

A System for Retrieval and Incubation of Benthic Sediment Cores at In Situ Ambient Pressure and under Controlled or Manipulated Environmental Conditions

KEITH JACKSON, URSULA WITTE, AND STEWART CHALMERS

Oceanlab, University of Aberdeen, Newburgh, Aberdeenshire, United Kingdom

ERIK ANDERS

Technical University of Berlin, Berlin, Germany

JOHN PARKES

School of Earth and Ocean Sciences, Cardiff University, Cardiff, United Kingdom

(Manuscript received 20 December 2016, in final form 13 March 2017)


ABSTRACT

The investigation of benthic biodiversity and biogeochemical processes in the deep sea is complicated by the need to conduct experiments at in situ pressures. Recovery of sediment samples to the surface without maintaining full-depth ambient pressure may damage the organisms that are of interest or cause physiological changes that could influence the processes being studied. It is possible to carry out in situ experiments using remotely operated vehicles (ROVs) or lander systems. However, the costs and complexity of ROV operations are significant and, for both ROVs and landers, the complexity and repeatability of the experiments are subject to the limitations imposed by these platforms. A system is described—the Multi-Autoclave Corer Experiment (MAC-EXP)—that has been developed with the aim of offering a new experimental approach to investigators. The MAC-EXP system is designed to retrieve sediment cores from depths down to 3500 m and to seal them into pressure chambers before being recovered so that they are maintained at their normal ambient pressure. After recovery the core chambers can be connected to a laboratory incubation system that allows for experimentation on the sediment without loss of pressure and under controlled conditions of temperature and oxygen concentration. The system is relatively low cost when compared to ROV systems and can be deployed using methods and equipment similar to those used for routine deployments of small unpressurized multicorers. The results of sea trials are detailed.

1. Introduction

Most of Earth's solid surface is deep seafloor lying at depths of more than 200 m, with 70% at a pressure of 38 MPa or above. Marine sediments are a major reservoir in the global carbon cycle, and the formation, cycling, or burial of organic matter (OM) in marine sediments are key processes in the global carbon, nitrogen, and phosphorous cycles. The continental margins of the world's oceans connect the shelf seas with the abyssal plains and are characterized by steep slopes,

rapidly changing oceanographic conditions, and often enhanced productivity. And while its remoteness and inaccessibility renders the deep ocean the least known ecosystem on Earth, they are also increasingly at the forefront of human resource exploration. Prokaryotes usually dominate benthic biomass in deep-sea sediments, and are regarded as the primary agents of OM demineralization, but to date our knowledge of the ecology of deep-sea organisms and deep-sea food webs is very limited and information is particularly scarce with regard to prokaryote diversity and ecosystem function.

 Denotes content that is immediately available upon publication as open access.

Corresponding author e-mail: Ursula Witte, u.witte@abdn.ac.uk



This article is licensed under a [Creative Commons Attribution 4.0 license](http://creativecommons.org/licenses/by/4.0/) (<http://creativecommons.org/licenses/by/4.0/>).

DOI: 10.1175/JTECH-D-16-0248.1

Most deep-sea organisms live in the piezosphere (the volume of the deep sea at more than 1000 m of water depth or more than 10-MPa pressure), and the main reasons for our limited knowledge of deep-sea biodiversity and ecosystem functioning lies in the combination of its remoteness and inaccessibility, and with the sensitivity to depressurization of deep-sea organisms and deep-sea biogeochemical processes. Pressure affects large organisms, which often do not survive depressurization (Pradillon et al. 2004; Shillito et al. 2008), and also affects microorganism (Lauro and Bartlett 2008) in many ways. It affects bacterial physiology and growth (Abe et al. 1999; Zobell and Oppenheimer 1950), membrane and storage lipid composition (Yayanos 1995; Mrozk et al. 2004; Grossi et al. 2010), and membrane proteins (Bartlett et al. 1989), with indigenous (piezophile or piezotolerant) microbial communities generally expressing higher activities under high pressure (Nagata et al. 2010). Biogeochemical processes such as total oxygen consumption (TOU), sulfate reduction, and the anaerobic oxidation of methane, for example, are also strongly influenced by pressure (Glud et al. 1994; Nauhaus et al. 2002; Kallmeyer and Boetius 2004). In consequence, meaningful experimentation must be carried out under in situ pressure, which results in major financial and technical constraints for ecological, biogeochemical, and geomicrobial deep-sea research. Technical advancements, such as the development of autonomous platforms (so-called landers) and, more recently, cabled or independent long-term observatories, have widened our ability to observe the deep sea, and the increasing availability of deep-sea remotely operated vehicles (ROVs) has enhanced our ability to conduct experimental research. But although now possible, in situ experimentation in the deep sea is very resource intensive, requiring sophisticated deep-sea ROVs that are operated by large crews from large vessels. In addition, samples in most cases suffer depressurization upon retrieval. As many piezophiles may only be culturable without depressurization (Yanagibayashi et al. 1999), this may explain why less than 1% of deep-sea prokaryotes can currently be cultured (Fuhrman et al. 1992; Rappe and Giovannoni 2003), and most of our knowledge of deep-sea microbial diversity comes from culture-independent studies.

Here we describe the design, construction, and testing of a flexible, cost-effective alternative to in situ experimentation: a pressure-coring experimentation and cultivation system that enables studies of deep-sea prokaryote biodiversity and ecosystem functioning, under ambient or manipulated pressure, temperature, and oxygen conditions from any medium sized oceangoing research ship with coring capability. In addition, the constant high

pressure chain from sampling to culture overcomes limitations of in situ experiments related to depressurization.

The methods of benthic sediment coring are long established and widely used but few groups have attempted to recover deep cores at their normal ambient pressures. Previous efforts have been dominated by geophysical investigations, particularly with respect to gas hydrate sampling, which require cores to be kept pressurized to maintain them in the solid state (Abegg et al. 2008; Dickens et al. 2003; Chen et al. 2013), whereas biologically orientated systems have focused on capture and recovery under ambient pressure of free swimming organisms (Drazen et al. 2005). The Hydrate Autoclave Coring Equipment Tools In New Tests on Hydrates (HYACINTH) gas hydrate corer (Schultheiss et al. 2006) is a drilling corer that could be connected to the DeepIsoBug subsampler (Parkes et al. 2009), allowing for small subsamples of the core to be isolated, manipulated, and cultured. However, this subsampling system has not found use in routine investigations, possibly because of the significant complexity and logistics involved in its operation.

Our aim was to produce a reliable and easily deployed pressure coring system that could be routinely used in biological investigations that require recovery and long-term incubation at in situ pressure and where environmental conditions may be controlled and manipulated.

2. Corer specification

The requirements for the Multi-Autoclave Corer Experiment (MAC-EXP) system were developed such that the device could collect and incubate useful quantities of sediment sample from working depths that cover a large proportion of the world's seabed. The system should be deployable from oceanographic vessels using general-purpose winches (Fig. 1). Practical design limitations were considered, particularly with respect to maximum working depth. Increasing the working depth leads to heavier and more costly construction, and so a value for maximum depth was specified based on what was known to be practical from previous geophysical pressure corer designs. General parameters are shown in Table 1.

It should be noted that the system could be used at depths that exceed the maximum pressure rating if a relief valve is fitted to the core chambers. On recovery, the valves would limit the pressure to 350 bar, so some depressurization of the cores would occur.

In addition to the general parameters, the following specifications were stipulated:

- The materials in contact with the samples should be “biologically inert” (i.e., they should have minimal effects on the organisms collected).

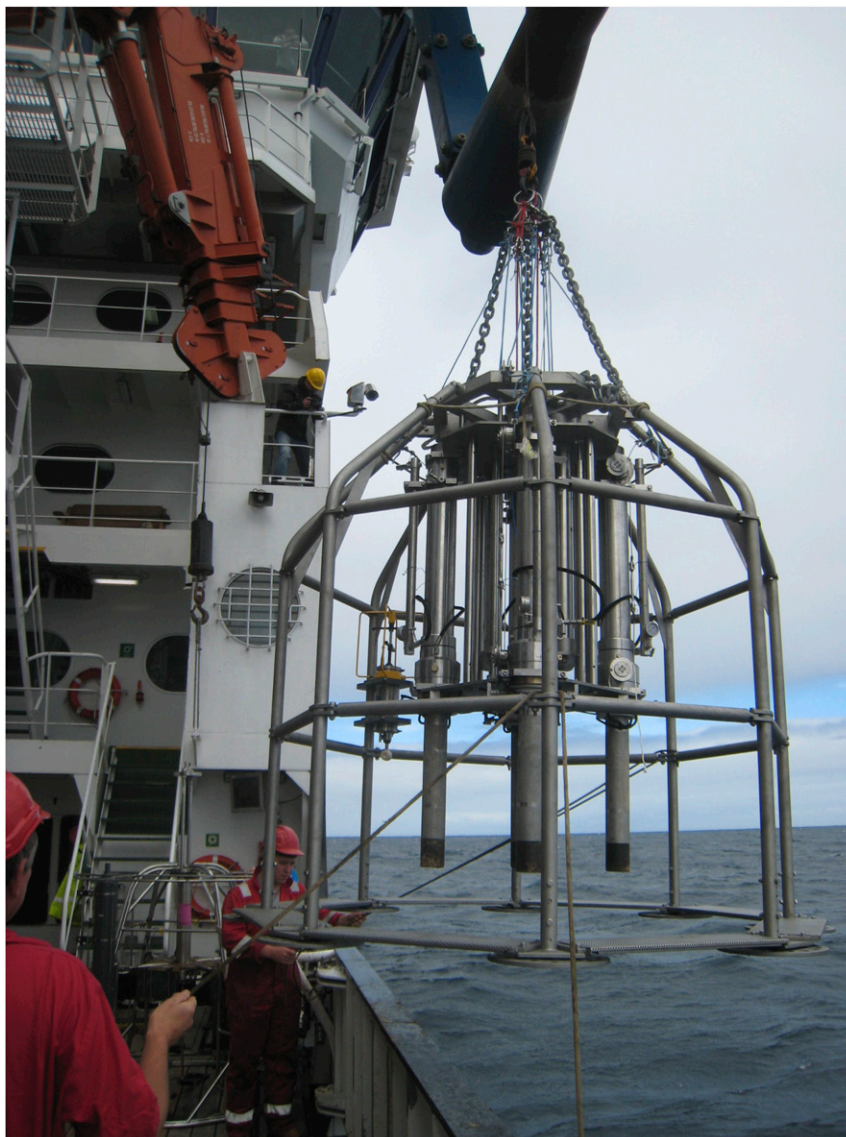


FIG. 1. MAC-EXP corer being deployed.

- A pressure accumulator should be fitted to each corer to maintain a constant pressure in the event of small leaks or changes in temperature.
- Two connections should be available to the interior of corer chamber to allow for connections to an external water circulation system that can refresh the water overlying the sediment core. This allows for control and manipulation of stable environmental conditions (O_2 , temperature).
- A facility is required to allow for the addition of tracer substances into the circulating water and for withdrawal of water samples for further analyses.
- A sensor to monitor temperature and oxygen concentration in the water above the sediment was required.
- A sleeve should be provided around the body of the corer pressure chambers to allow for circulation of water to maintain a stable temperature of the core in the laboratory. Such a sleeve also has the potential for being packed with ice to attempt to

TABLE 1. General specification of parameters.

No. of pressure corers	4
No. of reference corers	1
Core diameter	90 mm
Overall core length	700 mm
Seawater and sediments lengths	100–200-mm overlying water, 600–500-mm sediment
Maximum working pressure	350 bar
Corer action	Piston coring

maintain a low temperature of the sample during its recovery.

- On recovery, the cores will be sealed within metal housings, which makes it difficult to ascertain whether the coring process was successful. To avoid incubating an empty, disturbed, or otherwise unsuitable core, a translucent, unpressurized reference corer was required. Also, to allow for an assessment of the length of sediment core prior to incubation, a window positioned above the pressure chamber top valve was requested.
- The core chambers and their associated accumulators should be easy to remove from the frame, so they can be transferred to a laboratory without depressurizing.
- To control the rate of descent of the core liners into the sediment, the corer requires adjustable dampers.

Since biological gradients are particularly steep near the sediment surface, it was desired that the coring operation should cause as little disturbance as possible to this zone. Therefore, a piston corer was specified. This coring method is widespread and well tested (Kullenberg 1947), and utilizes a piston within the core liner that is held at a fixed height above the sediment surface. The core liner drops around this piston and is pushed into the sediment but friction between the liner and sediment can cause the sediment to be dragged downward into the liner, causing it to become compressed and distorted. However, the presence of the fixed piston means any downward movement of the sediment produces suction against the piston, which resists this movement. The water and sediment column within the liner is essentially locked in place as the liner is pushed down.

With regard to safety, it was required that the equipment conform to the European Pressure Equipment Directive (EU Parliament and Council 2014). The core chambers and accumulators were required to be tested and certified by an accredited test center.

3. Description of corer design and operation

Each of the four corers mounted in the system consists of the following major parts: a pressure chamber, a liner, a piston, and a pressure accumulator. Each part is now described.

The pressure chamber is a cylindrical steel vessel with large ball valves at the top and bottom. The interior of the chamber is of sufficient size to accommodate the core liner, which is sealed within the chamber after coring so that, on return to the surface, the ambient pressure at the seabed is preserved. Three threaded

ports are available in the sides of the vessel. One port allows for a burst disc to be fitted so it releases any dangerous overpressure, another port allows for connection of the pressure accumulator via a flexible hose, and a third port allows for one of the two connections required to the external circulation system. The second connection for the circulation system is via the top ball valve, but to enable the top ball valve to be opened without loss of pressure, a cap can be screwed onto the top of the chamber and pressurized before opening the valve (Fig. 2). The cap not only allows for connection to the circulation system but can also carry a small window to inspect the core through the top valve. Alternatively, the window can be replaced with an oxygen optode sensor to monitor the dissolved oxygen concentration in the water above the sediment. Another cap was developed for connection to a subsampling system that allows for the transfer of a subcore into the cultivation system, DeepIsoBug (Parkes et al. 2009), without depressurization. This has yet to be trialed.

The ball valves were originally fitted with plain valve seats manufactured from a polyoxymethylene polymer (POM) that has sufficient strength to resist deformation under the high forces acting on the valves when the system is pressurized (245 kN on the lower ball at 350 bar). The preferred material was polytetrafluoroethylene (PTFE), as this would have reduced the friction experienced when operating the valves but—like most plastics—it does not exhibit the necessary compressive strength. Trials revealed that the seals were not effective (for reasons discussed later), so additional O-rings were fitted.

The core liner (Fig. 3) is a tube that performs the actual coring operation. It is pushed into the sediment to collect the core and then retains it while being withdrawn into the pressure chamber on recovery. The construction material of the liner was intended to be acrylic, since it is relatively inert biologically and electrochemically. However, for the initial trials, stainless steel was used for its strength, as little was known about the forces involved in coring, particularly when pulling the core out of the sediment, as this could cause the collapse of the liner owing to the suction produced. The body of the liner is threaded top and bottom. At the lower end, a core catcher is fitted onto the thread to help retain the sediment within the liner when the holding action of the piston is released. At the upper end, a collar is fitted onto the thread, which reduces the internal diameter, producing a shoulder inside the bore against which the piston catches during recovery. Continued pulling on the piston then causes it to pull on this shoulder and lift the liner from the sediment and into the pressure chamber. Near the top of the liner is a shallow



FIG. 2. (a) Top cap with optode fitted. (b) Top of core chamber showing ball valve half closed. (c) Top cap fitted and hoses to gill connected.

annular groove on the external surface that is used to clamp it firmly while it is pushed into the sediment. The clamps are released so that the liner becomes free from the corer table before being pulled from the sediment.

The function of the piston is to produce suction to stabilize the core during coring stroke and, following this, to pull the liner from the sediment and into the pressure chamber. The piston consists of two mated parts, an inner and an outer section. These two parts will separate from each other when the piston is pulled against the internal top wall of the pressure chamber (Fig. 3). The inner section can then be retracted on a rope through the narrow top ball valve while the outer section holds the liner inside the pressure chamber by latching to an annular groove on the inside wall of the chamber. At deployment the two sections of the piston are mated and form a watertight seal within the liner but, when separated, the hole left in the outer piston allows access to the top of the sediment core through the top ball valve.

The pressure accumulator (Fig. 4) consists of a stainless steel tube with a freely moving piston inside. The section above the piston is charged with nitrogen to a pressure of around half the expected deployment pressure. The bottom section is attached to the corer

pressure chamber via a flexible hose. Before deployment, the core chamber is at atmospheric pressure, so the piston will be pushed hard against the bottom end of the accumulator. As the corer descends, water can flood the open core chamber and the resulting increase in pressure is transferred into the lower section of the accumulator so that at full deployment depth the piston will have been forced some way up the accumulator cylinder, which further compresses the nitrogen gas above. After the core chamber is sealed, the connection to the accumulator is maintained so that any small leaks of water or changes in volume due to temperature fluctuations or chamber deformation are compensated for by small movements of the piston driven by energy stored in the compressed gas. The accumulator effectively buffers the system to maintain a steady pressure once sealed. A pressure gauge is also fitted to the accumulator port to indicate the pressure inside the core chamber. This is a standard stainless steel industrial gauge filled with glycerin to prevent flooding by seawater. The accumulator is clamped to the side of the core chamber so that the chamber and accumulator can be quickly removed from the frame as a single unit and transported to a laboratory using a modified porter's trolley.



FIG. 3. (a) Assembled piston. Pushing down the outer sleeve (arrow) unlatches inner piston. (b) Outer sleeve is pushed down and ball bearings (arrow) are free to move outward, releasing the inner piston. Ball bearings also latch into the groove on the inside of the core chamber and are held out by an inner sleeve (just visible) that slides up on a spring as the inner piston is withdrawn. (c) Piston and liner assembled.

The assembled corers are mounted on a table that can move vertically within the deployment frame on fixed vertical guide bars. When at the seabed, the corer table slides down its guides after the frame has landed. This causes the liners, which are held by clamps on the

underside of the table, to be pushed into the sediment by the weight of the core chambers and table.

The operation of the ball valves is achieved using wires that are wrapped around pulleys mounted on the valve spindles. At their top end, these wires are

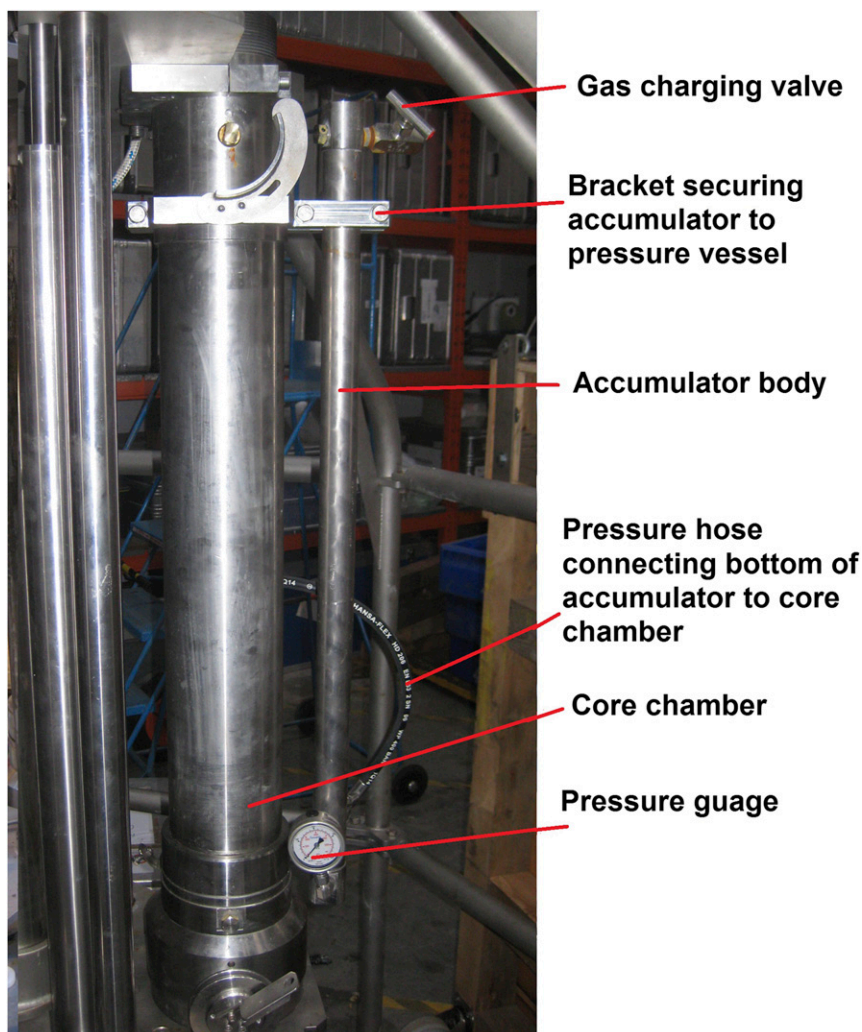


FIG. 4. Pressure accumulator mounted alongside core chamber.

connected to the winch point, which can pull on them, causing the pulleys to rotate, thereby closing the valves.

Details of the corer are shown in Fig. 5, and the sequence of steps in its operation is shown in Fig. 6.

4. Reference corer

A small lightweight corer (Fig. 7) is mounted in the center of the coring table with a 40-mm inner-diameter clear acrylic coring tube that allows for a quick assessment of the success of the coring operation. This is a simple gravity corer rather than a piston corer. The top of the coring tube is open on the downward coring stroke and sealed at the top when the upward recovery stroke begins. The corer was manufactured almost entirely of plastic, including the core catcher, and it proved sufficiently robust to collect cores in trials.

The reference corer is clamped to the coring table in the same manner as the main corer liners. When released from the clamps, it is withdrawn from the sediment by a rope connected to the winch point. The initial pull on this rope triggers the release of the spring-actuated plunger, which seals the top of the core tube.

5. Description of design, mechanisms, and operation of frame

The main frame and its associated mechanisms carry the corers to the seabed and coordinate the sequence of operations necessary to achieve coring. The main frame consists of the following major components: an outer frame, an inner coring table, dampers, a clamp plate, a fixed upper plate, hauling chains, and various ropes and wires (Fig. 8).

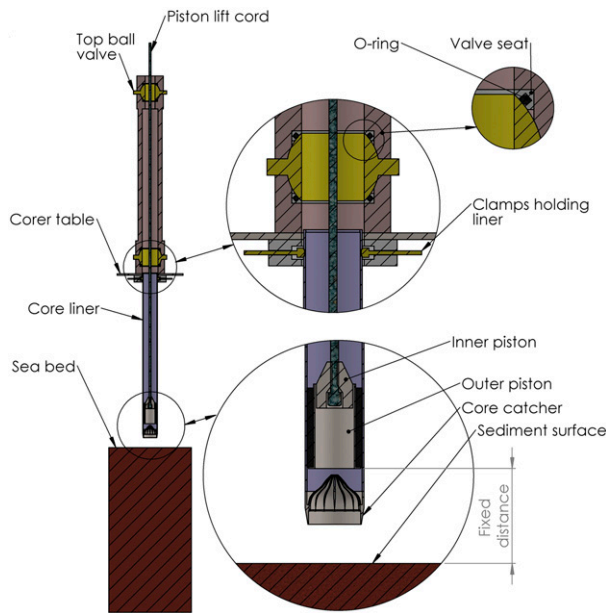


FIG. 5. Diagram showing section of corer at seabed prior to the coring operation. Insets show bottom ball valve detail, ball valve seals, and piston detail.

The outer frame is a large cage structure that is designed to sit squarely on the seabed so that the corers hung from inside it will operate vertically under gravity. Some existing geophysical corers feature a gimbal mechanism such that the internal workings of the system hang vertically even if the frame itself lands on a slope or is tilted by an uneven seabed. The MAC-EXP corer does not have a gimbal, so it may not function correctly if not vertical. The frame measures approximately 2 m high and 2 m in diameter, which allows it to fit within a standard U.K. lorry trailer fully assembled. The top of the frame consists of a substantial ring, the upper face of which has the recovery hauling points attached. On the lower face guide bars are fitted, guiding the movable coring table vertically up and down. If necessary, disassembly and reassembly of the frame is straightforward, since it consists of bolted sections. The total weight of the frame and corers at deployment was 1.0 tonne.

The coring table is a substantial flat plate on which the corers are mounted. It has a framework built on top of it to support the top of the corers and consists of four vertical linking bars and an upper table. Clamps on the underside of the coring table grip the top of the core liners. The whole table with its associated framework, corers, and liners can travel approximately 1 m up and down the guides, which protrude down from the top of the main frame. At deployment, the table is in its highest position such that its upper framework is pulled up

against stops on the underside of the top ring of the main frame. A hook on the upper plate of the table framework is used to lift the whole system into the water with the outer frame resting on top of the table framework. A central chain transfers the load from the hook to the winching eye. As the system touches the seabed, the outer frame will touch down and sit stably. Continued paying out from the winch means the coring table will continue to drop and slide down the guides, causing the liners to be driven into the sediment until a stop on the bottom of the guides is reached. This completes the downward coring stroke and continued paying out from the winch results in the cable going slack, causing the hook on the upper table plate to release from the central lifting chain. The hook falls backward under gravity if there is no load on it and disengages from a chain link.

The dampers are 1-m-long cylinders with internal pistons on rods. They control the rate of descent of the corer table and are connected between the corer table and the top ring of the main frame. Several threaded orifices allow water to enter and leave the internal chambers of the damper and by sealing some of these with screws allows for the adjustment of descent rate.

The clamp plate is a flat plate that controls the corer piston operation and which initially rests on the top of the main frame. The corer pistons are hung by ropes from this plate such that they are at a preset height above the sediment. Because the clamp plate rests on top of the main frame, it remains stationary while the corer table drops during the coring stroke. Since the piston ropes are attached to the clamp plate, the pistons also remain stationary while the liners are pushed down around them as the coring table descends. On recovery, this plate is pulled upward by tension transferred from the winch cable. As the clamp plate rises, tension is transferred from it by ropes to the pistons, which also rise, catch on the shoulder inside the liners, and pull them up until they are completely within the pressure chamber. During this phase the corer table and outer frame do not move. Continued winching causes the pistons to hit the top internal face of the pressure chambers, triggering the inner piston to detach and the outer piston to latch against the pressure chamber wall. The inner piston will be pulled clear of the chamber so that the top ball valve can close. To enable the clamp plate to move only on the upward core-recovery stroke, it is fitted with cam-operated cleats that allow ropes to pass freely downward but which grip the ropes when they move upward. The ropes attached between the winch point and upper corer table plate are pulled down through the cleats as the corers descend into the sediment. As the winch begins recovery, the same ropes are pulled upward, causing the cleats to grip and so causing

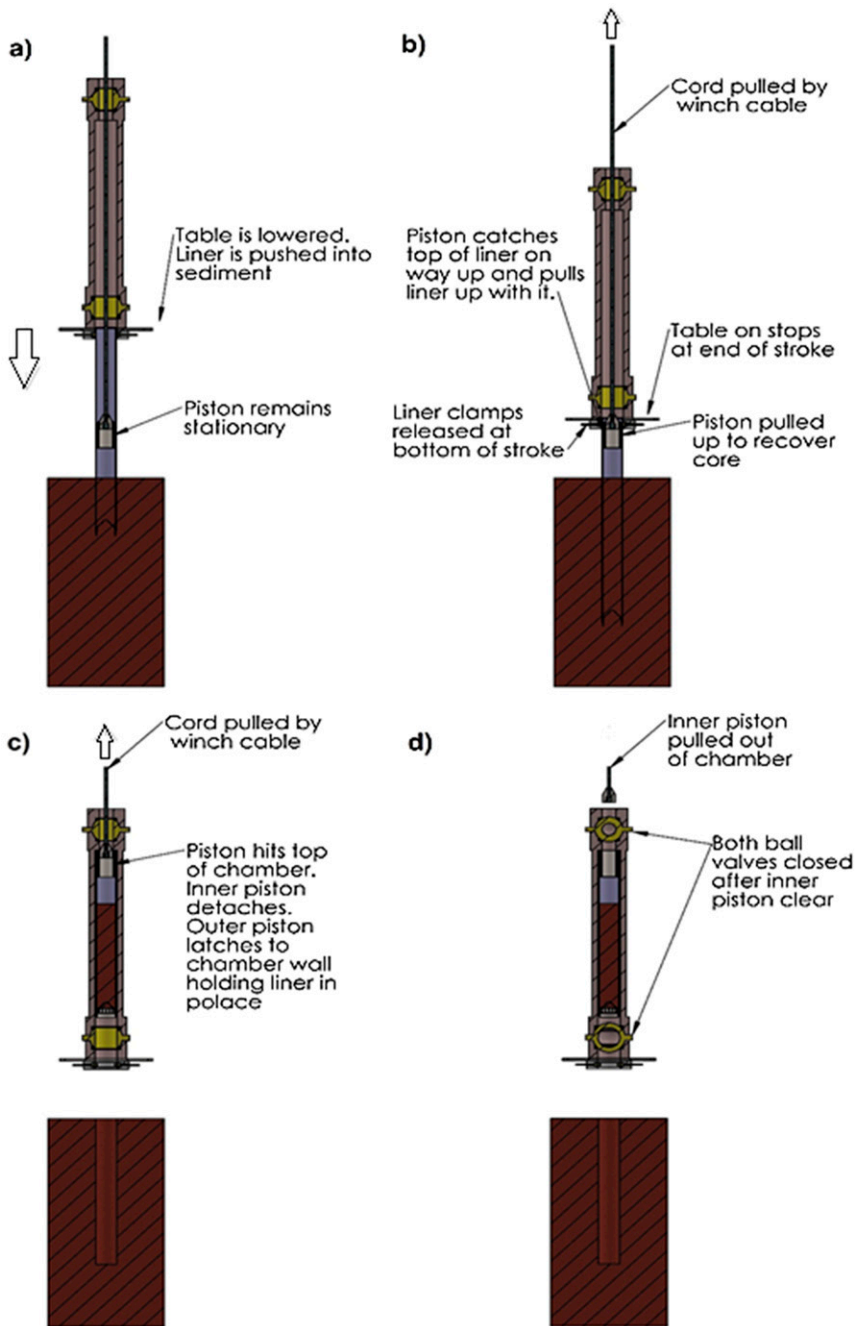


FIG. 6. Corer operation. (a) Coring in progress as the coring table is lowered, taking chamber and liner with it, but the piston remains at a fixed height above the sediment suspended on its cord. (b) End of coring stroke: once the table has reached the end, it stops. Recovery phase starts as the winch begins winding in and applies tension to the piston and the cord that pulls the liner from the sediment. The winch tension also triggers the release of the liner clamps, so the liner is free to be pulled upward. (c) Liner fully withdrawn and the piston hitting the top of the chamber. This triggers the piston to split such that the inner piston pulls free and the outer piston latches to the annular groove on the inside of chamber. Liner is then held fixed inside the chamber. (d) End of the process, where the inner piston and cord are pulled free from the top valve and then both valves are closed.



FIG. 7. Reference corer [computer-aided design (CAD) rendering].

the clamp plate to rise. The attachment of the ropes to the corer table is made using a weak breakable link and so the ropes detach readily when loaded during the upward coring stroke.

The upper plate is essentially an extension of the winching point that allows for attachment of numerous ropes and wires. On deployment, this plate is a fixed distance above the corer table until the end of the downward coring stroke, when the hook on the table detaches. After detachment, the upper plate can be pulled upward relative to the coring table and it will rise by approximately 1 m as the winch cable is taken in. It is attached to the outer frame by two substantial lifting chains that become taut and ultimately lift the whole system from the seabed. However, during its initial 1-m rise, a number of ropes attached to the plate are pulled. The first ropes pulled will release the liner clamps followed by others that pull the ball valves closed. The sequencing of these operations is achieved by adjusting the rope lengths.

The full sequence of operating steps of the system is shown in Fig. 9.

6. Design and operation of gill system

The purpose of the incubation system (referred to as the “gill system”) is to maintain or manipulate the oxygen level and temperature of the water overlying the sediment core (Fig. 10). Existing literature (Boetius and Damm 1998; Bourgeois et al. 2017) suggests the rate of oxygen demand by sediments can vary between 0.04 and 15.5 mmol m⁻² day⁻¹ across a range of shelf sites between 1000- and 4000-m depths. The gill was therefore designed to supply oxygen at a maximum rate adequate to cover the upper end of this range. The gill design followed that of similar systems (Morse et al. 1999; Sommer et al. 2008) but differs in that these systems were designed to work on the seabed during deployment, whereas for the MAC-EXP system the gill operates under pressure but at the surface after recovery of the cores. The gill operates by circulating the water above the sediment core via two high pressure hoses connected between the gill and the ports on the core chamber (Fig. 11). The gill itself is pressurized to the same pressure as the core chamber. The circulation flow rate is measured and stabilized by the control system at a set point value that can be varied from 0 to 30 L h⁻¹. Water enters the core chamber through a side port that is level with the piston. Channels within the piston direct water down and close to the surface of the sediment. The displaced water flows upward, through the center of the outer piston and the top ball valve and into the pressure cap screwed to the top of the pressure vessel, and returns

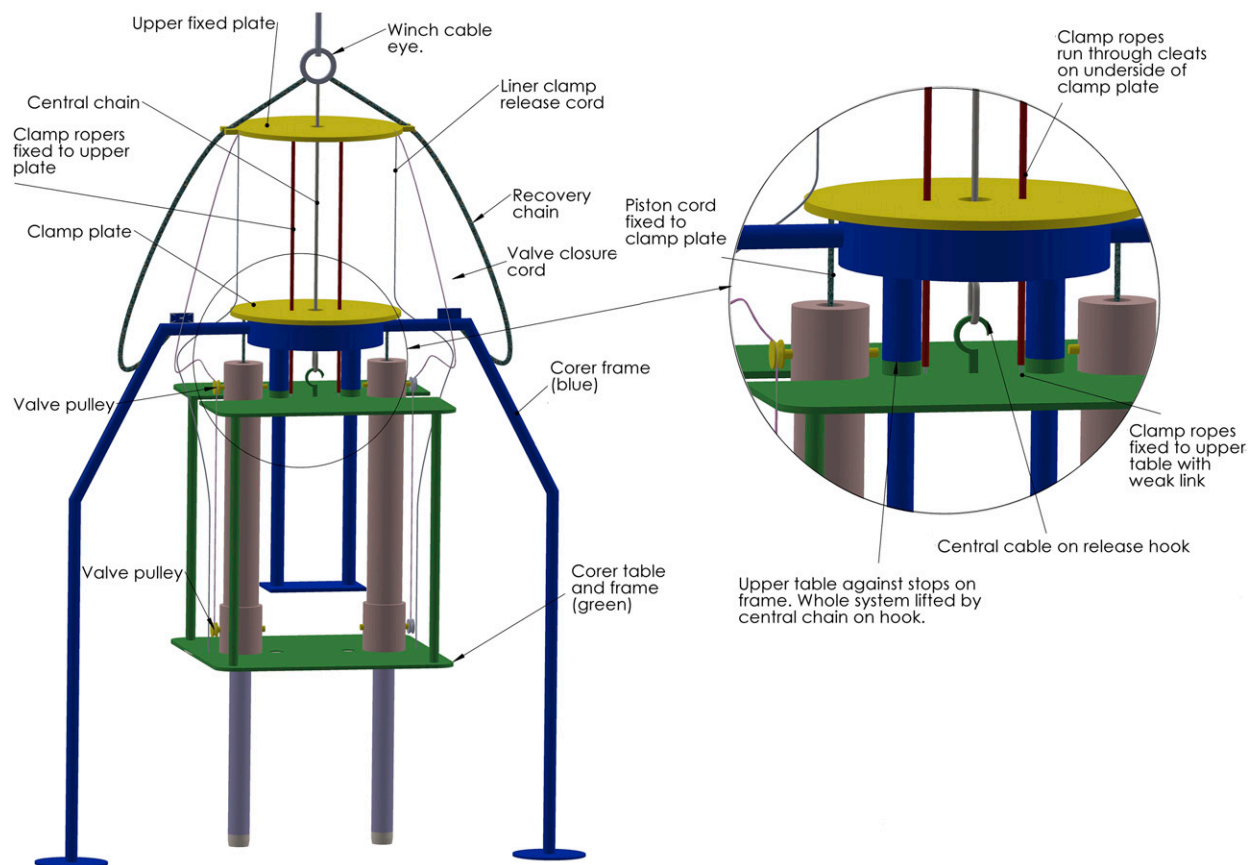


FIG. 8. Diagram of complete system with front and back of frame cutaway. System is shown in the state at deployment when being lowered through the water column.

to the gill through a port on the cap. The constant circulation ensures the water overlying the sediment is well mixed so that a static oxygen concentration gradient does not develop whereby the water immediately above the sediment could be heavily depleted while the optode sensor further up reports a much higher concentration. The top cap is attached, flooded, and pressurized from a hand pump such that the top ball valve can be opened without loss of pressure in the core chamber. The outward-flowing water passes the oxygen sensor in the cap. Connection to the gill is via flexible pressure hoses with isolating valves at both ends such that hoses can be connected and disconnected without having to depressurize either the gill or the core chamber. The adding of tracers or a collection of water samples can be achieved by isolating a hose, disconnecting and draining it, and then refilling it before reconnecting it to the system.

The gill itself consists of a substantial cylindrical pressure chamber (inner diameter: 98 mm, outer diameter: 102 mm, length: 500 mm) with piston-fit endcaps bolted to end flanges. It is filled with oxygenated water

that serves as an oxygen reservoir (Fig. 12). Water circulation is usually routed from the core chamber through the gill pump and immediately back to the core chamber. However, if the measured oxygen level in the core chamber drops, then a valve in the gill chamber can divert the return path of the circulation water through a coiled tube that is permeable to oxygen, thereby allowing it to absorb oxygen from the gill reservoir. This exchanger forms a barrier membrane, keeping the gill reservoir and core chamber fluids isolated from each other. A computer-controlled system monitors the oxygen level in the corer chamber and activates the diverting valve to achieve close regulation of the dissolved oxygen level at a preset value.

The circulation pump used is a small, low-cost, and low-voltage device that incorporates a brushless dc motor (TCS Micropumps Ltd., M500S-180) and is mounted inside the gill chamber. The use of an external pump with its own pressure housing was also an option, but these tend to be made for heavy industrial applications and the cost is significant. The TCS pump has an unusual feature that lends it to this application: the

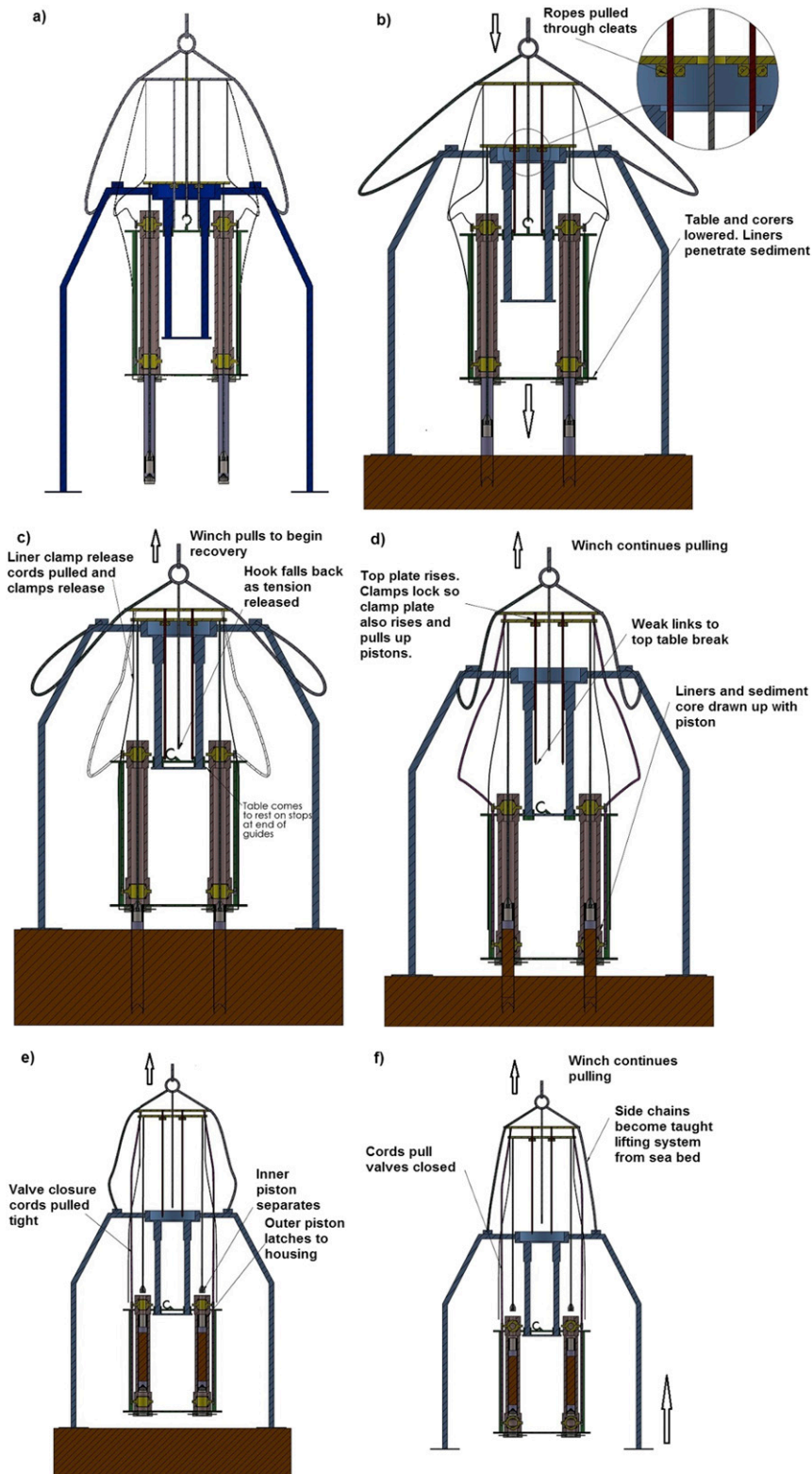


FIG. 9. Frame operation. (a) At deployment. (b) On seabed. Coring stroke in progress. (c) Coring stroke complete, recovery commencing. (d) Liners being drawn into chambers. (e) Cores in chambers, valves starting to close. (f) Valves closed, system leaves seabed.



FIG. 10. Image of gill system connected to pressurized core chamber that has been removed from corer.

entire pump body can flood with saltwater and still operate. The brushless pump motor consists of three stationary coils that are hermetically potted and arranged with radial offsets around a permanent magnet rotor. An electronic drive system is used to energize the coils at the correct times to rotate the rotor. Since there are no exposed electrical parts, the motor compartment can be left open to the pump impeller compartment and will flood, leaving no compressible air spaces within the device. The pump is not designed to operate at elevated pressures, but our own tests indicated it functions without any problems at 400 bar. Long-term exposure to saltwater did mean the nickel-plated rotor magnet eventually corroded but an operating life in excess of 6 months was observed. The pump is available in stainless steel bodies but for cost saving a PTFE-coated aluminum body was used.

A simple low-cost flow sensor was fitted following the outlet of the pump to monitor flow rates (Multicom S8011R). This again was not designed to operate at elevated pressure. It consists of a simple plastic body with a magnetic rotor and potted Hall effect magnetic sensor. There were no exposed electrical parts and testing at 400 bar showed it to function normally.

The changeover valve used to divert flow into the gas exchanger was a simple low-voltage, two-way solenoid pinch valve (Cole-Parmer, WZ-98302-46). This is a simple

contactless device that worked at 400 bar in tests, but the solenoid coil and its terminals are not potted and are open to being flooded, which would be problematic in saltwater. Consequently, the valve, the pump, and the flow sensor were all housed in an acrylic pressure-compensated chamber filled with an inert perfluorocarbon fluid (Flutec PP3). This was suspended inside the main gill vessel on a framework that also held the coiled gas exchanger tube.

The gas exchanger consisted of 2 m of silicon tube (Cole-Parmer, 06411-66). Experiments on this tubing (Morse et al. 1999) indicate oxygen can readily pass across this membrane, but it is virtually impermeable to all other common ions and molecules found in seawater. Small relief valves allow small quantities of water to move between the circulation system and the gill reservoir as required to balance the pressure between them to avoid bursting the membrane.

Electrical connections into the gill chamber were made using standard marine bulkhead connectors (SubConn circular) fitted on the internal faces of the endcaps. A port for an optode sensor (Aanderaa 4835) to monitor reservoir oxygen levels was provided, and a heat exchanger pipe was fitted to the inside of the bottom endcap to allow coolant to flow through the chamber at atmospheric pressure. A coiled plastic pipe and insulation were also wrapped around the chamber to carry coolant (not shown in the figure). Coolant was pumped from a chiller unit around the pipework under the control of a valve operated in response to the reservoir temperature as measured by the optode. A pressure sensor and a hand pump were connected to allow the vessel to be charged to the working pressure with a safety relief valve limiting at 350 bar. A small electric pump was mounted at the base of the chamber to stir the contents. External plumbing and valves used $\frac{1}{4}$ in. stainless steel tubing, stainless steel valves, and flexible nylon hoses—all rated above 350 bar.

The control electronics used a National Instruments USB-6001 data acquisition device that allows for several analog and digital channels to be monitored and provides both analog and digital outputs for control functions. The unit is controlled over a USB link to a laptop programmed using National Instruments LabWindows/C for virtual instrumentation (CVI). The optodes were connected to USB-to-RS232 converters and all items linked to a USB hub near the gill. Consequently, the only connection to the laptop was a single USB connection allowing the laptop to be located well away from the wet environment of the gill.

7. Materials

Most of the components of the corers and frame were manufactured using 316 (A4) grade stainless steel,

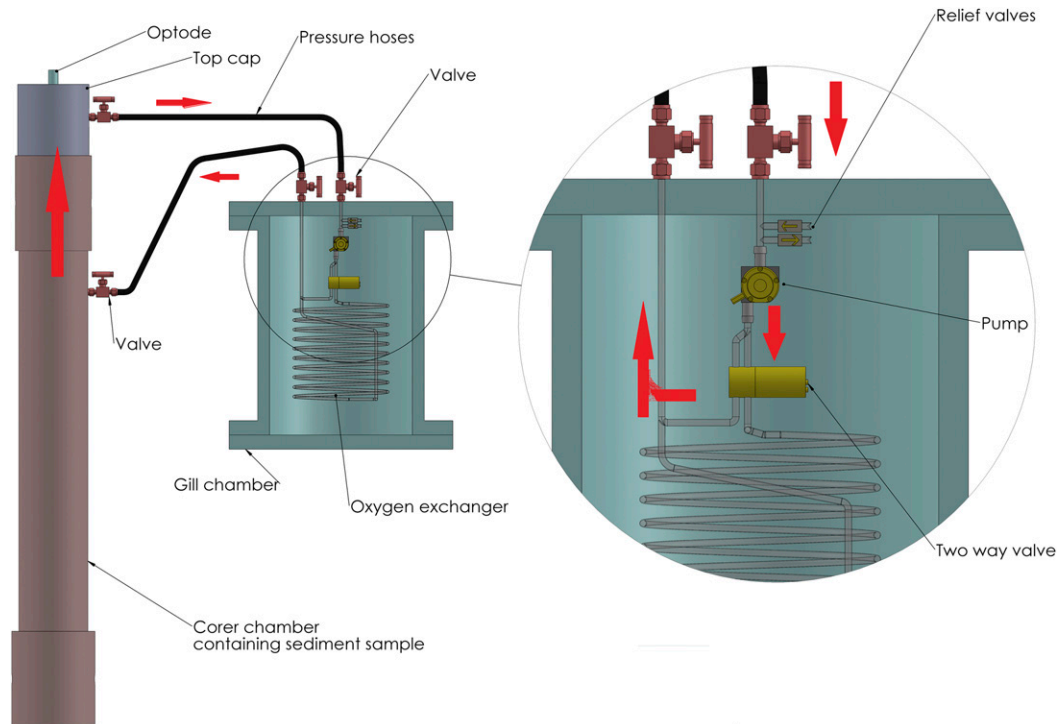


FIG. 11. Simplified diagram of gill system to show its connections to a pressure chamber and to illustrate its operation.

although some corer parts were made from 318 grade stainless steel. Some parts of the corer were also manufactured from aluminum bronze including the valve spindles, since the original stainless steel ones suffered from galling, causing the valves to seize. The aluminum bronze components had no surfaces open to the inside of the sealed chamber, since the copper content could be toxic to marine life and long-term contact with saltwater during incubations could potentially form an electrostatic cell with the neighboring stainless steel, causing corrosion.

The gill vessel was made using 17-4 (630) precipitation-hardened stainless steel, which provides higher tensile strength than 316 grade, which would have required a significant increase in wall thickness and weight.

Use was made of plastic 3D printing to produce many items, such as manifolds, caps, and structural parts (Fig. 13), but initial trials using tabletop extrusion printers were disappointing. The tolerances were found to be inadequate, and the need for scaffolding structures to form overhanging features made printing times very long. The surface finish was found to be poor and necessitated machining of sealing faces and O-ring grooves. Also, for fluid-carrying manifolds it was found that the printed parts were porous along the length of the extruded “sausages” and close inspection revealed that gaps occurred because the extrusions were circular

in section and did not completely tessellate after they fused, which left long open channels between them. To avoid the issues encountered with extruded printing, a commercial 3D laser sintering process was used instead that exhibits engineering-quality tolerances (typically $\pm 100 \mu\text{m}$) and allows for complex shapes to be produced without the need for scaffolding. The resulting parts in nylon PA2200 were very strong and one-off manufacturing costs are very low (approximately EUR 0.15 per cubic centimeter). The parts were found to be very slightly porous to water, but if they were first soaked in cyanoacrylate glue thinned with acetone, this sealed the pores. The material is not only strong but also flexible in thin sections (1–2 mm) and was successfully used to form the fingers of the reference core catchers.

8. Results of trials

The corer system was tested on a 3-week cruise aboard the British Royal Research Ship (RSS) *Discovery* (cruise DY051) in the spring of 2016 in the North Atlantic areas of Goban Spur and Rockall Trough. Much of the cruise was taken up with fault finding or undertaking modifications to get the corer functional, which left little opportunity to undertake cultivations once pressurized cores were retrieved. Twenty-five

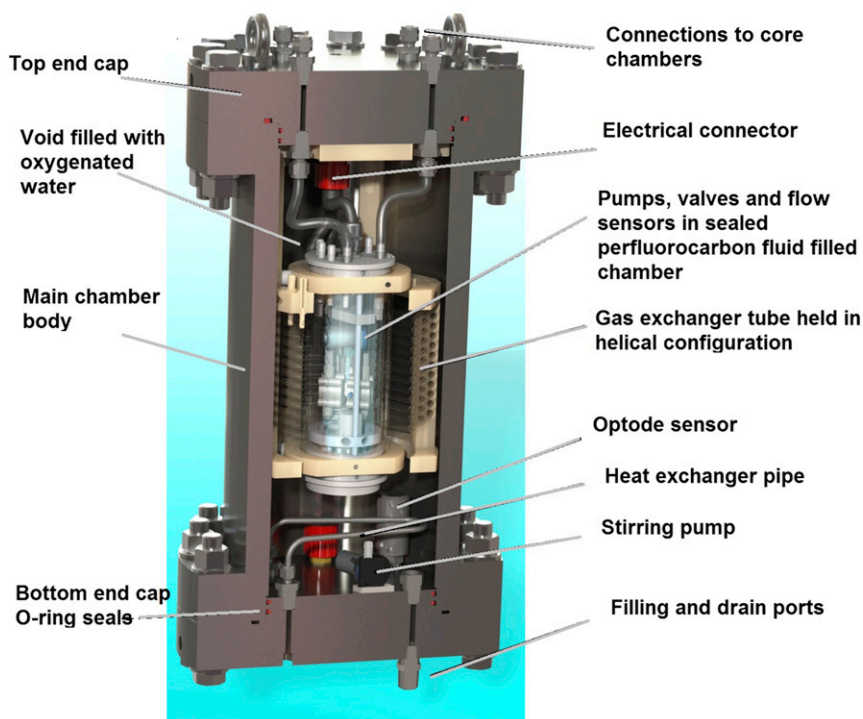


FIG. 12. Gill chamber in section.

modifications were made to the corer before it functioned correctly. These will not be detailed here but several significant issues are now discussed.

The force of extracting the corers from the sediment was much higher than expected. Examination of winch tension data suggested forces up to the equivalent of lifting 250 kg per core chamber were required. With all four corers fitted, this creates an apparent 1 tonne of extra weight on the winch cable and these forces proved too much for some components. In particular, the cleats on the clamp plates slipped and also the top plate became bent. Consequently, the number of corers used at one time was reduced to two and additional ropes and cleats were fitted on the clamp plate.

Ball valve sealing was problematic for three reasons. First, the angular position of the valves when supposedly closed was very variable and so stops were introduced to make this more accurate. Second, the torque required to close the valves became excessive unless the valves were regularly disassembled, cleaned, and lubricated. Using a bungee cord to preload the closing levers was found to help in overcoming friction. Third, the plain valve seats proved problematic, as a seal could be achieved only if the pressure in the chamber rose rapidly such that the ball was quickly pushed into its seat to close any gaps. The use of hand pumps in the laboratory could achieve a seal readily but during deployment the relatively slow rise

in pressure as the corer was winched up allowed water to leak out before sealing pressure was achieved, so at the surface it was consistently found that all pressure was lost. Several remedies were considered. Simply tightening down the seals to preload the force on the seats was not viable, as the turning forces to close the valves became too great. Another approach previously used in a geophysical pressure corer (Abegg et al. 2008) uses an overcharged pressure accumulator and valve to suddenly fire a burst of

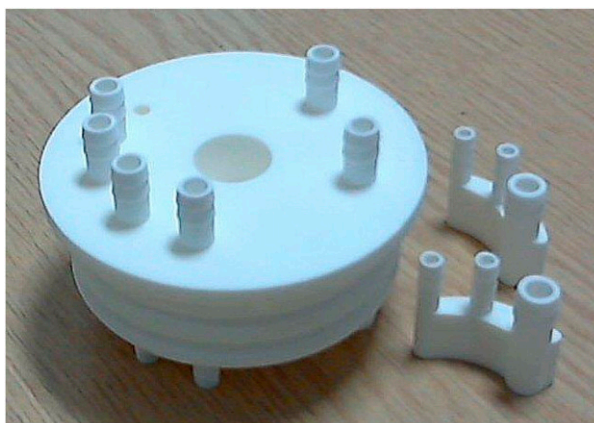


FIG. 13. 3D-printed cap with pipe connections through the body and O-ring sealing grooves. Also, small pipework connection manifolds.

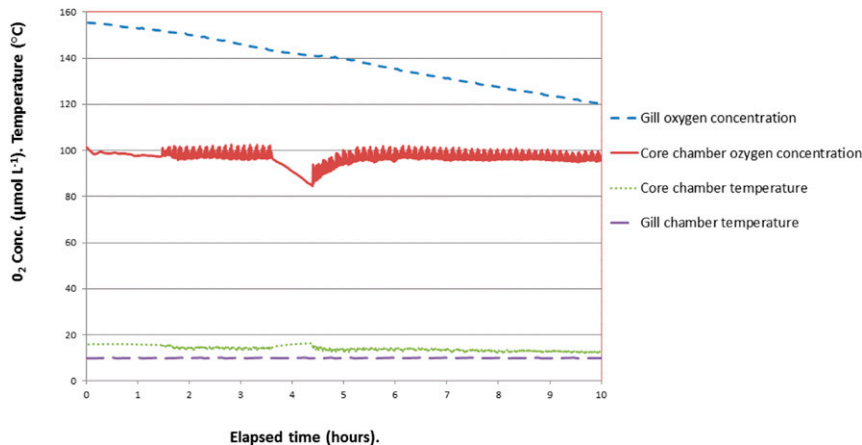


FIG. 14. Gill test. Solid line shows core chamber oxygen concentration controlled at $100 \mu\text{mol L}^{-1}$. Control (but not circulation) switched off around 4 h to measure sediment take-up rate. Sediment from Ythan Estuary, Scotland.

fluid into the chamber just after the valves close, causing a sudden change in the internal pressure that seats the ball and forms a seal. This was not adopted, as it involves considerable extra mechanisms to be fitted to open the accumulator valve and would require facilities on ship to charge the accumulators before each deployment. Also, the effect of sudden changes of pressure on biological samples is unknown and should therefore be avoided. The solution eventually adopted was to machine grooves into the valve seats and install O-rings. This can be problematic with ball valves because when the valve is rotating, some sections of the O-ring are exposed and not forcibly retained in the groove. Consequently, on closing the valve the leading edge of the ball can drag the O-ring from its groove. While this did occur, it was found that if the valves were kept sufficiently well lubricated, then the O-rings were seldom dislodged.

The system of numerous ropes to pull and operate the various mechanisms of the system is a seemingly simple approach but in practice this presented several problems. The large number of ropes is messy and many were initially slack at deployment or become slack during deployment, allowing for entanglement or catching on fixed parts. Adjusting rope lengths to ensure the correct sequence and timing of operations was very challenging, as it is difficult to accurately emulate the coring operation realistically on deck. Also, the effect of twisting as the system is lowered through the water column could potentially cause the large number of ropes attached to the top plate to wrap around the central lifting chain. A swivel was fitted on the winch line to help alleviate this issue.

Three pressurized cores were retrieved during two successful deployments of the system in an area of sea of 1000-m depth. Two of these cores were incubated in a controlled temperature room.

The gill system was initially tested on unpressurized cores that showed it can maintain a constant oxygen concentration in a core chamber to within $\pm 4 \mu\text{mol L}^{-1}$ (tested at a set point level of $100 \mu\text{mol L}^{-1}$) and a constant temperature to within $\pm 0.15^\circ\text{C}$ in the gill and $\pm 3^\circ\text{C}$ in the core chamber at a set point of 10°C (Fig. 14). The variation in oxygen concentration is almost equal to the level of hysteresis as set in the control software, which can be decreased to reduce the ripple produced by the actuation of the diverting valve. The tests were conducted in a very variable temperature environment, and no attempt was made to insulate the core chamber from the external environment. The core chamber was being fed with water at the set point temperature from the gill, but this alone does not achieve good regulation in the core chamber. However, incubations have been conducted in a controlled temperature room aboard ships with greatly improved regulation ($\pm 0.15^\circ\text{C}$). There are also the options of lagging the core chamber or fitting the outer sleeve around the core chamber through which controlled temperature water can be circulated. Either way, temperature variability could be greatly reduced.

The measurement of sediment oxygen uptake rate was achieved by suspending the oxygen control function while maintaining circulation for a period during which the decline in oxygen concentration was noted. For the sample under test, this was $72 \text{ mmol m}^{-2} \text{ day}^{-2}$, which is a high value for a deep benthic sediment but not atypical (Bailey 2005) for the estuarine sediment used in testing.

9. Considerations for future development

During trials the system was subject to many problems that required solutions before success was achieved.

Most of the issues were trivial, but it is suggested that in any future design iterations several aspects of the system could warrant more fundamental redesign. These are discussed below.

The use of ball valves was preferred to a flap system due to anecdotal evidence from designers of earlier geophysical corers that dirt and grit caught in the flaps prevented sealing. Ball valves have a wiping action that tends to preclude foreign matter from getting into the seals. However, as noted in this trial, the valves can be hard to rotate and an effective sealing requires significant back pressure. The fitting of O-rings did achieve successful sealing but problems with O-rings coming out of their seats were an issue. Consequently, some further effort to optimize the ball valve design would be justified to ensure the O-rings remain seated and that friction is reduced.

The use of many actuating ropes that are pulled by the winch eye to operate various mechanisms were the cause of many issues. Incidents of ropes breaking, stretching, and becoming entangled or twisted were common. There is scope for some significant reworking of how the various mechanisms operate and how they are actuated to reduce the number and lengths of these ropes.

The consumption of oxygen by stainless steel resulted in a significant rate of consumption in the gill system vessel and pipework (Fig. 14) that we believe is caused by electrochemical processes involving stainless steel (Cramer 1989). The oxygen level in the gill chamber was seen to drop rapidly and tests showed that even with the gill isolated from the core chamber, oxygen is consumed. It is presumed the same process occurs in the core chamber and liners. While the system is thus fully functional for the purpose of maintaining ambient or experimentally manipulated O₂ concentrations, a system of calibration or a control reference may be needed to allow for compensation of measurements of sediment oxygen demand. The use of plastic core liners and the use of alternatives to stainless steel for other parts (i.e., chambers, pipework, and valves) could be considered. The most obvious alternative is titanium, but this would have a very major impact on costs and we have not yet been able to ascertain if reactions like those between the stainless steel and dissolved oxygen would not still take place. Coating of steel surfaces is also a possible solution to consider.

The issue of regulating core sample temperature during recovery has not been fully investigated. The sediment will typically be collected at an ambient temperature of around 4°C, but this is likely to climb if the corer is winched up through warmer water over a period of typically 30–60 min. The corers were supplied with sleeves to surround the pressure chamber, which could be filled with ice to attempt to stabilize the corer temperature. However, the sleeves were found to provide

insufficient capacity for a useful amount of ice to be included and they could not be easily sealed against flushing through with seawater. Consequently, the sleeves were not used. In a future design iteration, this issue will need some consideration and perhaps other methods evaluated, such as insulation using syntactic foam. The possibility of inserting small dataloggers into the pressure chambers was considered [such as Star-Oddi data storage tag (DST), deep-sea fish tags], which can provide a continuous history of a sample pressure and temperature during recovery and incubation. However, this was not initially pursued because of cost constraints.

Future deployments will allow for testing the use of the DeepIsoBug system with the MAC-EXP system for taking subsamples for manipulations and isolation. Also, incubations using the gill within a full scientific experiment are needed to prove that the various processes required, such as taking samples and introducing tracers, are effective and can be undertaken in a safe and easy manner.

The addition of a video camera to the frame to observe the operation of the system would be useful for the purposes of adjustment and fault finding.

10. Conclusions

The MAC-EXP system has successfully retrieved multiple sediment samples at ambient pressure from depths in the region of 1000 m, and there are no indications to suggest it will not function at a full working depth of 3500 m. This success has demonstrated that the system has the potential for use as a practical method of undertaking onboard-laboratory-based investigations of benthic biological processes on samples that have not been depressurized. The prototype system described here is not without issues, but its operating principle is proven and refinements have been identified that could lead to improvement in the reliability and simplicity of operation of the system. The incubation system is capable of stabilizing oxygen and the temperature of retrieved cores but at present may not be able to allow direct measurement of oxygen consumption.

Acknowledgments. This work was undertaken using funding from the U.K. Natural Environment Research Council (NERC) under the MAC-EXP Project NE/1023465/1 awarded to U. Witte and J. Parkes. The manufacturing of coring vessels was undertaken by Corsyde GmbH, Berlin, and designed by them in collaboration with the Technical University of Berlin. The design and manufacture of the frame was undertaken by K.U.M. GmbH, Kiel. Ship time for trials aboard RSS

Discovery was provided by NERC through National Marine Facilities Sea Systems (NMFSS).

REFERENCES

- Abe, F., C. Kato, and K. Horikoshi, 1999: Pressure-regulated metabolism in microorganisms. *Trends Microbiol.*, **7**, 447–453, doi:10.1016/S0966-842X(99)01608-X.
- Abegg, F., H.-J. Hohnberg, T. Pape, G. Bohrmann, and J. Freitag, 2008: Development and application of pressure-core-sampling systems for the investigation of gas- and gas-hydrate-bearing sediments. *Deep-Sea Res. I*, **55**, 1590–1599, doi:10.1016/j.dsr.2008.06.006.
- Bailey, E. M., 2005: Measurements of nutrient and oxygen fluxes in estuarine and coastal marine sediments: Literature review and data report. Chesapeake Biological Laboratory, University of Maryland Center for Environmental Science Tech. Rep. CBL 05-091, 53 pp. [Available online at http://www.gonzo.cbl.umces.edu/documents/sediments/NChapter_Flux.pdf.]
- Bartlett, D., M. Wright, A. A. Yayanos, and M. Silverman, 1989: Isolation of a gene regulated by hydrostatic pressure in a deep-sea bacterium. *Nature*, **342**, 572–574, doi:10.1038/342572a0.
- Boetius, A., and E. Damm, 1998: Benthic oxygen uptake, hydrolytic potentials and microbial biomass at the Arctic continental slope. *Deep-Sea Res. I*, **45**, 239–275, doi:10.1016/S0967-0637(97)00052-6.
- Bourgeois, S., P. Archambault, and U. Witte, 2017: Organic matter remineralization in marine sediments: A Pan-Arctic synthesis. *Global Biogeochem. Cycles*, **31**, 190–213, doi:10.1002/2016GB005378.
- Chen, J.-W., W. Fan, B. Bingham, Y. Chen, L.-Y. Gu, and S.-L. Li, 2013: A long gravity-piston corer developed for seafloor gas hydrate coring utilizing an *in situ* pressure-retained method. *Energies*, **2013**, 3353–3372, doi:10.3390/en6073353.
- Cramer, A., 1989: A common artefact in estimates of benthic community respiration caused by the use of stainless steel. *Neth. J. Sea Res.*, **23**, 1–6, doi:10.1016/0077-7579(89)90036-7.
- Dickens, G. R., D. Schroede, and H. Kai-Uwe, 2003: The pressure core sampler (PCS) on ODP Leg 201: General operation and gas release. Initial reports, S. L. D'Hondt et al., Eds., Proceedings of the Ocean Drilling Program, Vol. 201, Texas A&M University, 22 pp., doi:10.2973/odp.proc.ir.201.2003.
- Drazen, J. C., L. E. Bird, and J. P. Barry, 2005: Development of a hyperbaric trap-respirometer for the capture and maintenance of live deep-sea organisms. *Limnol. Oceanogr. Methods*, **3**, 488–498, doi:10.4319/lom.2005.3.488.
- EU Parliament and Council, 2014: Directive 2014/68/EU: On the harmonisation of the laws of the Member States relating to the making available on the market of pressure equipment. *Off. J. Eur. Union*, **189L**, 164–259 [Available online at <http://data.europa.eu/eli/dir/2014/68/oj>.]
- Fuhrman, J. A., K. McCallum, and A. A. Davis, 1992: Novel major archaeobacterial group from marine plankton. *Nature*, **356**, 148–149, doi:10.1038/356148a0.
- Glud, R. N., J. K. Gundersen, B. B. Jørgensen, N. P. Revsbech, and H. D. Schulz, 1994: Diffusive and total oxygen uptake of deep-sea sediments in the eastern South Atlantic Ocean: *In situ* and laboratory measurements. *Deep-Sea Res. I*, **41**, 1767–1788, doi:10.1016/0967-0637(94)90072-8.
- Grossi, V., and Coauthors, 2010: Hydrostatic pressure affects membrane and storage lipid compositions of the piezotolerant hydrocarbon-degrading *Marinobacter hydrocarbonoclasticus* strain #5. *Environ. Microbiol.*, **12**, 2020–2033, doi:10.1111/j.1462-2920.2010.02213.x.
- Kallmeyer, J., and A. Boetius, 2004: Effects of temperature and pressure on sulfate reduction and anaerobic oxidation of methane in hydrothermal sediments of Guaymas Basin. *Appl. Environ. Microbiol.*, **70**, 1231–1233, doi:10.1128/AEM.70.2.1231-1233.2004.
- Kullenberg, B., 1947: The piston core sampler. *Svenska Hydrogr.-Biol. Komm. Skr.*, **3** (1), 1–46.
- Lauro, F. M., and D. H. Bartlett, 2008: Prokaryotic lifestyles in deep sea habitats. *Extremophiles*, **12**, 15–25, doi:10.1007/s00792-006-0059-5.
- Morse, J., G. Boland, and G. Rowe, 1999: A 'gilled' benthic chamber for extended measurement of sediment-water fluxes. *Mar. Chem.*, **66**, 225–230, doi:10.1016/S0304-4203(99)00032-8.
- Mrozik, A., Z. Piotrowska-Seget, and S. Labuzek, 2004: Cytoplasmic bacterial membrane responses to environmental perturbations. *Pol. J. Environ. Stud.*, **13**, 487–494.
- Nagata, T., C. Tamburini, J. Aristegui, F. Baltar, A. B. Bochdansky, S. Fonda-Umani, H. Fukuda, and A. Gogou, 2010: Emerging concepts on microbial processes in the bathypelagic ocean—Ecology, biogeochemistry, and genomics. *Deep-Sea Res. II*, **57**, 1519–1536, doi:10.1016/j.dsr2.2010.02.019.
- Nauhaus, K., A. Boetius, M. Krüger, and F. Widdel, 2002: *In vitro* demonstration of anaerobic oxidation of methane coupled to sulphate reduction in sediment from a marine gas hydrate area. *Environ. Microbiol.*, **4**, 296–305, doi:10.1046/j.1462-2920.2002.00299.x.
- Parkes, R. J., G. Sellek, G. Webster, D. Martin, E. Anders, A. Weightman, and H. Sass, 2009: Culturable prokaryotic diversity of deep, gas hydrate sediments: First use of a continuous high-pressure, anaerobic, enrichment and isolation system for subseafloor sediments (DeepIsoBUG). *Environ. Microbiol.*, **11**, 3140–3153, doi:10.1111/j.1462-2920.2009.02018.x.
- Pradillon, F., B. Shillito, C. Jean-Claude, G. Hamel, and F. Gaill, 2004: Pressure vessels for *in vivo* studies of deep-sea fauna. *High Pressure Res.*, **24**, 237–246, doi:10.1080/08957950410001699818.
- Rappe, M. S., and S. J. Giovannoni, 2003: The uncultured microbial majority. *Annu. Rev. Microbiol.*, **57**, 369–394, doi:10.1146/annurev.micro.57.030502.090759.
- Schultheiss, P. J., and Coauthors 2006: Pressure coring, logging and subsampling with the HYACINTH system. *Geol. Soc., London, Spec. Publ.*, **267**, 151–163, doi:10.1144/GSL.SP.2006.267.01.11.
- Shillito, B., G. Hamel, G. Duchi, J. Cottin, J. Sarrazin, P.-M. Sarradin, J. Ravaux, and F. Gaill, 2008: Live capture of megafauna from 2300 m depth, using a newly designed Pressurized Recovery Device. *Deep-Sea Res. I*, **55**, 881–889, doi:10.1016/j.dsr.2008.03.010.
- Sommer, S., M. Türk, O. Pfannkuche, and S. Kriwanek, 2008: Gas exchange system for extended *in situ* benthic chamber flux measurements under controlled oxygen conditions: First application—Sea bed methane emission measurements at Captain Arutyunov mud volcano. *Limnol. Oceanogr. Methods*, **6**, 22–33, doi:10.4319/lom.2008.6.23.
- Yanagibayashi, M., Y. Nogi, L. Li, and C. Kato, 1999: Changes in the microbial community in Japan Trench sediment from a depth of 6292 m during cultivation without decompression. *FEMS Microbiol. Lett.*, **170**, 271–279, doi:10.1111/j.1574-6968.1999.tb13384.x.
- Yayanos, A. A., 1995: Microbiology to 10,500 meters in the deep sea. *Annu. Rev. Microbiol.*, **49**, 777–805, doi:10.1146/annurev.mi.49.100195.004021.
- Zobell, C. E., and C. H. Oppenheimer, 1950: Some effects of hydrostatic pressure on the multiplication and morphology of marine bacteria. *J. Bacteriol.*, **60**, 771–781.