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Front Cover: Lee-side exposure of a fossil parabolic dune viewed from the Grahams Harbour side (west) of North Point, San Salvador, Bahamas. These Holocene carbonate eolianites have been assigned to the North Point Member of the Rice Bay Formation (Carew and Mylroie, 1995). The eolian cross-stratification dips below present sea level, proving that late Holocene sea-level rise is real. Top of the dune is about 7 meters above the sea surface. Photo by Al Curran.

Back Cover: Dr. Noel P. James of Queen's University, Kingston, Ontario, Canada, keynote speaker for this symposium. Noel is holding a carving of a tropical fish created by a local artist and presented to him at the end of the symposium. Photo by Al Curran.

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STERILE AND GENERAL SAMPLING TECHNIQUES FOR SUBMERGED CAVE ENVIRONMENTS

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ABSTRACT

Relatively simple and effective techniques have been devised for collection of water samples for sterile and non-sterile bacterial work and for collection of sediments and rock cores in submerged cave environments. These procedures allow for more rapid and organized sampling schedules and also reduce the chances of losing samples once collected. These materials, which in most cases are commercially available and relatively inexpensive, can reduce a diver's workload and improve collection time and sample numbers per dive once properly prepared for transport into the cave environment. All methods described here are cave-friendly and should leave little to no indication that any collection work has occurred.

INTRODUCTION

The need for new and improved sampling methods which could be used in submerged cave systems in the Bahamas, commonly known as "Blue Holes", (Benjamin, 1970; Palmer, 1984, 1986) became apparent following projects on which samples were collected exclusively for geochemical analysis. Blue Holes are laterally, and often vertically, complex and extensive overhead environments (i.e. a diver cannot ascend directly to air) in which special diving skills are required for both exploration and research.

Geochemical analytical results (Whitaker and Smart, 1993; Smart et al., 1988) revealed the possibility of biogenic activity and an apparent need to take a closer look at water samples for bacteria and bacterial activity. Water samples for ground-water analysis for earlier work were collected by using one meter long PVC tubing, approximately 10 cm in diameter. These tubes were held by the diver with both ends open. The diver would try to swim at exactly the same depth range while maintaining a steady forward speed, thus preventing water mixture of the water column ahead of the diver. This was especially important in cave environments with a fresh / salt water mixing zone and other forms of distinct vertical stratification.

Obviously, when the focus of a project is to look at the water chemistry and bacterial presence at 20 cm intervals within the water column, a non-sterile, 10 cm diameter pipe, one meter long, is not an acceptable tool. The tools and techniques which are described here are sterile, or can be sterilized, and they allow for precise placement and minimal to no disturbance of the water column to be sampled. Methods created for improving water sampling techniques were also used for collecting sediments with the same objectives of not contaminating or disturbing sample sites. Small 8 to 10 cm rock cores were recovered using a modified pneumatic socket wrench attached to a scuba cylinder. To achieve a straight entry during coring, the pneumatic socket wrench was used in conjunction with a Wheeler core base made out of PVC.

MATERIAL AND METHODS

Water Sample Tubes

Water samples collected for geochemical analysis were collected using 0.5 liter water sample bags, medically known as "intravenous bags". Larger bags can be purchased. Water samples were collected using these sterile intravenous bags, purchased from S.P. Services in Shropshire, UK (Figure 1) which came from the factory containing 0.5 liters of sterile 0.5% NaCl solution. The intravenous solution was drawn out of the bags and the bags were flushed with sterile distilled water to remove all remaining salt. Before use, sample bags were flushed four times, and then filled with sterile distilled water. Sub-samples were removed and conductance was measured. Usually 100 ml of distilled water injected into the bag three times was enough to create a salt free test. Four flushes gave additional security. Following the flushing, all water was evacuated from the bag. The injection port of the bag was cut away and a sterile 2-way stopcock (supplier Sherwood Medical) was attached.

A gas permeability test was performed on one bag prior to use. Water was boiled and cooled with filtered nitrogen. This water was further reduced with a few drops of a 12% sulfide solution. This was followed with a few drops of Resazurin blue (supplier Sigma Chemicals, UK), used as an oxygen indicator. The bag was then filled to capacity and left to sit on the work bench. Approximately 3 hours later the water within the bag began to turn pink, an indication that oxygen contamination had begun to occur. This was important to know, for some of the samples to be collected in the field were known to be sub-oxic to anoxic.

Prior to installing the pre-cut silicon sampling tubes (I.D. 4.8 mm, O.D. 7.9 mm, supplier BDH, UK.), the volume of the tubes was determined and the largest volume of the longest sample tube became the flushing volume used on all sample tubes. This made the task of flushing the tubes and bags easier and also ensured that tubes, bags and syringes were being fully flushed. Once in the cave, bags were attached to the sample tubes (Figure 2). Water was drawn up in 50 ml syringes, (supplier Becton-Dickinson, Ireland) (Figure 1) and expelled into the bag. The bags were shaken and the water was sucked out again. This was done three times for each bag to remove any potential contaminants.

Once the flushing procedure was done, the bag, sample tube and syringe all contained the water sample of interest; thus, sample recovery could begin. Samples were drawn in with the syringe, and with each syringe full, the 2-

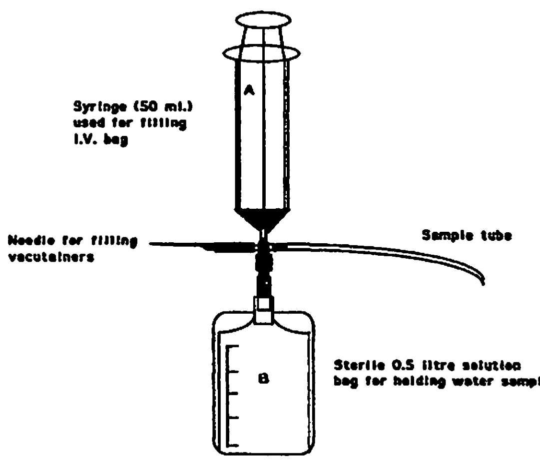

Figure 1 - Sample tubes with attached 50 ml syringe, water sample bag and/or needle.

way stop-cock that connected the sample bag and syringe to the sample tube was turned to close the sample tube and open the bag. The bags were filled to capacity by repeating this maneuver, which took approximately 5 minutes per bag.

Once the bag was full, it was removed and the tubes were once again flushed with sterile distilled water which was carried in a spare I.V.bag. After this procedure, the tubes were capped on both ends. The caps, which fit the ends of the silicon tubing, come with the stop-cocks. They are used as blanking-off caps on the stop-cocks. This was only done when all samples had been collected for the dive and the tubes were to be moved to another depth or site and used again. For long-term storage of the tubes, it is recommended that pipe cleaner, which can be purchased in lengths of several meters from BDH, UK, be used along with 95% ethanol to clean the sample tubes.

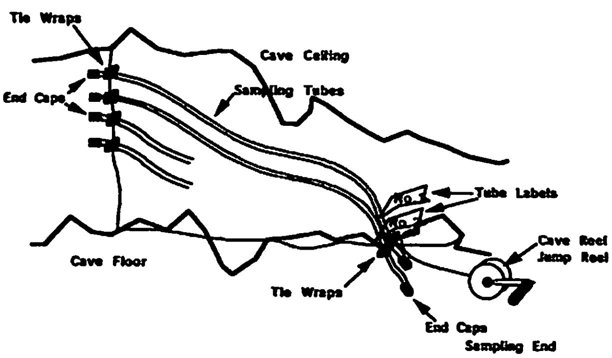

Figure 2 - Sampling tubes arranged in a cave passage.

In preparation for reusing the bags again for a different water sample, used bags were flushed with sterile distilled water, then flushed with 100 ml of 95% ethanol, flushed again three times with sterile distilled water and evacuated of all bubbles, leaving only a small amount of water in the bag. Leaving a small amount of water in the bag allowed a visual check for bubbles. It was necessary to check for bubbles since all samples were being measured for dissolved oxygen; we did not wish to introduce oxygen into our samples.

This water collection system can also be used in a non-overhead environment, i.e., a place without a hard ceiling. Instead of tying the string or rope supporting the sample tubes to a ceiling, it can be attached to an inflated marker buoy. The anchor end of the line should be tied to a rock or secured with lead weight. In areas where the sea may be rough, the marker should be set below the surface of the water. Another possibility for using this water sampling method in open water is to use a large, weighted camera tripod with a long dowel or PVC tube replacing the camera mount pole in the center. Here, as before, the sample tubes can be attached to the dowel and sampling can proceed as normal. What is especially convenient about both these systems is that the sample lines can be attached to the buoy line or the dowel prior to placement in the water. Since the dowel must be inserted into the tripod, having tubes attached to the base of the dowel will make insertion of the dowel into the tripod impossible, and so these lower tubes must be attached later.

Vacutainers

Smaller volumes of water samples for bacterial counts and bacterial activity were collected using 10 ml BD/sterile interior/no additive/red top vacutainers. These can be purchased from S. P. Services in Shropshire, UK. Tubes were taped together in strips (Figure 3) using silver cloth tape (duct tape), and labelled for different analytical processes. It was important to label both tube and tape. Should the tape be destroyed or removed before logging of the sample can occur, a sample may be wasted. Taping the tubes together prevents them from shooting to the surface and also reduces the number of individual items that needed to be handled by the diver. Attaching a weight to the end of the tape (Figure 3) allowed the strip to set firmly on the floor of the cave and the tubes to float straight up in the water column, making it very easy to recognize the different label tubes and making it easier to handle. Entanglement was never a problem.

These tubes were used for a variety of purposes, though primarily for acridine orange direct counts (AODC). These samples were used for determining bacterial numbers in the water; bacterial numbers in the water will dictate the volume of water needed for the main part of the study. A reconnaissance sample was collected prior to vacutainer usage for this part of the experiment. It was determined at that time that 10 ml was a sufficient volume for AODC. The AODC-marked tubes contained approximately 1 ml of 37% formaldehyde. Some tubes were marked for specific isotope use (isotope added later). In this case we were interested in measuring bacterial activity. Samples were collected for geochemical analyses, such as hydrogen sulfide. These tubes contain 1 ml of a 20% solution of zinc acetate. Zinc acetate binds with hydrogen sulfide and produces a white precipitate called zinc sulfide. The only substance which was discovered which could not be used in these red top tubes was acid. A silicon layer, which is used to keep blood from sticking to the inside of the tube, will dissolve and will mix with the sample.

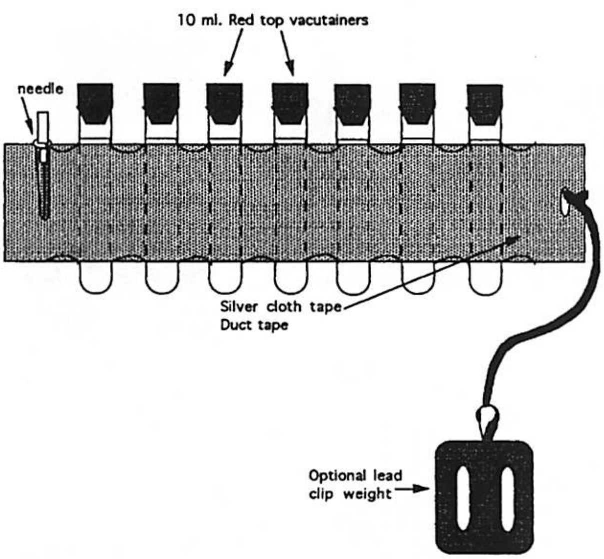

Figure 3 - Vacutainers on tape strip.

When attaching the tubes with silver cloth tape (duct tape), it is imperative to leave at least 3 cm of tube exposed at the red top end. This is to ensure, while underwater, that the diver can see where the water level is and not overfill the tubes. Head space must be left in the tubes or the red stoppers will blow off upon ascent. The loss of the red rubber tops occurs when gases coming out of solution expand during ascent. Filling occurs much more quickly at depth as ambient pressure is greater, so a diver must be able to see the water level at all times to ensure that the needle can be pulled out in time before overfilling can occur.

A 21 gauge disposable Sherwood Medical needle (Crawley, UK), taped along side the tubes, would be removed from the tape strip (Figure 3) and either attached to the sample tube (Figure 2) or to the 50 ml BD disposable syringe (Figure 2). Using the syringe allowed more control on the volume of water drawn into the vacutainer. Filling 10 tubes took less than one minute.

Plexiglas Stakes

Plexiglas stakes with several 7.9 mm in diameter holes drilled into them (Figure 4) can be used for collecting soft sediments and water samples. These can also be used for attaching items which are going to be left in situ for some time. The same diameter tube that was used for collecting water samples can be inserted into the drilled holes in the stake. To each tube, attach a 50 ml. syringe or the syringe size of choice. Once the stake is inserted into the sediment, the attached syringes which have been labelled according to the tube placement on the Plexiglas stake, will float just above the stake, making sampling very easy. Once the syringe is full leave the syringe attached, it will float in most cases where the sediment is not heavy. When sampling is finished, just pull the stake out and store in a bag or container for the trip out.

For long-term study, such as attaching rock pills, Whitaker and Smart (1993), items can be sewn into fine plastic netting and attached to the stake with tie wraps (Figure 4). Size of netting used will depend on experimental demand. Those in this study had been left in cave systems for three years without any sign of the materials used decaying. Coarse netting was used here since the experiment was designed to study weight loss of rock pills and large net spaces (though not so large to lose the pill) provided enough surface exposure not to interfere with the dissolution process. Fine close-woven netting would have reduced the exposed surface area of the pills, resulting in a possible compromising of the dissolution process.

Rock Corer

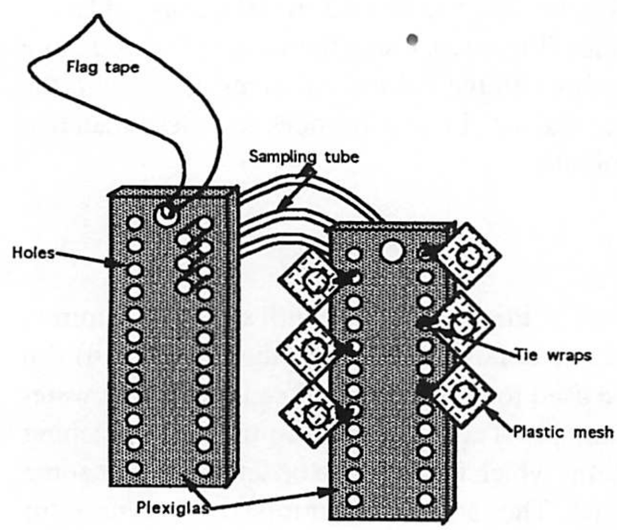

Figure 4 - Plexiglas stakes used with sample tubes and rock pills.

Rock core collection was made possible using a rebuilt pneumatic wrench. The air wrench (Figure 5) was purchased from a local hardware store in Bristol England. The original metal components found within the unit were composed of iron and aluminium, and found not to be compatible with marine environments. All metal parts were replaced with stainless steel. Being open to the water, lubricants were not needed for the air wrench, and this was a bonus, since we did not wish to contaminate the cave environment with any hydrocarbon-based oils. After each use, however, the drill must be opened, washed out with fresh water and dried thoroughly.

The air hose for the drill was fitted with an adapter which would allow the hose to be connected to a first stage of a scuba regulator. This permits the use of a standard 12 to 15 liter scuba cylinder as the air source for driving the air wrench. Another hose was attached to the exhaust port on the air wrench. At the end of this hose a dispersion unit(Figure 5) is attached. This dispersion unit is built from a piece of PVC pipe approximately 8 cm in diameter and approximately 4 cm deep. One end is sealed with a fitting to accommodate the exhaust hose, while the removale exhaust end has a plastic large-diameter screen. Inside this unit is inserted a large pore sponge which has been cut to fit the exhaust compartment.

The pore size of the sponge has to be large or the power of the drill will be reduced and it is likely that the exhaust hose will be blown off. When in use, this exhaust unit will float above the operator, well out of the way of

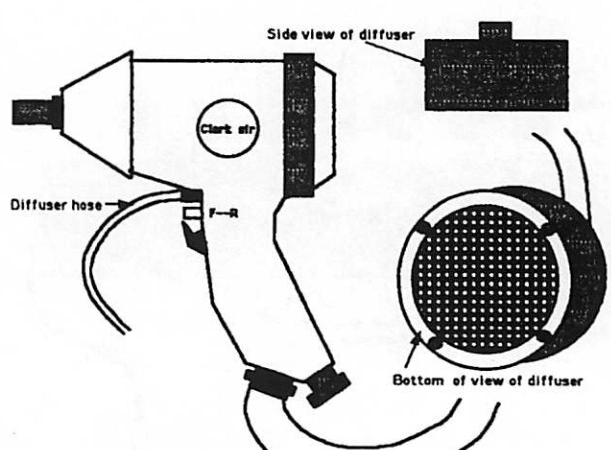

Figure 5 - Pneumatic power wrench which has been re-configured for underwater use.

the sampling site area. The object of having this dispersion unit attached to the end of the exhaust hose is to reduce the initial impact of gas bubbles on the cave ceiling, which will displace cave biota and sediments and thus reduce visibility. Visibility will be reduced in time anyway and it is for this reason that we suggest that drilling be the last project worked on in the cave, especially if you are measuring particulate organic carbon (POC), total organic carbon (TOC), or dissolved organic carbon (DOC).

The diamond-tipped corer itself was built into a PVC base designed by one co-author (Fred Wheeler). The base (Figure 6) was built with three stainless steel, independently movable pins. Movement of the independent pins is controlled by a cam located on the top of the corer base. The cam is released and this allows the pins to move independently when the base is placed against the wall. Once a site is chosen, the pins can be locked into position using the cam. The corer shaft (which is custom made by Durbin Metal Industries in Bowling Hill, UK) is set into the center of the base, allowing some free up-and-down movement. The corer shaft itself is fitted with a male luer hexagonal nut whereas the pneumatic wrench is fitted with the female luer hexagonal nut. Once the base is in place, the air wrench can be placed on the corer tip and drilling can proceed. Keep in mind it is not necessary to apply much pressure to the corer. The less pressure used, the better the corer will perform.

To remove the rock core from the wall a stainless steel tube approximately 10 cm long was made to exactly fit the rock core. Once the base is removed, this stainless steel tube can be fitted over the rock core and a gentle tap on the side of the tube will liberate the core from the wall. The rock cores were stored for transport in 30 ml., sterile, disposable polypropylene, clear tubes. Once the core is in the tube, a sterile polypropylene sponge, (supplier BDH) is inserted on top of the core. This is done to prevent movement and possible loss of orientation of the core. Most importantly, these sponges prevent damage to the core during transport. These tubes, like the vacutainers, can also be taped together with duct tape as shown in Figure 3.

DISCUSSION

Submerged cave environments can be particularly difficult areas to study in detail even under the best of circumstances and conditions. These environments can generate risks, (Balcombe et al., 1990; Prosser and Grey, 1992),

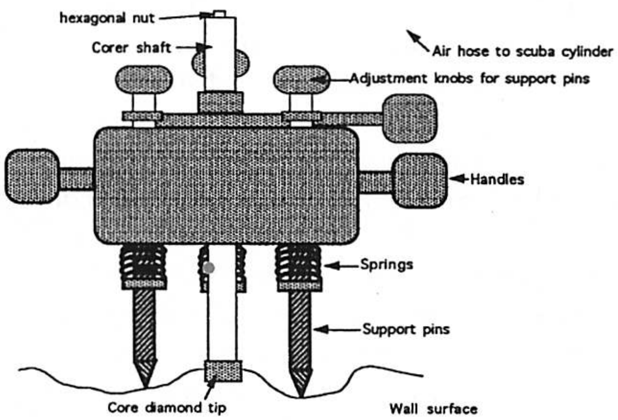

Figure 6 - Wheeler corer base.

which would keep a non-cave diver very occupied with the job of simply staying alive. To complicate this scenario, this environment combined with the tasks of collecting environmental samples has the potential of being a diving nightmare. It is under these pressures and within these settings where ideas are created out of necessity. For good sampling methods, several things need to be taken into account: (1) expense, (2) ease of handling in potentially tight, small environments, (3) sterile collection methods for specific demands of the sampling agenda, (4) reusability, and (5) tolerance to water pressure at depth. What is most important, however, is the ability to make adjustments in the sampling methods which will allow use in different cave environments such as a cave with larger rooms, smaller rooms, extreme depth, and highly ornamented passages and chambers.

Many may argue that these methods are

juvenile compared with remote sampling. They may very well be, but cave environments are not always the best place to use remote sampling methods. Cave environments vary in dimensions such as in passage size, length, and depth of the floor which, in some cases, may indeed be too deep for self-contained exploration. To complicate matters further, passages can be highly ornamented with speleothems which can be potentially damaged by the umbilical cord of an ROV and/or cause its lines to be hopelessly entangled, in which case a diver would still be needed to recover the equipment.

Automatic water samplers, which are commercially available, would still have to be placed by a diver in a cave environment and would still not allow the degree of freedom we have had using silicon tubes for water collection. Sampling was performed vertically throughout the water column every 20 cm in a very short time period, in a complex environment within a carbonate island fresh / salt water mixing zone. Automatic water sampling in this case would not at present be possible. Commercial samplers tend to be large, and in most cave environments the water body is hydrologically active with a flow range anywhere from 0 to 2 knots of water movement. One of the main concerns associated with using large instruments for delicate vertical water sample collection in flow settings is the turbulence created by the collection unit. It is especially critical in a complex site where there are bodies of water with different water chemistry and different lateral flows within the same water column. Turbulence would obviously mix the water sample prior to collection and therefore provide false information about the water chemistry. When the tubes were placed in the cave system for this project, there was a waiting period of 24 hours before divers went back in to collect samples, thereby allowing the water column to re-establish itself. The sample site was about 10 m away from the collection site, therefore the divers' pressure wave could in no way disturb stratification of the water column. On the day of collection, divers would swim carefully to the opposite side of the passage to the sample site.

Conductance measurements taken from recovered water samples range from virtually fresh water to marine water. Samples were collected and measured in February of 1994 in Lucayan Caverns and again in February of 1996 in exactly in the same chamber and exactly in the same place (Figure 7). Error bars in Figure 7 show potential tidal range and therefore a potential for change in conductance for samples collected from the same depth, but at different times, in the tidal cycle. The results demonstrate that the water collection techniques are reliable and reproducible and, most importantly, show that diver-induced turbulence was not a problem.

Other geochemical analyses generated from these water samples could possibly have been done by remote sensing. Instruments that are available now can measure dissolved oxygen, pH, conductance, temperature, turbidity and various other measurements. However, when funds are not available, the methods described in this paper can provide accurate results which can be tested repeatedly. We do agree that some hydrological measurements can only be properly done by remote sensing. The time involved in observing flow patterns within a cave system demands that a measuring source be in place for a minimum of at least 24 to 48 hours; ideally for longer periods of time. For obvious reasons, using a diver for this type of experimental data recovery is not practical.

Large commercially available hydraulic rock corers can be purchased, but to use these large and expensive units several hundred meters back in a cave passage is out of the question because of the need for umbilical support. With this particular project, there was no need for large diameter cores and in most cases, based on the instability of the cave environment, impractical. Small 1 inch (2.54 cm) commercial corers are not available for underwater use and therefore the need to find another means of collecting these cores was needed.

People have tried in the past to use pneumatic wrenches with little success. The problems they had were mostly associated with using the unit with the original parts. Usually after one or two uses the air wrench would stop functioning due to rusting of the mobile parts. Another problem was that the individuals did not have a supporting base, making the probability of collecting a good rock sample even less likely. The base is needed to provide support and stability to the core shaft. During drilling, divers have to be occupied with the business of maintaining buoyancy, being environmentally aware, and making sure that the angle of the shaft is straight. The base ensures that these conditions are met, otherwise the shaft will not turn and, as a result of this base, the shaft will also not be broken. The other problem that

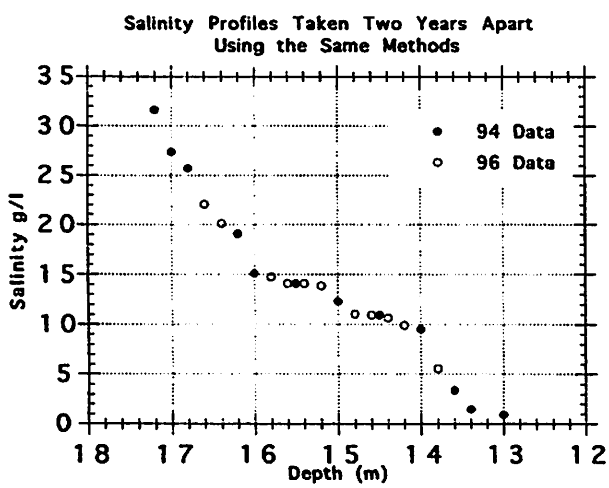

Figure 7 - Conductance profile for Wedding Hall Room, in Lucayan Caverns, Grand Bahama.

the diver has to contend with is the problem of pushing against a drill where in most cases there is little or nothing to hang on to. Driving a rock anchor into the wall and securing yourself to it using a short rope and quick release carabiner, is a possible solution, as is bracing yourself against floor, roof or an opposite wall.

In the future, as more and more remote sensing instruments become available, it is important to keep in mind why these instruments were created. Initially remote sensing devices were made available to be used in environments to replace the use of a human being because the environment was too dangerous and the risks too high, and the costs too great. As time moved on, it was recognized that remote sensing had more potentially different uses even in environments where humans could comfortably be placed. There are still, however, situations in which humans are and will always be the best instrument. The obvious reason for this is the individuals' capability to think, their manual and mental flexibility, which gives them the ability to respond to environmental alterations and thus make spontaneous changes in the sampling schedule which few remote sensing devices to date can do. There is no question that combining remote sensing and human intervention is really the best way to get the most out of the environments being studied. However, when funds are not available for buying remote sensing instruments, or when the environment precludes their use, then simple collection methods as presented here, if done properly, generate good and reliable data cheaply and effectively.

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