

Submersible operated peepers for collecting porewater from deep-sea sediments

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Abstract

Peepers are a common alternative to push cores for collecting porewater from shallow aquatic environments such as lakes, streams, and wetlands but are infrequently used to sample deep-sea sediments due to design limitations. In this study, a peeper was developed to obtain porewater from precisely located positions on the deep-sea floor using remotely operated or manned submersibles. The sample cells of the peepers can be sealed closed after they are deoxygenated on board a ship to ensure that they remain anoxic until peepers are deployed at the deep-sea sampling location. Similarly, peeper cells can be closed at the end of an incubation period to maintain sample integrity during submersible recovery and until sample processing. Each peeper can be used to collect samples from 10 cm above the sediment water interface and at 10 cm intervals down to 60 cm depth in the sediment, and can provide sufficient volume for multiple chemical analyses, including stable isotope ratio determinations.

Seepage of methane gas and/or oil across the seafloor is widespread at active and passive margins worldwide, and sustains chemosynthetic communities dominated by vesicomyid clams, mytilid mussels, and vestimentiferan tubeworms (Sibuet and Olu 1998). These cold seep macrofaunal communities are often fueled by hydrogen sulfide produced in the sediment by microbial associations that couple anaerobic methane and hydrocarbon oxidation with sulfate reduction (Boetius and Suess 2004; Joye et al. 2004; Levin 2005). Collection and analysis of porewater from these sediments is crucial for understanding the geochemistry as well as the biology at these sites (Arvidson et al. 2004; Levin 2005; Treude et al. 2003). A majority of classic cold seep communities are found at depths greater than 400 m (Sibuet and Olu 1998), and porewater sampling at these sites is typically conducted using manned or remotely-operated submersibles.

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Push cores and peepers are two of the most common methods for collecting sediment porewater. Peepers were introduced by Hesslein (1976) and are based on equilibration of fluid contained within a cell with the surrounding medium across a dialysis membrane. Sediments collected using cores need to be centrifuged or squeezed under pressure to obtain porewater samples, which can lead to serious post-sampling artifacts (Bollinger et al. 1992; Holcombe et al. 2001). On the other hand, samples collected using peepers are already particle free and require minimal post-collection processing (Bollinger et al. 1992; Hesslein 1976). This has made peepers a popular alternative to push cores for sampling sediments in a variety of aquatic environments such as lakes (Bollinger et al. 1992; Carignan et al. 1994), wetlands (Laforce et al. 2000), and salt marshes (Howes et al. 1985; Weston et al. 2006).

Sampling using push cores in deep-sea cold seep environments suffers from several drawbacks. Sediment profiles obtained using push cores collected with submersibles are usually limited to less than 30 cm in depth. Moreover, push cores do not normally retrieve good samples from sediments that contain mussel or clam beds, bivalve shell hash, tubeworm roots, or carbonate rubble, all of which are common in cold seep environments. Since seepage is usually associated with robust animal communities, this is a serious limitation of relying on push cores for characterizing porewater chemistry of cold seep communities. Also, decompression or warming of cores during submersible retrieval can rupture bacterial cells and change concentrations of some porewater constituents (Aller et al. 1998). This is compounded if gas

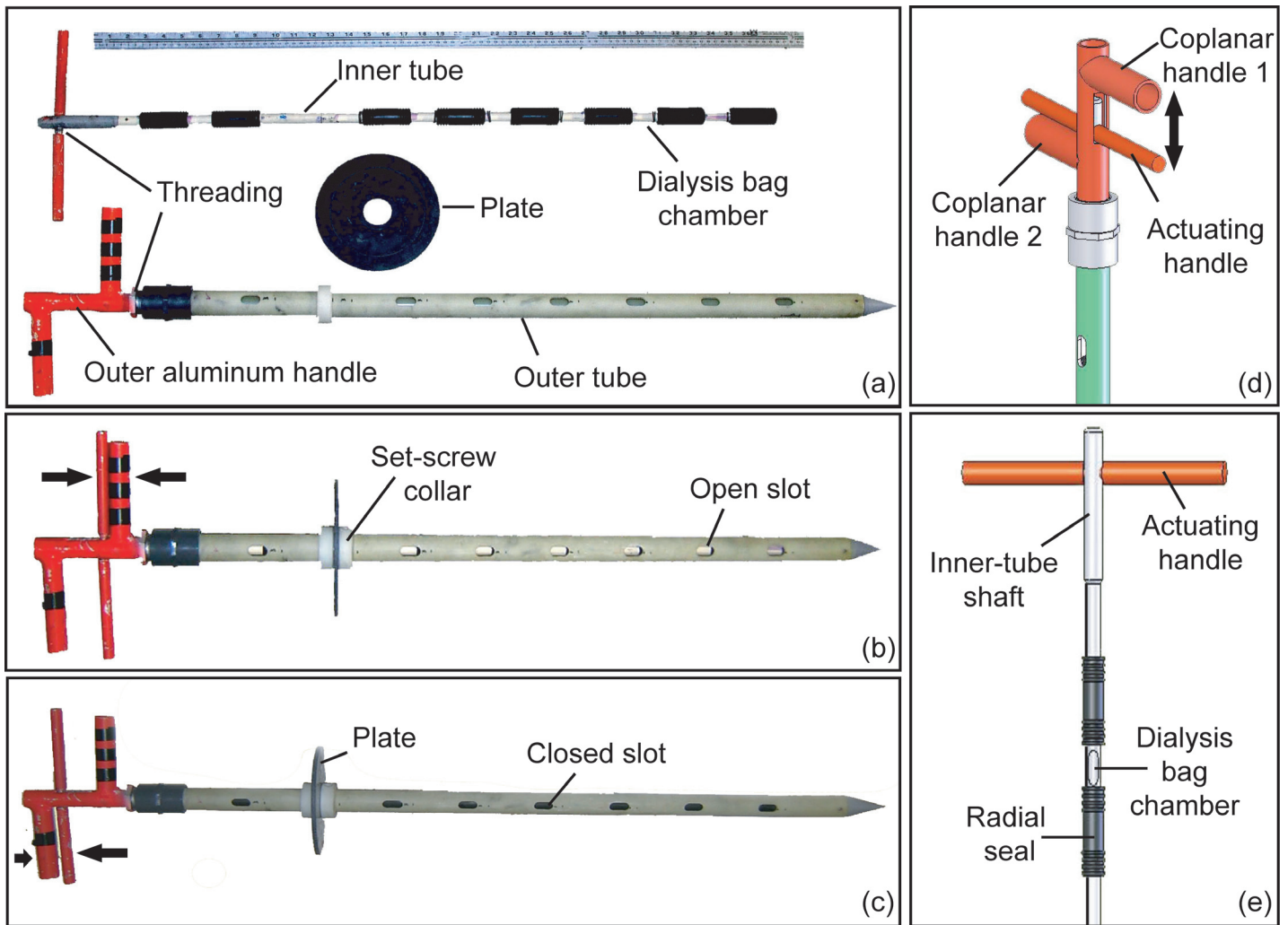


Fig. 1. (a) Peeper dismantled to show its component parts. The meter stick on the top of the picture is included for scale. (b) Peeper in “open” position; (c) peeper in “closed” position; (d) schematic view of peeper handle assembly, and (e) schematic view of inner tube showing actuating handle and details of radial seals.

hydrates are present in the cores as their decomposition and the resultant gas expansion can compromise core integrity. Therefore, there is a need for an effective alternative to using cores for collecting sediment porewater from biogeochemically active deep-sea sediments.

Although the use of peepers can overcome some of the problems associated with push cores, using peepers in the deep-sea can be problematic because samples might back-equilibrate with ambient seawater during the time between collection on the sea floor and processing on board a ship (Aller et al. 1998; Hashimoto et al. 1995; Holcombe et al. 2001). In this study, peepers were specifically designed for sampling biogeochemically active deep-sea sediments. They can be opened and closed to maintain sample integrity, and are amenable to operations conducted using a single robotic arm of a remotely operated or manned submersible.

Materials and procedures

Overall design and construction of peepers—Peepers were comprised of two coaxial cylindrical tubes (each with seven sampling slots at 10 cm intervals), an actuating handle assembly, a circular plate, and a conical substrate-penetrating tip (Fig. 1). The inner tube housed the sample cells and the outer tube formed an encasing sleeve. Sample cells contained dialysis tubing that was sealed at both ends to form cylindrical bags (hereafter referred to as “dialysis bags”). Peepers were designed to toggle between two modes. In one mode, the slots of the inner and outer tubes aligned so that sample cells were open to the environment. In the other mode, the inner tube was repositioned so that its slots no longer aligned with the outer slots, and the sampling cells were sealed off from the external environment. Peepers were toggled between open and closed modes using a submersible manipulator by squeezing together

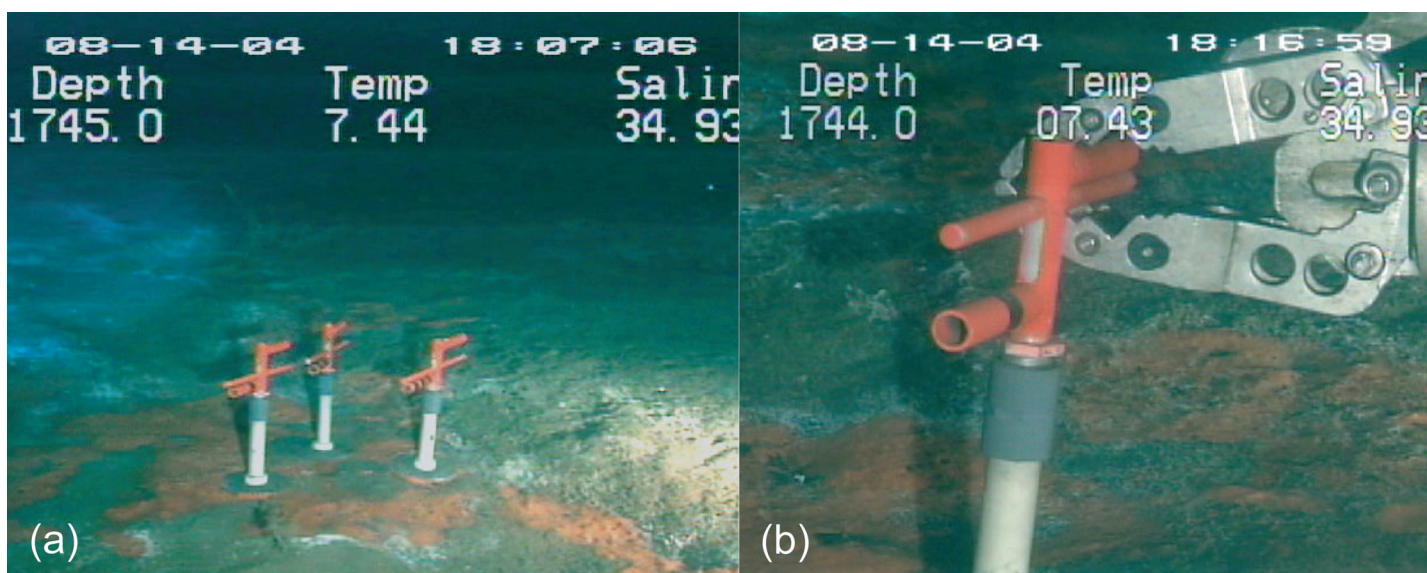


Fig. 2. (a) Peepers deployed in situ with plates level with the sediment-water interface, in sediment underlying a *Beggiatoa* bacterial mat. Handle assembly is in the “open” position. (b) Peeper handle being “closed” by the robotic arm of the Johnson Sea-link submersible during collection of the device.

the appropriate handles at the top of the two cylinders (Figs. 1b, 1c, 1d, and 2b). Peepers were also designed so that they could be easily and quickly assembled from and disassembled into their component parts.

The chambers of the inner tube were constructed using 10 cm sections of half an inch nominal diameter chlorinated polyvinylchloride (CPVC) hollow pipe, with 1 cm by 4 cm windows milled into them (Figs. 1a, 1e). Consecutive chambers were separated using solid PVC plugs that served to prevent fluid flow between the chambers. Seven chambers were stacked axially so that the centers of their windows would be positioned 10 cm apart from each other, except the first and second windows, which were 20 cm apart. Custom-made butyl rubber radial seals (Iris Rubber Company, Cicero, Indiana, USA), with ribs at the top and bottom, were cemented to either side of each inner tube window (Fig. 1e). When fitted over the inner tube, the seals had an outer diameter (OD) of 24.2 mm, and the ribs had an OD of 24.8 mm, which allowed a tight seal with the inner diameter (ID) of the peeper outer tube.

The outer tube was a 90 cm long hollow tube with 1 cm by 2.5 cm slots milled into its side and positioned to align with the inner tube windows (Fig. 1). The outer tube was constructed using G-10 Garolite, which was chosen due to its high tensile strength and stringent ID tolerance (± 0.008 cm). The handle assembly was threaded into the top of the outer tube, and a solid PVC cone was fastened to the bottom end using a removable stainless steel split pin. A circular PVC plate with a hole in its center was secured circumferentially on the outer tube by sandwiching it between two shaft collars. The plate was positioned halfway between the first and second slots of the outer tube. During sampling, the plate was seated at the

sediment-seawater interface (Fig. 2a) to ensure that the first slot was 10 cm above the interface, whereas the remaining slots were at 10 cm depth increments below the interface. The plate also served to prevent channeling of overlying seawater down the length of the peeper.

The handle assembly (Fig. 1d) was comprised of three parts: (i) a solid PVC shaft attached to the inner tube assembly, (ii) a two-part actuating aluminum handle threaded through a hole in the inner shaft and the slots in the outer tube, and (iii) an outer aluminum handle with slots on the side and two welded coplanar handles. The actuating handle could be moved to shift the position of the inner tube with respect to the outer tube. When the actuating handle was squeezed together with coplanar handle 1, the windows in the inner tube were aligned with the slots on the outer tube. In this position (the “open” position; Fig. 1b), dialysis bags placed inside the inner tube chambers would be open to the environment. Conversely, when the actuating handle was squeezed together with coplanar handle 2, it aligned the radial seals with the slots in the outer tube. In this position (the “closed” position; Fig. 1c), the sample cells were sealed closed. The handle assembly could be disassembled quickly to facilitate removal of samples from the inner tube chambers for processing.

Preparation of dialysis bags—Noncellulose-based, heat sealable, polyvinylidene difluoride (PVDF) membrane (500 kilodalton molecular weight cut off, 16 mm wide tubes, Spectrum Laboratories, Inc.) was used to make dialysis bags. The PVDF membrane material was chosen as it was resistant to degradation by aromatic and nonaromatic hydrocarbons that might be present in hydrocarbon seep sediments (Joye et al. 2004). After purchase, the dialysis membrane was stored in cold (4°C) sulfate-free artificial seawater (SF_ASW) containing 0.05%

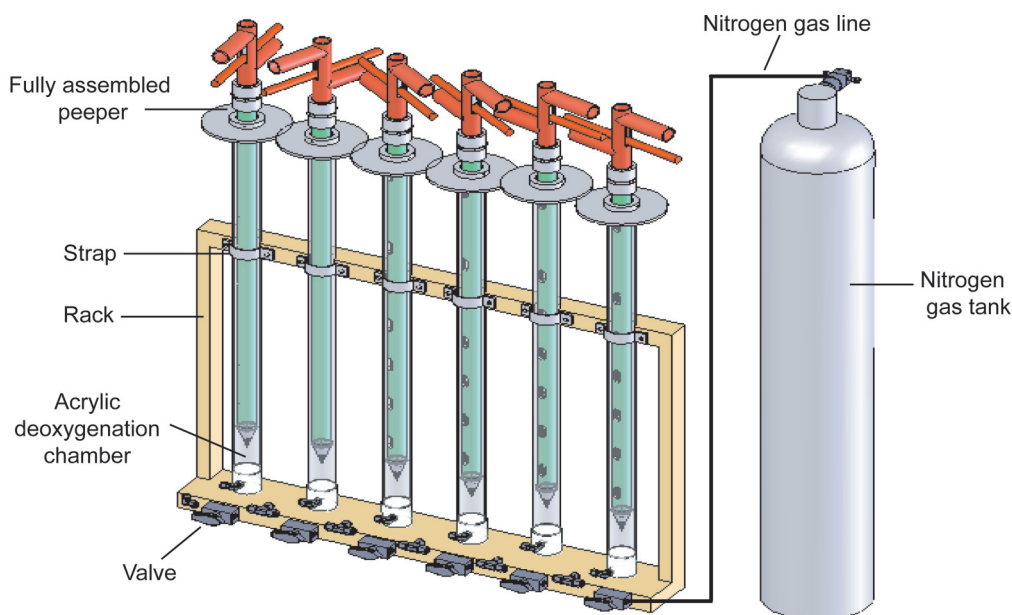


Fig. 3. Schematic representation of the set-up used to de-oxygenate peepers before deployment.

sodium azide to prevent bacterial growth. To prepare the dialysis bags, 6 cm sections of the PVDF membrane tube were cut and soaked in 20× volume of distilled de-ionized water (DDI water) for one hour, with two intermediate water changes, to remove the sodium azide. The insides of the tubes were flushed ten times with DDI water before they were heat-sealed at one end. The tubes were then filled with 4 mL of SF_{ASW} (salinity = 36‰, pH = 8.0) containing 500 mmol L⁻¹ sodium chloride, 9 mmol L⁻¹ potassium chloride, 9.3 mmol L⁻¹ calcium chloride, 48.5 mmol L⁻¹ magnesium chloride, and 2.5 mmol L⁻¹ sodium bicarbonate. SF_{ASW} was used instead of distilled water, as peeper compartments filled with water less dense than the surrounding porewater could cause shifts in concentration profiles (Grigg et al. 1999). Air bubbles from the SF_{ASW} in the dialysis tubing were removed, and the other end of the tube was heat-sealed to form a cylindrical bag. One bag was placed inside each chamber of the inner tube of a peeper, and covered with a 1.5 × 5 cm² piece of nylon mesh held by rubber bands to protect the membrane from damage. The peeper was then assembled and placed inside a de-oxygenation chamber.

De-oxygenation procedure—Fully assembled peepers were de-oxygenated in the “open” position inside chambers comprised of 1.5 m long, 4 cm ID acrylic cylinders with PVC end caps (Fig. 3). A gas inlet was threaded into a hole in the PVC cap to allow introduction of a constant stream of nitrogen gas. Six de-oxygenation chambers were mounted upright onto a rack, and one peeper was loaded tip first into each chamber. The PVC plates were moved on the peepers up above the top windows so that they served to loosely cap the top of each chamber. The de-oxygenation chambers were filled with SF_{ASW} and continuously bubbled with nitrogen gas for approximately 6 h. After 2 h bubbling with nitrogen gas, the oxygen

level in the SF_{ASW} inside each de-oxygenation chamber was determined to be zero using a portable oxygen meter. The pH of the SF_{ASW} inside the chambers increased from 8 to approximately 8.6 as a result of stripping CO₂ during the degassing process. Before deployment, the peepers were closed while they were inside the de-oxygenation chambers and loaded onto the submersible approximately 10 min before commencing the dive.

Deployment and recovery of peepers—Twelve peepers were deployed and recovered in the summer of 2004. On a single dive, six peepers were placed inside individual PVC quivers mounted onto the *Johnson Sea-link* submersible for transport to the sampling location, site GC234 located at a depth of ~540 m on the upper Louisiana slope of the Gulf of Mexico (27°44.7'N, 91°13.3'W). This site is dominated by large tubeworm aggregations that occur at varying densities over an area of several square kilometers (Bergquist et al. 2003; Macdonald et al. 1990). Once at the sampling location, peepers were inserted tip first in a vertical orientation into the sediment until the PVC plates of the peepers were level with the sediment-seawater interface (Fig. 2a). The robotic claw of the submersible was then used to manipulate the actuating handles of the peepers to “open” them. Peepers were allowed to equilibrate for 5 weeks, at the end of which they were “closed” (Fig. 2b), collected, and brought to the surface where they were processed on board the ship.

Post collection sampling and analyses—Once on board the ship (*Seward Johnson II*), peepers were dismantled one at a time and sampled in a coordinated manner so that each peeper was processed completely within 2 min of its opening. Water samples were used for analysis of pH and salinity, sulfate, sulfide, and dissolved methane gas concentrations, and sulfur stable isotope ratios. After dismantling the peepers and extracting

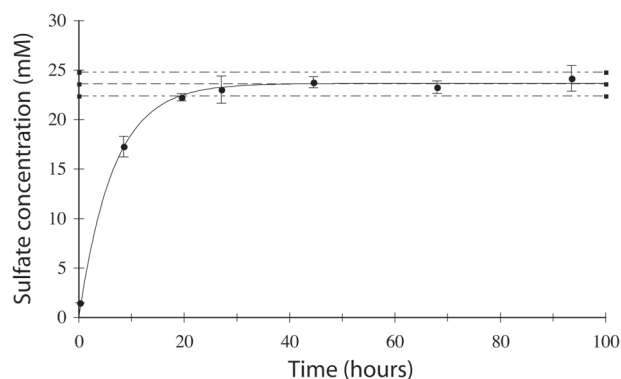


Fig. 4. Average \pm standard deviation (SD) of sulfate concentrations ($n = 3$) inside dialysis bags at various time points during an equilibration experiment. The dashed lines show the average \pm SD of sulfate concentration in the solution outside the bag, which did not vary over the course of the experiment. The solid line shows the best-fit curve corresponding to the equation $C_i = 23.6 * [1 - \exp(-0.153 t)]$, where C_i is the sulfate concentration inside the bags at time, t .

the inner tube, the mesh covering from each window was removed. Syringes were used to draw samples for pH, salinity, and methane analyses. After syringe samples were withdrawn, dialysis bags were removed from the inner tube chambers, and the contents were emptied into vials containing zinc acetate fixative solution. These samples were mixed well, centrifuged, and the supernatant was transferred to a different vial for sulfate concentration and stable isotope analyses. The zinc sulfide precipitate was used for sulfide concentration and stable isotope analyses.

Assessment

Twelve peepers were successfully deployed and collected from a hydrocarbon seep site located at 540 m depth on the seafloor of the Gulf of Mexico, using the manned *Johnson Sea-Link* submersible. Sediment depth profiles of pH, salinity, methane, sulfate, and sulfide concentrations were obtained from all 12 peepers. Moreover, stable sulfur isotope compositions of both sulfate and sulfide were determined whenever porewater concentrations were sufficient for isotope analyses (unpubl. data). Ambient seawater values were determined using dialysis bags positioned 10 cm above the sediment-seawater interface. The average sulfate concentration was found to be 29.5 mmol L⁻¹, and the average sulfate stable isotope composition to be 20‰, similar to previously reported values of ambient seawater in the Gulf of Mexico (Aharon and Fu 2000).

The peeper design was extremely effective for rapid sampling of the porewaters after recovery by the submersible. Samples were obtained and processed from six peepers within 15 min of retrieval. The rubber radial seals maintained excellent sample integrity after collection. The effectiveness of the radial seals was tested using an experiment in which two peepers loaded with dialysis bags filled with DDI water were attached in the “closed” position to the outside the Johnson

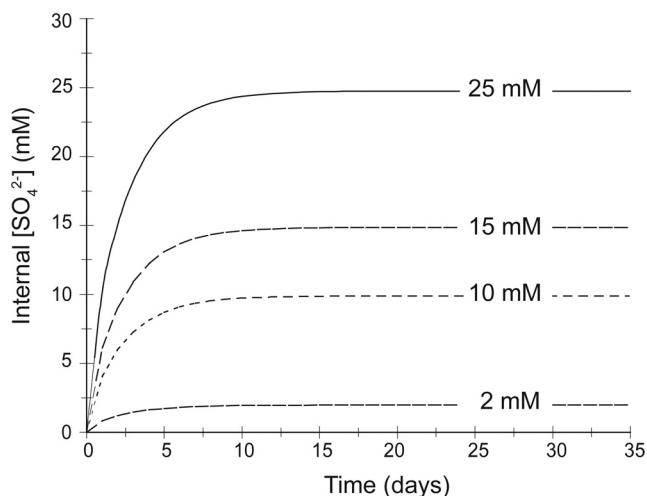


Fig. 5. Results of a model in which sulfate diffusion from the surrounding sediment into a dialysis bag was simulated at various porewater sulfate concentrations.

Sea-link submersible, and carried to depths up to 650 m during a 4 h dive. Since the peepers were exposed to seawater during the entire dive, there was a strong gradient for diffusion of chloride ions into the bags. However, the radial seals prevented diffusion of seawater into the sample compartments, and the salinity of the water contained within the 12 dialysis bags remained at 0‰ when sampled at the end of the dive.

To estimate equilibration times for the peepers, a model was constructed to simulate diffusion of sulfate (one of the major ions of interest) through the sediment into the peeper cells. Since diffusion coefficients of methane and sulfide in seawater are greater than that of sulfate (Iverson and Jorgensen 1993; Stumm and Morgan 2000), the estimates obtained for sulfate ions are conservative. The sediment diffusion coefficient of sulfate was calculated using the equation $D_s = D_o / (1 + n(1 - \phi))$ (Iverson and Jorgensen 1993), where D_s is the sediment diffusion coefficient, $D_o = 0.501 \text{ cm}^2 \text{ day}^{-1}$ is the diffusion coefficient in seawater (Li and Gregory 1974), $n = 3$ for clay-silt sediments, and $\phi = 0.765$ is the porosity of the sediment at our study site (Cordes et al. 2005). The diffusion coefficient of sulfate through the dialysis membrane was determined using an equilibration experiment performed with the dialysis bags inside a cold room maintained at 6°C (Fig. 4), using previously described calculations (Sten-Knudsen 1978). For this experiment, dialysis bags were filled with SF_ASW and suspended inside a container with 2 L of SF_ASW containing 24 mmol L⁻¹ magnesium sulfate. The simulation results of the diffusion model indicated that the peeper cells would reach steady state, at least with respect to sulfate concentration, within 15–20 d (Fig. 5). This estimate agrees well with the 20 d equilibration time recommended by Carignan (1984) for cold (4–6°C) sediments. In our study, the peepers were incubated in situ for 15d, in excess of the time required to reach steady state.

Comments and recommendations

One of 12 of our peepers broke at the shaft of the inner handle during the closing process. The inner handle shafts had been constructed using solid PVC, and replacing that part of the handle with stainless steel might be better for use with submersibles. The material for the outer tube should be chosen carefully, as materials such as PVC that are extruded into tubes have too large a variation in inner diameter to be used for this design. If reduction in costs were a priority, sections of rubber tubing could replace custom radial seals, and cheaper noncellulose based dialysis membranes tied on either end with nylon string could replace the PVDF membrane. The vertical resolution (10 cm) of our peepers might not be sufficient for certain applications, in which case our design could be modified to make peepers with improved depth resolution. It is important to note that channeling of water around the shaft of the peeper could occur if the devices are not deployed vertically until the top plate is sealed against the sediment.

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