q water; then, following drying, the bags are double-bagged and heat-sealed prior to sterilization by gamma irradiation. Depending on anticipated usage, the bags may be flushed with inert high purity N_2 gas. Bags can also be pre-deployment inoculated with tracers or preservatives.

5. Results and discussion

5.1. GeoMICROBE

The GeoMICROBE was deployed for a12-hour period during summer 2010 and for a year-long deployment from August 2010 to July 2011 at the U1301A CORK prior to being turned-around for a one-year re-deployment (July 2011 to July 2012) at the U1362A CORK. Preliminary results from both of the U1301A deployments indicate that the system as a whole performed remarkably well (Table 1). The RCM3100 microcontroller and software performed as intended in coordinating the sequence in which pumps and sensors were powered up or down; especially notable was the flawless synchrony of the primary pump with the McLane manifold port selection, the powering up and down of the secondary McLane fluid sampling pumps and the proper timing of the electrochemical analyzer (ISEA) components (Fig. 4a,b; Tables 1 and 2). For example, the effective communication between components and the central RCM3100 microcontroller is demonstrated by the consistent rapid transition to the successive port operation upon the premature completion of a previous port's operation during the short-term deployment (Fig. 4); this necessitates the central RCM3100 microcontroller be automatically alerted that one operation has finished early and then to command the system to proceed immediately to the next operation, whether to a system-wide rest or for the subsequent sampling operation.

The variability in some of the sensor data (e.g., temperature, O_2, pH) indicates an imperfect seal between the CORK and the umbilicum 'Aeroquip' connector. An imperfect seal is verified by the physical and chemical data from the in-line sensors and the fluids collected during the 12 h time-series deployment (Fig. 4, or Table 2). Generally, samples collected early and late in this deployment were contaminated by bottom seawater (e.g., high Mg^{+2}, Table 2; low temperature and high O_2, Fig. 4a,c); whereas the fluids collected via ports 15 and 16 were excellent high integrity crustal fluid samples, as confirmed by the chemical

Table 1
Performance summary for short-term GeoMICROBE deployment at borehole CORK U1301A.

Date	Time (hr:mm)	Battery (V)	T (°C)	Pump Speed (RPM)	Current (A)	Volume (mL)	Port
6/23/2010	13:47	24.2	8.2	375.5	0.4	189	1-GFF/POC
6/23/2010	13:49	24.2	7.6	377.1	0.6	7808	2-PC/TEM
6/23/2010	16:16	24.1	8.4	314.0	0.5	250	3-PC/SEM
6/23/2010	16:20	24.1	8.3	314.0	0.5	189	4-bag/non-filter
6/23/2010	16:22	24.1	8.7	314.0	0.5	190	5-bag/GFF
6/23/2010	16:24	24.1	(not programmed properly)			0	6–0.22 um Cartriage
6/23/2010	16:28	24.0	7.7	470.0	0.9	189	7-GFF/POC
6/23/2010	16:30	24.0	8.1	470.1	0.7	9481	8-PC/TEM
6/23/2010	19:28	24.0	8.7	470.0	0.7	250	9-PC/SEM
6/23/2010	19:33	24.0	8.4	470.0	0.7	189	10-bag/non-filter
6/23/2010	19:35	24.0	7.4	470.5	0.7	189	11-bag/GFF
6/23/2010	19:37	24.0	(not programmed properly)			0	12–0.22 um Cartriage
6/23/2010	19:41	24.0	8.7	401.0	0.6	189	13-GFF/POC
6/23/2010	19:43	24.0	8.5	503.1	0.9	3475	14-PC/TEM
6/23/2010	20:47	22.6	22.7	1795.6	7.2	250	15-PC/SEM
6/23/2010	20:52	22.7	24.9	1836.5	6.9	189	16-bag/non-filter
6/23/2010	20:54	22.7	25.0	1841.0	6.9	189	17-bag/filter
6/23/2010	20:56	22.7	(not programmed properly)			0	18–0.22 um Cartriage
6/23/2010	21:19	24.0	10.6	472.5	0.3	189	19-GFF/POC
6/23/2010	21:21	24.0	8.4	383.0	0.4	6223	20-PC/TEM
6/23/2010	23:18	23.8	7.9	332.0	0.5	250	21-PC/SEM
6/23/2010	23:22	23.8	8.5	329.7	0.5	189	22-bag/non-filter
6/23/2010	23:25	23.8	8.8	328.5	0.5	189	23-bag/GFF
6/23/2010	23:27	23.8	(not programmed properly)			0	24–0.22 um Catriage

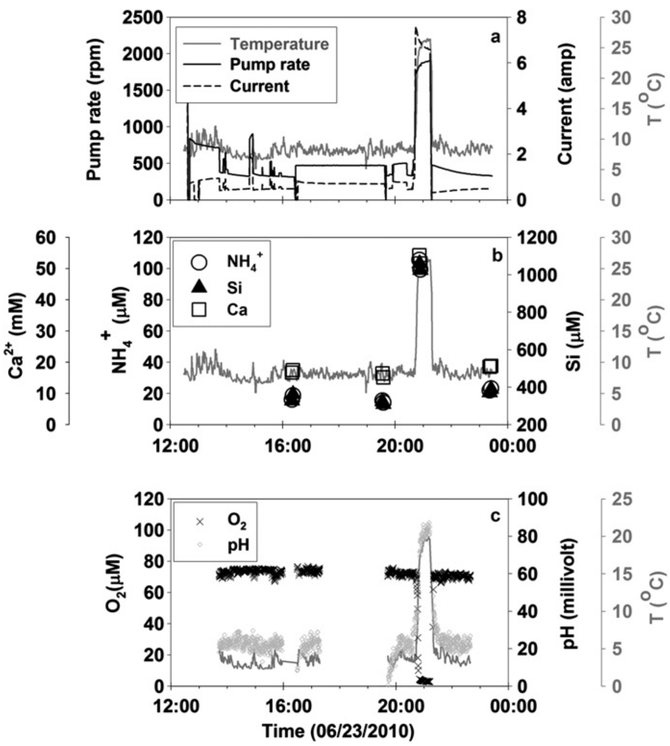

Fig. 4. Selected results of GeoMICROBE 12 h deployment data: (a) Time-series data for temperature (SBE11) of fluids down-stream of primary pump, primary pump rate, and current draw for overall system; (b) Time-series data for Ca^{2+}, Si and NH_4^+ concentrations in discrete whole fluid samples collected by GeoMICROBE's multi-sampling system superimposed on the SBE11 continuous temperature record; (c) Time series data for dissolved oxygen (Optode), pH and temperature.

composition (e.g., low Mg^{++}; Table 2, Fig. 4b; low O_2, Fig. 4c) and the constant elevated temperatures (SBE sensor; Fig. 4a) recorded during their collection. The temperatures measured downstream of the primary pump averaged 22–25 °C during this period, comparable to the temperatures observed during collection of the other pristine crustal fluid samples at U1301A using the MPS.

This fluctuation behavior was noted with several of the other stainless steel connectors following this deployment, including the new connector used for the year-long deployment (Fig. 5). However, the recently modified Ti connector (Wheat et al., 2011) achieves an excellent seal and consistently exhibits a very steady rise in temperature to a constant plateau of >23 °C coupled to high integrity basement fluid collections. All subsequent GeoMICROBE deployments will employ modified 'Aeroquip-type' connectors (see Wheat et al., 2011), or the new style connectors introduced with new L-CORK deployments during IODP Expedition 327 and subsequent CORK deployments (Wheat et al., 2011). For example, the GeoMICROBE deployed in July 2011 for a year-long period at borehle CORK U1362A uses the new style connector.

Whereas the SBE temperature probe, Optode temperature and O_2 sensors, pH sensor, pump motor speed sensor, and current recorder worked well (Fig. 4a–c), the (previous generation) flow sensor failed on both the GeoMICROBE. The failure occurred because the small reservoir of the sensor's pressure-compensated oil-filled housing was not adequate to prevent seawater from circumventing the neoprene jacketed, fibrous filled cables that powered the flow sensors. The new flow sensor, described earlier, employing Seacon bulkhead connectors to protect sensors from pressure-induced water intrusion, performed brilliantly during 2011 MPS operations (Figs. 6 and 7).

As noted, the fluid sampler and in situ filtration system sequenced properly during the short-term deployment. The sampling pump started and drew fluids past filters and/or into sample collection bags as commanded until either the programmed volume or minimum pump rate was reached. The only exceptions were the 4 ports loaded with MilliQ 0.22 μm cartridges (nos. 6, 12, 18, and 24; Table 1) whose pumps failed to turn on due to an operator programming error; this issue was recognized immediately and fixed prior to the GeoMICROBE's 1-year redeployment. In contrast, the 0.2 μm polycarbonate filters for TEM (ports 2, 3, 14, 20; Table 1) continued to filter until the minimum programmed pump rate (50 ml min^{-1}) was reached, having filtered from 3.4 to 9.5 L in 58 to 178 min. The filters all were visually discolored with abundant particle loading for further processing for TEM. Pumping at the ports loaded with

Table 2
Time-series sample chemistry data for short-term GeoMICROBE deployment.

2010 short term						Cl⁻ corrected										
Date	Time	Port #	Filter	Cl^- (mM)[1]	Cl^- (mM)*	Mg^{2+} (mM)[2]	Fractional purity	Ca^{2+} (mM)[2]	NO_3^- (µM)[3]	NH_4^+ (µM)[4]	SO_4^{2-} (mM)[1]	Na^+ (mM)[1]	K^+ (mM)[1]	Alkalinity (meq/L)[2]	Si (µM)[3]	PO_4^- (µM)
6/23/2010	16:20	4	None	500	552	47.5	12%	16.7	30.8	16	27.3	478	9.7	2.23	328	2.3
6/23/2010	16:22	5	GFF	464	552	46.9	13%	17.4	30.0	19	27.3	480	9.8	2.18	362	2.6
6/23/2010	19:33	10	None	485	552	47.8	11%	16.4	31.4	16	27.5	479	9.8	2.26	325	2.7
6/23/2010	19:35	11	GFF	492	552	49.1	9%	15.1	31.7	14	27.7	479	10.0	2.20	309	2.6
6/23/2010	20:52	16	None	501	552	4.8	94%	54.2	0.3	105	18.0	471	5.9	0.53	1059	0.2
6/23/2010	20:54	17	GFF	516	552	6.8	91%	51.5	1.5	99	18.6	469	6.2	0.58	1023	0.2
6/23/2010	23:22	22	None	491	552	45.0	17%	18.5	28.5	22	26.8	478	9.6	2.05	371	2.4
6/23/2010	23:25	23	GFF	486	552	44.7	17%	18.8	29.1	23	26.9	476	9.5	2.04	391	2.2

* All chemical compositional data presented were corrected for prime DI dilution based identical Cl^- concentrations in basement fluid and bottom seawater (552 mM).
Analytical methods:
[1] Ion chromatography.
[2] Titration.
[3] Colorimetry.
[4] Fluorescence.

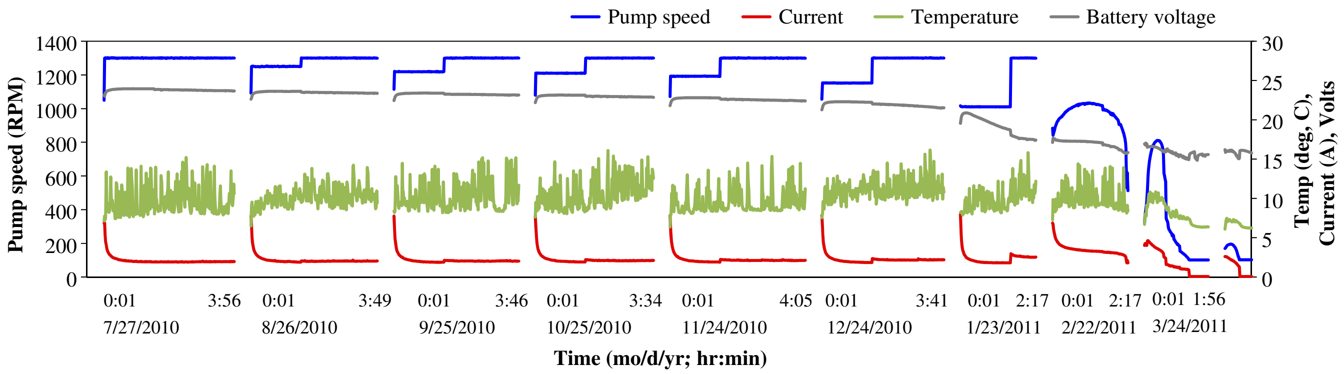

Fig. 5. Selected results of GeoMICROBE 12 month deployment data. Time-series data for temperature (SBE11) of fluids down-stream of primary pump, primary pump speed, battery voltage and current draw for overall system. Note that time axis is discontinuous, with the month long 'rest' periods removed.

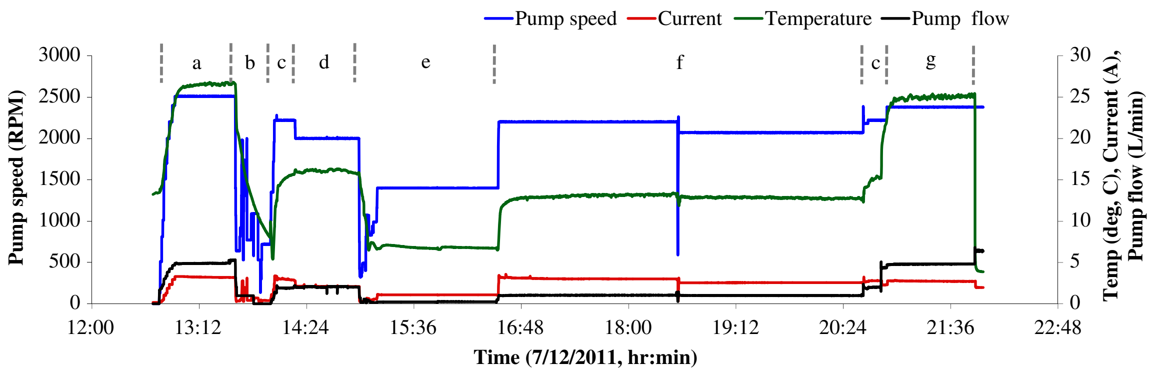

Fig. 6. Selected system status data for Mobile Pumping System while connected to 0.5 in. ETFE fluid delivery line of CORK at U1362A. Parameters shown include primary pump motor speed (RPM), system current, temperature (SBE probe), and pump fluid flow (flow sensor). Letters at top of figure refer to time spent flushing (a), a temporary problem with a mechanical valve (b), flow diverted to electrochemical flow cell (c), filling all six 15-liter bags (d), sequential filtration through two 25 mm diameter GFF filters (e), sequential filtration through two Steripac filters (f), flow diverted to the common port with fluid trap for Ti-Major fluid collections.

0.2 µm polycarbonate filters intended for SEM analyses (ports 3, 9, 15, and 21) proceeded until the programmed volume (250 ml) was reached in each case. At the ports intended for large volume POC filtration using glass fiber filters (GFF) (ports 1, 7, 13, and 19) the pumps started per command, but prematurely slowed to their programmed minimum pump rates, resulting in only 189 ml being filtered in each case instead of the expected >10 L. The ports that led to 500 ml bag samplers (ports 4.5 10, 11, 16, 17, 22, and 23), with or without GFF filters, also collected 189 ml of fluid in each bag, the pumps having stopped when their pump rates also prematurely slowed to the programmed minimum rates. The premature slowing of the pumping rate occurred only for those ports that were programmed to start at 125 ml min^{-1} with minimum pump rates of 75 ml min^{-1}; ports with programmed start and minimum rates of 80 ml min^{-1} and 50 ml min^{-1}, respectively, were not similarly affected. Subsequent bench tests revealed that the root cause was sensitivity to the viscosity of oil used in the oil compensated housings; a lightweight mineral oil had to be used instead of the

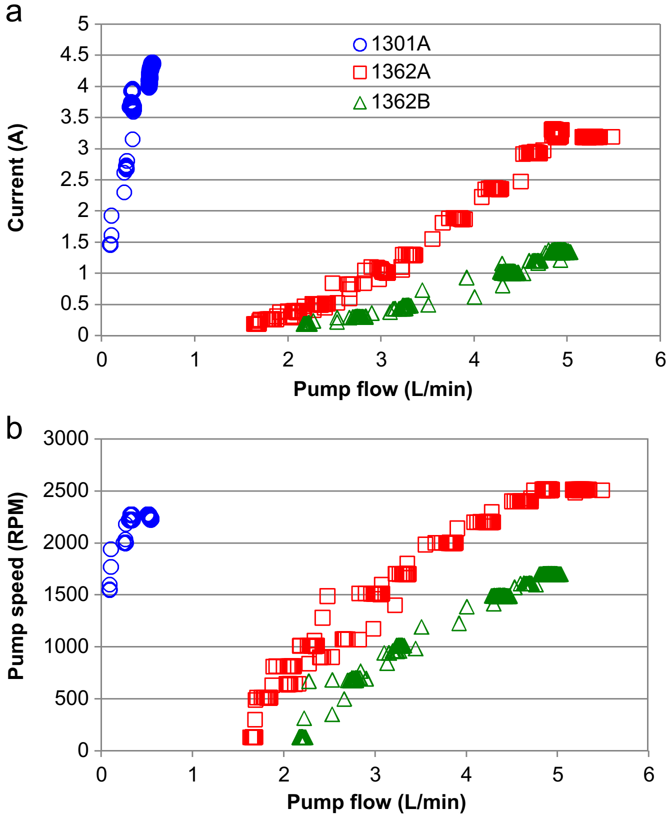

Fig. 7. Effect of fluid delivery line diameter and length on system current demand (a) and primary pump speed (b) versus pump fluid flow for the Mobile Pumping System during flushing phase, with unimpeded flow to open common port. Shown are data for CORKs at U1301A (0.125 in. ID, 280 m; open circles), U1362A (0.5 in. ID, 440 m; open squares) and U1362B (0.5 in. ID, 280 m; open triangles).

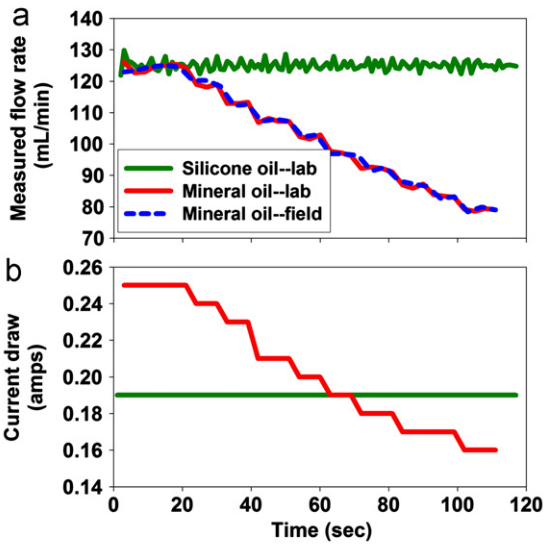

Fig. 8. (a) Effect of pressure housing oil type on secondary (McLane) pump rate versus time. Red and blue lines represent pump results using mineral oil in laboratory bench test and short-term field deployment of the GeoMICROBE, respectively; the green line represents results of a bench test using low viscosity silicon oil. (b) Comparison of current draw (amps) versus time for the same bench tests in 'A'.

even lighter (low viscosity) silicone oil due to the long-lead time for the latter. Further bench tests, using the same programmed initial and minimum flow rates (125 ml min^{-1} and 75 ml min^{-1}, respectively), showed that the pump immersed in the mineral oil slowed down almost immediately, identically to its behavior during the short-term deployment, whereas, when immersed in the silicon oil the pump continued to operate at the initial higher flow rate for the duration of the experiment (Fig. 8a). The higher resistance of the more viscous mineral oil drew a higher current, as it would with an overloaded in-line filter, causing the firmware to slow the pump rate to protect both the pump and the integrity of fragile filtered particles (Fig. 8b). Use of the silicone oil in the secondary and primary pumps also will conserve power.

Preliminary chemical composition of the fluid samples collected in the 500 ml bags is generally consistent with sensor data. Magnesium is much depleted in crustal fluids from the region of Hole 1301A (Wheat et al., 2000) and its concentration is a useful indicator of the sample integrity (Lin et al., submitted to). Plots of Ca^{++}, K^{+} and SO_4^{-2} versus Mg^{++} show that the samples collected in the 500 ml bags during the GeoMICROBE short-term deployment exhibit differing concentrations of Mg^{++} (Fig. 9). The two most Mg^{++}-depleted samples (4.6 and 6.8 mM) are from ports 16 and 17; the average temperatures measured by the GeoMICROBE's temperature probe during sampling through these ports were 24.9 and 25.0 °C, respectively (Table 1). The other less depleted samples (42.1–46.9 mM Mg^{++}) corresponded to collection temperatures of 7.4–8.7 °C, relative to bottom seawater Mg^{++} concentration of 51.2 mM and temperature of <2 °C. These compromised samples likely leaked at the 'Aeroquip-like' connectors. The fractional purity of the fluids collected by the GeoMICROBE with respect to recent dilution with unreacted seawater is calculated relative to Mg^{++} or Ca^{++} concentrations in end-member bottom seawater and Mg^{++}-depleted formation fluid (Lin et al., submitted to) (Table 2).

Following the recovery of the 12 h deployment at U1301A, the GeoMICROBE was redeployed to the same CORK for 1 yr (August 2010 to July 2011). The software and programming problems experienced with the 12 h deployment were fixed within the 3-day turnaround period; fluid collections and filtrations proceeded as programmed (Table 3). However, since this recovery and redeployment cycle occurred during the same research

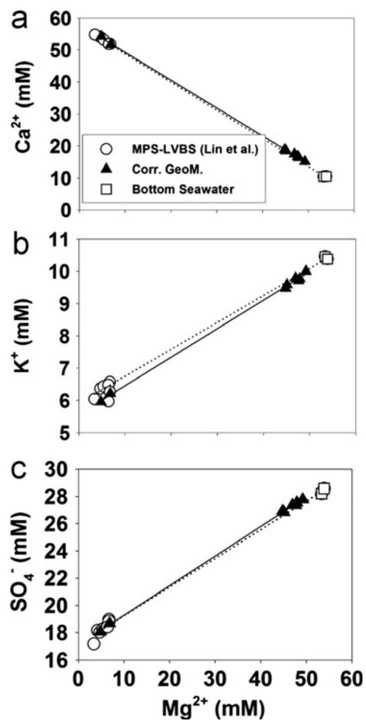

Fig. 9. Plots of the concentrations of Ca^{+2}, K^{+}, and SO_4^{-2} versus Mg^{+2} concentration for 500 ml bag samples collected the GeoMICROBE's mult-sampling system during the short-term deployment at 1301A.

Table 3
Performance summary for year-long GeoMICROBE deployment at borehole CORK U1301A.

Date	Time (hr:mm)	Battery (V)	T (°C)	Pump Speed (RPM)	Current (A)	Volume (mL)	Port
07/27/2010	3:25	23.71	8.28	1301	1.96	500	1-bag/non-filter
08/26/2010	3:25	23.38	11.08	1299	2.04	500	2-bag/non-filter
09/25/2010	3:25	23.15	14.91	1301	2.08	500	3-bag/non-filter
10/25/2010	3:25	22.89	12.85	1301	2.11	500	4-bag/non-filter
11/24/2010	3:25	22.45	11.53	1298	2.18	500	5-bag/non-filter
12/24/2010	3:25	21.59	11.64	1299	2.19	500	6-bag/non-filter
01/23/2010	Program terminated due to low battery						7-bag/non-filter
02/22/2010							8-bag/non-filter
03/24/2010							9-bag/non-filter
04/23/2010							10-bag/non-filter
05/23/2010							11-bag/non-filter
06/22/2010							12-bag/non-filter
07/27/2010	1:44	23.79	12.9	1299	1.97	5228	13-PC/Microsphere
08/26/2010	1:44	23.50	11.03	1301	2.00	5195	14-PC/Microsphere
09/25/2010	1:44	23.24	8.38	1302	2.09	5081	15-PC/Microsphere
10/25/2010	1:44	22.95	11.62	1300	2.12	5103	16-PC/Microsphere
11/24/2010	1:44	22.61	8.64	1301	2.18	4829	17-PC/Microsphere
12/24/2010	1:44	22.02	10.96	1300	2.23	4717	18-PC/Microsphere
01/23/2010	Program terminated due to low battery						19-PC/Microsphere
02/22/2010							20-PC/Microsphere
03/24/2010							21-PC/Microsphere
04/23/2010							22-PC/Microsphere
05/23/2010							23-PC/Microsphere
06/20/2010							24-PC/Microsphere

cruise, neither a functional flow sensor nor the low viscosity Silicon oil was yet available. Nevertheless, the battery voltage lasted over 6 months (Fig. 5) despite using the more viscous mineral pump oil and the high drag of the small-bore (0.125″ ID) FDL (see data for U1301A in Fig. 7). The primary pump continued to function into the ninth or even the tenth month (Fig. 5), since the Vicor power supply that steps the battery voltage up to 48 V for the primary pump can deal with input voltages lower than 18 V. On the other hand, no fluid samples were collected or filtered after month six because the McLane controller will not allow the McLane subsystem to operate if input voltages drop below 18 V as it did during the flushing step in month 7 (Fig. 5).

The noisy temperature signal throughout the 8 months is a result of the leaky, though previously unused, stainless steel "Aeroquip-style" connector. The leakage is confirmed by the Mg^{++} chemistry that indicates that the fluids collected are 15% crustal fluids. This GeoMICROBE deployment was largely in support of the "Tracer Transport" project (Fisher et al., 2011a); fluid samples and filters will be used to detect fluorescent microspheres and other gas and dissolved chemical tracers injected into the crust via a nearby CORK during the IODP Expedition 327 in August 2010 (Fisher et al., 2011a). These analyses are underway and will be reported separately.

5.2. ISEA subsystem

During the 12-hour GeoMICROBE deployment at IODP 1301A in 2010, the ISEA collected voltammetry (Fig. 10) in addition to pH, temperature, and dissolved O_2 data noted above (Fig. 4c). Data for in situ voltammetric scans (Fig. 10) are in agreement with other GeoMICROBE sensors and analyses from discrete samples (see above), indicating that for much of the short-term deployment the fluids delivered were contaminated with oxygen-rich background bottom seawater, with the exception for about a one hour period at ~21:00. During this time, significantly warmer, oxygen-deplete fluids, of lower pH were sampled, suggestive of high-integrity subsurface formation fluids (Figs. 4 and 10). Also encouraging was the flawless coordination between the RCM3100 microcontroller and ISEA for delivery of sample fluid, Mn^{2+} standard, or DI rinse & storage fluid to the voltammetric flowcell.

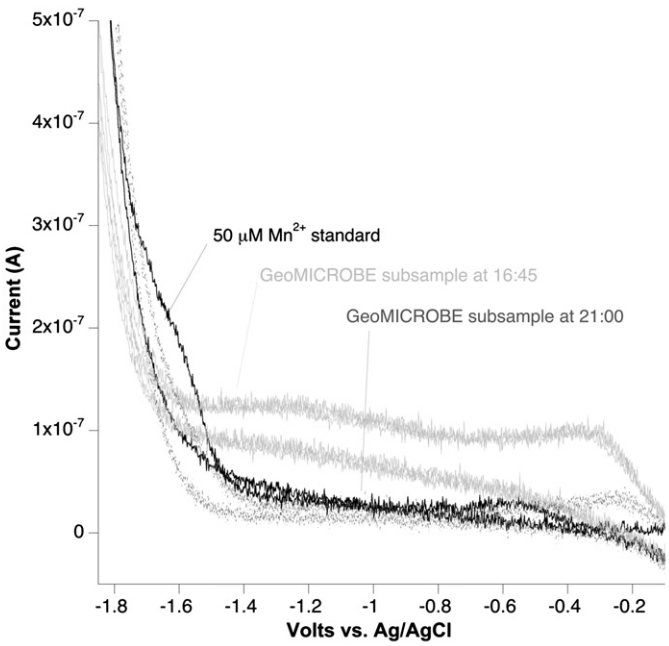

Fig. 10. Representative voltammetric scans collected during GeoMICROBE short-term deployment. Replicate scans are shown during routine automated Mn^{2+} standardization, a time point (16:45 h) during oxic fluid sampling (~70 μM O_2), and a time point (21:00 h) during sampling of low-oxygen fluid sampling (< 4 μM O_2).

5.3. The mobile pumping system

The Mobile Pumping System, coupled to a CORK FDL and operated in real-time by ROV or HOV has evolved into a dependable and versatile system by which to routinely draw crustal fluids to the seafloor for collection into a variety of samplers. Results from analyses of multiple MPS-LVBS sample collections over three field seasons (2008, 2009 and 2010) are reported separately (Lin et al., submitted to; S.P. Jungbluth, J. Grote, H.-T. Lin, J. P. Cowen, and M. S. Rappé, manuscript in preparation, 2011;

M. Matzinger, B.T. Glazer and J.P. Cowen, manuscript in preparation, 2011; J.P. Amend, J. Boettger, M. Henscher, H.-T. Lin, and J.P. Cowen, manuscript in preparation, 2011; A. Robador and J.P. Cowen, manuscript in preparation, 2011). Most recent field efforts in July 2011 employed the MPS in its MPS-McLane-MVBS configuration; the MPS included the new low flow sensor that functioned very well (Figs. 6 and 7). Detailed chemical analyses of MPS samples from all years demonstrate that if the umbilical connection at the CORK is secure, the system collects large volumes of crustal fluids uncontaminated by collection procedure or materials (Lin et al., submitted to). Fluid pump rates, and therefore collection times, depend on the length and diameter of the FDL and the specific operation (e.g., flushing, filtration, filter type). Maximum pump rates at the 1301A CORK, through its ~280-m-long 0.125 in. ID FDLs, were on the order of 0.3–0.6 L min^{-1}, while flow rates through similar lengths of 0.5 in. ID FDLs (e.g., U1362A: 440 m and U1362B: 280 m) are considerably faster, to greater than 5 L min^{-1} (Fig. 7). The MPS (or GeoMICROBE) pump can deliver fluid flow rates through 0.5″ ID FDLs (e.g., U1362A and U1362B) that are ~10 times faster at one third the power demand than a similar length FDL of 0.125 in. ID (e.g., U1301A) (Fig. 7).

6. Conclusions

The GeoMICROBE instrument sleds and the MPS have been designed to couple with fluid delivery lines (FDLs) of ODP and IODP CORK observatories to draw fluids from upper basaltic crust to the seafloor. Both integrate powerful, trace element, and microbiologically 'clean' positive displacement pumps with in-line samplers and sensors, and are controllable in real-time via interactive communication. The GeoMICROBE is specifically designed for autonomous long-term sampling and is cable network ready (e.g., http://www.neptunecanada.ca/sensors-instruments/locations/corks.dot). Immediate applications for the GeoMICROBE include (1) autonomous sampling for multiple parameters requiring large volumes and/or otherwise requiring large amounts of time; (2) time-series microbial geochemistry/ecology sampling in association with physicochemical sensor data; and (3) monitoring large-scale ocean crust tracer injection experiments (Fisher et al., 2011a).

Important advantages of these seafloor samplers relative to downhole samplers include the capability to rapidly collect very large sample volumes and replicates, significant reduction in constraints on instrument/sampler size, simultaneous collection of multiple real-time (in-line) sensor data, high sampling frequencies within a single dive or dive series and avoidance of the complicated and submersible time-consuming recovery and re-instrumentation of downhole samplers. CORK FDLs can be thoroughly flushed prior to sampling. Large sample volumes permit reliable analyses of multiple complementary parameters requiring large fluid volumes, biomass or general particle mass, as well as providing adequate volumes for enrichment, metabolic or other experimental studies. Disadvantages include potential changes in temperature and pressure between downhole and seafloor environments; for example, fluid temperature at the 3.5 my old JdFR flank sites decrease from ~64 °C in upper crust to 20–40 °C upon reaching the seafloor depending on fluid pumping rate, or <2 °C if allowed to sit at the seafloor. Temperature changes at the North Pond sites, are expected to be much smaller due to cooler upper crustal temperatures (~2–15 °C) (Becker et al., 1984). Proportionally, the degree of decompression (~4–11%) from upper crust to seafloor are less than for temperature and likely do not have strong influence on biogeochemical processes.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.dsr.2011.11.004.

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