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A full-ocean-depth rated modular lander and pressure-retaining sampler capable of collecting hadal-endemic microbes under *in situ* conditions

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ABSTRACT

The hadal zone remains one of the least studied environments because of its inaccessibility, in part because of hydrostatic pressures extending to 110 MPa. Few instruments are capable of sampling from such great depths. We have developed a full-ocean-depth-capable lander that can be fit with sampling packages for the collection of still images, video, motile megafauna, and hadal seawater. One payload includes a pressure-retaining sampler (PRS) able to maintain seawater samples under *in situ* pressure during recovery. We describe the technical specifications of the lander and the PRS and preliminary results from three deployments at depths in excess of 10,700 m in the Mariana Trench. Seawater from full-ocean depth was recovered at 81% of the *in situ* pressure. This facilitated the collection of microbial genomes affiliated with the family *Flavobacteriaceae* within the *Bacteroidetes* and the phylum *Marinimicrobia*. We show that these microbes are specifically enriched in hadal zones, representing novel trench lineages, and describe their adaptations for living in hadal environments. These findings highlight the utility of this lander system, which facilitates scientific inquiry at depths greater than 6000 m.

1. Introduction

Hadal trenches, found at depths exceeding 6000 m, represent only ~0.21% of the volume but 45% of the depth range of the ocean (Jamieson, 2015). Hadal sites remain relatively unexplored due to technological constraints, most notably brought about by high hydrostatic pressures that can reach 110 MPa. While remotely operated vehicles (Momma et al., 2004; Bowen et al., 2008; Nunoura et al., 2015) and submersibles (Leon-Zayas et al., 2017) have been used to study hadal zones, most samples have been collected *via* free-vehicle landers, unmanned instruments that are not tethered to a ship and which descend into the deep ocean, collect samples of interest, release ballast, and return to the surface autonomously. These devices allow the deployment of scientific payloads to locations where other instrumentation is unable to go (Jamieson, 2016). Landers have facilitated the collection of hadal samples, including seawater (Eloe et al., 2011a, 2011b; Tarn et al., 2016; Peoples et al., 2018), sediments (Glud et al., 2013), and bait-attending fauna such as fishes (*e.g.* Fujii et al., 2010; Jamieson et al., 2011; Linley et al., 2016) and amphipods (*e.g.* Hessler et al., 1978; Blankenship et al., 2006; Eustace et al., 2016). Hadal megafaunal communities are distinct from those in the abyss, including many taxa that appear endemic to hadal zones (Wolff, 1970; Beliaev, 1989). The high rate of endemism is in part the result of specific adaptations to high hydrostatic pressures (Yancey et al., 2014) and increased abundances of organic matter due to topographical funneling (Ichino et al., 2015) that lead to niche differentiation (Gerringer et al., 2017).

Hadopelagic microbial communities are also distinct from those found at abyssal depths (Eloe et al., 2011b; Nunoura et al., 2015; Tarn et al., 2016; Peoples et al., 2018). Culture-independent 16S rRNA gene high throughput sequencing has identified taxa related to the genus *Aquibacter* within the *Bacteroidetes*, the phylum *Marinimicrobia*, and members of the *Rhodobacteraceae* and *Rhodospirilliceae* as enriched in hadal pelagic communities when compared to abyssal sites (Peoples et al., 2018). However, little is known about members of these hadal lineages. More is known about isolated piezophiles, cultured microbes

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that show optimum growth under high hydrostatic pressure conditions. These isolates belong to a narrow set of genera within the *Gammaproteobacteria*, including *Colwellia*, *Shewanella*, *Moritella*, and *Psychromonas* (*e.g.* Nogi et al., 1998; Nogi et al., 2002; Nogi et al., 2004; Nogi et al., 2007), which appear to make up only a small portion of hadalopelagic communities (Eloe et al., 2011b; Tarn et al., 2016; Peoples et al., 2018). The lack of diverse, *in situ*-abundant cultured isolates may be in part due to the collection of samples under decompressed conditions. Decompression can lead to morphological changes and inactivation of known microbial piezophiles after only a few hours (Yayanos and Dietz, 1982, 1983; Chastain and Yayanos, 1991). Significant shifts in community composition (La Cono et al., 2015) and gene expression (Edgcomb et al., 2014) at meso- and bathypelagic depths have been noted as a result of decompression. Therefore pressure-retaining samplers, or devices capable of collecting and maintaining samples under *in situ* pressure conditions, have been developed to allow for the collection of samples without decompression (*e.g.* Macdonald and Gilchrist, 1969; Jannasch et al., 1973; Yayanos, 1977; Bianchi et al., 1999; Yanagibayashi et al., 1999; Tamburini et al., 2013). However, these devices have not been used in great detail for microbiology in hadal zones.

Here we report the development of a new lander system capable of sampling from full-ocean depth. The modular design allows for the rapid deployment of a number of scientific packages, including instrumentation for collecting bait-attending motile megafauna, water, and still images and video. We also describe a new pressure-retaining sampler (PRS) capable of maintaining high hydrostatic pressure during ascent from full-ocean depth. In both cases the technical specifications of these devices are presented, along with preliminary data collected from their successful deployment to the deepest depths in the ocean, including genomic properties of the hadal-enriched *Flavobacteriaceae* and *Marinimicrobia* lineages. These technologies will facilitate future scientific investigations of the deepest parts of the global ocean.

2. Lander technical specifications

2.1. The floatpack module and deployment instrumentation

The lander is composed of two payloads; an upper floatpack payload that provides positive buoyancy and retrieval instrumentation and a lower payload that delivers scientific sampling equipment (Fig. 1). The upper payload frame is made of high-density polyethylene plastic and contains interlocking cogs on all sides. These cogs provide a flush fitting with the lower payloads and are held together with titanium pins in a quick fit, modular manner. Enclosed within the payload frame is Isofloat® syntactic foam (Ron Allum Deepsea Services, St. Peters, Australia). This syntactic foam provides positive buoyancy and structural support for the lander. Mounted on top is instrumentation for deployment and recovery. This includes a bale for retrieval and a carousel containing a GPS beacon (XMI-11k, Xeos Technologies Inc., Dartmouth, Nova Scotia), a pressure sensor (RBR Ltd, Ottawa, Canada), a strobe light (Xeos Technologies Inc.), a radio directional finder (Xeos Technologies Inc.), and a flag. The instrument has a timer with six burn wire channels that can be programmed to trigger at different time intervals. For retrieval of the lander, one of the channels can be configured to release the weight stack while on the seafloor. Inside the timer housing is a thermistor for measuring external temperature to within 0.5 °C. The instrument can also be released acoustically using a BART board (EdgeTech, Wareham, MA) and hydrophone. All electronics are housed within a titanium pressure case. The weight of the upper payload is 125 kg. Additional buoyancy can be added depending on the scientific payload configuration.

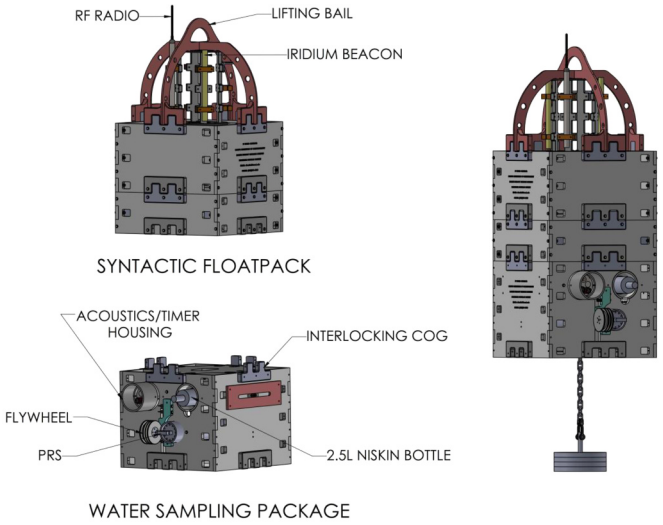

Fig. 1. The lander with the water sampling package. Upper left; The upper syntactic floatpack containing instrumentation for deployment and recovery. Lower left; The lander with the water sampling package, including a 2.5 L Niskin bottle and a pressure-retaining sampler (PRS). Right; The complete instrument with the water sampling package.

2.2. Scientific payload

2.2.1. Water package and pressure-retaining sampler

The lander can be fit with a package for collecting water samples and retaining *in situ* high hydrostatic pressures upon collection using a pressure-retaining sampler (PRS; Fig. 1). This device collects 135 mL of seawater that can be compared against decompressed water collected in an accompanying 2.5 L Niskin bottle. The PRS is made of Ti-6Al-4V titanium alloy and when fully assembled is 69.8 cm in length and 9.2 cm wide (Fig. 2). It weighs 10.2 kg in air and 6.8 kg in water. At the front of the sampler is a 316 stainless steel needle valve with an orifice

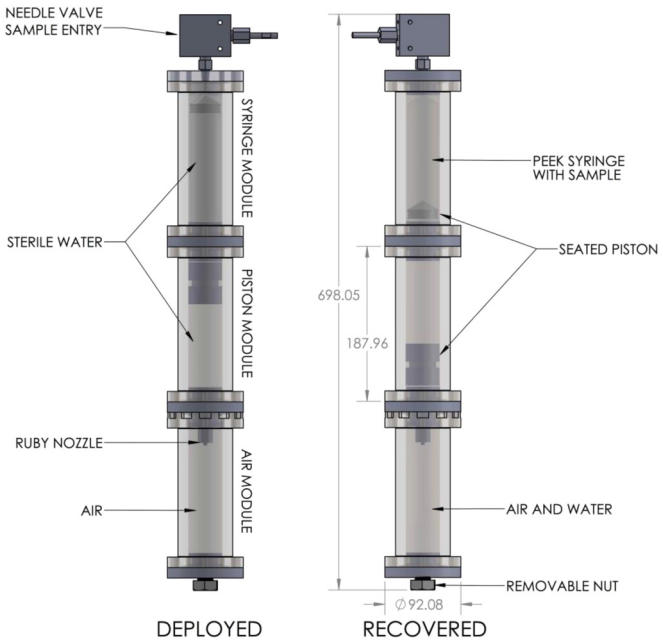

Fig. 2. Design of the pressure-retaining sampler (PRS). Left; The PRS orientation during deployment. Right; The PRS orientation after recovery. The valve is opened at depth, allowing the water sample to enter. The PEEK syringe piston moves, displacing water and moving the titanium piston, which seals against the endcap and retains the *in situ* pressure. The valve is then closed.

size of 13/64″ (~0.52 cm; High Pressure Equipment Co., Erie, PA). The PRS is composed of three modules: a syringe module, a piston module, and an air module (Fig. 2). Within the syringe module sits a sterile polyetheretherketone (PEEK) syringe for collecting the sample of interest. A titanium piston resides in the piston module. Each module is connected *via* an end-cap and is sealed internally with 70 durometer O-rings and from external pressure with face-seal 90 durometer O-rings (Supplementary Fig. S1). The end-cap between the hydraulic fluid module and the air module contains a ruby blast nozzle with an orifice size of 0.005″ (~0.127 mm; H2O Jet, Tumwater, WA). The final air module end-cap contains a threaded removable nut sealed with an O-ring. All sections are held together using twelve ¼ inch (6.35 mm) steel Grade 8 bolts. The sampler is rated to ~138 MPa (20,000 pounds per square inch (PSI)). Prior to deployment it was tested at ~103 MPa (15,000 PSI) internal pressure in the lab and in mimicked, sinking conditions to ~69 MPa (10,000 PSI) in a pressure-tank at Scripps Institution of Oceanography (San Diego, CA) where it a) withstood an external pressure of 69 MPa with an internal pressure of 0.1 MPa (14 PSI), b) external and internal pressure of 69 MPa after filling, and c) external pressure of 0.1 MPa and internal pressure of 69 MPa after recovery.

The sampler, with the exception of the air module, is assembled prior to deployment submerged in sterile ultrapure (MilliQ) water to eliminate any compressible air space. Care is taken during assembly to minimize the introduction of microorganisms into the internal compartments of the sampler. Full-arm gloves and sterile MilliQ water are used at all times. The titanium sections are wiped down with isopropanol and the PEEK syringe is sterilized with 10% HCl prior to assembly. To confirm the biological compatibility of the needle valve, we tested the affect of shear on cells passing through the sampling orifice of the PRS during one 90° turn of the valve handle. No significant difference in cell numbers was observed using seawater collected from Scripps Pier (n = 3; *T*-test, $p > 0.26$).

When deployed the sampler functions as follows (Fig. 2). The valve is opened at depth using a flywheel. The flywheel is connected to three aircraft cable wires and is held under tension in both the clockwise and counterclockwise directions. The first wire is static and is connected to a burn wire that holds the valve closed. The second wire is attached to a burn wire *via* a 'strong' spring (overriding the tension of the third, weaker spring), providing tension in the 'open' direction. The third wire is connected to a 'weak' spring that also provides tension in the 'closed' direction of the valve. At depth the first burn wire is triggered, releasing the static wire. The 'strong' spring then pulls the valve open 360°, overcoming the 'weak' spring and filling the sampler. As seawater enters the syringe, the syringe piston moves and displaces MilliQ water within the syringe module. The displaced water then flows through the end-cap and pushes a titanium piston, which in turn pushes water within the piston module. This water is displaced through the ruby nozzle, which acts to maintain a consistent and slow flow rate, and enters the air module. Because of the pressure differential present on either side of the titanium piston, the sampler continues to fill until the piston reaches the end of the hydraulic fluid module and seats against the end-cap, sealed by the O-ring. The water/air mixture in the air module is therefore sealed from the remainder of the pressurized PRS, holding a pressure of 0.2 MPa, approximately twice atmospheric pressure. Contained within the PEEK syringe is the water sample of interest. The second burn wire is then triggered after at least five minutes, releasing the 'strong' spring and allowing the 'weak' spring to pull the valve shut.

Upon recovery the sampler is immediately placed on ice to maintain the sample near *in situ* temperature during sample processing. Pressure in the air module is released by removing the back nut (Supplementary Fig. S1). A high-pressure pump and pressure gauge are hooked up to the sampling valve, pressurized to 50% of the predicted *in situ* pressure to avoid contamination of the sample, and the valve opened. The change in gauge pressure provides an estimate of the retained pressure. The air module and the ruby nozzle are removed, revealing the back of the seated titanium piston. The hydraulic pressure pump is then hooked up to the hydraulic fluid module in place of the ruby nozzle and pressure is applied against the titanium piston to the estimated pressure at collection depth. The front valve is then opened, allowing water to flow out at a controlled rate. As it does so, pressure is applied using the hydraulic pump, thereby moving the titanium piston and maintaining the pressure within the system. In this way pressurized seawater can be subsampled into 'subsamplers' in 5 mL volumes that allow for the addition of fixative or other chemicals while maintaining *in situ* pressure. The stainless steel subsamplers function in the same manner as the PRS, with a PEEK syringe holding the sample of interest and a titanium piston displacing sterile hydraulic fluid (Supplementary Fig. S1). The subsamplers are connected to the PRS using a three-way needle valve, with one port for the PRS, one port for the subsampler, and one port for the addition of chemicals or removal of sample of interest *via* syringe.

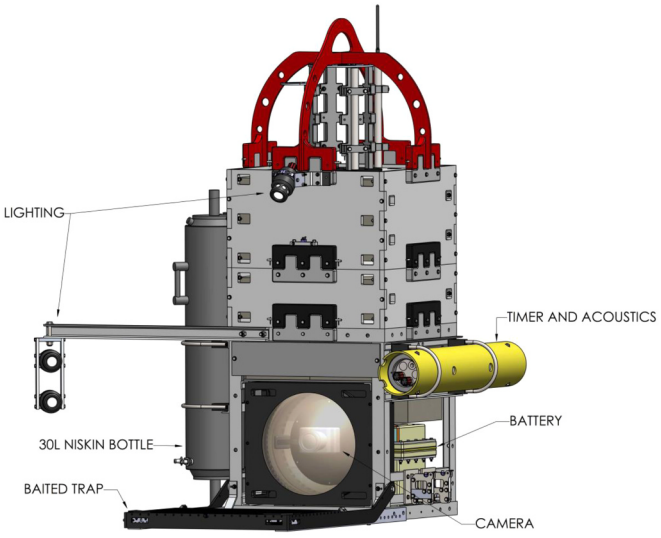

Fig. 3. The lander with the camera payload, including a baited trap and camera.

2.2.2. Camera and baited trap system

The lander can be configured with a modular package to take video, still images, and collect motile megafauna (Fig. 3, Supplementary Fig. S2). This scientific package has a netted trap that can be baited prior to deployment. A screen is released *via* burn wire that falls on top of the trap and captures any fauna feeding on the bait. The baited trap and surrounding environment are imaged by a Canon 5D Mark II DSLR camera (Canon USA, Inc, Melville, NY), as described elsewhere (Hardy et al., 2013), placed within a polished Vitrovex 17-in outer diameter x 23 mm wall thickness glass sphere (Nautilus Marine Service GmbH, Buxtehude, Germany). The field of view is illuminated by three Sealite Sphere white 5700 k lights (SLS-5100, DeepSea Power and Light, San Diego, CA), two placed on a boom arm deployed to the side and one directly above the trap. The timing of the camera and lights are controlled by an Arduino microcontroller (Freetronics Pty Ltd, Victoria, Australia). A gear attached to the zoom lens allows for dynamic focusing over varying distances. Power is supplied by a lead-acid oil-compensated battery (SB-2440, DeepSea Power and Light). All instrumentation for the baited trap system and camera is housed within a 6061 aluminum alloy frame. 30 L Niskin bottles can be mounted on the side of the frame and are triggered by burn wire. The weight stack is positioned directly below the lander and its length can be adjusted, allowing for either a pelagic mooring or a benthic deployment where the lander sits directly on the seafloor. The complete weight with this package is 478 kg. The lander travels at approximately 39 m per minute during both ascent and descent through the water column.

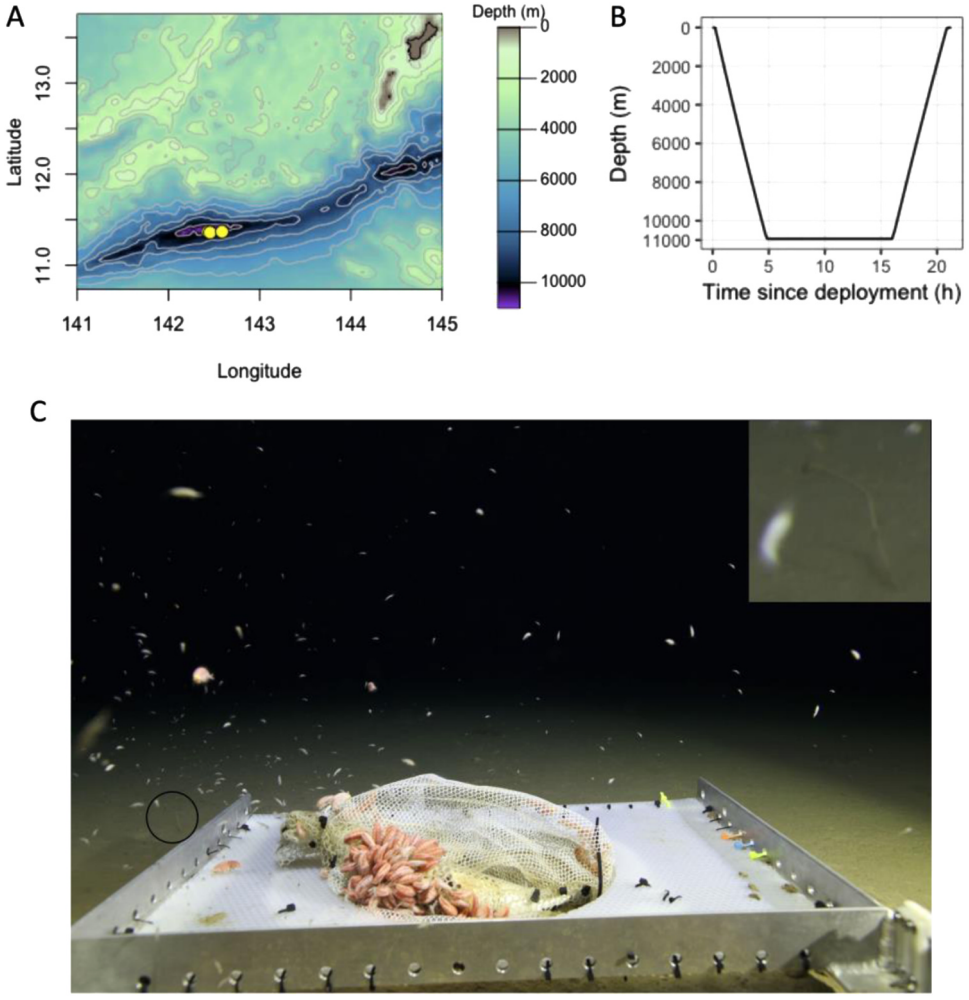

Fig. 4. A; Three deployment locations within the Challenger Deep in the Mariana Trench. B; Depth over time based on the pressure sensor during the deployment to 10,929 m. C; Image obtained from the camera at a depth of 10,929. The inset image (upper right) shows the circled, stalked organism, potentially a feather duster worm, in more detail.

3. Deployments and methods

Three deployments were conducted in the Mariana Trench (Fig. 4), two with the camera system and one with the PRS. The first deployment conducted with the camera system and baited trap was within the middle portion of the Challenger Deep at a depth of 10,778 m (location 11.359575 142.456135). The lander was deployed in a 'pelagic' orientation approximately 1 m above the seafloor with jack mackerel as bait. The second camera deployment was performed within the eastern portion of the Challenger Deep at a depth of 10,929 m (location 11.368537 142.587517). During this deployment the lander and baited trap were positioned directly on the seafloor. On both deployments the camera was cycled to focus and take still photos and video at 10′, 7′ and 3′ distances, with each video 30 s in length. The lights and camera were then turned off for six minutes, followed by further camera cycling as described. Collected amphipods were stored frozen and in RNALater. Type specimens were deposited in the Scripps Institution of Oceanography Benthic Invertebrate Collection (catalog numbers C12056-12063) and the identification of some specimens is described elsewhere (Zhang et al., 2018).

One deployment was performed using the water package and PRS at a depth of 10,970 m within the middle portion of the Challenger Deep (location 11.366393 142.432555). Water samples were collected approximately 3 m above the seafloor. The valve was triggered to open and was closed after 15 min. Upon return to the surface samples were placed on ice and immediately subsampled into glycerol/TE buffer (Rinke et al., 2014) without decompression and stored at −80 °C prior to single-cell sorting at the J. Craig Venter Institute (JCVI, La Jolla, CA). The temperature of the sampler upon collection was estimated based on an accompanying 2.5 L Niskin bottle.

Single-cell genomics was conducted by staining cells with SYBR Green I (Invitrogen, Carlsbad, CA) and sorting them using a FACS-Aria II flow cytometer (BD Biosciences, San Jose, CA) into 384 well microtiter plates containing 2 μL of TE Buffer. Multiple displacement amplification was performed using the Repli-G kit (Qiagen, Hilden, Germany). Amplified products were cleaned with Exonuclease I and Shrimp Alkaline Phosphatase (Thermo Fisher Scientific Inc., Waltham, MA). Wells were screened for positive amplification using the 16S rRNA gene primers 515f-926r (Parada et al., 2015). Amplified genomes of interest were further purified using Agencourt AMPure XP beads (Beckman Coulter, Brea, CA) and sent for sequencing on an Illumina HISeq. 4000 (Institute for Genomic Medicine Genomics Center, University of California San Diego, La Jolla, CA). Raw reads were quality trimmed with Trimmomatic (Bolger et al., 2014) and paired end reads were assembled using SPAdes v3.9 (Bankevich et al., 2012). Only contigs longer than 5 kb were retained for further analysis. Contigs were screened against the NCBI nr/nt database (NCBI Resource Coordinators, 2016) using DIAMOND (Buchfink et al., 2015) and MEGAN (Huson et al., 2007) to remove sequences typical of contamination during multiple displacement amplification, including those

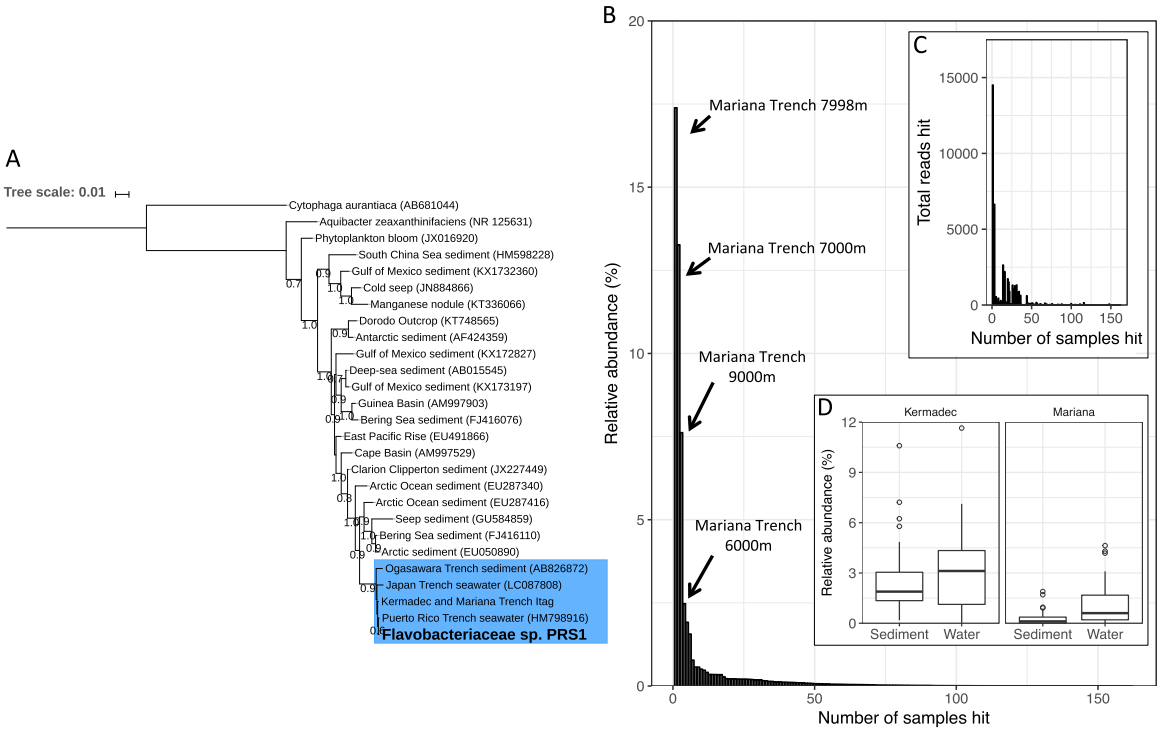

Fig. 5. Identification of sequences related to *Flavobacteriaceae* sp. PRS1. A; Ribosomal 16S RNA gene phylogenetic tree of *Flavobacteriaceae* sp. PRS1 with closely related taxa. B; The relative sequence abundances of 16S rRNA gene sequences at 99% similarity to *Flavobacteriaceae* sp. PRS1 within samples queried within the IMNGS SRA database (211,968 samples queried), with samples ordered by decreasing relative sequence abundance. Labeled Mariana Trench samples are from Nunoura et al. (2015). C; The total number of sequences at 99% similarity to *Flavobacteriaceae* sp. PRS1 that were identified within each sample queried within the IMNGS SRA database (211,968 samples queried), with samples plotted in the same order as B. D; Relative sequence abundances per sample of a hadal V4-V5 16S rRNA Itag sequence related to *Flavobacteriaceae* sp. PRS1 within the Mariana and Kermadec trench sediment and water communities from Peoples et al. (2018) and Peoples *et al.*, unpublished. Sequences can be found in Supplementary Table 1.

related to *Actinetobacter*, *Pseudomonas*, and *Propionibacterium*. Genome completeness and phylogenetic placement was estimated using single-copy marker genes identified with CheckM (Parks et al., 2015). For phylogenetic analysis, 16S rRNA gene sequences were aligned using the SINA aligner (Pruesse et al., 2012) and trees built using FastTree (Price et al., 2010), which were then visualized using the Interactive Tree of Life (iTOL; Letunic and Bork, 2007). To evaluate the distribution of the genomes obtained, samples within the NCBI SRA database were queried for sequences at 99% similarity to the 16S rRNA genes of the genomes presented here using the Integrated Microbial NGS Platform (Lagkouvardos et al., 2016). Genomes were annotated with Prokka (Seemann, 2014) and compared against other related genomes using Roary (Page et al., 2015) at a protein similarity cutoff of 40%. Carbohydrate-active enzymes were identified using dbCAN (Yin et al., 2012). Genomes were also annotated with IMG/ER (Markowitz et al., 2012). Genomes can be accessed at the NCBI BioProject PRJNA497607 and IMG Genome IDs 2770939648 and 2778260900.

4. Results and discussion

4.1. Camera package

Three deployments were successfully conducted in the Challenger Deep in the Mariana Trench in excess of 10,700 m (Fig. 4). For all deployments acoustics were lost at ~10,000 m and the instruments relied on timers and burn wires for operation. One deployment was conducted with the camera system and baited trap within the middle portion of the Challenger Deep at a depth of 10,778 m. Although the camera was out of focus, very few amphipods were seen throughout the eight hours the instrument was moored above the seafloor (Supplementary Fig. S3). No amphipods were collected. In contrast, a large number of amphipods were seen during the second camera deployment located directly on the seafloor (Fig. 4; Supplementary Fig. S4). Genetic identification indicated they were affiliated with *Hirondellea gigas* and the genus *Halice* (Scripps Institution of Oceanography Benthic Invertebrate Collection catalog numbers C12056-12063; Zhang et al., 2018). *H. gigas* is commonly found in baited traps in large quantities at depths greater than 10,000 m and has been found in trenches of the northwestern Pacific, including the Mariana Trench (Hessler et al., 1978; France, 1993; Glud et al., 2013; Ritchie et al., 2015). *Halice* spp. have also been identified in at least four trenches in the western Pacific, including the Tonga and Kuril-Kamchatka trenches (Jamieson, 2015). The identification and collection of these taxa in the benthic but not the pelagic orientation of the lander is consistent with *Hirondellea* spp. being demersal amphipods (Blankenship and Levin, 2007; Fujii et al., 2013), which colonize bait to a lesser extent with increasing distance from the sea floor. Current flow was evident as the amphipods were in higher abundance to the left of the bait and were visibly carried in that direction. Bioturbation was also seen in the background and has been previously noted within the Challenger Deep (Glud et al., 2013). A vertically-positioned organism, potentially a feather duster worm, was visible to the left of the trap (Fig. 4, inset). This organism is similar to one seen in the Kermadec Trench at a depth of ~ 9000 m (HADES program, unpublished data).

4.2. Pressure-retaining sampler single-cell genomics

A third deployment was conducted using the pressure-retaining sampler within the middle portion of the Challenger Deep at a depth of 10,970 m. The PRS held ~90 MPa (13,000 PSI), ~81% of the *in situ* pressure at sample collection depth. Pressure retention of 70–80% is consistent with previous results of deep-ocean samplers that have shown pressure losses due to seal movement and metal elasticity

Table 1
Characteristics of the two single-cell genomes described in this study.

Genome	Completeness	Contamination	Genome size (Mbp)	Number of contigs	Number of genes	Longest contig	IMG Genome ID
Flavobacteriaceae sp. PRS1	90.85%	0.94%	2.42	61	2359	158472	2770939648
Marinimicrobia sp. PRS2	57.78%	0.00%	1.4	89	1254	109386	2778260900

(Macdonald and Gilchrist, 1972; Yayanos, 1977; Bianchi et al., 1999). The water recovered from an associated 2.5 L Niskin bottle was 15.4 °C, significantly warmed from the *in situ* temperatures of 2.6 °C, because surface water temperatures were ~30 °C. Regardless, we identified members affiliated with the *Marinimicrobia* and the family *Flavobacteriaceae* within the *Bacteroidetes* using single-cell genomics. The 16S rRNA gene sequences from these cells showed greatest similarity to other sequences recovered from deep-sea locations, and in particular trenches, including the Puerto Rico, Kermadec, Mariana, and Ogasawara trenches (Fig. 5; Supplementary Fig. S5). Mining 16S rRNA gene datasets revealed that these genomes show a limited distribution and are specifically enriched in hadal zones (Fig. 5; Supplementary Fig. S6). Therefore these genomes are representative of abundant hadal microbes and likely have specific adaptations to the conditions found within trenches.

Whole genome sequencing performed on the *Flavobacteriaceae* single-amplified genome yielded an estimated completeness of 91% (Table 1). The genome is composed of 61 contigs with 2359 genes and a total length of 2.4 Mbp. When classified using the SILVA 16S rRNA gene database (Quast et al., 2013), *Flavobacteriaceae* sp. PRS1 is identified as a member of the genus *Aquibacter*. However, strain PRS1 and *Aquibacter zeaxanthinifaciens* (Hameed et al., 2014) only share 94% 16S rRNA sequence identity and both a 16S rRNA gene tree (Supplementary Fig. S7) and a concatenated single-copy marker gene tree (Supplementary Fig. S8) show that strain PRS1 is distinct from other members of the *Flavobacteriaceae*. Therefore, strain PRS1 likely represents a novel genus in the family *Flavobacteriaceae*. Interestingly, most of the 16S rRNA gene sequences closely related to strain PRS1 were found associated with sediments. However, members related to strain PRS1 and the genus *Aquibacter* have been found in high abundances in both trench water (Nunoura et al., 2015; Peoples et al., 2018) and sediments (Fig. 5), indicating this microbe may colonize both environments. A pangenomic comparison between 37 members of the *Bacteroidetes* showed that 410 genes were unique to *Flavobacteriaceae* sp. PRS1, with the majority being hypothetical proteins. *Flavobacteriaceae* sp. PRS1 was only one of three compared genomes that lacked a DNA photolyase. The conspicuous absence of a DNA photolyase within known piezophiles has been noted (Lauro and Bartlett, 2008; Lauro et al., 2013, 2014) and may be a conserved attribute of deep-ocean microbes. Strain PRS1 appears to be heterotrophic and capable of breaking down particulate organic matter. The genome has 59 carbohydrate-active enzyme (CAZymes) genes for the attachment to and the degradation of particulate organic matter. These include glycosyl transferases to generate polysaccharides for particle attachment and four susC/susD pairs involved in binding and transporting polysaccharides into the cell. PRS1 appears capable of gliding motility (*gldB-E*, *gldH-N*, *sprA*, *sprE* genes) which may facilitate movement on particles. PRS1 also has an alkane monooxygenase (*alkB*) involved in hydrocarbon degradation. Overall, these findings are consistent with other members of the phylum *Bacteroidetes* that show an affinity to utilize particulate organic matter (Fernandez-Gomez et al., 2013). The enrichment of this genome within the hadal zones of trenches is consistent with hadal *Flavobacteriaceae* breaking down particulate organic material that is funneled (Glud et al., 2013; Ichino et al., 2015) or resuspended (Oguri et al., 2013) in hadal trenches.

A genome affiliated with the *Marinimicrobia*, *Marinimicrobia* sp. PRS2, was also obtained. This genome is 1.4 Mbp in size and estimated to be 58% complete (Table 1). While the final assembled genome obtained here lacked a 16S rRNA gene, the gene obtained during multiple displacement amplification screening (Supplementary Table S1) indicated that strain PRS2 is affiliated with an OTU enriched in the hadal zone of the Mariana and Kermadec trenches (Supplementary Fig. S5) that can reach abundances of up to 10% within hadal samples (Supplementary Fig. S6; Peoples et al., 2018). It has been noted that a large diversity exists within the *Marinimicrobia*, with specific lineages capable of using different electron acceptors (Hawley et al., 2017). Ribosomal 16S RNA gene sequences previously collected from the Mariana and Kermadec trenches showed a large diversity of *Marinimicrobia*, with some clades showing a deep or hadal-specific distribution (Peoples et al., 2018). Based on the partial 16S rRNA gene, strain PRS2 is related to clade HF770D10 (Supplementary Fig. S5). Consistent with the putatively –oxic distribution of this clade (Hawley et al., 2017), the genome encodes a NADH dehydrogenase but no genes for alternative electron acceptors. No genes suggesting autotrophic carbon fixation were identified, indicating a potentially heterotrophic lifestyle, although we acknowledge the incompleteness of this genome. However, in contrast to *Flavobacteriaceae* sp. PRS1 only four CAZyme genes were identified in *Marinimicrobia* sp. PRS2. This may be consistent with the distribution of *Marinimicrobia*, which are enriched in the free-living, rather than the particle attached fraction in deep-ocean communities (Eloe et al., 2011b; Salazar et al., 2015; Tarn et al., 2016; Peoples et al., 2018). Relative to other members of the *Marinimicrobia*, strain PRS2 appears enriched in leucine-rich repeats, MORN repeats, and Por secretion system proteins, including a gene related to gingipain. Por secretion system proteins, which are typically found in the *Bacteroidetes*, may function in gliding motility or pathogenesis (Sato et al., 2010; McBride and Zhu, 2013). The *Marinimicrobia* sp. PRS2 genome includes the *paa* gene cluster that functions in the degradation of aromatic phenylacetate, which can be derived from the amino acid phenylalanine and other related substrates (Teufel et al., 2010). Both strain PRS1 and PRS2 have cyanophycin synthetase and cyanophycinase. Cyanophycin synthetase is involved in the synthesis of the polymer cyanophycin (CGP), which consists of aspartic acid and arginine (Simon and Weathers, 1976) and can function as a temporary nitrogen, energy and carbon reserve in *Cyanobacteria* (Sukenik et al., 2015). Cyanophycin is in turn degraded by bacterial cyanophycinases. CGP has also been implicated in spore formation in *Clostridium* (Liu et al., 2016) and the use of cyanophycin as a means of dealing with temperature stress was suggested in psychrophilic *Colwellia* (Methe et al., 2005). Therefore cyanophycin may function as an energy store and source in *Marinimicrobia* and *Flavobacteriaceae* under deep-ocean conditions.

5. Future directions

We have developed new full-ocean depth instrumentation and report their successful deployment at depths exceeding 10,700 m in the Mariana Trench. One deployment used a pressure-retaining sampler that held 81% of the *in situ* pressure. Pressure retention of 70–80% is consistent with previous results of deep ocean samplers, which have shown similar pressure losses due to seal movement and metal elasticity (Macdonald and Gilchrist, 1972; Yayanos, 1977; Bianchi et al., 1999). If full-ocean depth pressure is to be retained, future deployments will need pressure accumulators to compensate during recovery (Bianchi et al., 1999). Some piezophiles are able to grow at pressures within ~60% of their optimum pressure range of growth (Cao et al., 2014;

Kusube et al., 2017), suggesting the amount of pressure-retention achieved here is able to yield novel piezophiles. A more important concern may be the recovery of water samples under *in situ* temperature conditions using these devices, as cultured piezophiles are sensitive to changes in temperature (Yayanos and Dietz, 1982). Future deployments will need additional insulation to maintain low temperatures and avoid inactivating obligate deep-sea psychrophiles. Other important improvements to this lander will be the addition of an oil-compensated actuator to open and close the PRS valve, the addition of a pressure and temperature sensor to monitor the conditions inside the PRS throughout deployment and retrieval, and a hadal-rated pump for the filtration of large volumes of water *in situ*.

Landers and associated payloads such as the PRS described here provide versatile and low cost options when it comes to addressing unresolved issues in deep-ocean microbiology. The effects of decompression on the integrity, viability and activity of hadal microbes are largely unknown. Even at the greatest ocean depths only modest technological investments are required to be able to pursue a variety of scientific objectives. This includes testing the effects of decompression on the integrity, viability and activity of hadal and other deep-sea microbes, which are as yet largely unknown.

CRediT authorship contribution statement

Logan M. Peoples: Conceptualization, Investigation, Formal analysis, Writing - original draft. **Matthew Norenberg:** Investigation. **David Price:** Investigation. **Madeline McGoldrick:** Investigation. **Mark Novotny:** Investigation. **Alexander Bochdansky:** Conceptualization, Investigation. **Douglas H. Bartlett:** Conceptualization, Investigation, Writing - original draft.

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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.2018.11.010.

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