

DIVING FOR SCIENCE...1993



CALIFORNIA
SEA GRANT

PROCEEDINGS OF THE AMERICAN ACADEMY OF UNDERWATER SCIENCES THIRTEENTH ANNUAL SCIENTIFIC DIVING SYMPOSIUM

September 19-22, 1993
Asilomar Conference Center
Pacific Grove, California

John N. Heine
Nicole L. Crane
Editors

American Academy of Underwater Sciences
430 Nahant Road, Nahant, MA 01908

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Proceedings of the
American Academy of Underwater Sciences
Thirteenth Annual Scientific Diving Symposium
"Diving for Science...1993"

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This publication was partially supported by the California Sea Grant College Program under Grant # NA89AA-D-SG138 from the National Oceanic and Atmospheric Administration. Opinions presented at the Symposium and in the Proceedings are those of the contributors, and do not necessarily reflect those of the American Academy of Underwater Sciences, NOAA, or any of its sub-agencies.

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INTRODUCTION

The *Proceedings of DIVING FOR SCIENCE....1993* contain 23 papers and/or abstracts presented at the 13th Annual American Academy of Underwater Sciences Scientific Diving Symposium held Sept. 19-22, 1993 at the Asilomar Conference Center, Pacific Grove, California.

The Symposium was hosted by the Diving Officers of Monterey Bay: John N. Heine, Moss Landing Marine Laboratories, Nicole Crane, Hopkins Marine Station of Stanford University, Randy Wilder of the Monterey Bay Aquarium, and Kim Reisenbichler of the Monterey Bay Aquarium Research Institute. Many thanks are owed to Ms. Lynn McMasters, who performed the heroic task of taking computer diskettes of all types and transforming them into readable text. She also designed many of the figures and logos which appear in these *Proceedings*. We thank the California Sea Grant College Program for their support in preparation of these *Proceedings*. We also thank Mr. Gordie Heck of SCUBAPRO who donated funds towards the evening reception at the Monterey Bay Aquarium.

John N. Heine
for the Symposium Committee

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EVIDENCE OF PREHISTORIC MAN AT RAY HOLE SPRINGS: A DROWNED SINKHOLE LOCATED 32 KM OFFSHORE ON THE CONTINENTAL SHELF IN 12 M SEAWATER

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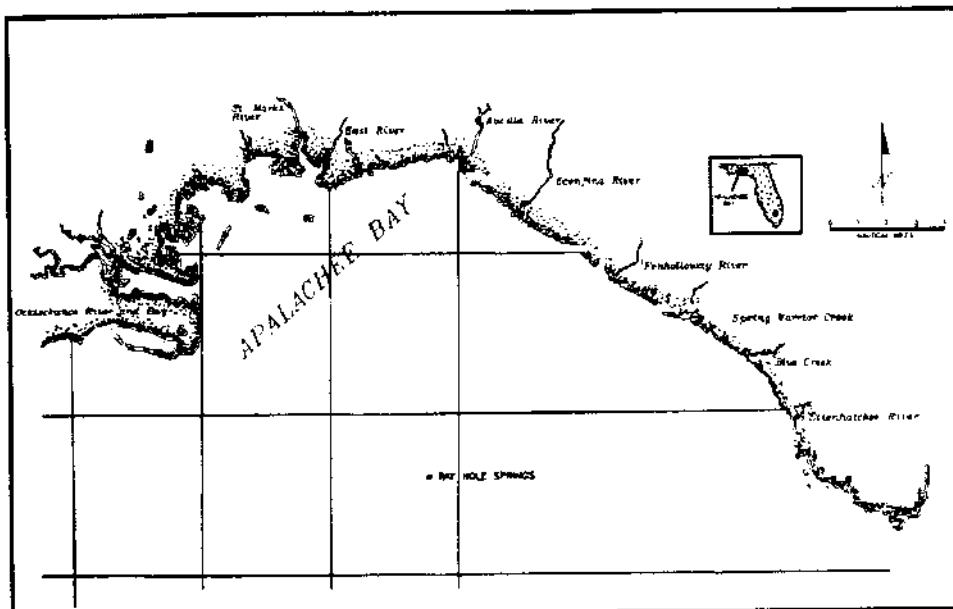
*This paper will discuss a cooperative research effort between academic, State, and Federal agencies that researched and found evidence of prehistoric activity at Ray Hole Spring, a submerged sinkhole. This drowned karst feature is also located in Apalachee Bay, Florida, about 32 km (21 mi) offshore in 12 m of water. Evidence presented in this paper will focus on analysis of carbon dates from organic samples, live oak (*Q. virginiana*) and articulated oyster shell (*C. virginica*), collected at the site. These organic materials were used to reconstruct a sea level curve for this area of the Gulf of Mexico. In addition, scientists have recovered lithic debitage in the form of secondary reduction thinning flakes. This debitage, interpreted as cultural indicators from prehistoric man, was found buried in crevices associated with the rim of this sinkhole feature. These cultural indicators provide irrefutable physical evidence that this submerged sinkhole was utilized by prehistoric man.*

INTRODUCTION

The study of Paleoindians in the New World has been the focus of anthropological research with researchers attempting to answer questions about "where" these prehistoric settlers lived and "how" they survived in the late Pleistocene to early Holocene environment. In the past, the majority of Paleoindian archaeological studies have been in the terrestrial arena and were founded in the scientific method with a strong theoretical background. In the recent past archaeological researchers have been asking these same "where" and "how" questions and finding some interesting answers on submerged inundated sites.

Sites "inundated by the sea are the most elusive sites to locate. These sites may be deeply buried and inaccessible in some regions of the continental shelf...."(Dunbar

submerged early prehistoric sites (Dunbar 1991), but only in recent years have significant discoveries begun to reinforce such views (Dunbar et al. 1992:124-125). The majority of known inundated Early-Archaic sites discovered in the United States are located in the eastern Gulf of Mexico on the Florida Tertiary limestone shelf (Faught 1988 a&b; Dunbar and Waller 1983; Dunbar et al. 1988, and Anuskiewicz 1987 and 1988). This offshore area of karstified limestone deposits has little sediment cover, which enhances the chances in locating and accessing prehistoric sites on the continental shelf. The Apalachee Bay Region of Florida is an ideal geographic and geologic area to search for submerged evidence of prehistoric man (Map 1). This area is located in the northern part of the Florida karst shelf with several spring-fed, low-sediment-load rivers flowing into the bay along with numerous naturally occurring underground caves and surface sinkholes (Dunbar et al. 1989:25).



Map 1. Apalachee Bay Region of Florida.

KARST AND THE SIGNIFICANCE OF PALEOHYDROLOGY AND LITHIC RESOURCES TO PALEOINDIANS

To locate "where" the stone tool dominated Archaic or Paleo-indian sites depends on being able to predict site locations based on models of past human behavior and on geological criteria (Dunbar and Faught n.d.). For prehistoric populations to maintain their lifeways during post-glacial times, they sought out areas rich in varied natural resources. These resources included potable water, food sources (terrestrial, freshwater and marine), and suitable lithic and bone material for tool production.

During low sea level stands, potable water sources on the Florida shelf may have included freshwater rivers, streams, and springs that emptied into the Gulf of Mexico, although some researchers believe most of the karst rivers, those inland, did not have continuous flow until the mid-Holocene. Another important source of water in the

Apalachee Bay area occurs in sinkholes intimately tied to the underground Floridian Aquifer freshwater system. Florida's topographic character in the Tertiary karst region is a well-developed, mature karst with complex underground channel systems. Where the Tertiary limestones are near the surface, numerous sinkholes and other openings connect to the surface. According to Cooper et al. (1953), and Davies and LeGrand (1972), the Floridian Aquifer in the these karstic limestones is one the nations largest groundwater aquifers (Figure 1).

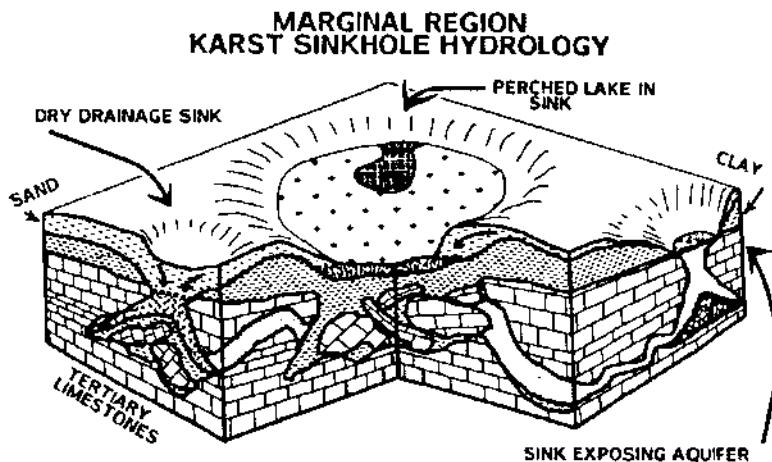


Figure 1. Geologic block diagram of marginal region karst sinkhole hydrology (from Dunbar 1991).

One might now ask how does the Floridian Aquifer system relate to Paleoindian settlement patterns on the continental shelf? Today the Floridian Aquifer is near the surface throughout much of the mainland Tertiary Karst Region. However, during the Paleoindian time frame, the aquifer, like sea level, was lower, and Paleoindian site clusters were concentrated in areas where numerous karst features gave access to persistent sources of water (Dunbar 1991).

Other than the dramatic effects of sea level transgression, it is also important to consider human adaptations from the perspective of the paleohydrology that existed on the continental shelf prior to present inundation. Regardless of yearly precipitation, karst terrains elevated high above groundwater levels tend to be usually dry and support xeric habitats because the porous rock permits water to drain quickly to the level of the aquifer. Therefore, climate, which generally exerts the dominant influence on ecology, is somewhat nullified in limestone regions where karst plays an equally influential part with ecology (LeGrand 1973).

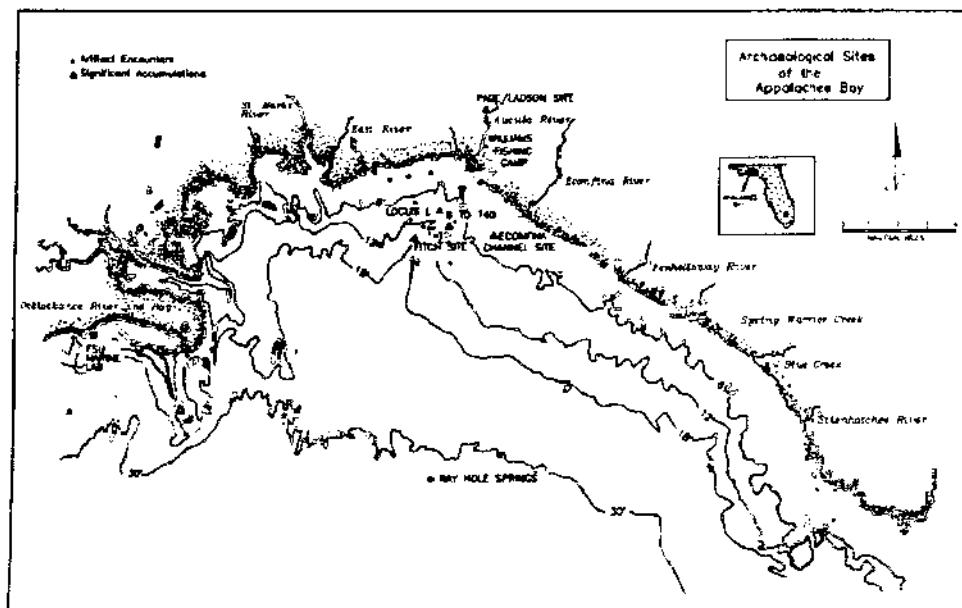
In Florida, the late Pleistocene decline in sea level lowered the karst aquifer about 26 meters or more in some places (Webb 1974; Clausen 1979), and water levels in deep lakes marginal to the Tertiary Karst Region dropped around 18 to 20 meters (Watt 1983). During the late Pleistocene, the Tertiary karst shelf is believed to have displayed a gradation of the ecological variation depending on the elevation of the ground surface above the water table. This in turn was largely affected by the level of the sea. Areas now far out to sea (i.e., Ray Hole Spring-32 km) and nearer to the Clovis shoreline would have been located where the Floridian Aquifer was near the surface.

Lithic resources were also important to Paleoindian and Early Archaic subsistence patterns. Generally, chert bearing limestones comprise a slightly undulating karst plain sculpted by higher sea level stands. The distribution of chert in the karst plain is irregular but frequent. Chert boulders are exposed when the limestone surrounding them dissolves and leaves behind pinnacles of erosion resistant chert rock.

Offshore rock outcrops are one of the most easily identifiable sea floor features on the Tertiary karst shelf. These exposed surface expressions are not hidden by sediment cover and are somewhat easy to detect because the rock is erosion resistant chert or dolomitic limestone.

THE OFFSHORE SURVEY IN APALACHEE BAY AND RAY HOLE SPRINGS

The search for offshore prehistoric sites becomes easier when inundated sinkholes, river channels and chert rock outcrops can be used as convenient guideposts. The irregular, topography-associated features such as submarine sinkholes provide excellent habitat to attract fish and other marine life (Dunbar et al., 1989; Serbousek 1988). Many of the offshore prehistoric sites in the Apalachee Bay area have been discovered as a result of work with fisherman and sport divers who frequent their favorite fishing hole. In Apalachee Bay only a few submerged topographic targets have been inspected, and the results have been extremely fruitful with the discovery of 15 prehistoric sites (Anuskiewicz 1987, 1988; Faught 1988 a&b; 1989, 1990 a&b, 1992, Garrison 1992; Stright 1992, and Dunbar per. com., 1993). Near shore archaeological surveys (inside the State of Florida's 16-km limit) conducted by Faught and Dunbar located 15 archaeological sites (Faught 1992:6) from 1 km to 10 km offshore at depths ranging from 0.5 m to 5.5 m below sea level (Map 2).



RAY HOLE SPRINGS GEOLOGY

In 1976, The Florida Bureau of Geology Bulletin described Ray Hole Springs as "an occasionally flowing spring lying in 11.6 m of water and measuring 7.6 m in diameter. The north side of the sink slopes southeast and the southeast side has a nearly vertical limestone wall to a depth of 18 m. A cave strikes down and southeast from the 18 m depth to approximately 30 m."

The outer rim of the sink was described by Dunbar et al.(1989:28) as limestone, with minor amounts of silicified rock along the rim of the sinkhole and in the collapsed zone around the rim. Exposed rock, including limestone, and what appear to be dolomitized limestone and chert, was pitted by marine organisms capable of dissolving and implanting themselves into the rock.

Dunbar et al. (1992:131) further describes the geology around Ray Hole Springs as,

The natural bottom surrounding Ray Hole sink consists of a thin veneer of bioclastic detritus and sand above the flat limestone bedrock of a karst plain. Similar to pocked surface of the onshore karst plain, small crevices and solution holes are probably abundant, but hidden by sediment fill and difficult to identify without subbottom profiling equipment....The sinkhole was the only topographic interruption in the otherwise monotonous marine-scape.

1986 FIELDWORK AT RAY HOLE SPRINGS

In the fall of 1986, a cooperative research effort sponsored by the Department of the Interior, Minerals Management Service, Gulf of Mexico Region with support from the Florida Bureau of Archaeological Research and the Academic Diving Program and Marine Laboratory of Florida State University, investigated Ray Hole Springs. Initial diving investigation and reconnaissance revealed that the sink was deeply filled with recent marine sediment. The diving investigation of the sink included diver swimming reconnaissance, site mapping, attempts at auger testing, and water-jet induction dredging at a few selected locations. Auger testing was not successful because the auger could not penetrate the thick marine sediment, and the testing was therefore abandoned.

Next, a 4-inch water induction dredge was employed at random areas around the margins of the sink in an effort to locate sediment pockets that may have trapped lithic artifacts. Several chert-like flakes (pseudo artifacts) were recovered. Initial interpretation of the pseudo artifacts recovered from Ray Hole Springs was that they may represent poorly preserved lithic debitage.

During an excavation of a large crevice filled with typical marine sediment consisting of sand and marine shell detritus, two interesting discoveries were made in the same narrow, deep-test unit. The crevice tested measures approximately 15-20 cm in width and is oriented in a southwesterly direction towards the southern rim of the sink. The first 50 cm or so yielded some possible pseudo artifacts. At about the 70-75-cm level the crevice yielded a lens of oyster shell (*C. virginica*). Samples of the oyster were carefully collected. Continued excavations produced a large piece of waterlogged wood at the bottom of the oyster shell level and lying on the rock bottom at 1 m deep. The wood was collected along with an oyster shell sample. Below the point where the wood

was collected, the crevice narrowed and bottomed out, and archaeological testing was terminated (Anuskiewicz 1987:417; 1988:96). The discovery of wood not damaged by teredo worm and the oyster shell above the wood suggests that wood was deposited in a wet, probably freshwater environment. Later analysis of the wood determined it was live oak (*Q. virginiana*) species. Dunbar et al. (1989:28; 1992:30) states that the discovery of the live oak is strongly suggestive of a coastal hammock environment where salt water intrusion would not likely occur. The oyster shell is indicative of a brackish water environment where saltwater becomes diluted by the influx of freshwater adjacent to the coast, and certain species, notably oysters, flourish. The sand/marine shell detritus, articulated oyster shells, and live oak wood order of deposition indicates that a marine transgressive sequence is present at Ray Hole Springs.

Carbon-14 on both oak and oyster shell strongly support this interpretation. The Carbon-14 date for the oak wood yielded an age of $8,220 \pm 80$ years and for the oyster shell $7,390 \pm 60$ years, carbon-13 adjusted $7,740 \pm 60$ years B.P. (Anuskiewicz 1987:417, 1988:184; Dunbar et al. 1989:28, 1992:31). The carbon dates indicate that brackish coastal environment replaced terrestrial/ freshwater habitats by about 7,700 years ago (Figure 2).

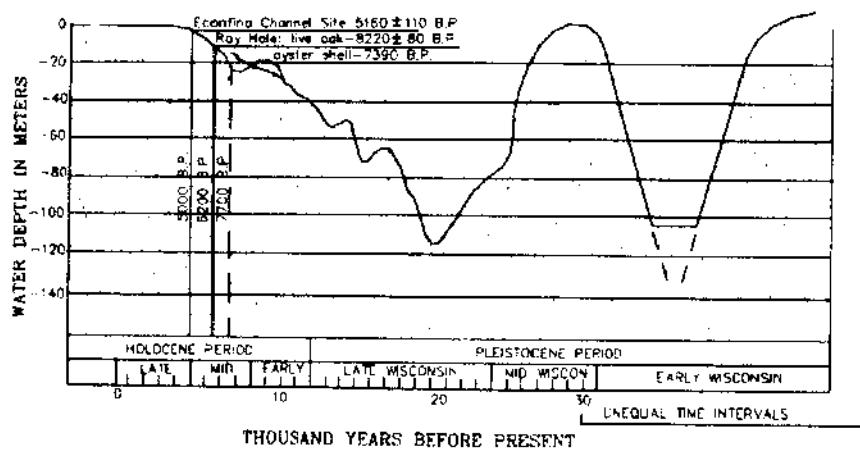


Figure 2. Relative changes on the level of land and sea during the late Quaternary for the Gulf of Mexico. This sea level curve indicates the relative position of Ray Hole Springs based on live oak and oyster samples that were radiocarbon-dated (sea level curve was developed and modified from Coastal Environments, Inc. 1977 revised 1978 and 1982).

ADDITIONAL FIELD WORK AT RAY HOLE SPRINGS

Since 1986, several diving expeditions have been made to Ray Hole Springs to collect additional scientific data. Three of the dive trips in, 1989, 1990, and 1992, involved dredging activities at the site to look specifically for diagnostic lithic artifacts to support carbon dates for the live oak. In addition, 1990 and twice in 1992 vibra-core testing was attempted to gather a stratigraphic sediment sample of the sinkhole sediment cone for paleo-environmental supportive information. Unfortunately, bad weather and mechanical problems with the vibra-core resulted in gathering a 1.3 m core sample. This specific research was conducted in an attempt to gather supporting paleo-environmental sea level curve data to supplement existing information for this area of the Gulf. These

diving operations were a cooperative research effort between the MMS and the National Park Service, Southwestern Region, and the Florida State University, Department of Geology.

INITIAL ANALYSIS AND RESULTS OF TESTING

Testing prior to 1990 at Ray Hole Spring gave us evidence of past brackish water and terrestrial environments from a 1-m deep, sediment-filled crevice. Other tests produced a number of pseudo-artifacts recovered from shallow marine sediments around the rim of the sink. We considered the specimens recovered pseudo-artifacts because they were too corroded by the marine environment for their origins to be certain. These findings raised our hopes that evidence of prehistoric human activity might also be found at the site and that further testing was warranted and pursued (Figure 3).

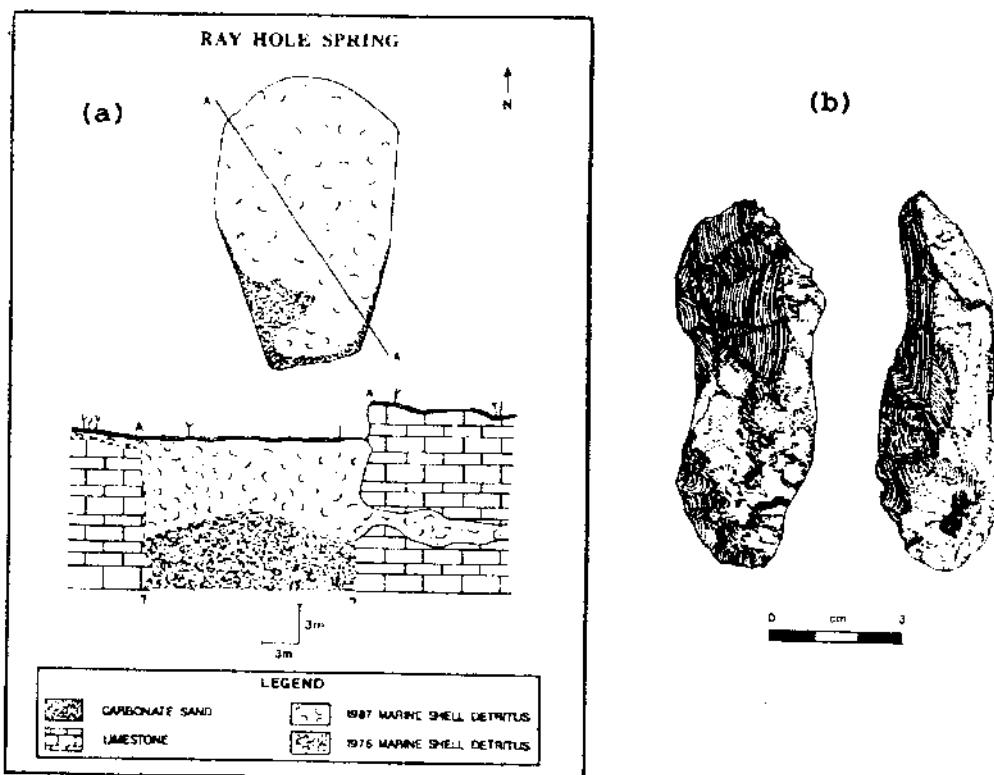


Figure 3. (a) A plan and profile view of Ray Hole Springs, (b) a pseudo-artifact collected from a crevice located at the north-end of the sinkhole (from Anuskiewicz 1987).

Continued testing in 1990 and 1992 was primarily focused in the rim area of the sink in hopes of finding another deep sediment-filled crevice in the limestone bottom. Although no deep crevices were located, several shallow, sediment-filled crevices were encountered and two debitage flakes were recovered. The debitage flakes also displayed a form of corrosion but, unlike the other samples, their exterior remained intact

and allowed them to be positively identified as artifacts. The two debitage flakes, one recovered in 1990 and the other in 1992, have a patina-like corrosion that has transformed the interior of the flakes into an almost pure, caulk-like tripoli. The flakes are similar to chert artifacts recovered in near-surface deposits from sites in Apalachee Bay closer to the present shoreline. The degradation of the flakes by corrosion weakened them, and our collection technique of filling a nylon-mesh sample bag with bulk quantities of shell detritus and rock rubble caused the flakes to crumble around their thin edges. Both flakes show the scars of more than one concoidal flake detachment, and one has a surviving bulb of percussion. Both are also the result of secondary reduction, extracted from a core in an area previously cleared of outer cortex by primary reduction.

ARCHAEOLOGICAL CONTEXT

In 1986, our initial test in the sink at Ray Hole Spring indicated that the marine sediment was too deep for continued excavation. The subsequent discovery of the 1-m deep crevice with sediments indicating sea level transgression changed our focus to tests around the sinkhole's rim. Although no other deep crevices were located, a number of pseudo-artifacts and eventually two debitage flakes were recovered. The context of these specimens can be traced to two stratigraphic horizons. The first is a thin level, usually less than 10 cm thick, of transient, surficial sand deposits that dominate the bottom terrain surrounding the sink along with less frequent natural rock outcrops. A number of pseudo-artifacts, some with multiple flake scars, were recovered from the surficial sand, but displayed decomposed exterior surfaces due to presumed frequent exposure and marine growth. The second sediment type consists of rubble deposits that occur under the surficial sand deposits in crevices and depressions in the limestone that average about 25 cm to 35 cm deep. The rubble deposits consist of a mixture of bioclastic shell detritus and pebble to cobble-size rock mixed with sand, silt and clay. Pseudo-artifacts were recovered from the rubble deposits and include specimens that appeared to have undergone exterior corrosion followed by erosion that removed the corroded exterior and left behind a "ghost" core of a former tool. The two debitage flakes were also recovered from this context and, although it cannot be proven, we believe they were recovered from the deepest recesses of the limestone crevices where they were protected from the effects of erosional events.

Finally, in 1992 a second test (Test 2) was opened in the sink adjacent to the rim. This test proved to be much more interesting in that another deep section containing brackish water sediment was identified. Both oyster and mussel shell dominated the strata with a number of occurrences of still articulated bivalves, which indicate primary deposition. As frequently happens, Test 2 was never completed due to project time constraints, and the brackish water sediment column was never penetrated to expose potential lower stratigraphic levels. The Test 2 area has stratigraphic depth and promises to have in-situ cultural site component(s) still buried. It will be our next target for continued testing.

DISCUSSION AND CONCLUSION

Normally, a terrestrial location would not be recorded as an archaeological site on the basis of two debitage flakes, even though the artifacts demonstrate evidence of past

human activity. There are two reasons why we decided to record Ray Hole Springs as such a site at the end of the 1992 testing session. First, it is the farthest offshore location at the greatest depth below present sea level to have produced prehistoric artifacts in the northeastern Gulf of Mexico. Second, the two debitage flakes, along with several pseudo-artifacts, suggest the site has greater evidence of prehistoric cultural activity. Now that a second area of brackish water sediment has been identified, further testing promises to give us an opportunity to identify potential undisturbed site components.

Future research at Ray Hole Springs should determine how extensively prehistoric peoples used the site. In this paper it has been our purpose to report this archaeological site's existence. Along with the 15 other prehistoric sites in Apalachee Bay it is confirmation of a larger resource base inundated by Holocene transgression. It is a marine resource, once a subject of speculation, that can now be confirmed in the Apalachee Bay region of the Gulf of Mexico. It is a resource base we are just beginning to understand (Figure 4).

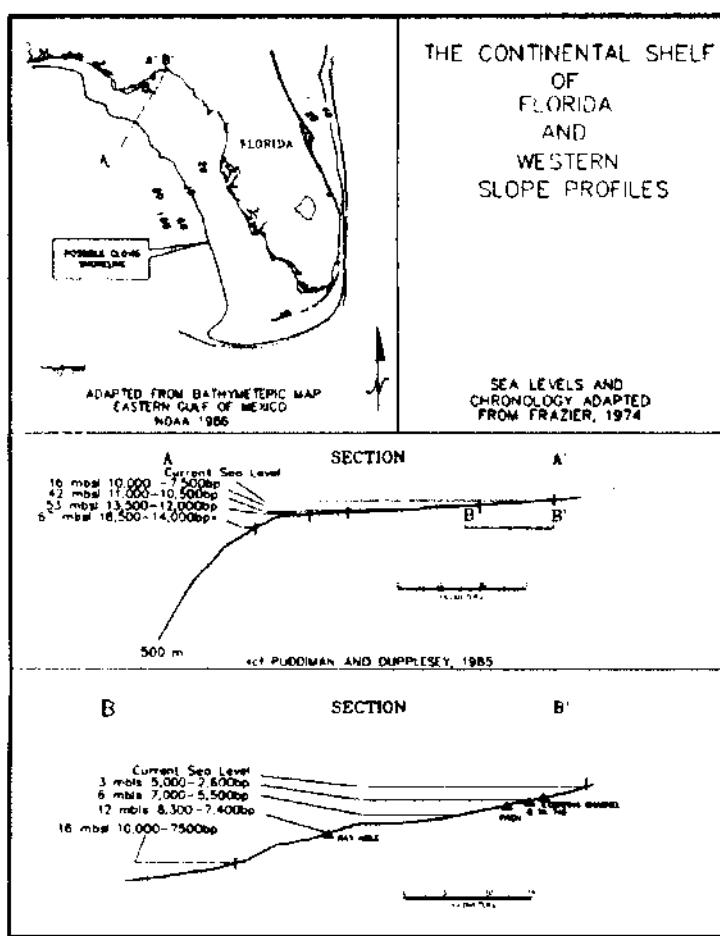


Figure 4. Western slope profiles of the Florida continental shelf (from Faught 1992).

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TRAINING AND CERTIFICATION OF THE SCIENTIFIC DIVER

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An analysis of procedures and methods to prepare and sustain participation of individuals in underwater study and research under institutional auspices. Discussion of prerequisite requirements, importance of free diving training and experience, selection and management of life support equipment in the pool and the ocean environment, introduction to research methods, evaluation and testing, certification and maintenance of active status within institutional guidelines. This discussion includes the relationship of scientific diving training, maintenance of certification, and assistance for research to the effectiveness and safety in the conduct of in-water research, expectations for reciprocity and adherence to federal/state mandate and AAUS standards.

INTRODUCTION

There are four major groups in diving practice: recreational, commercial, military and scientific. Each group operates under specific rules with specific purposes.

The modern era of scientific diving began in the early 1950's. This occurred with the development of scuba. These early aqualung divers at the University of California at Los Angeles and Scripps understood the hazards and adopted rules of conduct to protect their health and safety and promote effective research. These rules were the basis for the first Scripps training classes and Diving Safety Manual. These rules have been the foundation for scientific diving fostering training/certification, certification maintenance and research support.

PREREQUISITES FOR TRAINING

Medical/physical fitness is evaluated by a physician and swim testing. This system was developed independently by many institutions. Standards for both are outlined in the Diving Safety Manuals of institutions and in the AAUS Standards.

Free diving (breath-hold diving) skills should be evaluated prior to training. This should include snorkel and mask clearing, surface dives, fin kicks, weight belt and mask ditch and recovery etc. Evaluating these skills allows one to determine how comfortable the person is underwater. Some very good swimmers are not comfortable underwater, therefore, not suited for diving. If free diving skills are weak, free diving training should be taken prior to starting scientific diving training.

Scuba experience or recreational certification can be an important prerequisite. Understanding of equipment and its management in the ocean environment may,

however, be minimal.

Orientation to, selection and organization of his/her diving equipment prior to the start of training is extremely important. Equipment must be carefully evaluated by the instructor and the student to insure proper selection, fit and adjustment. Equipment should be simple, durable and reliable. Proper sizing of equipment is very important. For example, custom made exposure suits and properly sized air cylinders (50 cu/ft cylinder for persons under 130 lbs, 70 cu/ft cylinder for person 130-170 lbs and 80 cu/ft cylinder for persons over 170 lbs). Equipment such as air cylinder, backpack, regulator, pressure gauge, depth gauge, watch, compass and buoyancy compensator must be tested and certified. Student ownership of equipment is an important prerequisite to learning how to use it and care for it. All equipment should be marked for identification.

Students should be encouraged to become physically fit prior to the start of training. Swimming with fins, mask and snorkel is the best conditioning for the legs and ensures more in-water time, therefore, greater in-water comfort. Women students should be encouraged to lift small free weights for upper body conditioning. Interval training using fins will also improve cardiovascular condition.

It is important to orientate students to the time, money and commitment necessary to accomplish training. Students must be willing to limit the number of other academic units and other activities. These discussions allow the student to determine the level of their motivation for evolvement in training. This early motivation is key to success in training and later success in research. A written contract for participation may be used.

CPR and First Aid certification are required.

TRAINING

Training must ensure academic excellence, physical and technical ability and psychological, social and environmental awareness. There should be enough time (10-15 weeks -- lecture, 2 hrs/week; pool 4 hrs/week and 7 to 8 ocean diving days) so that protocols can be repeated often enough to ensure a satisfactory level of learning.

Lectures should include all the basic material included in the NOAA/US Navy Manuals, institutional regulations included in the "Diving Safety Manual" and the AAUS standards. There should be quizzes, at least two midterms and a final examination. Students should receive an overall score of 75% with correct answers to specific key questions for certification. Special testing should be required for air table use.

All exercises should be practiced in the pool and then in the ocean. All diving equipment (full ocean dress), mapping equipment, lift bags, boats, research equipment and methods are used in the pool and then in the ocean. Every four to six students should be supervised by a senior instructor with one or more assistant instructors. There should be progression from simple/easy skills to more complex/difficult skills and from pool to ocean environments. Students who cannot do simple skills (mask/snorkel clearing, weight belt recovery) by the fourth week should be dropped from training. A contract or agreement prior to training is important. During midterm and final testing all tests should be satisfactorily completed in the pool before they are attempted in the ocean. Circuit training, meant to be difficult and stressful physically, technically and psychologically should only be done in the pool. Difficult/stressful exercises ie: rescue must be done in the ocean, through the surf zone, but with close supervision. The first one and a half days of ocean diving should be used for free diving only to promote free diving, surfing skills and ocean environment awareness. It is extremely important that

the student be given the opportunity to become comfortable in the ocean without the "burden" of the air cylinder. Students should use life vest style buoyancy compensators for rescue techniques.

During training students should experience easy dive sites and then progress to more difficult sites; protected to exposed sites; sandy beach to rocky headland:small boat entry and exit etc. Research methods should include mapping, water sampling etc. Each student should plan and execute an individual research project using a simple format with one or two dives with an instructor as a buddy.

Instructors should be career professionals with long term commitment to teaching scientific diving at your institution.

Learning is ensured with excellent instructors, few students per instructor and time. It is extremely important to only certify students who develop the physical, technical, psychological and social strength to be safe and effective divers. Social learning is very important. Students must learn how to be self reliant, but at the same time learn to help others. They must know and trust themselves and others in and out of the water. A true buddy system works only when both divers are well trained and are willing and able to take care of themselves as well as each other. It is the only way to ensure productive research and prevent accidents.

MAINTENANCE OF CERTIFICATION

Certified divers maintain certified status by having medical examinations every two to three years (depending on age), completing cardiopulmonary testing, maintaining CPR certification, diving frequency of usually one dive every six months with twelve dives each year and conduct or assist in research and/or training. Active status allows the diver to maintain skills and diving fitness. It also allows the Diving Officer reference to current diving practice when making recommendations regarding reciprocity.

CONCLUSION

Each institution should take the responsibility for training and certification to ensure administrative awareness and responsibility. The excellent safety record presented to the Federal government in the late 1970's was a direct result of individual institutions ability to take responsibility for safe and effective scientific diving training and practice.

INVESTIGATIONS OF COLD SEEP COMMUNITIES IN MONTEREY BAY, CALIFORNIA, USING A REMOTELY OPERATED VEHICLE

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Cold seep communities are sites of biological productivity based upon chemosynthesis by free-living or symbiotic bacteria, which utilize reduced inorganic or organic compounds (e.g. hydrogen sulfide, methane) as an energy source. Because these communities do not depend upon photosynthesis they are decoupled from solar energy, and are of considerable scientific interest. Cold seeps have been discovered in Monterey Bay associated with 4 distinct geologic settings.

Studies of geology, ecology, and biology of cold seeps have relied upon the remotely operated vehicle, Ventana, operated by Monterey Bay Aquarium Research Institute. These studies indicate that seep communities include obligate species (e.g. bacterial mats; Beggiatoa sp., and vesicomyid clams) which require close association with sulfide-rich fluids, and non-obligate species (e.g. anemones, gastropods, crabs) distributed regionally, but not wholly dependent upon chemosynthetically-based production. Ratios of stable carbon isotopes in tissues indicate the relative importance of seep-derived, versus non-seep food sources.

Mechanisms of recruitment of vesicomyid clams to cold seep sites are poorly known. Preliminary studies of larval development indicate that eggs and larvae float. Larvae develop as test-cell larvae, but have not been observed to metamorphose to an adult shelled form.

INTRODUCTION

The discovery of cold seeps and hydrothermal vents on the sea floor since 1977 has generated considerable scientific interest in their geology, geochemistry, biology, and ecology (Corliss et al. 1979; Paull et al. 1984). Hydrothermal vents are sites of rapid flow of warm to hot (~5-380 °C), mineral-rich water from the sea floor, found at isolated sites in regions of thin oceanic crust and accretionary wedges of active continental margins

(e.g. tectonic spreading centers and active volcanic areas associated with subduction zones; Gage and Tyler 1991). Cold seeps are similar to hydrothermal vents in that mineral-rich waters emanate from the sea floor at sites along both active and passive continental margins. Unlike hydrothermal vents, however, cold seeps are not always associated with active tectonic processes, and occur under a variety of geologic settings. Nor are fluids found at cold seeps generally colder (or warmer) than the surrounding seawater. Unique biological communities exist at cold seeps and include autotrophic species wholly dependent upon inorganic or organic compounds in fluids released, and other heterotrophic species which rely on organic material produced at the vent or seep sites.

The discovery of warm and cold seeps was largely a result of advanced technology that increased human access to the deep sea. Hydrothermal vents were first suspected to exist after hot water 'spikes' were detected near the sea floor at abyssal depths (Lonsdale 1977). The actual discovery of vents and seeps occurred during dives of manned submersibles (Corliss et al. 1979; Paull et al. 1984). Later discoveries of such sites have come about from direct observations of the sea floor using manned or unmanned submersibles (remotely operated vehicle - ROV). Cold seeps have since been documented at sites around the world, including Monterey Bay, and associated with hydrocarbon seeps and methane hydrate deposits (Kennicutt et al. 1988), groundwater seeps (Paull et al. 1984; Robison and Greene 1992), accretionary wedges (Jollivet et al. 1990), and tectonic subduction zones (Ohta and Laubier 1987). Fluid flow from cold seeps is generally much more diffuse than at hydrothermal vents, and is related to a variety of geologic processes.

In the Monterey Bay region, cold seep communities were first discovered in the axial valley of the Monterey Submarine Canyon during dives in the research submersible *ALVIN* (Embley et al. 1990). However, the presence of these communities was suspected in Monterey Bay from shells of vesicomyid clams, associated with seeps, dredged from the bay during research cruises (J. Nybakken, personal observation). Additional seeps have recently been discovered at 3 sites under distinctly differing geologic settings using the remotely operated vehicle (ROV) *VENTANA*, operated by the Monterey Bay Aquarium Research Institute (MBARI). These sites are presently being investigated by scientists from MBARI, Monterey Bay Aquarium, U.S. Geologic Survey, Hopkins Marine Station, and Moss Landing Marine Laboratories. Research topics include the distribution and extent of cold seeps in Monterey Bay, geologic settings under which they exist, and characteristics of biological communities found at seeps. Geochemical aspects of the seeps are also in question. Fluids released may or may not contain sulfide and methane, or other inorganic and organic compounds required for chemosynthetic production, and thus, result in formation of seep-associated biological assemblages. Biological studies include surveys of the species composition of various seep assemblages, including 1) obligate species [bacterial mats, bivalve taxa (Vesicomyidae, Solemyidae, Tellinidae), and vestimentiferan worms], 2) potentially obligate species (unidentified columbellid gastropods, limpets, and an unknown galatheid crab), and 3) non-obligate species that utilize seep-derived production (anemones, brachyuran and galatheid crabs, gastropods, and soft corals), but are cosmopolitan in distribution.

Reproductive patterns of seep fauna are also poorly known. Because these sites are often highly isolated from similar habitats, dispersal mechanisms of these species may exhibit particular specializations to facilitate successful recruitment and colonization of new habitats. However, mechanisms of fertilization, larval development, and larval

ecology of vesicomyid clams, the dominant group of clams for hydrothermal vents and cold seeps, are poorly understood.

Other aspects of the ecology and energetics of these communities are also poorly understood. For example, the extent to which chemosynthetic production at these underwater oases fuels secondary productivity by the local non-seep biological assemblage is unknown. Our observations indicate that seep communities may act as underwater oases in a relatively energy-poor seafloor landscape. A variety of species of cosmopolitan benthic fauna appear to benefit from foraging at cold seeps. Thus, we postulate that seep communities constitute seafloor oases.

Little or no information is available concerning ecological processes that influence demographic rates of biological populations at cold seeps. Predation, competition, and disturbance likely play a major role, but few hypotheses regarding these population processes have been addressed. Thus, there are a constellation of questions concerning the geologic and biologic structure and function of cold seeps. The presence of four geologically distinct seep sites in Monterey Bay provides a unique opportunity for these investigations.

METHODS

ROV Ventana

Recent studies of cold seep communities in Monterey Bay have been accomplished with the use of the ROV *Ventana*, operated by the Monterey Bay Aquarium Research Institute (MBARI). The *Ventana* is an International Submarine Enterprises HYSUB 40 with a Siemens computer-based control system, seven function Magnum manipulator, Mesotec 971 sonar, and multi-camera system. Power and video or other data are transferred through the 1000 m kevlar-covered tether through hard wire and optical fibers. The ROV is powered by a 40 hp motor, using hydraulic thrusters and hydraulic actuators for a variety of sampling devices, and is rated to 1650 m depth, but is presently limited to a maximum of 1000 m by tether length. The primary camera is a high resolution SONY DXC-3000 Betacam with an 8:1 zoom, focus and iris control. Images are transmitted via optical fiber to the surface, and coupled to a Betacam recorder. Additional peripheral cameras include 2 Osprey wide angle color cameras, an Osprey SIT camera, and 1 Panasonic peripheral cameras. Sensors and samplers include a conductivity-temperature-depth (CTD) sensor and fluorometer, broad band hydrophone, high and low flow suction samplers with a carousel-type sample chamber system, a Photosea 35 mm still camera and strobe, tube-cores, a sample drawer with dividers, paired green lasers spaced 10 cm apart for size measurement, a Sevonius rotor current meter, and detritus samplers. During this study the *Ventana* has enabled submarine surveys of cold seep sites and surrounding areas, and has been used as a platform to deploy and recover experimental and observational structures, and collect seafloor rocks, sediments, and biological samples.

Survey Methods

Surveys have been performed at several dive sites selected according to geologic characteristics that suggested the existence of cold seeps. ROV surveys were performed

along transects, oriented either horizontally at a constant depth along flat-lying or gently dipping strata, or vertically from deeper to shallower depths, during which observations of the bottom allowed detection of cold seeps from the presence of bacterial mats or other seep-associated fauna. Horizontal transects ranged in length from 100 to 3000 m, and were performed by flying the ROV along a straight line or constant depth. Potential targets were also located along transects using sonar (Mesotec); ranging from 50 to 100 m in all directions allowed detection and investigation of rock outcrops. Transect tracks were occasionally altered to investigate topographic features identified by sonar that were potentially associated with seeps.

Once cold seeps were identified along an ROV transect the latitude and longitude of the site were recorded and marked as a 'contact' on the navigation system and a more complete survey of the local region was performed to identify the vertical and horizontal extent of the seep locality. In addition, sites with abundant seep biological communities were documented using video and still cameras. Collections of representative fauna were made using the robotic arm and placed in the sample drawer for later analyses (see below).

Geological Studies

Geologic characteristics of cold seep sites, such as the type of lithology and presence of structures associated with each seep site (e.g. evidence of faults, fault-scars, and bedding) were identified during ROV surveys. Rock samples were collected and placed in the ROV drawer for further analyses which included petrologic, paleontological and chemical analyses. Vertical and horizontal surveys around known seep sites enabled the identification of diagnostic geologic features that indicate seep locations. These features consist primarily of sedimentary rock outcrops with fault and fracture zones.

The geochemical composition of seep-related fluids has been investigated using gas chromatographic techniques on samples collected from cores at selected seep locations. Cores of sediment were collected using 7.6 cm diameter by 35 cm long push-cores. Five PVC push-cores were mounted in sleeves on the ROV frame and operated by the robotic arm. Cores up to 25 cm deep were taken within aggregations of vesicomyid clams and black surficial muds indicative of high sulfide levels. Additional cores were collected from the margins of clam aggregations, and in 'non-seep' locations approximately 5 to 10 m from clam aggregations. Upon recovery of the ROV, push-cores were placed expediently in a nitrogen-filled chamber (glove-bag), and subcores samples were taken using an open-ended 10 cc syringe (1.3 mm diameter x 4.0 cm length), oriented either vertically or horizontally. Vertical subcores were sampled from the surface of the main cores and horizontal subcores were taken at 3, 6, and 10 cm depths in the main core. Syringes were capped with rubber stoppers and spun in a nitrogen-filled centrifuge at 3600 rpm for 20 minutes, to separate pore waters from sediment. Following centrifugation, pore waters were extracted using a 500 μ l glass syringe via a hypodermic needle inserted through the wall of the 10 cc subcore syringe. Syringes containing pore water samples were then dropped into liquid nitrogen to inhibit oxidation of sulfide prior to analysis. Samples were thawed and analyzed using a Hewlett Packard Model 5890A Series II Gas Chromatograph, modified for extraction and quantitative analysis of carbon dioxide, sulfide, oxygen, nitrogen, and methane, dissolved in fluid samples.

Biological Investigations

Community Structure

The composition of biological communities at cold seeps was examined using video and photographic imagery, supplemented by specimens collected by the ROV. Close up video imagery was collected at several locations in each seep site using the SONY DXC-3000 camera. Still photographs were taken using the Photosea 1000 camera. Samples collected using the robotic arm were stored in the sample collection drawer of the ROV, then sieved (sediment) and sorted following each dive. Specimens of vesicomyid clams were measured (size, weight) and transported live to laboratory aquaria and held in chilled seawater (4 to 6 °C) for further analyses.

Stable Isotopic Analyses

Because chemosynthetic bacteria are known to fractionate ratios of naturally occurring stable isotopes of carbon (^{12}C and ^{13}C), and heterotrophic metabolism of carbon usually has little or no effect on isotopic ratios, analyses of the stable carbon isotope ratio is a useful indicator of the source of production. We analyzed tissues of vesicomyid clams as well as other organisms from the surrounding benthic habitats in order to assess the source of food for these clams. Several individuals of *Vesicomya gigas* were dissected, dried, and powdered, then analyzed using a mass spectrometer to detect the ratios of ^{12}C and ^{13}C ($\delta^{13}\text{C}$). Specimens of other benthic megafauna (fragile urchin - *Allocentrotus fragilis*; soft coral - *Anthomastus ritteri*; ophiuroid - *Asteronyx loveni*; giant kelp - *Macrocystis pyrifera*; gastropod mollusc - *Neptunea* sp.; sea star - *Rathbunaster californicus*; and sea cucumber - *Psolus* sp.) were similarly analyzed.

Vesicomyid Clam Biology

Vesicomyid clams, the dominant megafaunal group at cold seeps in Monterey Bay, were used in laboratory experiments focusing on characteristics of larval development and settlement processes. Clams maintained in chilled water aquaria were used for spawning experiments in which groups of clams were subjected to various treatments known to stimulate gamete release in related molluscan groups (Strathmann 1987). Treatments included short term elevation of temperature (+5 °C), soaking in solutions of hydrogen peroxide (H_2O_2 ; 5 mM solution in sea water at pH 9.1), potassium chloride (KCl; 1-2 g/l), sperm solution (extracted by dissection), or oxygenated and deoxygenated seawater, mechanical agitation, or injection of KCl (0.5 ml of 0.5 mM solution) or serotonin (crystalline 5-hydroxytryptamine, creatine sulfate complex; 0.25 to 0.5 ml of 2 mM solution) into the anterior adductor muscle. Gametes were also obtained from dissected gonads of male and female individuals shaken in filtered seawater.

Male and female gametes obtained from these methods were combined in filtered seawater (1 μm) and held overnight in 300 ml culture dishes submerged in chilled seawater. Dishes were inspected after 24 h to assess fertilization and developmental rates. Counts of 100 or more eggs/embryos were made, summing into categories of unfertilized eggs, 2 cell, 4 cell, or 8 cell embryos. Counts were repeated each day, including higher developmental stages. Samples of embryos were collected when possible, and preserved

in Bouin's solution for detailed microscopic inspection. Approximately every other day, embryos were transferred to clean culture dishes with filtered sea water.

Recruitment rates of clam larvae and stimuli for settlement of larvae were evaluated in field experiments in which sets of settlement treatments contained in sediment-filled trays were deployed at or near active seeps and clam assemblages. The presence of conspecific clam shells and sulfide seeps were chosen as potential settlement stimuli. Four treatments were arranged in each of three subdivided plastic trays (30 x 30 x 10 cm). Treatments included 1) sediment alone, 2) sediment + clam shell fragments, 3) sediment + artificial sulfide seep, and 4) sediment + clam shell fragments + artificial sulfide seep. Artificial sulfide seeps were created using a combination of homogenized macroalgae (mainly *Macrocystis pyrifera*) and CaSO₄ (plaster of paris) mixed with sediment. Shell fragment treatments were created by inserting shells of vesicomyid clams partially into the sediment. Trays were filled with seawater and frozen prior to deployment. Trays were transported to the in situ experimental site via the sample drawer of the ROV and placed using the manipulator arm. After 3 to 6 months, trays were recovered using the ROV. Upon recovery sediments from each treatment were sieved through 0.5 and 1.0 mm sieves, and the retained material was fixed in 10% formalin for 2-3 weeks, rinsed, and transferred to 10% isopropyl alcohol for storage. Samples were sorted on a dissecting scope and all biota were identified and counted.

Growth rates of individuals for vesicomyid clams at cold seeps are being studied using labelled individuals maintained in situ and confined to 'corrals' placed over individual seeps. Clams (approximately 50 individuals) were collected from small aggregations at 1 to 3 isolated seeps at 3 seep sites (Mt. Crushmore, Clamfield, Mud Volcano) using the manipulator arm, and placed in the sample collection drawer. Corrals (circular plastic rings 35 cm diameter x 25 cm deep) were then placed into the sediment over the site of collection. Collected clams were brought to the surface, where each individual was measured and labelled with numbered plastic tags glued to scrubbed shell surfaces. Attempts were made to minimize the time of emersion for each individual. Labelled clams were placed in the sample collection drawer, returned to the bottom and deposited into corrals at the location where they had been collected. Growth rates will be assessed by repeated measurement of labelled clams, and growth curves will be constructed for different cold seep sites and clam species.

RESULTS AND DISCUSSION

Distribution and Geologic Setting of Cold Seeps

ROV surveys of several sites in Monterey Bay resulted in discovery of 3 sites with cold seep communities, each associated with distinct geologic conditions (Figure 1).

Monterey Bay Cold Seeps

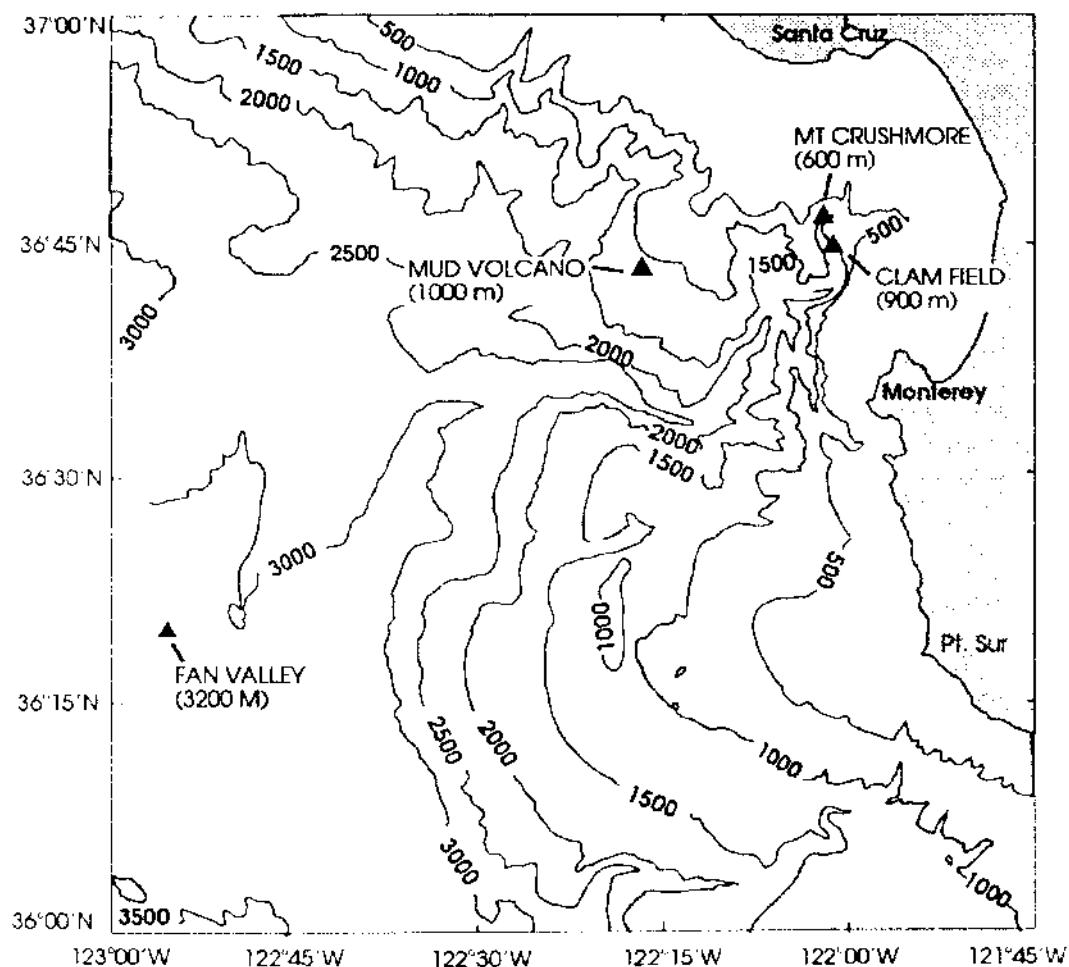


Figure 1. Map of Monterey Bay region, indicating location of cold seeps. Bathymetry in meters.

Seeps generally consisted of small (c. 0.25 - 1 m diameter) isolated locations of fluid flow, indicated by the presence of bacterial mats or clams aggregations, or both. Numerous small aggregations were scattered throughout a general area c. 1 km across and covering a range of depth near 100 m. Depending upon the site, seeps were found in crevices, fractures, and faults on vertical or near-vertical cliffs, sediment at the base of layered sedimentary rock cliffs, or in open regions away from areas of high relief.

One site, the 'Mt. Crushmore' cold seep site was the first discovered by MBARI's ROV. It consists of a cluster of seeps ranging from approximately 580 to 700 m depth and 1 km wide, associated with the exposed Purisima Formation near the junction of the Monterey and Soquel submarine canyons (36°46.9'N, 122°2.6'W; Figure 1). The Purisima formation is a highly permeable, well-layered sandstone unit that dips seaward from the Santa Cruz Mountains, and is a freshwater aquifer. We suspect that these shallow seeps derive substance from aquifer-driven fluids recharged from rainfall in the Santa Cruz Mountains. Fluid flow is downdip in the aquifer and fluids eventually exit at seeps exposed at the Mt. Crushmore site, some 1000 m difference in elevation. Tectonic motion has fractured and faulted rocks at this site. These fractures and faults intercept and concentrated fluid flow within the Purisima Formation.

A second seep (Clamfield; 36°44.2'N, 122°1.8'W; Figure 1) was discovered approximately 6 km south of Mt. Crushmore in the Monterey Formation. The Monterey Formation in this locality is a highly fractured, hydrocarbon-bearing shale/sandstone unit that underlies the Purisima Formation, and is exposed from near 760 m depth to greater than 1000 m. The Clamfield seep is restricted to about 890 to 930 m depth, and approximately 0.5 to 1 km wide. The main site is a long narrow band (c. 3 m wide by 150 m long) located at the base of a small cliff. Aggregations of live clams occur throughout the band, and shells of dead clams litter the sea floor to about 30 m below the active seep. Several other seeps are in close proximity to the main Clamfield site, found in detritus that appears to have been eroded from a thin cliff on the slope about 20 to 40 m above.

Cold seeps were also found between 900 and 1000 m depth on a smooth ridge located on the continental slope near 36°45'N, 122°17'W. The existence of this cold seep site (named 'Mud Volcano') was suggested from side scan sonar imagery of the area, indicating the presence of mud volcanoes, small mounds on the sea floor associated with compression-driven fluid flow from sediments. Compression is caused by thrust fault motion along the oblique convergent margin of the Pacific and North American plate, leading to 'squeezing' of near-surface sediment and outflow of CO₂-saturated interstitial fluid. Fluid flow results in carbonate formation and release of sulfide and methane-rich fluids at the sediment-water interface. Communities of vesicomyid clams and bacterial mats, indicative of seeps, were found at this site.

A fourth site known to have cold seep biological communities was discovered in 1988 during dives in the manned submersible, *ALVIN* (Embley et al. 1990). This was the first documentation of cold seeps in Monterey Bay. Dives along the axial valley of the canyon in the Monterey and Ascension Fan Valleys (36°23.1'N, 122°53.0'W; Figure 1) near 3200 m depth encountered clam communities including vesicomyid clams and *Solemya*. These communities were found in gently sloping to nearly flat areas along the margins of the axial valley and are potentially related to out-gassing (via mineral-rich fluid flow) of organic material buried during turbidity flow events over the past 100s to 1000s of years. Fluid flow may be driven by tectonic compression from oblique convergence of the Pacific Plate against the North American Plate, perhaps along older décollements.

Pore-water Chemistry of Cold Seep Sediment

Analyses of pore waters using gas chromatography indicate a highly heterogeneous distribution of dissolved gases (O_2 , H_2S , CO_2 , CH_4 , N_2) at three seep sites (Mt. Crushmore, Clamfield, Mud Volcano; Table 1). Cores for sub-samples of pore waters were collected from areas suspected to have high sulfide concentrations (e.g. central and marginal locations in clam aggregations, locations with black surficial sediment) and at control sites several meters from cold seeps. Sulfide concentrations were highest (7,000-11,000 μM) in pore waters from sub-cores taken 3 to 10 cm below the sediment-water interface at cold seep locations, and much lower at non-seep sites (0-20 μM). Highest sulfide values (11,300 μM) were found at the Clamfield site in sediment underlying a small open patch of black surface deposits which were surrounded by live clams. Sulfide within this patch may have exceeded the tolerance of vesicomyid clams. Oxygen values also varied considerably, and were lowest where sulfide concentrations were high, and vice versa. Methane was found at 2 of 3 sites investigated (Mud Volcano, Clamfield), but was not detected in pore waters from the suspected aquifer-related seep (Mt. Crushmore). Carbon dioxide were remarkably high, ranging up to 11,071 μM (normal seawater is c. 2,000 μM), and was positively correlated with sulfide concentrations.

Table 1. Summary of pore water gas concentrations (μM) at cold seeps and nearby control sites. Sample sizes for each site vary from 2 to 9.

Cold Seep Samples Site	CO_2	H_2S	O_2	N_2	CH_4	
<i>Mud Volcano</i>	3442	0	52	482	28	low
	5393	862	122	532	119	high
	4418	432	87	507	74	mean
<i>Mt. Crushmore</i>	2508	0	39	279	0	low
	3893	571	57	447	0	high
	3306	129	45	378	0	mean
<i>Clamfield</i>	3536	108	0	311	0	low
	11071	11263	3	628	62	high
	8475	5914	1	502	16	mean
Control Samples Site	CO_2	H_2S	O_2	N_2	CH_4	
<i>Mud Volcano</i>	nd	nd	nd	nd	nd	low
	nd	nd	nd	nd	nd	high
	nd	nd	nd	nd	nd	mean
<i>Mt. Crushmore</i>	2254	0	37	333	0	low
	2546	0	63	444	0	high
	2444	0	53	393	0	mean
<i>Clamfield</i>	2499	0	153	501	0	low
	2526	0	162	501	0	high
	2513	0	158	506	0	mean

Biological Communities of Cold Seeps

Biological assemblages associated with cold seeps were distinct even though several species also occur throughout the surrounding non-seep habitat. The most obvious organisms were bacteria (*Beggiatoa* sp.) and vesicomyid clams, which appeared to be highly localized and in direct association with fluid flow or sediment with high sulfide content (black muds) at seep sites. In addition to these groups, other species that appear to require close affinity with mineral-rich fluids (obligate species) were vestimentiferan worms, tellinid and solemyid clams. Some species, including an unknown species of galatheid crab, a columbellid gastropod, an unidentified limpet and chiton species, occurred only at seep sites, but their status as obligate or non-obligate species remains unclear. Not all groups were found at all seeps. Non-obligate species occurred at cold seeps, but were not restricted to these sites, and included many common species of benthic megafauna (e.g. sea stars, soft corals, fishes, anemones, gastropods, urchins, bivalves, brachiopods). Table 2 lists the relative abundance and species composition of obligate and non-obligate biological communities at each cold seep locality, including the clam communities reported by Embley et al. 1990.

Table 2. List of obligate and non-obligate megafauna associated with cold seeps in Monterey Bay, California. Obligate species occur only in direct contact with seeps. Non-obligate species occur at seeps and other benthic habitats. A - abundant, C - common, O - occasional, R - rare, X - present.

ORGANISM	Obligate?	Mt. Crushmore	Clamfield	Mud Volcano	Fan Valley
Monera					
<i>Beggiatoa?</i> sp.	Y	A	A	A	X
Cnidaria					
Anthozoa					
Alcyonacea					
Alcyoniidae					
<i>Anthomastus ritteri</i>	N	R	R		
Pennatulacea					
Umbellulidae					
<i>Umbellula lindahli?</i>	N	R	R		X
Actinaria					
Actinostolidae					
<i>Stomphia</i> sp.	N	C	C	O	O
<i>Paracinostola</i> sp.	N	C	C	O	O
Unknown sp.	N	O	O	O	X
Mollusca					
Polyplacophora					
Unknown chiton species	?	R			
Gastropoda					
Patellogastropoda					
Unknown limpet sp.	?	R			
Neogastropoda					
Neptuncidae					
<i>Neptuna</i> sp	N	C	C	C	
Columbellidae					
Unknown sp.	Y	A	A	C	
Bivalvia					
Solemyoidea					
Solemyidae					
<i>Solemya</i> sp.	Y		R	R	X
Nuculida					
Nuculanidae					
<i>Nuculana</i> sp.	N	R	R	R	
Yoldiidae					
<i>Yoldia</i> sp.	N	C	C	C	

ORGANISM	Obligate?	Mt. Crushmore	Clamfield	Mud Volcano	Fan Valley
Venerida					
Tellinidae - Unknown sp.	Y	R	O	O	
Vestimentidae					
<i>Calyptogena pacifica</i>	Y	A	O	O	
<i>Vesicomya gigas</i>	Y	R	A	A	
<i>Vesicomya phaeoliformis</i>	Y				X
<i>Vesicomya</i> sp. A	Y		R	R	
Unknown sp. B ("sharp butt")	Y	A	R	R	
Unknown sp. C (fan valley)	Y				X
Annelida					
Polychaeta					
Several unknown sp.	N	C	C	C	
Sipuncula					
Unknown sp.	N	O	O	O	
Pogonophora					
Theconephria					
Polybrachiidae					
<i>Polybrachia</i> sp.	Y				X
Vestimentifera					
Basibranchia					
Lamellibrachiidae					
<i>Lamellibrachia</i> sp.	Y		R		
Arthropoda					
Crustacea - Malacostraca					
Decapoda					
Paguridae - unknown sp.	N	C	C	C	
Lithodidae					
<i>Lithodes aequispina?</i>	N	O	O	O	
<i>Lithodes comes?</i>	N	O	O	O	
Majidae					
<i>Chorilia longipes</i>	N	C	C	C	
<i>Chionoecetes</i> sp.	N		O		
Galatheidae					
<i>Munidopsis?</i> sp.	N	C	C	O	
Unknown sp.	Y?	C			X
Brachiopoda					
Articulata					
Terebratulida					
Terebratulidae					
<i>Terebratulina</i> sp.	N	O			
Laqueidae					
<i>Laqueus californianus californica</i>	N	C			
<i>L. c. vancouverensis</i>	N	C			
Inarticulata					
<i>Glossidium albida?</i>	N			R	
Echinodermata					
Crinoida					
Comatulida					
Amidoniidae					
<i>Florometra?</i> sp.	N	R			X
Asteroides					
Unknown sp.	N	O	O	O	
Forcipulatida					
Asteriidae					
<i>Rathbunaster californicus</i>	N	R			
Zorocallida					
Zorasteridae					
<i>Zoraster</i> sp.	N	O	O		
Ophiuroidea					
Unknown sp.	N	O	R	R	
Phrynophiunda					
Asteronychidae					
<i>Asteronyx loveni</i>	N	O	O		

ORGANISM	Obligate?	Mt. Crushmore	Clamfield	Mud Volcano	Fan Valley
<i>Echinoides</i>					
<i>Echinida</i>					
<i>Strongylocentrotidae</i>					
<i>Allocentrotus fragilis</i>	N	R			
<i>Spaniogida</i>					
<i>Schizasteridae</i>					
<i>Brissaster latifrons</i>	N	O	O		
<i>Holothuroidea</i>					
<i>Dendrochirotida</i>					
<i>Psolidae</i>					
<i>Psolus sp.</i>	N	C	O		
<i>Elasipodida</i>					
<i>Lactognathidae</i>					
<i>Pannychia moseleyi</i>	N	R			
<i>Chordata</i>					
<i>Agnatha</i>					
<i>Myziniidae</i>					
<i>Eptatretus stoutii</i>	N	C	C	O	
<i>Chondrichthys</i>					
<i>Squaliformes</i>					
<i>Scyliorhinidae</i>					
<i>Apristurus brunneus</i>	N	R	R		
<i>Osteichthyes</i>					
<i>Gadiformes</i>					
<i>Macrouridae</i>					
<i>Coryphaenoides acrolepis</i>	N	O	O	O	
<i>Nezumia stegidolepis?</i>	N	R	R	R	
<i>Zoarcidae</i>					
<i>Aprodon cortezianus</i>	N	O	O	O	
<i>Perciformes</i>					
<i>Scorpaenidae</i>					
<i>Sebastolobus alascanus</i>	N	C	C	C	
<i>Sebastolobus alivelis</i>	N	C	C	C	
<i>Pleuronectiformes</i>					
<i>Pleuronectidae</i>					
<i>Emblemaria bathybius</i>	N	O	O	O	
<i>Microstomus pacificus</i>	N	C	C	C	

Stable Carbon Isotope Analyses

Analyses of stable carbon isotopes indicated that tissues of vesicomyid clams had isotopic ratios indicative of chemosynthesis based upon sulfide oxidation (Figure 2). The $\delta^{13}\text{C}$ of the vesicomyid tissues ranged from -35.6 to -38.9, while non-obligate benthic megafauna values were from -12.7 to -19.1. The low $\delta^{13}\text{C}$ of the clam tissues are similar to those reported by Rau et al. (1990) for *Calyptogena phaseoliformis* from the Ascension Fan-Valley. Further studies will indicate the relative importance of seep-derived production to non-obligate megafauna at seep sites and near cold seep localities.

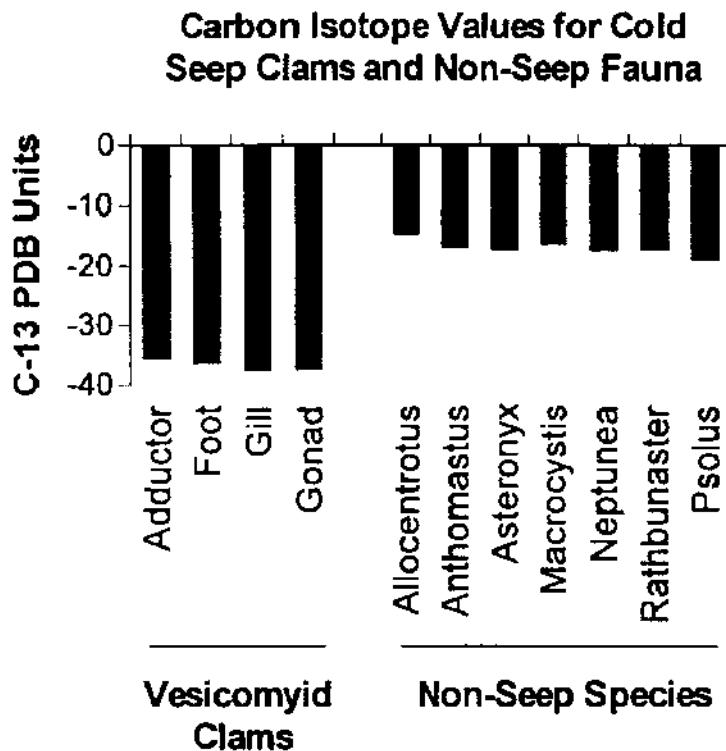


Figure 2. Results of stable carbon ($^{13}\text{C}/^{12}\text{C}$) analyses, in PDB units. Vesicomyid clam tissues are isotopically depleted in ^{13}C , relative to ^{12}C , compared to non-seep species, indicating chemosynthesis as a source of production.

Biology of Vesicomyid Clams

Reproduction and Development

Individuals of three species of vesicomyids (*Calyptogena pacifica*, *Vesicomya gigas*, unknown sp.) were used for investigations of spawning and larval development. For all 3 species, gamete release was stimulated by injection with serotonin, but no response was evoked by other treatments (KCl, H_2O_2 , temperature variation, agitation, pH, sperm solution). Eggs near 200 μm diameter were shed for approximately 1 hour in a weakly viscous fluid from the outcurrent siphon into the water column. Most eggs floated. Fecundity of females showing strong response to serotonin injection released approximately 3000 eggs. Males exhibited less response to gamete release treatments, but some individuals did release sperm in a fine cloud. Sperm for most fertilization trials was obtained via dissection of gonads.

Fertilization rates varied from 0 to 40% and development was quite slow. After 1 day, embryos reached a 4 to 8 cell stage, and at 2 days, embryos were mostly 32 cell stages. Embryos became ciliated and began swimming at approximately 5 days. This

stage for protobranch bivalves is termed a 'test-cell' larvae (pericalymma), a non-feeding larva that metamorphoses into a shelled juvenile (Strathmann 1987). Developmental rates for these larvae are slow compared to shaltower lamellibranchs (e.g. mussels), which develop to a trochophore within about 24 h (Field 1922). However, larval morphology is similar to more closely related protobranch bivalves (e.g. *Solemya*), which develop to a test-cell larva in about 18 to 24 h, and metamorphose after c. 5 days (Gustafson 1985). Survival rates of embryos were low, and the longest survival was 12 days, at which time it was a ciliated swimming larva, with little apparent external change from day 5.

We remain puzzled by the low rates of fertilization and survival, and have initiated multi-factor experiments to evaluate parameters that may influence these rates, including pH, temperature, oxygen saturation, antibiotic compounds, presence of dissolved organic and inorganic compounds (e.g. sulfide, methane), salinity, and pressure.

Larval Recruitment of Vesicomyid Clams

Recruitment of larvae of vesicomyid clams to experimental trays placed in cold seep habitats was low, and showed no statistically significant affinities for particular treatments (Table 3). Inspection of experimental trays during deployment identified the presence of bacterial mats on each treatment containing an artificial cold seep, indicating that such treatments were similar to sulfide seeps. A total of 8 juvenile clams were collected from 4 experimental trays deployed at 2 cold seep sites. Clams were found in all treatments, so no trends were indicated. Further experimentation is planned to identify settlement cues for larvae and seasonal or non-seasonal patterns of recruitment.

Table 3. Summary of vesicomyid recruitment experiments from cold seeps. -S: without artificial seep, +S: with artificial seep, -C: without vesicomyid clam shell, +C: with vesicomyid clam shell. Values are the number of juvenile clams found in each treatment replicate.

Treatment	-S-C	-S+C	+S-C	+S+C
Replicate				
1	0	0	1	0
2	2	3	1	1
3	0	0	0	0
4	0	0	0	0
Total	2	3	2	1

Growth Rates of Vesicomyid Clams

Experiments concerning growth rates of vesicomyid clams are still in progress, and will be analyzed during fall, 1993. Clams of known initial size, labelled with plastic numbers, have been inspected during repeated visits to these sites, and appear alive and well inside 'clam corrals'. One individual was recaptured 58 days after its initial

measurement and had increased in total length from 38.0 to 38.3 mm (0.005 mm/d), and from 19.97 to 20.55 mm in height (0.01 mm/d).

SUMMARY AND CONCLUSIONS

Investigations of cold seep communities in Monterey Bay have been possible using the ROV *Ventana*. Without a manned or unmanned submersible, these habitats would be nearly inaccessible owing to their isolation and rugged submarine terrain, which prohibit the use of towed camera systems. The major conclusions of these studies are:

- 1) Cold seep habitats have been discovered at 3 additional sites in Monterey Bay, under differing geologic settings, and appear to be related to fluid flow produced by markedly differing processes.
- 2) The dominant fauna of the seep sites include obligate species, mainly vesicomyid clams and bacterial mats, and non-obligate species (a variety of benthic megafauna)
- 3) Geochemical analyses of pore waters from cold seep sediment indicated high levels of sulfide and carbon dioxide, and lower levels of methane at 2 of 3 sites sampled.
- 4) Stable carbon isotopic analyses indicated a $\delta^{13}\text{C}$ from -38.9 to -35.6 for vesicomyid clam tissues, indicative of chemoautotrophic production, compared with $\delta^{13}\text{C}$ values of -19.1 to -12.7 for benthic megafauna and macrophytic detritus from non-seep sites.
- 5) Biological studies of vesicomyid clams indicate this group has fairly large (c. 200 μm), buoyant eggs, which develop as a 'test-cell' larvae, a non-feeding, relatively short-lived planktonic stage.

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PRELIMINARY INVESTIGATIONS IN ANCHIALINE CAVES OF CUBA

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A series of scuba dives conducted in fifteen anchialine caves in two distinct regions of Cuba in September 1992 resulted in the collection of a new gastropod species, a new Remipedia (Crustacea) species, as well as other cave adapted organisms. Caves in the extreme west area of Cuba were Aston type collapses similar to the sinkholes of Florida and blue holes of the Bahamas, while those in the Bay of Pigs area were slumping fracture cracks like those on Andros Island. In conjunction with the scientific studies, Cuban cave diving researchers were assisted with equipment development and evaluation, and with training in techniques utilized by American cave divers.

INTRODUCTION

In September 1992, the Second Caribbean Speleological Symposium was held in Cuba. This symposium was attended by approximately 400 participants from more than 25 countries. Three members from Island Caves Research Center (ICRC) were invited to speak at the week long program. The conference was hosted in the town of Vinales, about 20 kilometers from Pinar del Rio and a four hour drive west of Havana.

ICRC objectives included: (1) an initial assessment of the geology and biology of anchialine caves in Cuba, which have not previously been studied, and (2) assisting local diving speleologists in developing personal diving techniques and improving cave diving equipment so as to improve the safety of cave diving in the country. To accomplish these goals, ICRC researchers conducted diving operations in two different areas in Cuba, working with different groups of Cuban cave divers in each of those areas. In addition, a special cave diving session was conducted during the general symposium, which was split about equally between scientific and safety presentations.

During the symposium, one day was taken to travel to Maria de Gorda to conduct an evaluation of the caves in that vicinity. Three American divers were joined by two Cuban cave divers for the three hour drive from Vinales to the western shore of Cuba. Three dives were conducted in two sites in this area.

A special post-conference excursion was organized to allow a group of five American and three Cuban divers the opportunity to dive in numerous caves in the Bay of Pigs region of Cuba. We planned and executed a nine day trip based from Playa Giron, a several hour drive southeast of Havana. The primary objective for ICRC participants was biological collection for continuing ICRC research. Of particular interest was searching for Remipedia, a class of crustacea first described by Yager in 1981. These animals were originally discovered in the Lucayan Cavern System on Grand Bahama Island, and have since been described from anchialine caves on other Bahamian Islands (Yager, 1987a), in the Turks and Caicos Islands (Yager and Schram, 1986), and

the Yucatan peninsula (Yager, 1987b). Because of similarities in the geology and geographic location of Cuba, we expected to find them in Cuba as well. Nineteen dives in nine sites were conducted in Bay of Pigs caves.

Logistics

Infrastructure: As the exchange of currency in Cuba is restricted by the government of the United States, ICRC participants were required to obtain visas from the Department of State to attend the sessions. Scientists conducting or involved in professional activities are eligible for such visas upon demonstration of need. Permission was eventually granted, with the necessary paperwork arriving less than twenty-four hours before departure. All applications were handled by Marazul Tours, the travel agency authorized to organize the excursions.

Due to the poor economy in Cuba, the availability of commercial electricity for charging batteries was uncertain. In Vinales, where the conference was held, the community reportedly has only four hours a week of electricity commercially available. Conducting effective research while cave diving requires adequate lighting, so the ability to recharge the high capacity batteries is critical.

Gasoline is a rationed commodity in Cuba. This limited travel within the country, and prevented the team from investigating several sites which, based on the information available, had high potential for significant biological findings. Due to the rationing, some sites were accessed on foot (distances up to 4 kilometers), and others were visited by bicycle (distances to 15 kilometers). In these cases standard equipment configurations were altered to make the mode of travel viable.

Diving Equipment: The availability of scuba equipment and extent of supporting infrastructure in Cuba was unknown before the group left the United States. To save on excess baggage fees (\$2.00 per pound for each pound over 44 pounds, including carry-on baggage) the divers carried only three sets of cave diving equipment, plus their personal wetsuits, masks, and fins. This meant that everyone shared major equipment, and were not be able to dive simultaneously.

The cylinders used in Cuba are all equipped with DIN fittings, so the team used compatible Poseidon and Sherwood regulators. The tanks were configured as dual singles on standard backplates, negating the need to install dual valve manifolds. This also saved weight for the plane flight, and provided an additional level of redundancy should an air system failure occur.

Because of concerns about availability of 120-volt alternating current, several 7.2-volt wet cell nicad lighting systems were carried. These batteries can be charged from any available 12-volt automobiles, and thus provided a level of flexibility otherwise unavailable. Three Dive Rite Manufacturing Star Bright 12-volt lights, which recharge from 120-volt alternating current or 12-volt automobile systems, were also utilized.

Aladin Pro (50% of all dives) and Parkway (15%) dive computers and US Navy dive tables (35%) were used to regulate dive profiles.

Biological Sampling and Data Collection: Swimming crustaceans, including amphipods, remipedes, and shrimp, were collected using Sket bottles and plastic bags. Hand towed plankton nets were utilized to collect copepods, mysids, and planktonic organisms. Fish were collected by hand with plastic bags. Gastropods and sessile organisms, such as clams and mussels, were hand sampled. Bacteria colonies suspended in the water column were collected using plastic bags. Information on general collection

techniques is described by Bozanic (1985).

Yellow Springs Instruments (YSI) of Yellow Springs, Ohio provided a prototype device which would record salinity, conductivity, dissolved oxygen, redox potential, temperature, and depth throughout the dives on which it was carried. The information was later uploaded into an IBMcompatible personal computer for analysis. The unit itself was a cylinder about 15 cm in diameter, and approximately 60 cm long. It was carried by the divers on the back of the cylinders, nestled in the hollow between the two tanks. This was the first time this instrument has been carried by scuba divers, and YSI was provided with an evaluation of its performance and problems experienced. Salinity measurements were checked using a temperature corrected refractometer, and dissolved oxygen values were confirmed *in situ* using Chemetrix indicator ampules.

Underwater photography was accomplished using Nikonos cameras and Ikelite strobes. Only a limited amount of underwater photography was conducted because of the nature of the excursions.

Site Descriptions and Findings

The following is a brief summary of the caves examined by the group, along with a site description and collections. The caves are organized by the two regions of Cuba in which they exist. Maximum cave depths observed (in meters of sea water (msw) and feet of sea water (fsw)), salinities (in parts per thousand (o/oo)), and depths of density interfaces (DI)/haloclines (fsw) are summarized in Table 1.

Maria de Gorda Caves

Pozo de Juan Claro--This cave is located at the shoreline, about 20 km north of the Maria la Gorda resort on the Gulf of Guanahacabibes. It is a fairly typical reversing current blue hole, like those described by Williams (1978). The blue hole empties into a small lagoon which is connected to the sea via a small ria. This cave was accessed during the excurrent cycle, to maximize diver safety. The site had been previously explored by a French Expedition in 1991. Maximum penetration was about 200 meters. One species of white shrimp, currently unidentified, was collected. The cave was biologically very sterile in comparison to undisturbed ocean blue holes in the Bahamas. To the south another smaller surface boil was sighted, but not investigated.

Pozo Azul--This cave is located about a 4 km walk from the village of Guana. It is a typical Aston-collapse type sinkhole with expected bellshaped walls and breakdown pile in the center, as described in Zumrick, et al (1988). The surface pool was about 100 m in diameter. Entry was a 6 m giant stride into the water. Collections included the blind cave fish *Lucifuga dentatus*, cave adapted shrimp *Troglocubanus* sp., another as yet unidentified shrimp, copepods, planarians, sponges, and cirroanid isopods. All collections were made in diffuse light from the surface, as no lightless passages were found.

Bay of Pigs Caves

All of the remaining dive sites were on the eastern side of the Bay of Pigs. With the exception of Caleta Buena, geomorphically they all appear to be slumping fracture cracks comparable to those seen on Andros Island (Palmer and Williams, 1984). They are oriented approximately parallel to the shoreline. Typical expression of the cave is a completely submerged, extremely tall (often 60 meters) and narrow (1-3 meters) wide

crack which extends sometimes for several hundred meters in either direction from the surface pool. The surface pools were generally linear ponds where the crack was open at its ceiling. Elevations were typically 0.5 to 4 m above water level. Visibility ranged from zero to over a hundred meters. Mung-like sediments were common, as were hydrogen sulfide (H₂S) layers and soft bottom silts. The walls were usually very durable, and often were coated with flowstone. Drapery and other speleothems were seen in many of the caves. Boulders wedged in the cracks, and areas where the crack narrowed to the point where a diver could not pass made these a two-dimensional maze oriented perpendicular to the Earth's surface. Water temperatures ranged from about 25-28°C.

Ilona Cave--Collected two species of shrimp, amphipods, copepods. An American bomb from the Bay of Pigs invasion lies at 40 fsw.

Cueva XXXV Anniversario--High densities of Eliotris (a type of fish commonly found in anchialine caves) in the surface pool, and an unidentified shrimp were found in the cave. In some cases there were in excess of 20 shrimp per square meter of wall. Collected Eliotris sp., shrimp (four different species), mussels, sea stars, a form of Cyclostrema (a small snail) as yet undescribed (Cosman, 1993), and rock boring clams. A new species of land snail was also collected from inside the cave, which is currently waiting description (Cosman, 1993). Walls were highly decorated with flowstone.

Casimba el Brinco--This cave is the only one seen in this area which had an aerial expression of the slumping fracture crack, with the sides of the crack extending about five meters above the water level. The entry is from a cement platform in a small cavern formed by these walls, and is a two meter jump into water 60 m deep. Visibility in this system is in excess of 100 m. The entire east side of the cave is an intact fossil reef, complete with corals, sponges, etc., which has been left behind after the surrounding matrix was preferentially dissolved away. Flowstone coats the other side of the cave. This cave has significant potential for further paleontological and geological research, but is extremely prone to damage by careless dives as described by Bozanic (1992). Collected mysids, copepods, an eyed cirratulid isopod, and three species of shrimp, including one *Macrobrachium*.

Casimba Susanna--The surface water layer is a greenish, tannic water with 1 m visibility. The 3-6 m (10-20 ft) layer is a very red tannic layer. The 6-15 m (20-50 ft) layer contains clouds of dissolved H₂S, dissolved O₂ levels exceeding 1 ppm, colonial bacteria suspended in the water column, and numerous shrimp in clear water. The deepest layer has about 15-m visibility, with lower dissolved O₂ levels (<0.05 ppm). Collected bacteria, shrimp, thermosbaenaceans, and *Lucifuga dentatus*. Cave extends in excess of 100 m in both directions.

Laguna Larga--Large lake, with several small caverns. Two caverns too small for a diver to enter. One additional cavern has a 12 m tunnel extending from the end. Large breakdown blocks line the bottom. It might be possible to find going passage under some of this breakdown, given sufficient effort. Low visibility H₂S water layer seen at deepest point in lake.

Humo--Cave extends about 40 m in at 294°, and 50 m at 108° (the other side of the pool). H₂S layer at 12 msw (41 fsw). The top of the talus mound was at 22.5 msw (75 fsw) in surface pool, with the floor extending to greater than 40 msw (120 fsw) in the southeast passage. Collected shrimp (three species), cirratulid isopods, medusae, copepods, hadziid amphipods, copepods, and thermosbaenaceans.

Casimba Juan Jose--A shallow, muddy collapse with no going passage. Located near the Caleta Buena Restaurant.

Tarpon Cave--Another small collapse northeast of Casimba Juan Jose. Two 50 cm

tarpon seen. No significant cave passage seen, although a small possibility exists that passage could be found in this site.

Caleta Buena--This cave is anomalous for this region, as it is not a slumping fracture type cave. This cave resembles those of the Yucatan Peninsula and Cozumel (Bozanic, 1984), in which a continuous excurrent emerges from a low oval passage (1.5 m high by 2 m wide). In the Yucatan caves, the lower water layer is typically marine, the flow of which tidally reverses. While only a cursory examination of this cave was made, this appeared to be true in this site as well. According to the Cuban divers, the cave extends approximately 150 m from the entrance before becoming too small for divers to pass (Nieto, 1992).

Cueva del Trueno--This was the first time this cave had been dove using scuba, according to the Cubans. The water has extremely high H₂S concentrations at depths of 6-20 msw (21-67 fsw). The dive was turned based on planned bottom time at a talus mound which might indicate another surface opening, at a penetration distance of 170 m in going passage. Visibility about one meter. Collected one mysid.

Lacrina de Catalina--A very small (2 x 5 m) pool clogged with trees, trash, electrical cable, fishing line, etc. Cave could be seen to go at least another three meters deeper than penetratable by a diver. It is possible that it could be cleaned out, resulting in a diveable site. Located about 150 m behind a group of houses in a community called Giron. Visibility about three meters.

Cueva de los Carboneros--Clear water (50 m visibility), with a new species of remipede found swimming in sight of daylight. This new remipede closely resembles Speleonectes lucayensis (Yager, 1993), but has not yet been described. Also collected were thermosbaenaceans, copepods, blind cave fish, and shrimp. Almost all of the cave animals were found immediately below the 12 m (40 ft) halocline. Large quantities of mung in the cave. Visibility dropped to less than 1 m as a result of bubbles disturbing sediment and bacterial colonies on the roof. Going passage in both directions, explored to distances of about 150 m in each direction.

Cueva 1900--This opening is located in the middle of a swamp, about 200 m from the nearest solid ground and 500 m from Cueva de los Carboneros.

The area consists of a dense growth of mangroves, ferns, and plant life, all growing in water a few centimeters deep. Walking to the site requires wading through mud which is usually ankle to calf deep, and occasionally knee deep. The surface pool appears like any other place in the swamp, except instead of being knee deep the water was 5 m deep. The opening is just larger than one meter in diameter, with the walls and bottom consisting of mud and peat. As there was no solid place to tie off the guideline, it was "secured" to base of three individual ferns. A solid tie was obtained at a depth of 4.5 msw (15 fsw), out of sight of surface light. Water was red in color from tannin on the surface, with visibility of about one meter. There was a weak H₂S layer at 10 msw (30 fsw). Remipedes and thermosbaenaceans were found at depths of 18-21 msw (60-70 fsw) and 13-22.5 msw (42-75 fsw) respectively. The cave only goes in one direction, but it may be possible to dig out the other side. We limited the maximum depth to 27 msw (89 fsw) (because of residual nitrogen from previous dives), but could see a minimum of 18 m downward from that point, yielding a maximum depth in excess of 45 msw (150 fsw). As this dive was conducted literally minutes before the group left for Havana to return to the United States, it was not possible to conduct further exploratory dives in this cave.

Table 1--Summary of Salinities and Density Interfaces (DI) of water strata in caves: Asterisks (*) denote data which was not collected, dashes (--) denote information not applicable to that cave.

Water Strata (right)	Max Depth fsw	#1		#2		#3		#4		#5
		Shallow		H ₂ O ‰	DI fsw	H ₂ O ‰	DI fsw	H ₂ O ‰	DI fsw	Deep H ₂ O ‰
Cave Name (below)										
Pozo de Juan Claro	11.6	38	-10	37	-	-	-	-	-	-
Pozo Azul	37.5	1.5	9.1	1.5	22.9	4.5	28	20	34.1	33
Ilona Cave	42.7	15	1.8	32	-	-	-	-	-	-
XXXV Anniversario	66.7	4.5	9.1	37	-	-	-	-	-	-
Casimba el Brinco	36.6	0	-1	3.5	12.2	35	21.3	36	-	-
Casimba Susanna	30.5	*	3	*	6.1	35	15.2	*	-	-
Laguna Larga	14	4	-14	*	-	-	-	-	-	-
Humo	33.8	2	3.7	*	12.5	37	-	-	-	-
Casimba San Juan	3	*	-	-	-	-	-	-	-	-
Tarpon Cave	4.6	*	-	-	-	-	-	-	-	-
Caleta Buena	4.6	*	-3	37	-	-	-	-	-	-
Cueva del Trueno	20.4	*	-	6.4	*	-	-	-	-	-
Lacrina de Catalina	7.6	3.5	-	-	-	-	-	-	-	-
Cueva Carboneros	61	3.5	4.3	6	12.2	35	-	-	-	-
Cueva 1900	45.7	*	9.1	*	12.8	*	17.7	*	-	-

Water Strata (right)	Max Depth msw	#1		#2		#3		#4		#5
		Shallow		H ₂ O ‰	DI msw	H ₂ O ‰	DI msw	H ₂ O ‰	DI msw	Deep H ₂ O ‰
Cave Name (below)										
Pozo de Juan Claro	11.6	28.5	-10	37	-	-	-	-	-	-
Pozo Azul	37.5	1.5	9.1	1.5	22.9	4.5	28	20	34.1	33
Ilona Cave	42.7	15	1.8	32	-	-	-	-	-	-
XXXV Anniversario	67	4.5	9.1	37	-	-	-	-	-	-
Casimba el Brinco	37	0	-1	3.5	12	35	21	36	-	-
Casimba Susanna	31	*	3	*	6.1	35	15	*	-	-
Laguna Larga	14	4	-14	*	-	-	-	-	-	-
Humo	33.8	2	3.7	*	12.5	37	-	-	-	-
Casimba San Juan	3	*	-	-	-	-	-	-	-	-
Tarpon Cave	4.6	*	-	-	-	-	-	-	-	-
Caleta Buena	4.6	*	-3	37	-	-	-	-	-	-
Cueva del Trueno	20	*	6.4	*	-	-	-	-	-	-
Lacrina de Catalina	7.6	3.5	-	-	-	-	-	-	-	-
Cueva Carboneros	61	3.5	4.3	6	12.2	35	-	-	-	-
Cueva 1900	45.7	*	9.1	*	12.8	*	17.7	*	-	-

DISCUSSION

One of the reasons ICRC personnel were invited to attend the symposium was to assist with the development of scientific cave diving within Cuba. This effort proceeded along two major avenues. First, several lecture style presentations were made as part of a workshop session on underwater speleology. These included presentations on techniques, equipment, and philosophies, and eventually lead to several informal practical training sessions in the pool of the hotel in which the conferees were housed. Second, several teams of Cuban cave divers participated in the research dives with the American visitors.

The skill level of the Cuban divers was unknown prior to the trip, so part of the initial activities was to informally evaluate them so as to make best use of their abilities during the post-conference excursion. We found that they were very accomplished divers, but were forced to work under severe equipment handicaps, because they could not get modern scuba equipment. As an example, one of the divers tied his [extremely rusty] double cylinders together with rope, lacking any better means of uniting them. Even such things as cave diving line and batteries were coveted, as they simply were not available in Cuba. When we left, we left with them a new edition of the NSS Cave Diving Manual (Zumrick, et al, 1988), all of our remaining line, several back-up lights, two reels, and much miscellaneous equipment.

We also found that skills commonly taught in cave diving courses in the United States, such as anti-silting kicks, weighting and trim, and running reel were often new concepts for Cuban diving speleologists. During the course of research activities, much was taught to these divers, primarily by example. Tentative plans were made to have some of the American participants return to teach a full cave diving course, subject to obtaining governmental permission and funding.

Maximum dive depths in the caves ranged to 66 msw (219 fsw), with a median of 31 msw (104 fsw). On dives below 45 msw (150 fsw), one diver remained at that depth while the other descended to the maximum depth for a bottom time at depth of one minute, enabling collection of data on the YSI probe. 67% of the dives required stage decompression, which was based on either dive computers or US Navy dive tables (at the individual diver's discretion).

Several situations occurred which potentially could have jeopardized the safety of the divers. Two of these were related to waters with high naturally occurring H₂S concentrations. Cubans had previously explored Casimba Susanna using 1 cm manila rope as their permanent guideline. It is interesting to note that this line could be easily pinched in half between thumb and finger, due to the corrosive action of the H₂S. The rock walls in this cave were noted to be very friable, as evidenced by a very large piece of wall which fell onto one of the divers during a decompression stop. The influence of the H₂S in this event is unknown.

The concentration of H₂S in Cueva del Trueno was high enough to cause extreme discomfort to divers. Sufficient H₂S was absorbed through the skin so they could not see at times because of the burning it caused in the eyes. Had some other problem occurred, this could have contributed to a fatal incident.

Another incident occurred while exploring Cueva de los Carboneros. A new reel was used by the divers on the dive. In the course of their exploration, the end of the guideline on the reel was reached. Normally, the guideline is tied to the reel to prevent its being lost in this situation. This had not been done in this instance by the manufacturer of the reel, resulting in its being left behind the divers as they advanced. Loss of the line

was noted after about 10 m, and a standard lost line search was instituted in the half-meter visibility. The guideline was located, and the divers exited without further incident. This points to the need to check the security of line termination to the reel.

CONCLUSIONS

Preliminary investigations in anchialine caves in Cuba resulted in the collection of several new species of animals, including one mollusk, one remipede, and potentially several other crustaceans. As only a relatively few number of Cuban caves were examined, it seems reasonable to expect that further work would yield other new organisms. In addition, collection of remipedes in Cuba should contribute to understanding the geographic distribution of the class, and possibly of other cave adapted fauna as well. Based on these findings, continued inquiries in these anchialine systems is indicated.

Several potentially hazardous incidents related to waters with naturally high concentrations of H₂S were noted. While H₂S is known to be toxic to humans, the amount of H₂S which is absorbed and the amount which can be safely tolerated by a diver remains unknown. It seems that these amounts would be a function of the concentration, which can vary significantly in natural environments, exposure time, and protective suit utilized. Studies to define safe exposure levels for divers would benefit researchers diving in some anchialine cave environments, as well as those working in the stratified lakes of Palau.

ACKNOWLEDGEMENTS

Funding and logistical support for this project were provided by the Government of Cuba, the Explorer's Club, Yellow Springs Instruments (YSI), the National Speleological Society (NSS), and the Cave Diving Section of the National Speleological Society (NSS-CDS). This paper was read in manuscript by Dr. Thomas Kline of the University of Alaska, Fairbanks; I am grateful for his valuable comments. This paper is No. 42 of the Island Caves Research Center, Inc. Safety and Education Series.

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PHOTOGRAPHY OF UNDERWATER SCIENTIFIC STUDIES: EQUIPMENT AND METHODS FOR THE 90'S.

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Many subsea experiments depend on photographic documentation. When photographs are unclear, interpretation of data is compromised. In the past five years there has been a welcome proliferation of high quality films and advanced moderately priced microcircuit camera equipment. Scanning photos into computers is a powerful tool. In addition to documenting primary data, photographs convey information to research assistants prior to dives. Valuable bottom time is saved by familiarizing team members with topographic features, task sequences (e.g. laying down transect lines, retrieving equipment), and important marine life or geological features. Photographs can be published, projected in slide shows, organized in subject notebooks with handwritten captions, or used in poster sessions. The ingredients of an effective photo are clear detail, accurate (or skillfully manipulated--e.g. infrared, uv) color, and a coherent storyline. Success depends on careful pre-planning, compulsive attention to detail while diving, and fallback techniques for adverse conditions. This paper will review the tools and techniques of underwater photography for the 90's.

Many brilliant lectures are illustrated by nearly incomprehensible photographs. It amazes us that fuzzy communication is acceptable to those who abhor fuzzy thinking. If the purpose of a presentation is funding, or swaying public opinion--good luck. The world, thanks to TV and the print media, is visually sophisticated, easily bored. Before you contemplate appearing before a committee asking for a hundred or so thousand dollars, take a look at a Coke or Pepsi ad where they ask for around fifty cents. Beyond that, most subsea experiments depend on photographic documentation. When the documentation is unclear, interpretation of data is compromised.

In the past five years there has been a welcome proliferation of high quality films and advanced moderately priced microcircuit camera equipment. In the ten thousand dollar range Nikon has introduced an auto-focus, zoom lens, camera and strobe setup. In the under two thousand dollar range there are several autofocus, highly accurate strobe metering cameras with a full compliment of lenses that may be installed in underwater housings. With a data back, photos can be imprinted at the time of exposure.

In the time between the writing of the present paper and its presentation no doubt another new film will be introduced. Films for 35mm cameras are divided into three categories:

Color positive, aka Slide/Transparency/or E-6
Color negative, aka Print/or C-41
Black & white, aka Black & white.

In Slide films during 1993 Kodak unveiled Ektachrome Underwater film. They also delivered 100 ASA Ef6 Elite film with resolution rivaling Kodachrome 25. Kodak is readying shipments of a red enhanced version of this film as well. Photographers owe Fuji film of Japan a debt of gratitude for prodding Kodak. The introduction of Fujichrome 100, & 50 films several years ago, as well as their more recent Velvia film took market share--and prestige--away from Kodak.

The ongoing improvements in color and grain properties of films are especially important to underwater photography. Landscape, fashion, architectural, or most scientific photographers have always been able to use slower speed, larger format films to achieve high resolution and accurate color. Tripods, 8x10 cameras, and 10,000 watt/sec A/C strobe banks are impracticable for a SCUBA diver. The advent of new grain structures for film have driven the improvements. At the high end of the ASA spectrum there is Kodak 3200 ASA T-Max black and white film that produces natural light photographs of large underwater structures or schooling fish in murky water where strobes would create unacceptable back-scatter. Fuji provides a 1600 ASA comparable black & white film. Many manufacturers produce 1000 ASA and faster color negative films that are surprisingly sharp due to the new grain technology. There is one 1000 ASA color slide film manufactured by Agfa. It is available at larger professional film dealerships--and somewhat expensive.

Once the film is exposed, it is important to carefully choose a photolab. Quick photolabs eliminate the wash step, counting on squeegees and chemical neutralization. If you want the prints to last, go to a professional lab. Cost cutting labs use slide chemistry long past its prime. Color balance and density suffer. Go to a professional lab. If there is no local lab, ship the film via FedEx to a major lab.

Many of the Autofocus , thru-the-lens (TTL) strobe metering cameras only work with ASA 400 or slower films, so don't leave the dock without testing your strobe equipment with any new high-speed film. Which brings us to the topic of USING the new films and cameras. I made my living for fifteen years inspecting and photographing underwater structures. The clients generally picked up daily expenses anywhere from five to twenty thousand dollars, and inspections lasted weeks or months. They didn't accept excuses for blank film. **ALWAYS TEST EVERY NEW PIECE OF PHOTO EQUIPMENT AND EVERY NEW FILM BEFORE LEAVING THE DOCK (OR THE AIRPORT).** Jump into a swimming pool with it. If time is short, hook all the equipment together and photograph in the shade outdoors, inside a room, or anywhere the sun can't override the strobe. Check the yellow pages for a lab that will process your film. If the film looks good, be sure to pack the instruction manuals and plenty of spare batteries. The new computer cameras have a lot of features, and precise key-strokes to invoke them.

Scanning photos into computers is a powerful tool. I first heard about computer enhancement when NASA developed programs to improve the resolution of space images. Today the most popular program is Photoshop. Focus and contrast can be enhanced somewhat. Colors can be manipulated. Sections of one photo can be combined with sections of other photos. The potential for creating more dramatic slide presentations is high, but the ability to eliminate back-scatter, put detail where lights failed to illuminate, or totally rescue the failed photograph is not yet upon us. Careful technique is not yet obsolete. At present the great use for computers for the underwater

photographer lies in cataloging images. Photo Database files such as PhotoTrak for IBM, or StockView for Mac's, are efficient time savers when slides must be located or labels applied. The computers ability to automatically cross-references photos beats any card file I've seen.

In addition to documenting primary data, photographs convey information to research assistants prior to dives. Valuable bottom time is saved by familiarizing team members with topographic features, task sequences (e.g. laying down transect lines, retrieving equipment), and important marine life or geological features. Having a photographer document each task can get information across to new team members more effectively than verbal description alone. An "album" can be carried on the cruise for reference, and as a reminder of what photos need updating. Although this paper does not address video, a combination of still and video coverage is ideal for sequential tasks.

Photographs can be published, projected in slide shows, organized in subject notebooks with handwritten captions, or used in poster sessions. It is important to store photos properly. Moisture, dust, and organic fumes destroy both slides and prints. I store slides and negatives in ARCHIVAL transparent preservers. I store 20 sheets per hanging file folder. The folders hold either a subject, such as fish, or a location such as Gulf of Mexico. Folders are purchased in colors: blue = underwater, yellow = desert, green = landscape, etc. A four-drawer steel file cabinet holds over 10,000 slides. The room is air conditioned for temperature and humidity control. Valuable original slides of not easily re-photographed subjects should not be projected or lent out. Duplicate slides cost one dollar. Another expedition, ship time, the likelihood of seeing the subject--more than one dollar. Each showing in a projector degrades the image. For highest quality, take what are called in camera dupes: if a subject is obviously important, take several photos of it.

The ingredients of an effective photo are clear detail, accurate (or skillfully manipulated--e.g.infrared, UV) color, and a coherent story line. The best way to learn how to effectively photograph is to study published photos. Then compare yours. Go back with improvements in mind. I practice on my aquarium fish. The best way to assure the image is in focus is to carefully take several photos. Subjects and divers are in motion. Banking all on one exposure is rarely effective. Be careful with pointing the strobe light. Built-in modeling lights are a great help in aiming strobes. Slide films are notoriously difficult to meter correctly -- the 36 out of 36 myth. If the subject is important then bracket your exposures. Underexpose and overexpose by one f/stop. These additional exposures are useful for slide shows, and they will save you many, many times. If your technique is good, then cut back to + & - one-half stop brackets. The new generation cameras automatically bracket.

Success depends on careful pre-planning, compulsive attention to detail while diving, and fallback techniques for adverse conditions.

UTILIZATION OF THE MINERALS MANAGEMENT SERVICE OPERATIONS AND SCIENTIFIC DIVING PROGRAM FOR OPERATIONS MONITORING AND INSPECTION A CASE STUDY

Les Dauterive

U.S. Department of the Interior

Minerals Management Service

Gulf of Mexico OCS Region

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The Minerals Management Service (MMS) Gulf of Mexico (GOM) Operations and Scientific Diving Program gathers underwater scientific data in support of the GOM Region's mandate to prepare environmental impact statements and environmental assessments for oil and gas lease sale and operations-permitted activity. Monitoring and inspecting operations-permitted activity are also functions of the Diving Program. An underwater inspection for operations-generated debris reported to have been discarded near satellite oil and gas platforms was done using the MMS GOM Region's (GOMR) Diving Program divers. The MMS divers accomplished and documented a visual search of the seafloor adjacent to, and at 7.6 meter (25 feet) and 15.2 meter (50 feet) circles around 16 satellite platforms and 4 multi-well production platforms in the Bay Marchand Oil and Gas Field (BMF) immediately offshore Grand Isle, Louisiana. Minimal debris was found near the satellite platforms. Substantial debris was found near and underneath the multi-well platforms. Corrective measures have been taken to address discarded debris associated with oil and gas operations in the GOMR.

THE MINERALS MANAGEMENT SERVICE

The MMS, a Federal regulatory agency within the U.S. Department of the Interior (USDOI), was created in January 1982, from the Conservation Division of the U.S. Geological Survey (USGS), to improve management of Federal leasing revenues. The management of Federal offshore minerals—another Federal responsibility deemed to be in need of organizational change—became part of the MMS. Functions related to managing Federal offshore energy and mineral resources were fragmented among the USGS, the Bureau of Land Management, and the USDOI Office of Outer Continental Shelf (OCS) Program Coordination.

In May 1982, the USDOI combined these offices and all functions concerned with managing energy and mineral resources on the Federal OCS into a single organization—the MMS.

Management of Federal offshore mineral resources is guided by the OCS Lands Act of 1953 and amendments made in 1978 and 1985. This Federal statute mandates the missions of the MMS:

- make OCS energy and mineral resources available expeditiously to meet the Nation's energy needs;
- protect human, marine, and coastal environments;
- ensure that State and local governments have timely access to information and opportunities to participate in OCS planning and policy decisionmaking; and
- get a fair and equitable return on resources while preserving and maintaining free enterprise competition.

The MMS has its headquarters office in Herndon, Virginia, and its offshore Atlantic, Gulf of Mexico, Pacific, and Alaska OCS Regional offices in Herndon, New Orleans, Camarillo, Calif., and Anchorage, respectively.

THE GULF OF MEXICO REGION'S DIVING PROGRAM

The MMS Operations and Scientific Diving Program is active only in the Gulf of Mexico Region (GOMR). Diving programs in the other Regional offices are inactive because leasing and further operations in these OCS Regions have been temporarily suspended. The Diving Program is managed by the MMS Diving Officer and Regional Dive Masters, and is guided by the MMS Diving Procedures and Safety Practices Operations and Scientific Diving Manual.

The MMS GOMR Diving Program activities have involved gathering underwater scientific and operations data for the Region's Environmental Studies Program and the Region's mandate to prepare environmental impact statements in support of leasing submerged OCS properties for oil and gas resource development and production. The data are also used to prepare environmental assessments for permitting exploration, development, production, transportation, and platform removal (site clearance) operations. Monitoring of OCS biological resources associated with natural and artificial reefs, assessment of prehistoric and historic archaeological sites on the OCS, and inspection for debris (bottom obstructions) associated with oil and gas operations, are examples of diving operations activities the GOMR's Diving Program have conducted.

INSPECTION

In response to an alleged knowing and willful violation of discarding batteries from satellite platforms in the BMF, divers of the GOMR Diving Program were directed by the GOM Regional Director to conduct a visual inspection for batteries and other debris near BMF platforms. Sixteen (16) satellite and four (4) multi-well platforms were selected at random for the visual inspection.

INSPECTION STUDY SITE

The BMF, located offshore Louisiana (Figure 1), is one of the oldest oil and gas fields in the GOM.

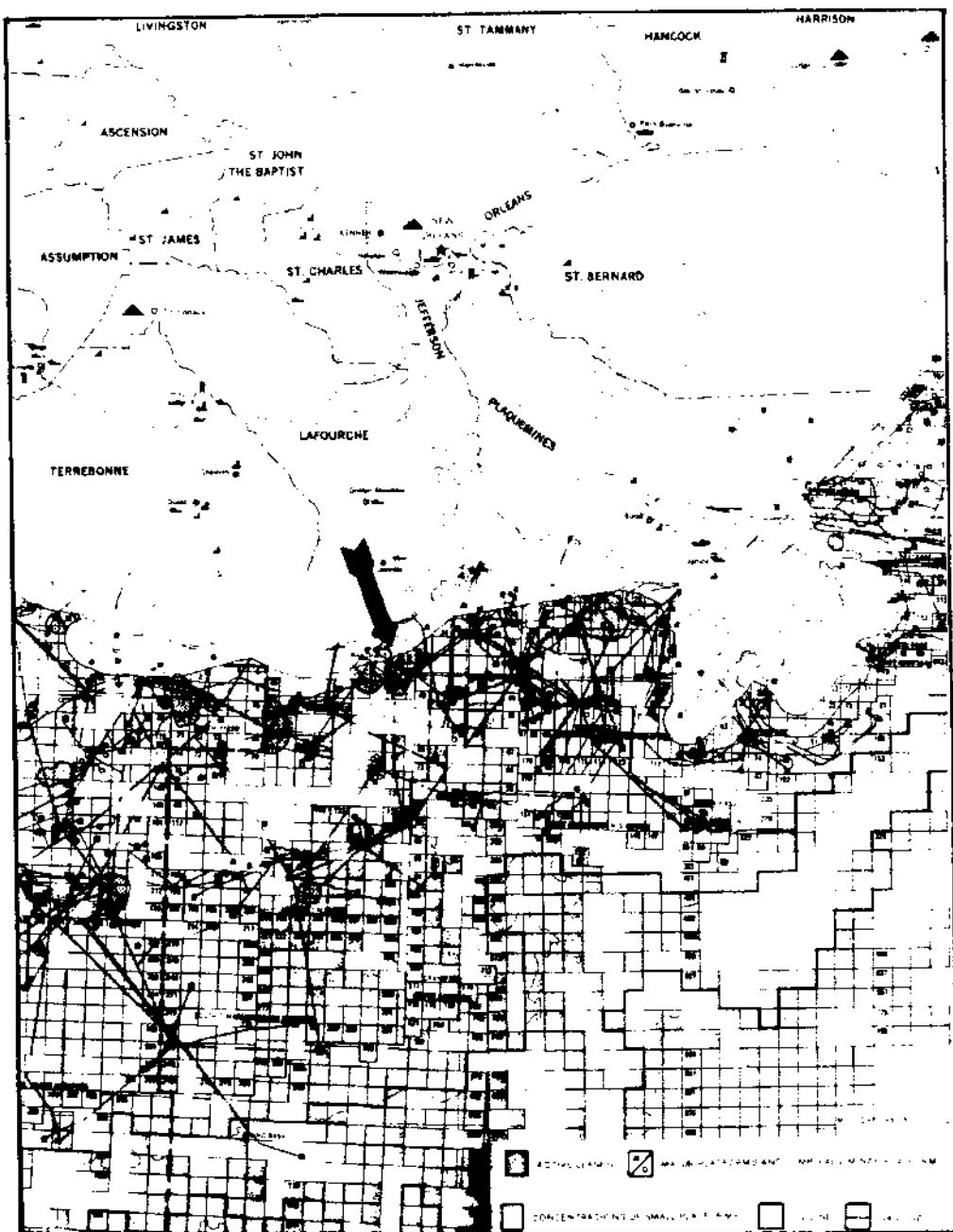


Figure 1. Location of the Bay Marchand Oil and Gas Field Offshore Louisiana.

The BMF, partially in State and Federal waters, is encompassed by 3 Federal oil and gas leasing areas, and 12 Lease Blocks—i.e., Bay Marchand Area Blocks 1, 2, and 3; South Timbalier Area Blocks 23, 24, 25, and 26; and Grand Isle Area Blocks 25, 26, 37, 38, and 49 (Figure 2).

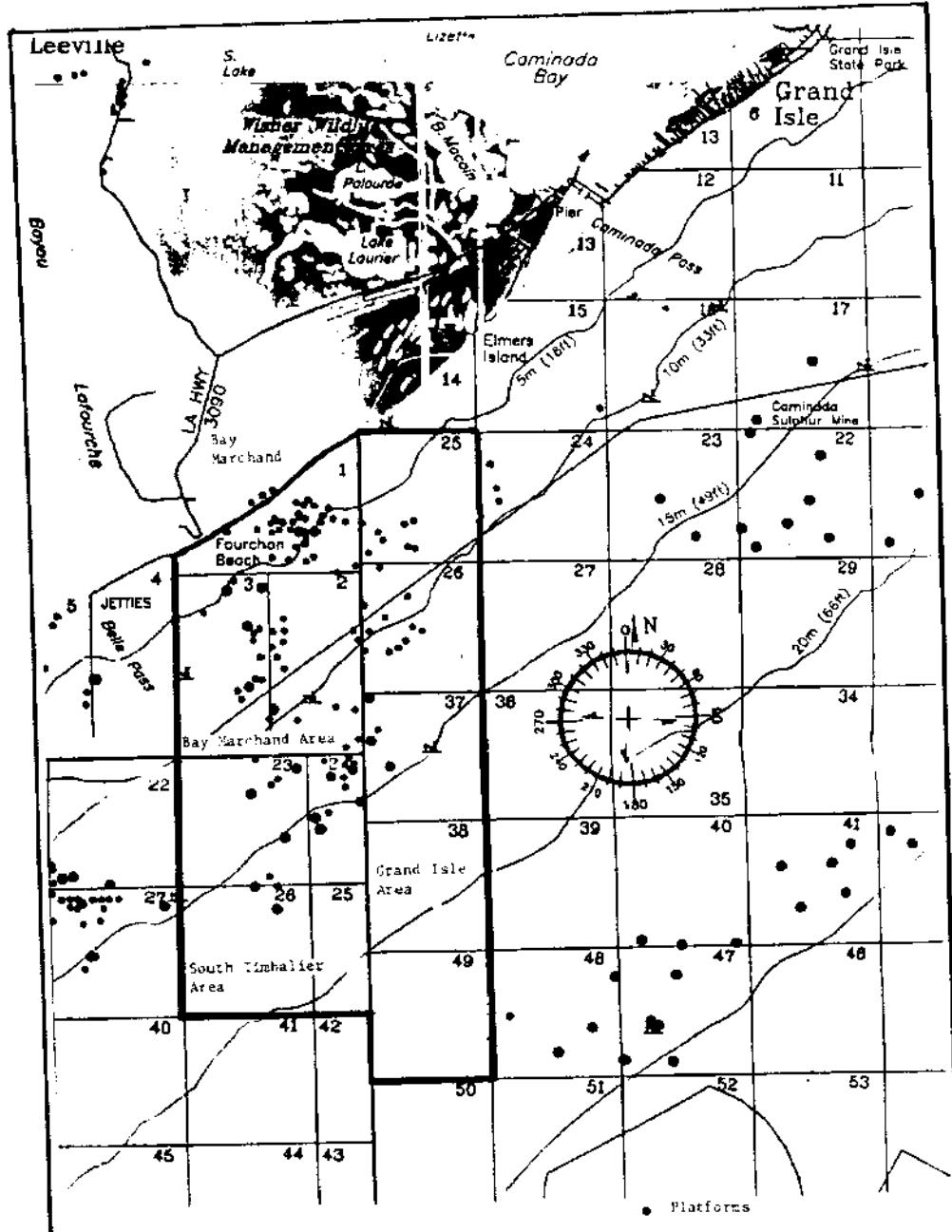


Figure 2. Lease areas and blocks of the Bay Marchand Oil and Gas Field ---: BM Area Blocks 1, 2, and 3; ST Area Blocks 23, 24, 25, and 26; GI Area Blocks 25, 26, 37, 38, and 49.

Sixty-two (62) oil and gas platforms, mostly 1 well satellite platforms (Figure 3), exist within 9 of the field's 12 lease blocks. No platforms exist in South Timbalier Block 25 or in Grand Isle Blocks 38 and 49. There are a few multi (4-8) well platforms (Figure 4), in production in the BMF.

Water depths at the satellite and multi—well platforms range from 5 meters (16 fsw) to 20 meters (66 fsw).

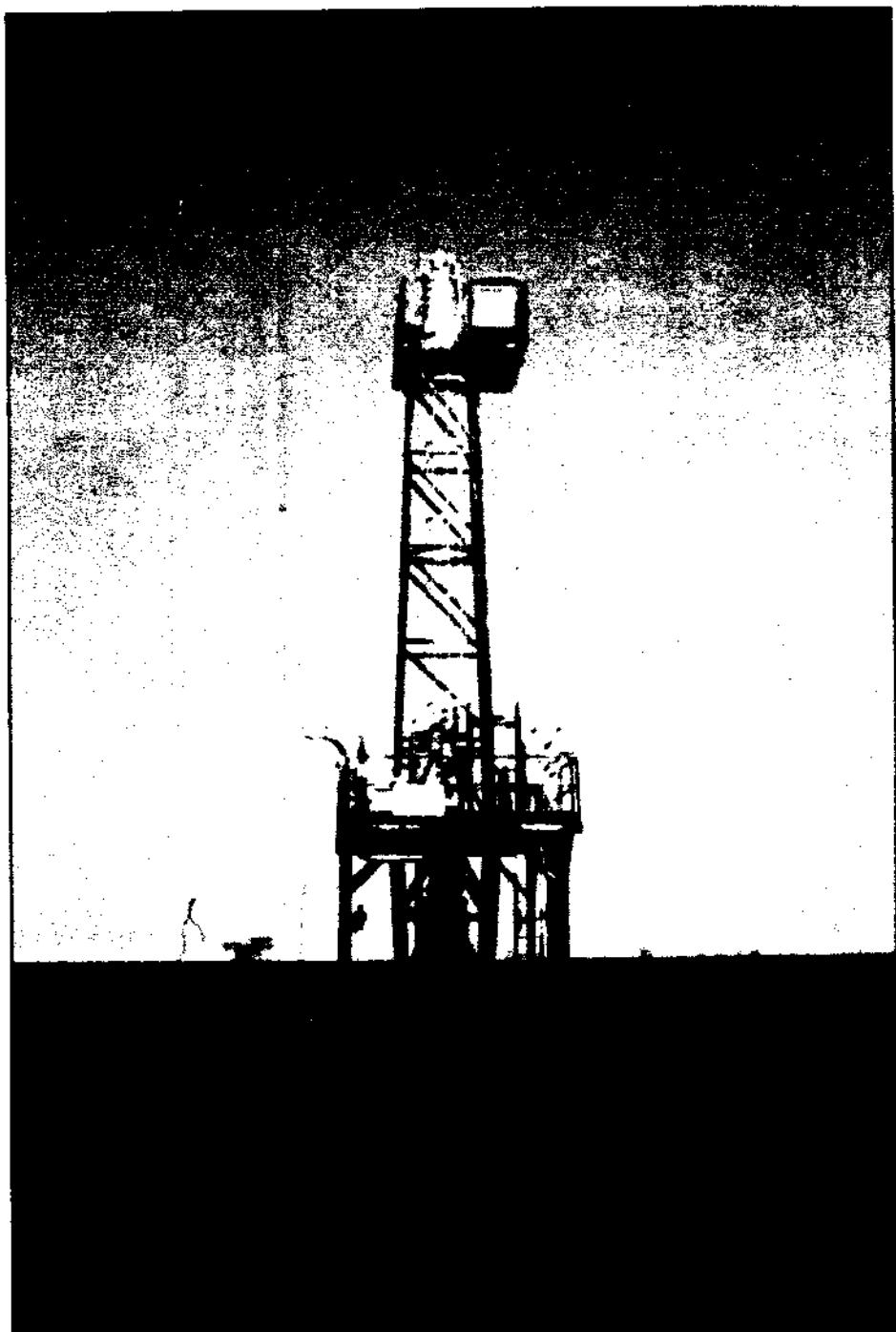


Figure 3. Satellite Platform in the Bay Marchand Field.

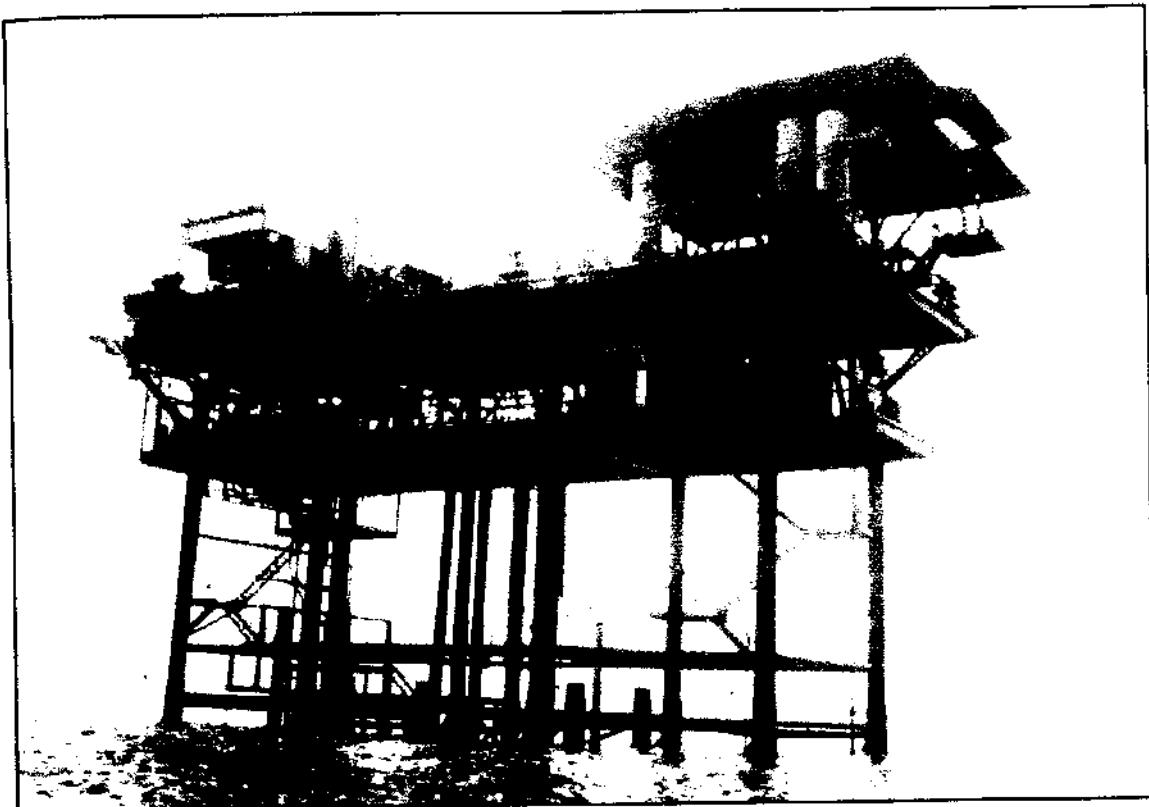


Figure 4. Multi-Well Platform in the Bay Marchand Field.

INSPECTION DISCUSSION AND RESULTS

Before the use of solar panels, batteries were used on satellite platforms to power lights and fog horns (aids-to-navigation), a requirement of the U.S. Coast Guard. In response to the alleged violation of discarded batteries from satellite platforms in the BMF, GOMR divers inspected 20 (16 satellite and 4 multi-well) production platforms over a two-day period. The 20 platforms, a representative sample of platforms in the BMF, were selected at random for the visual inspection. This number accounts for one-third of the BMF's 62 platforms.

The seafloor sediment in the BMF is a very soft black mud. Immediately adjacent to the platforms, a thin layer of barnacle hash overlays the mud. Ten feet from the platform, the mud had the consistency of a fine powder. Consequently, MMS divers had to exercise care and good buoyancy control above the seafloor, particularly away from the platform, so as not to hamper visibility and the visual inspection in an already low (<10 feet) visibility environment.

A visual inspection of the seafloor using the underwater circle search pattern was conducted adjacent to, and at 7.6 meters (25 ft.) and 15.2 meters (50 ft.) circles around, the 16 satellite platforms. The "U" search pattern was applied adjacent to and underneath the 4 multi-well production platforms.

The visual circle search around the satellite platforms found a total of four batteries and a small assortment of other debris, mainly pieces of grating, cable, rope, tubing, and pipe within the 15.2 meter (50 ft) circle at 9 of the 16 satellite platforms. The remaining 7 satellite platforms were essentially clean and free of debris. Most of the debris found near the satellite platforms were partially buried in the mud. It is likely that some debris are completely buried because of the unconsolidated nature of the sediment at the surface of the seafloor. No probing for debris possibly buried in the soft mud was done because this would have made the visual search impossible in the already low visibility environment.

A large assortment of debris was found underneath and immediate to the outside perimeter of the 4 multi-well production platforms. In addition to the debris found at the satellite platforms, an assortment of nuts and bolts (Figure 5), stainless steel tubing, and a welder's toolbox and tools were found at these platforms.



Figure 5. Nuts and Bolts Found Underneath a Platform.

Visibility at the multi-well platforms was somewhat better 6.1 meters (20 ft.) because the debris served in stabilizing the sediment at these platforms. The kinds of debris found at each platform (i.e., satellite and multi-well), the platform location, and the corresponding dive at each platform are given in Table 1.

Table 1. Debris Found at Satellite and Multi-Well Platforms. Dive Number and Location of Platform.

Dive #	Location	Platform	Debris Found
1	ST 26-E	Satellite	No Debris Found
2	ST 23	Satellite	No Debris Found
3	ST 23	Satellite	Cable,Rope,Grating
4	ST 23	Satellite	No Debris Found
5	ST 24	Satellite	No Debris Found
6	BM 2	Satellite	Batteries
7	BM 2	Satellite	No Debris Found
8	BM 1	Satellite	Battery,Grating, Winch Drum,Cable
9	BM 3	Multi-Well	Batteries,Pipe,Rope, Nuts and Bolts
10	BM 3SL	Multi-Well	Batteries,Pipe,Rope, Cable,Toolbox
11	BM 2	Satellite	No Debris Found
12	BM 1SL	Satellite	Concrete Rubble
13	GI 37	Satellite	Battery,Grating, Gauge
14	GI 37	Satellite	Ladder, Cable, Rope,Winch Drum
15	GI 23	Satellite	Battery
16	GI 26	Satellite	Tubing,Grating,Rope
17	GI 26	Satellite	No Debris Found
18	ST 24	Satellite	Tubing,Grating
19	ST 24	Multi-Well	Battery,Pipe,Rope
20	ST 23	Multi-Well	Tubing, Grating,Pipe

BM-Bay Marchand ST-South Timbalier GI-Grand Isle

CONCLUSION

Minimum requirements for clearance of a site subjected to oil and gas operations of all bottom obstructions were instituted in 1981. However, there have been an increase in platforms removed from the OCS in recent years and growing concerns by the GOMR over debris and construction material left behind following clearance of sites where OCS platforms and wellheads had been removed. The GOMR therefore designed a program to ensure that any object (i.e., well-heads, platforms, etc.) installed on a GOM OCS lease is properly removed and the site cleared so as not to conflict with other uses of the OCS. Furthermore, the GOMR's Diving Program provides the Region with the hands-on and ground-truthing capability for inspecting sites for verification that a site has been cleared of all bottom obstructions resulting from oil and gas operations.

**MISSIONS OF THE MINERALS MANAGEMENT SERVICE
MANDATED BY THE OUTER CONTINENTAL SHELF LANDS ACT OF 1953
AND AMENDMENTS MADE IN 1978 AND 1985**

- MAKE OUTER CONTINENTAL SHELF (OCS) RESOURCES AVAILABLE EXPEDITIOUSLY TO MEET THE NATION'S ENERGY NEEDS**
- PROTECT HUMAN, MARINE, AND COASTAL ENVIRONMENTS**
- ENSURE THAT STATE AND LOCAL GOVERNMENTS HAVE TIMELY ACCESS TO INFORMATION AND OPPORTUNITIES TO PARTICIPATE IN OCS PLANNING AND POLICY DECISIONMAKING**
- GET A FAIR AND EQUITABLE RETURN ON RESOURCES WHILE PRESERVING AND MAINTAINING FREE ENTERPRISE COMPETITION**

**FLOWER GARDENS OCEAN RESEARCH PROJECT: CONVERSION OF AN
OFFSHORE PRODUCTION PLATFORM TO A FULL TIME TRAINING AND
RESEARCH STATION IN THE GULF OF MEXICO**

Quenton Dokken
Center for Coastal Studies
College of Science and Technology
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Corpus Christi, Texas

The Flower Gardens Ocean Research Project (FGORP), a cooperative program between Mobil Exploration and Producing U.S. Inc. and several academic and resource management agencies in Texas and Louisiana, is investigating the feasibility of converting Mobil platform High Island-A389A to a full time training/research station.

A full time offshore training/research station could be utilized to instruct students and scientists in underwater research technologies such as the use of remote operated vehicles, manned submersibles, advanced SCUBA technology, surface supplied diving, hyperbaric chamber operations, and survey techniques. Training programs meeting certification requirements of state and federal agencies, the American Academy of Underwater Science, and academic research institutions could be conducted in a safe and cost effective training environment.

HI-A389A (25°54'30" N, 93°35'06", 161 km south of Cameron, Louisiana, 185 km east of Freeport, Texas, and 1.5 km east of the East Flower Garden Bank) is located in a water depth of 125 m, with the nearby reefs of the Flower Garden Banks National Marine Sanctuary cresting at 17 m. The surrounding water mass is tropical with surface temperatures ranging from 19° C to 30° C and visibility ranging from 20 to 60 m. Normal conditions should allow for in-water training for at least 300 days/year.

A METHOD FOR QUALITATIVE ASSESSMENT OF BACTERIAL FLORA ON THE SKIN OF OCTOPUSES IN NATURE.

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There is no information available on the bacterial populations inhabiting the skin of cephalopod molluscs in nature. One of the problems inherent in collecting such data is sample contamination from collecting gear and animal handling. A method was developed for taking bacterial swabs underwater without handling animals and the method was evaluated during a research diving project in the Channel Islands off Southern California. Agar slants were prepared in screw-top glass tubes that were then filled with sterile mineral oil. Tubes were carried by SCUBA divers in a simple rack. When an Octopus bimaculatus was found near the mouth of its lair, a sterile cotton-tipped swab was used to sample the dorsal mantle of the octopus and then inoculate the agar slant. A sea water control tube was also inoculated. Fifteen tubes were inoculated underwater at several sites. For habitat comparison, trap-caught octopuses from Long Beach Harbor were swabbed as trap lines were brought to the surface, thus requiring no handling of the animals. A month later, the slants were processed and representative isolates identified. Nine genera of gram-negative bacteria were identified, including six species of Vibrio. No gram-positive isolates were cultured. The method proved to be logically feasible, cost effective and potentially suitable for bacterial sampling of other aquatic species.

INTRODUCTION

Our institute is involved in the intensive laboratory culture of cephalopod molluscs for the purpose of making them widely available to the biomedical and physiological research community (Hanlon and Forsythe, 1985). We have cultured five species of octopuses, four species of squids and two species of cuttlefishes through the complete life cycle. With each of these species we have had to deal with bacterial disease problems ranging from minor to severe (Hanlon and Forsythe, 1990). Gram-negative bacteria of the family Vibrionaceae and specifically the genus Vibrio are the most commonly implicated pathogens in disease outbreaks. We and others (Leibovitz et al., 1977; Ford et al., 1986) found that in the laboratory these potential pathogens are ubiquitous on all environmental surfaces as well as on healthy animals in culture. The mechanisms by which these bacteria switch from being unobtrusive symbionts to deadly

pathogens are not understood. This symbiont-to-pathogen switch has been shown to be a widespread and common problem with all marine molluscs brought into the laboratory (Elston and Lockwood, 1983; Elston, 1984).

Any microbiological sampling of the skin of cephalopods in captivity produces cultures of Vibrio and other potential pathogens. This led to the question of whether or not the case is the same in nature. A search of the literature revealed only one paper dealing with sampling the bacterial flora of wild cephalopods (Ford et al., 1986). The method employed was to sample freshly trawled and seine-caught squids. Obviously, the squids sampled were mixed with the other organisms in the catch, touched the netting itself and were exposed to a large amount of ambient water passing through the net during trawl-capture. Nothing was said of how the squids were handled on site for the taking of swab samples of the skin (i.e. were squids handled with gloves or laid on a sterilized surface, etc.). The likelihood of contamination seemed high from this method or any other requiring capture and handling.

A new method was devised to take bacterial samples underwater without handling or even moving the animal being sampled. The method allowed a diver to approach a resting octopus, swab the surface of the animal and immediately inoculate an agar slant underwater. It was hoped that this would reduce the extent of contamination from non-animal sources. Octopuses were sampled in the pristine waters of the Channel Islands. For comparison, octopuses being trap-caught from a marina in Long Beach Harbor were also sampled instead. The results indicate that this method is a feasible, inexpensive and practical way of sampling the microbiological fauna of submerged organisms and surfaces without having to bring the sample substrate (be it living or non-living) to the surface.

METHODS

Microbiological methods. Agar slants were prepared in 20mm diameter X 125mm long screw-cap glass tubes. The culture media was Trypticase Soy Agar supplemented with three salts (6.94 g MgSO₄•7H₂O, 0.75 g KCl and 12.0 g NaCl per liter of media). This media was prepared as described by Colwell and Wiebe (1970). Approximately 25 ml of sterile media were aliquoted into sterile tubes and the tops screwed on tightly. The tubes were tilted and the agar allowed to solidify at room temperature. At the study site, the tubes were filled completely with sterilized mineral oil and the caps replaced tightly. Upon returning to the laboratory, the slants were swabbed and plated out on T-soy + 3 salts agar plates for identification. From these plates, isolates were made of representative colony types to determine the range of bacteria types sampled by this method. Standard microbiological methods and protocols were utilized to identify representative isolates (Buchanan and Gibbons, 1974; Lennette et al., 1985). Logistical limitations prevented isolating and identifying all bacterial species obtained.

Study site. Diving trials to test this sampling method were conducted during a Channel Islands Research Program cruise aboard the R/V Cormorant in October, 1988. Dives were made at Catalina, Santa Barbara and Sulil islands in the Channel Islands group off southern California. All samples were taken at depths of less than 10 m.

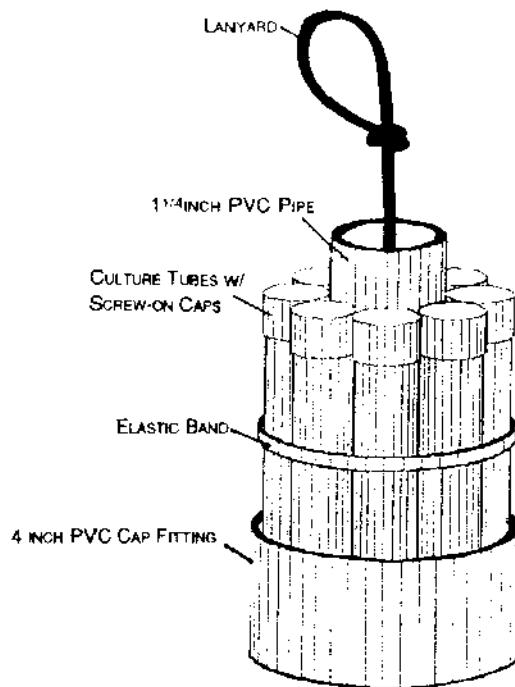


Figure 1. Tube holder used for carrying inoculation tubes during dives. The lower base portion is a 4 inch PVC cap fitting with an 8 inch length of 1 1/4 inch diameter PVC pipe heat welded into the center of the cap. A lanyard was passed upward through the central pipe.

Sampling. A simple PVC tube-holder was fabricated that could carry up to 8 sample tubes (Fig. 1). The lower base portion was a 4-inch PVC cap fitting with an 8-inch length of 1 1/4 inch diameter PVC pipe heat welded into the center of the cap. The space left between the edge of the cap and the vertical piece of pipe was exactly equal to the diameter of the glass culture tubes. A rubber band was used to secure the tops of the tubes against the vertical piece of pipe and keep them from falling out during dives. A lanyard was passed upward through the 1 1/4 inch diameter PVC pipe and clipped to the diver's Bouyancy Compensator. During dives, a concerted effort was made to locate octopuses in lairs that offered an exposed part of the animal's body, a fairly common occurrence with this species (Ambrose, 1982). When such an animal was located, first a control tube was inoculated. This was done by removing a sterile swab from a glass tube, swirling it in the water within 1 m of the octopuses lair and inoculating a new agar slant. If other octopuses were found within a 20m radius of the first animal, no new control was made. The agar slant tube was held upside down with the screw-cap down, the cap was unscrewed carefully. This upside-down position kept the mineral oil from floating out of the tube since the oil is lighter than water. The oil therefore also prevented the ingress of raw sea water. The inoculating swab was then passed upward into the tube, through the oil and swabbed across the agar surface. The swab was then broken off (since its wooden handle was longer than the tube) and left in the tube and the cap was screwed back on. The animal was then sampled with a second swab and agar slant tube

using the exact same procedure. It was possible to pass the swab over the skin surface of the octopus without the octopus attempting to retreat (Fig. 2). Usually the octopus attempted to grab the swab. Different animals presented different dorsal surfaces of the body, in some cases the dorsal mantle was sampled while with other animals the arm surfaces or head might be sampled. The inoculated tubes were placed in the tube holder for the remaining duration of the dive. Up to six tubes were inoculated on a single dive. A slate was used to record information on sample site depth, temperature and tube number inoculated. On board the boat, the tubes were stored at ambient room temperatures ranging from 15-20 °C.



Figure 2. Diver sampling the dorsal mantle of *Octopus bimaculoides* in the Channel Islands with a cotton-tipped swab.

A second group of octopuses was sampled in a marina in Long Beach Harbor using the same culture tubes filled with mineral oil. At this site, a local marine specimen collector had a series of trap lines laid along the bottom to catch *Octopus bimaculoides*. The trap line consisted of short pieces of PVC pipe or small ceramic flower pots attached at 2m intervals along the line. As the collector retrieved the trap line from the bottom (3-4 m depth), it was possible to swab the dorsal mantle of octopuses residing in the various pots without having to handle the animals themselves. The octopuses held on tightly to the sides of the container they were in and offered no resistance to the swabbing procedure. Again, a sea water control was taken on site.

RESULTS AND DISCUSSION

Fifteen tubes were inoculated underwater: 10 from octopuses and 5 as sea water controls. This procedure was performed easily by two divers in cold-water (15-18 °C) wearing neoprene gloves. The screw-cap tubes were pressure safe and water tight to 13m, the maximum depth of exposure. Two tubes leaked during dives and this apparently was the result of not tightening them sufficiently at the beginning of each dive. Often 6-8 tubes would be taken down on a dive but only 2 or 3 actually used. This made it possible for a tube to go on several dives without being used, allowing repeated pressurization and depressurization to loosen the cap. Another tube was broken against a rock during a dive and one other tube was lost from the holder during a dive. Overall, the holder worked effectively and did not interfere with other activities such as photography and areal surveys. We found that it worked best to have one person handle the inoculation tubes while the second diver did the swabbing. The "swabbie" diver would take the glass tube containing swabs, open it and remove one swab for the control sample. The other diver held two un-inoculated tubes. As the "swabbie" finished the control swab, the "tuber" would invert the first tube, remove the inoculation tube cap allowing the swabbie to insert the swab and pass it over the agar slant. The second diver replaced the cap once the excess swab handle was broken off. This was repeated to swab the octopus and inoculate the second tube. After one or two inoculations, the dive team became proficient at the procedure and it required about a minute per tube to complete the inoculation procedure. The samples taken from octopuses trap-caught at the Long Beach Harbor site were done easily by a single individual using the same type of tubes with mineral oil.

Bacterial growth on most slants became visible in about three days. After the expedition, the tubes were stored at 4 °C for approximately one month before attempts to plate out isolates from the slants were begun. All slants produced viable colonies. This simple system seems capable of collecting a representative range of bacterial species. The culture media proved effective at supporting the standard array of gram-negative marine bacteria, although other media could be substituted easily such as specialty media for specific bacterial types.

Only gram-negative bacteria were cultured and identified because they are selected for by the culture media utilized. Table 1 lists the genera of bacteria identified and the general location from where they were cultured. Logistical and fiscal limitations precluded identification of all isolates from all samples. Only representative colony types from each locale were worked through all the way to genus or species identification. Note that the isolates identified only to genus do represent a single species. Although this was not an exhaustive study, there are indications that this methodology can provide specific and useful information. Comparing the island samples, ten species were identified from octopuses and sea water controls but only two of the ten species, Vibrio vulnificus and Moraxella sp., were found on both octopuses and sea water controls.

Likewise, at the inshore site, seven total species were identified but only two, Aeromonas hydrophila, were shared between animals and controls. Both comparisons suggest that the swabs from octopuses sample different populations of bacteria than the sea water controls despite the potential for cross-sampling. Also, comparing offshore to inshore samples, of fourteen total species sampled, only three were found both inshore and offshore. More vigorous studies with replication would be required to substantiate such differences, but the results suggest that such studies would be worthwhile.

Table 1. Comparison of bacteria genera and/or species isolated from general locations of study. The offshore locations were in the Channel Islands and the Inshore site was Long Beach Harbor.

Gram Negative Bacterial Flora	Offshore (Channel Islands)		Inshore (Long Beach Harbor)	
	Octopuses	Controls	Octopuses	Controls
FERMENTERS				
<i>Aeromonas hydrophila</i>			X	X
<i>Vibrio alginolyticus</i>			X	
<i>V. damsela</i>	X			
<i>V. harveyi</i>		X		
<i>V. pelagius</i>	X		X	X
<i>V. splendidus</i>			X	
<i>V. vulnificus</i>	X	X		
NON-FERMENTERS				
<i>Alcaligenes</i> sp.	X			
<i>Achromobacter</i> sp.	X			X
<i>Cytophaga</i> sp.				X
<i>Flavobacterium</i> sp.		X		
<i>Flexibacter</i> sp.	X			
<i>Moraxella</i> sp.	X	X		
<i>Pseudomonas</i> sp.	X			X
Total Species	10		7	

It was also noted that viable bacterial cultures could be initiated from the original inoculation tubes for at least six months after collection when stored at 4 °C. It was not determined if all bacterial genera were still present after this time period. Since it is a standard method to cover bacterial isolates with mineral oil for long-term archiving, the overall method is well suited to storage of samples after their collection.

This method does not eliminate all possible sources of contamination. Obviously, the swab that contacts the octopus must still pass through the surrounding sea water. However, this contact is negligible compared to the amount of sea water contact a sample specimen encounters during capture and transport to the surface by any conventional collection method. The sea water controls used in this study seemed to give some useful indication of the bacterial flora in the surrounding water. The second source of contamination is encountered when the screw-top cap is removed from the inoculation tube underwater. Some amount of contact occurs where the mineral oil interfaces with the ambient sea water. Also, a small volume of water (<1 ml) remains in the cap when it is screwed back on although most is forced out since the mineral oil leaves no space for water to enter the tube until the cap is tightened. Two tubes that leaked 1-2 ml of sea water during dives due to loose caps were kept to evaluate contamination potential. After one month there was no growth on the agar slants

indicating that residual water in the caps when they are screwed back on the vials underwater may have negligible impact on the sample results. The case could be different in polluted waters having high bacterial counts.

It is possible for divers to perform microbiological sampling of living and non-living substrates without having to handle or move the substrates. The simple system described here should be adaptable to different applications and could be modified easily for specific needs. For example, different media could be used for species-specific studies. Differential media such as Blood Agar or Colistin, Nalidixic Acid (CNA) Agar could be substituted to select for gram-positive bacteria only. Differential media such as McConkeys Agar could be used to sample coliform bacteria. This sampling system can minimize sample contamination inherent in conventional capture methods such as trawling, hand netting or line fishing.

ACKNOWLEDGEMENTS

The authors wish to thank the Tatman Foundation for the continued support of the Channel Islands Research Program and operational support for the R/V Cormorant. We also thank the crew of the R/V Cormorant for their excellent support and in particular the Scientific Dive Leader, Dr. Jack Engle. Special thanks go to Mike Lang for organizing this cephalopod expedition and inviting us to participate. Mr. Chuck Winkler was most cooperative in the capture of octopuses in Long Beach Harbor. Travel funding was provided by the Marine Medicine general budget account of the Marine Biomedical Institute.

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APPLICATION OF A REMOTELY OPERATED VEHICLE IN GEOLOGIC MAPPING OF MONTEREY BAY, CALIFORNIA, USA

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Monterey Bay Aquarium Research Institute's Remotely Operated Vehicle (ROV) is being used to fill data gaps in the geologic maps of Monterey Bay. With the adaptation of classical field geology methods to ROV technology, preliminary detailed geologic mapping has been successfully undertaken in Monterey Canyon since 1991. The steep relief of this area has been nearly impossible to map using conventional techniques.

Our efforts have integrated biological and geological approaches to mapping geology. We delineate faults by the distribution of cold seep communities that are supported by venting sulfide-rich fluids. Conversely, we characterize biological assemblages associated with particular geomorphologies through structural and lithological mapping. Newly discovered rock types have been collected and rock cores have been obtained with the use of an ROV-mounted drill.

INTRODUCTION

Monterey Bay, a nearly crescentic embayment that indents the coastline of central California by nearly 25 km, is located approximately 115 km south of San Francisco (Fig. 1). Submarine topography of the bay is dominated by a large submarine canyon (Fig. 2). Monterey Canyon deeply incises the Cretaceous crystalline basement rocks and overlying Tertiary sedimentary sequences of the bay, exposing a geological chronology that to date has not been fully investigated. Using the Monterey Bay Aquarium Research Institute's (MBARI) remotely operated vehicle (ROV) *Ventana* (Fig. 3) has enabled us to directly examine canyon walls and sample the constituent rock types in order to determine the geologic and tectonic history of the region.

The geology and tectonic history of the Monterey Bay region is complex, owing to its position within the tectonic boundary marking the convergent (transformational) margin of the Pacific and North American plates. Here the San Andreas fault system is

over 100 km wide, comprising several fault zones, located both onshore and offshore (Fig. 4). Two major fault zones, the Palo Colorado-San Gregorio and Monterey Bay fault zones, are part of the offshore fault system in Monterey Bay (Greene, 1990). Movement along these fault zones has dismembered and introduced exotic lithologies in the Monterey Bay region and has fractured and deformed Cretaceous and Tertiary rocks. Cretaceous granitic rocks that underlie the entire Monterey Bay are out of place, belonging to the allochthonous Salinian block that has been transported on the Pacific plate northward along the San Andreas fault proper (Page, 1970). Benthic communities are occasionally found along these faults where nutrient-rich fluids are discharged.

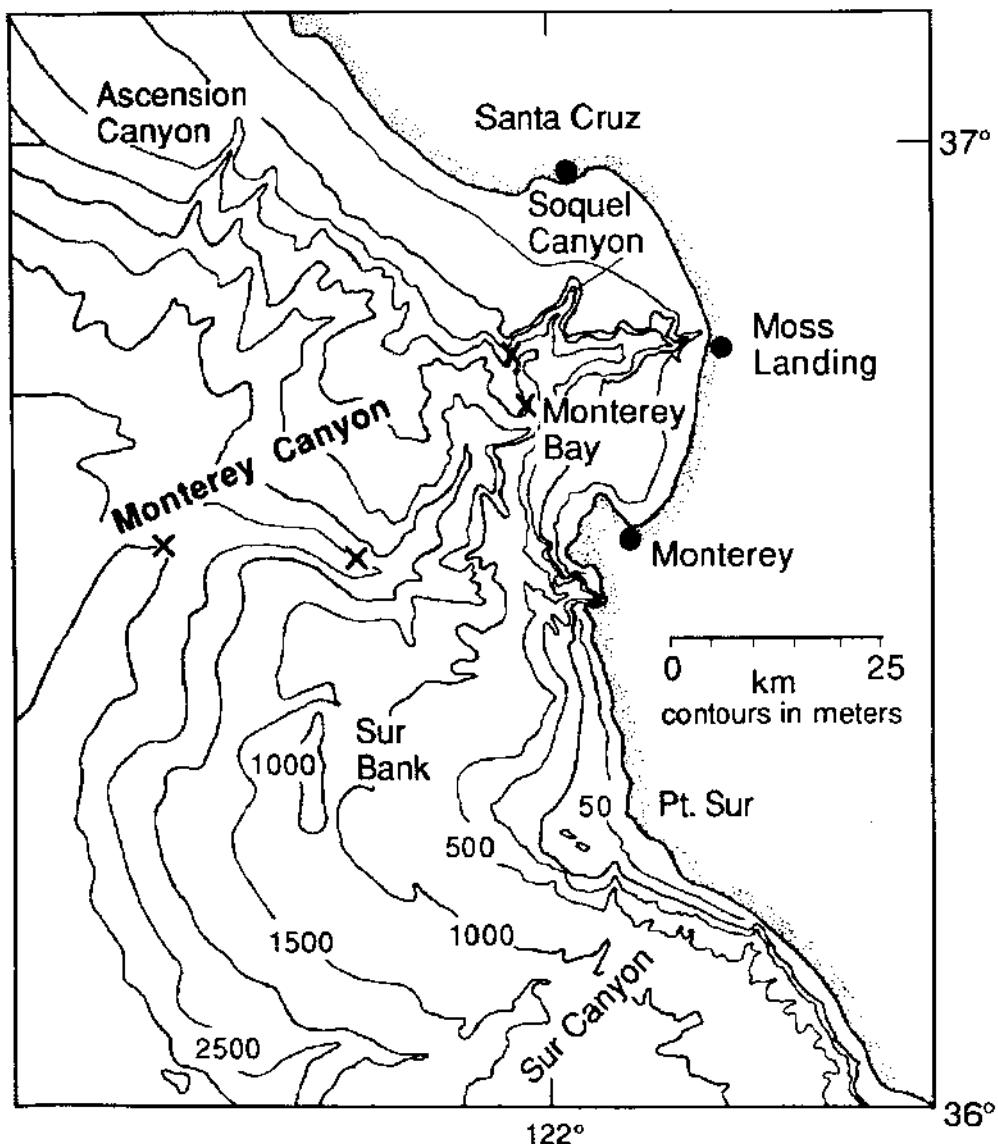


Figure 1. Location map showing general bathymetry and geographical points of references in the Monterey Bay region. Xs mark cold seep community sites.

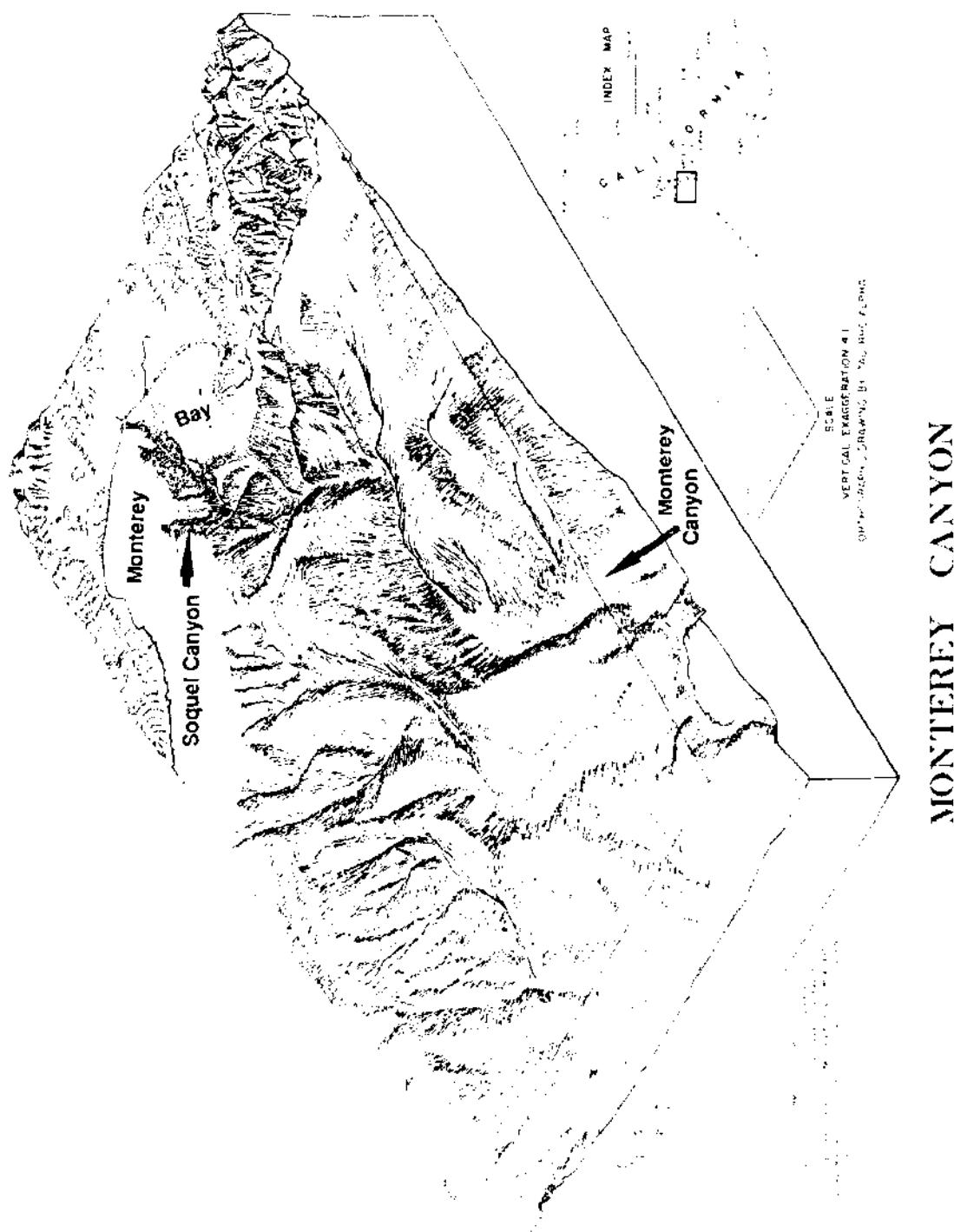


Figure 2. Physiographic drawing of Monterey Canyon.

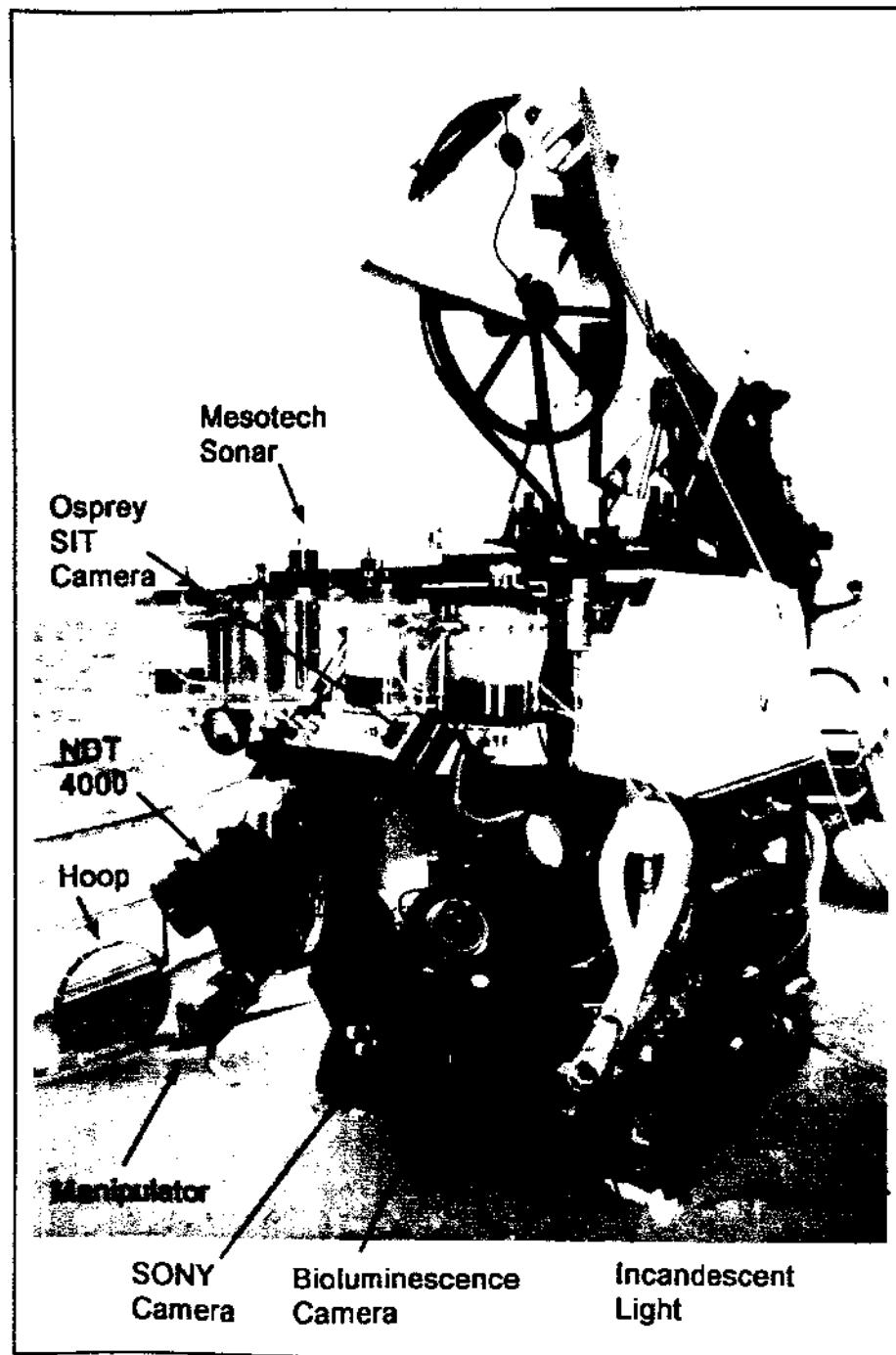


Figure 3. Photograph of MBARI's ROV *Ventana*.

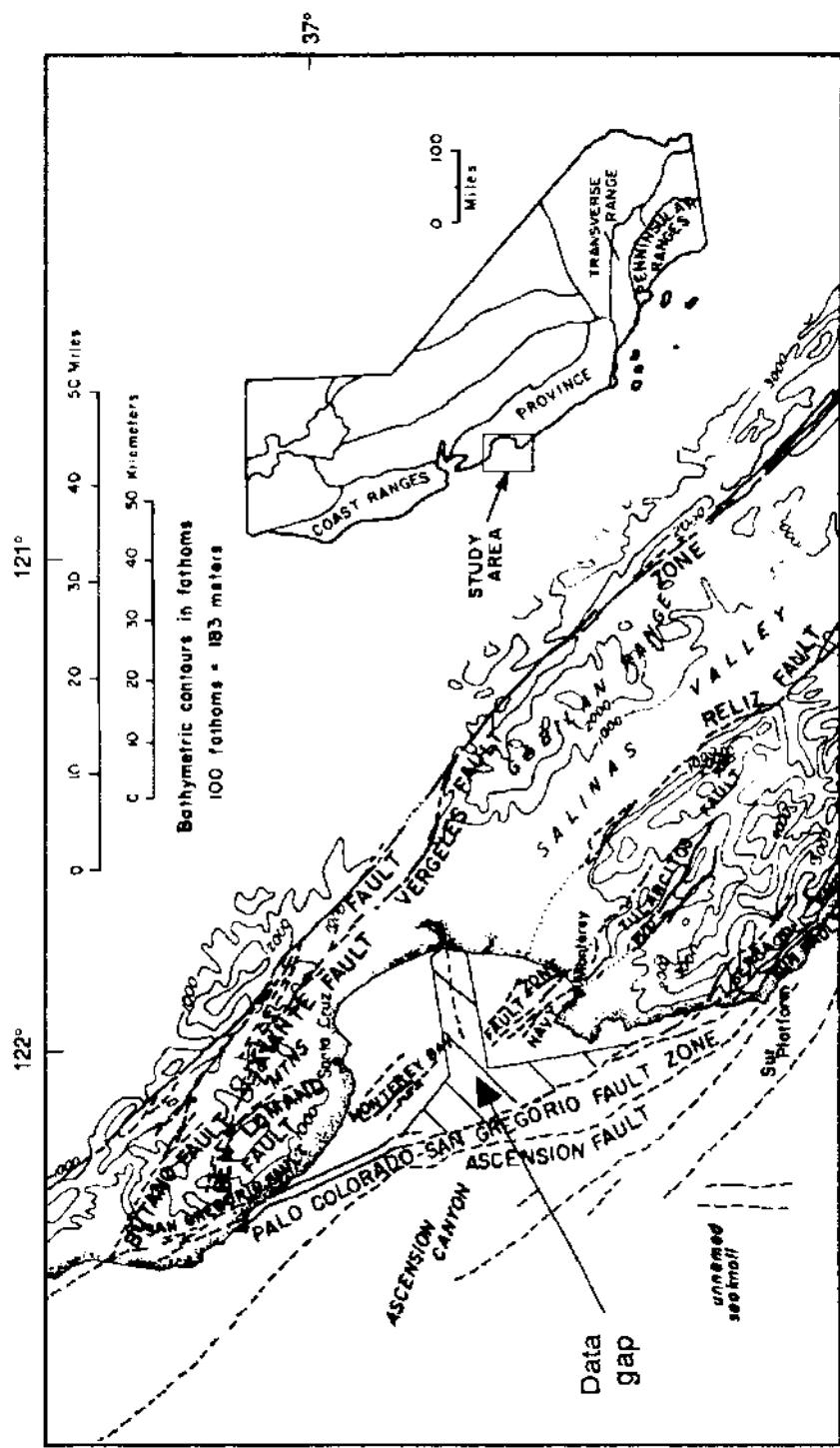


Figure 4. Fault map of the Monterey Bay region showing fault zones that compose the San Andreas fault system. Hachured area represents geologic data gaps in canyon area where present-day mapping is being done using the ROV *Ventana*.

The structural complexity of the bay and its relationship to the emplacement of exotic lithologies, to fluid flow through geologic conduits, to the support of biological communities, and to chemical flux are important to document if a comprehensive evaluation of the biogeochemical interactions within Monterey Bay is to be accomplished. We have initiated a unique multi-disciplinary (geological, biological, chemical, and hydrologic) systems approach to mapping the geology of the Monterey Bay region using the MBARI ROV as the principal tool. The physiography of steep terrain composed of near-vertical to vertical and overhanging relief that exists in Monterey Canyon is nearly impossible to image using conventional shipboard geophysical techniques. The use of ROV technology is necessary to complete a comprehensive map of the region. This paper describes briefly the technology and methodologies used in our mapping project and discusses some of our preliminary results.

TECHNOLOGY

Presently, the principal tool used in mapping the geology of Monterey Bay is the ROV *Ventana* (Fig. 3). This vehicle is of moderate size (2.51 m long, 1.42 m wide, 1.35 m high), has a syntactic foam flotation module attached to the top, is propelled with 6 hydraulic thrusters (2 verticals, 2 laterals, 2 fore and aft), and weighs approximately 4800 lb. The thrusters are powered by a 40 hp, 2300 VAC electric motor that drives a 25 gpm, 3000 psi hydraulic pump and the vehicle can travel at a speed of 1 to 1.5 knots across the ground and ~3/4 knots vertically (Robison, 1993).

Although *Ventana* is capable of diving to a depth of 1850 m, its operational depth limit, because of the length of its tether, is 1000 m. Generally a dive lasts for 5 to 6 hours, although the vehicle's submergence time is generally unlimited. The tether is a fiber-optic umbilical (cable) that transmits power via hard wire to the ROV and returns video signals, telemetry, and control data along individual fiber-optic conductors, providing real-time communication between the control room on the mother ship (R/V *Point Lobos*) and the vehicle. This umbilical includes five #12AWG wires, eight multimode optical fibers, and two single-mode optics encased with inner and outer layers of TPR with four intermediate layers of contrahelically wound Kevlar (Robison, 1993; Stakes et al., 1993).

Ventana is controlled by a GESPAC telemetry system aboard the vehicle communicating with a 486 surface unit; a 68000 processor is used aboard the vehicle. The primary information obtained with the ROV is video; the vehicle can be outfitted with several video cameras including a Sony¹ DXC-3000 broadcast-quality three-chip camera, an Osprey 1323 SIT camera, two Osprey 1359 low-light monochrome cameras, a Panasonic GP-CD1 and an EOS ultra low-light monochrome filterable zoom system. In addition, a Photosea NDT 4000 (macrovideo sighted stereo still) camera or Photosea 1000, 35mm (wide angle still camera with strobe) can be used. Lighting for both video and 35 mm still camera images are provided with up to four sodium-scandium lamps and four incandescent lights all powered through the connecting tether. *Ventana* also has a robotic manipulator arm and a compartmentalized sample drawer.

¹Any use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the U. S. Government.

The recently developed Holloway-Stakes-Tengdin-Rajcula (HSTR) drill system was deployed by the *Ventana* with great success and will be used as a principal hard rock collecting tool in the future. This system is composed of a small diameter (5 cm) double-barreled drill with a custom diamond bit. The drill is rotated by a hydraulic motor plumbed directly into the ROV's hydraulic network through a spare servo valve, which provides hydraulic flow up 10 gpm at 3000 psi (Stakes *et al.*, 1993). Salt water circulates through the space between both barrels, forced down between them by a hydraulic rotary pump/saltwater pump that runs in series with the coring motor to flush chips and other debris past the bit and allow for smoother rotation of the drill (Stakes *et al.*, 1993). Rates of drill rotation and water flow are variable; drill speeds are adjustable to greater than 1200 rpm. Cores recovered from this drill are 3.18 cm in diameter and can be as long as 35.56 cm.

A bank of video screens and computer keyboards located in the control room of the mother ship, the *Point Lobos*, is the working center for ROV operations. The *Point Lobos* is a 33.5 m long vessel used as a day boat out of Moss Landing. The control room seats an ROV pilot and co-pilot, a chief observer, and two assistant observers. The pilots control or "fly" *Ventana* by utilizing a joy-stick, a push-button instrument panel, and touch screen computer while the chief observer controls the primary video camera with a computer connected instrument box. Navigation for the *Ventana* is by differential GPS satellite navigation integrated to the *Point Lobos* with an ultra short base-line system used to position the ROV. Repositioning accuracy is variable (approximately 100 meters). A Mesotech 971 azimuthal and forward looking sonar with a 100 m range aboard the ROV are used to locate rock outcrops and steep slopes.

A microwave link between the *Point Lobos* and shoreside scientific facilities allows real-time audio-video-data communications between shipboard scientists and land-based scientists. This technology facilitates the participation of many more scientists than can be accommodated aboard ship and allows for real-time mapping outside of a cramped control room aboard ship, on a stable, large lay-out table.

METHODOLOGY

Seafloor mapping with an ROV differs from the classical forms of seafloor mapping, using marine geophysical (acoustical) and geological methodologies. These traditional methodologies produce a series of swath maps and cross sections that in turn are used to construct geologic maps. Rather, ROV mapping methodology is similar to that used on land and consists of *in situ* observations, measurements, and sample collection. Basically two types of mapping can be done with an ROV: (1) reconnaissance mapping and (2) detailed mapping. When mapping in a reconnaissance mode, selected seafloor sites, generally spaced far apart (1-10 km), are investigated in a relatively rapid fashion and sample collection, as well as lengthy examinations, is kept to a minimum. The detailed mode of mapping consists of careful sample collection, measurement of sections, and detailed examination of outcrops, structure, and seafloor morphology.

Like all geologic mapping methodologies, offshore mapping requires base maps. In the Monterey Bay region, NOAA full-resolution SeaBeam digital bathymetric data are currently being used to construct the base maps (Greene *et al.*, 1989). Spatial scale is dependent upon the type of mapping to be undertaken (i.e., reconnaissance or detailed mapping). Sidescan sonographs are used in place of aerial photographs, which are

commonly used onshore. Bathymetric maps are used to identify and locate areas of steep relief where rock exposures are most likely to occur and to identify linear features that likely are the expression of faults, fractures, and folds. Areas of varying reflectivity in sidescan sonographs are investigated to determine the types of substrata and structures that compose the seafloor. In addition to acoustical seafloor mapping tools used to construct the base maps, seismic reflection profiles are used to locate structures and seafloor exposures and to provide a third dimension (cross sections) to the mapping process.

Reconnaissance Mapping

For reconnaissance mapping, linear to zigzag transects across the seafloor are run coupled with continuous updates of vehicle position via the ultra-short baseline/differential GPS integrated navigation system (Meridian Oceans Systems - MOS). Coordinates, in latitude and longitude, or UTM units, or both, are noted and recorded for sample stations, photo stations, and changes in course and are plotted aboard the ship at the end of the day's dive as way points. Continuous video is collected during the transect for future referencing and construction of photomosaics for specific sites of interest. A Mestotech azimuthal sonar is also used as a mapping tool, and sonar images are recorded on video tape for future referencing. For geologic transects, the vehicle heading, depth, altitude above bottom, pitch and roll of the vehicle, direction with pan and tilt of video camera, time (GMT), dive number, and current or vehicle speed are continuously recorded on the video tape. Real time observations and descriptions are recorded on the audio track of video tapes as well as in the observer's notebook. Particularly significant observations are signified on video tapes with a distinct sound spike intended to draw the attention of the curator of tapes and other researchers doing archiving or reviewing of tapes.

Presently, the attitudes of bedding planes, fault planes, and other structures, inclinations of slopes, scarps, and cliffs, and alignments of structural and morphological features are measured by aligning the ROV parallel or perpendicular to the feature of interest and estimating orientation. Dip and strike are measured by leveling the vehicle (pitch and roll = 0), leveling and aiming the master video camera straight ahead, and orienting the vehicle so that the camera is aimed along strike. A protractor placed against the video screen is used to estimate dip, and the heading of the ROV gives the strike. This arduous and primitive method of measuring attitude will soon change as an upgrade to a more sophisticated and accurate laser-based tool is in progress.

Detailed Mapping

The procedure for detailed geologic mapping with *Ventana* is very similar to reconnaissance mapping. A 100% video coverage of the transect is obtained with enhanced positioning and more fixes taken. Attitudes of structures are estimated in the same fashion, with care to obtain accurate navigational data. A principal difference between reconnaissance and detailed mapping is that more time is spent along the transect to note and record the details of the geology i.e., more stations are occupied. Stratigraphic sections are measured in detail either by using a laser rule (10 cm between laser points) or measured with the vehicle's depth meter. Detailed sampling is undertaken

with the collection of rock samples at every distinct lithologic boundary or at regular spacing along an outcrop. In areas where structure is particularly complex, detailed examination is made with the ROV and the manipulator can be used to clean outcrops where close-up observations are needed.

Where it is not possible to collect rock samples with *Ventana*'s manipulator, such as on sheer steep cliffs of hard rock, the HSTR drill is used. Prior to the development of the drill, selected lithologies could not be routinely sampled as the manipulator is limited to just picking up fractured rock pieces. Areas for drilling are identified from previous reconnaissance dives and are relocated using the *Point Lobos*' positioning system. Once a drill site is located, the ROV transits to the location on the outcrop where coring is desired and using the thrusters, *Ventana* is pushed up to the outcrop with three-pointed docking bars snug against the rock. Drilling with forward thrusters used to maintain pressure on the outcrop requires skillful operation by the ROV pilots.

DISCUSSION

Preliminary detailed geologic mapping of Monterey Bay using MBARI's ROV *Ventana* was initiated in 1991 and continues today. The methodologies described above are currently being used in this investigation. New underwater mapping technologies and methodologies are continually being developed and applied to this mapping project, thus improving the techniques used with ROVs.

Our mapping activity is concentrated in the central part of Monterey Canyon, in the Monterey meander area near the intersection of Monterey and Soquel Canyons (Fig. 5). This is a region of steep relief and complex structure where faults of the Monterey Bay fault zone cut and deform Cretaceous basement and Tertiary sedimentary rocks (Fig. 6). Survey mapping indicates that many faults within the Monterey Bay fault zone vent cold fluids with sulfide concentrations (perhaps including methane) that support cold seep biological communities. Consequently, we use these communities to delineate faults and fractures within the bay. *Vesicomya* and *Calyptogena* spp. clams, some vestimentiferan tube worms, and various colored (orange, yellow, white, black, and gray) bacterial mats are concentrated along faults and fractures where fluids emanate. These communities appear to be restricted primarily within the fault zone, along the down canyon, western meander wall (Fig. 1 and 5), between 400 and 900 meters below sea level (mbsl).

The source of fluids is presently unknown. Since most of the cold seep communities are concentrated along faults and fractures within the Pliocene shallow-marine sandstone of the Purisima Formation, we suspect that fluids are aquifer driven along the Purisima Formation, with a recharge zone high up in the Santa Cruz Mountains. However, sulfide-rich fluids may also be derived from the Miocene Monterey Formation, an organic-rich shale and mudstone unit that is locally a source for petroleum; the Monterey Formation underlies the Purisima Formation and fluids may travel up along the faults. Future chemical sampling of the seeps should delineate the sources of fluids and promote our understanding of the hydrogeologic regime of the bay.

Faults mapped in detail are of two types, normal and thrust. Normal faults are vertical faults, many outlined with bacterial mats. Thrust faults are defined by areas of



Figure 5. Computer generated physiography of Monterey Bay using NOAA full resolution digital SeaBeam data and showing Monterey meander and Soquel-Monterey Canyons intersection. Detailed mapping is presently being undertaken in the area of this image.

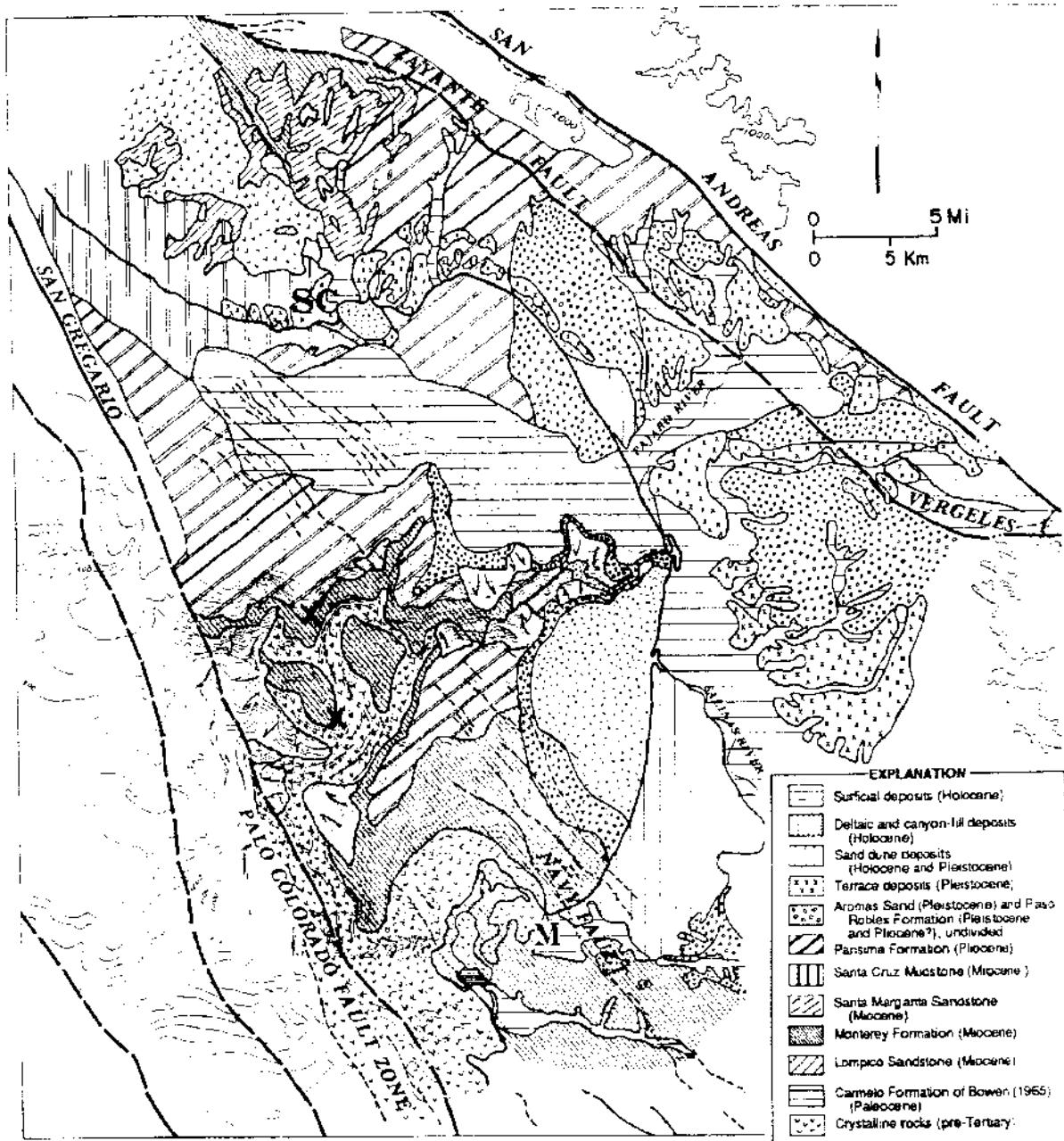


Figure 6. Geologic sketch map of seafloor in Monterey Bay region based on bottom samples (Martin and Emery, 1967; Greene, 1977) and from acoustical imaging (Greene, 1977) traditional mapping techniques. Rock contacts in canyon area are not well located due to difficulty in acoustical imaging in submarine canyons. This area is being re-mapped using the ROV *Ventana*. M = Monterey. SC = Santa Cruz. Landslides shown with curved arrows, faults by heavy and lightly dashed lines. Xs mark location of cold seep communities.

highly fractured rocks and steep eastward-dipping fractures. The majority of the faults are vertical and trend generally NW-SE. Many of these faults are outlined with bacterial mats (Fig. 7). In two places high (~400 mbsl) on the western meander wall, fractured and highly deformed Purisima sandstone appear to be broken by steep eastward-dipping thrust faults (Fig. 8). All of these faults occur along a section of the canyon that has not been mapped accurately due to its steep relief. Likewise, the occurrence of these faults is not detectable in the high-resolution SeaBeam data.



Figure 7. Photograph of a vertical fault in the Monterey Bay fault zone cutting the Purisima Formation and outlined by bacterial mats that thrive on fluids seeping from faults. Photo taken from ROV *Ventana*.

In addition to mapping structure, we have been mapping rock types (lithologies) that are exposed in the canyon walls. The contact between the Cretaceous granitic basement rocks and the overlying Tertiary sedimentary sequence (here the Monterey Formation) has been located and observed directly and is found to lie at a water depth greater than previously mapped (Greene, 1977; Fig. 6). However, the contact between the Miocene Monterey Formation and the Pliocene Purisima Formation has not been observed directly or located accurately. It has, however, been constrained to depths between 450 and 410 mbsl along the western meander wall, also much deeper than previously mapped. Evidently, the contact is buried everywhere beneath Quaternary erosional debris and pelagic muds. Recently, exotic greenstones and serpentinite rock types were collected *in situ* in Monterey meander with *Ventana* and indicate a more complex western boundary to the Salinian block than had previously been mapped. Video can usually be used to identify lithology, but not always, especially when rocks are deformed and altered in fault zones or are heavily encrusted with biota. In these areas, selective sampling is required.

Continued ROV mapping will lead to the construction of a comprehensive geologic map of Monterey Bay, especially in Monterey Canyon (Fig. 4). The ROV technologies and methodologies described above now enable mapping of complex physiographic areas, such as submarine canyons, in the detail commonly restricted to land mapping.



Figure 8. Photograph of crushed and fractured Purisima Formation sandstone that has been compressed from thrust faulting along faults in the Monterey Bay fault zone. Bacterial mats and clams, representing the cold seep communities, mark fractures where fluids are venting. Photo taken from ROV *Ventana*.

CONCLUSIONS

ROV technologies and mapping methodologies developed using MBARI's ROV *Ventana* have revolutionized the format of marine geologic mapping being carried out today. Mapping the great detail that is commonly accomplished on land is now possible. Moreover, to some extent, mapping offshore with ROVs can be considerably more effective and expedient than onshore mapping. The ability to collect 100% video coverage along tracklines, rock samples, still photos, and drill cores in hard, steep outcrops makes the *Ventana* an excellent and efficient tool for mapping geology.

Mapping with *Ventana* has filled data gaps in geologic maps of Monterey Bay. Areas in Monterey Canyon difficult or nearly impossible to image using conventional geophysical mapping techniques are now being mapped in detail using *Ventana*. We initially concentrated our mapping efforts in the Monterey meander of the canyon and are in the process of mapping the faults of the Monterey Bay fault zone exposed along the canyon wall. Here, two different types of faults exist, normal and thrusts. Sulfide-rich fluids vent from many of these faults and support cold seep communities of clams, tube

worms, and bacterial mats. Often these biological communities are used to delineate faults, resulting in a biological-geological systems approach to mapping. The future chemical analysis of sulfide-rich fluids will assist us in determining the sources of fluids and increase our understanding of the hydrologic continuity of faults.

Ventana mapping has located the contact between Cretaceous granitic basement rocks and the overlying Miocene Monterey Formation, found to be lower on the canyon wall than previously mapped. Newly discovered exotic greenstone and serpentinite rocks in the Monterey meander indicate a more complex and deformed western boundary of the Salinian block than had been mapped in the Monterey Bay region before. Future mapping with MBARI's ROVs should reveal new and exciting geology and lead to the production of detailed geologic maps for the region.

ACKNOWLEDGMENTS

We thank the MBARI marine operations manager, Steve Etchemendy, and the *Ventana* pilots, Craig Dawe, Chris Grech, and Jim McFarlane, for their support and assistance in collecting data. Also, we appreciate all of the help that the captain and crew of the *Point Lobos* have given to us in the past. Lynn Lewis and Judith Connor of MBARI assisted us in organizing and processing video tapes. Thanks are extended to Susan Kalb of the USGS for her assistance in typing the manuscript and to Lynn McMasters of Moss Landing Marine Laboratories for the production of the illustrations. We particularly appreciate reviews by Brian Edwards and Bruce Jaffe of the USGS and Chris Gech of MBARI; their comments markedly improved the manuscript.

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DISTRIBUTION, ABUNDANCE, AND UTILIZATION OF DRIFT MACROPHYTES IN A NEARSHORE SUBMARINE CANYON SYSTEM

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The potential importance of exported drift macrophyte material from kelp forests to adjacent deep-sea benthic communities near Monterey Bay, California, was investigated using a deep-diving remotely operated vehicle (ROV). Temporal and spatial variation in drift macrophyte and megafauna abundance and distribution were determined at continental shelf (Pt. Joe) and submarine canyon (Carmel Submarine Canyon) study sites. Twenty-two different types of macrophytes were encountered during the surveys, most of which were brown algae, including the giant kelp Macrocystis pyrifera. The carbon biomass of M. pyrifera alone ranged from less than 1 mg C m⁻² at Pt. Joe to over 10000 mg C m⁻² in the canyon. These data, combined with estimates of drift kelp decomposition rates, suggest that drift M. pyrifera may account for up to 60% of the carbon flux to 500 m depth in canyon habitats. M. pyrifera was the primary component in the guts of Allocentrotus fragilis from the Carmel Submarine Canyon while detritus and other non-plant material dominated the guts of animals from Pt. Joe. Both gut fullness indices and gonad indices were higher from canyon animals compared to their Pt. Joe counterparts. Our data therefore suggest that macrophytes transported from kelp forests play a potentially important ecological role in adjacent deep-sea benthic communities.

PUBLIC AQUARIUMS AND THE AAUS: STRANGE BEDFELLOWS, OR THE SAME KETTLE OF FISH?

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Currently, elevated environmental public awareness, concerns for selected animals and ecosystems, and a negative association with outdated institutions of the past mandate that zoos and aquariums, of today and tomorrow, reflect a higher level of scientific credibility and purpose than in previous years. Aquariums are expected to be fun, informative, and enlightening with regard to environmental issues, and one of the ways this can be accomplished is through active research and educational programs. Additionally, aquariums must function as regional resource centers for their biogeographical regions. This new emphasis on conservation through educational programs, research, and exhibiry is not only the goal of aquariums and oceanariums into the 21st century but provide the links to the more classically scientific and to research communities associated with government and academia. "Scientific diving" and "diving for a public aquarium" are, by new definitions, synonymous and reflect commonalities of purpose and opportunities not previously recognized.

Zoos and aquariums have changed drastically in the last twenty years. Previously, numbers of species were far more important than individual animals--spectacles and oddities were sought out--and the aquariums themselves were organized like the art galleries they emulated. "Galleries," "jewel tanks," "keepers," and "collections" were terms which reflect this attitude and persist even today. The Audubon Zoo in New Orleans still has its primate section and hoofstock area. Changing this way of thinking and organization is an uphill battle, and this "old" way of exhibiting animals is, unfortunately, typical of the way we have taught the public to view them.

Fortunately, today zoos and aquariums tout a more naturalistic exhibit and provide enclosures which more closely resemble biocomplexes. They feature a much wider and occasionally more representative assortment of animals, with the intent being to imply the interdependent link between the animals and the environment in which they live.

This "naturalistic" movement is certainly an improvement, but what does naturalistic mean, and is it real? The unfortunate reality is that all too often we go out of our way to portray animals living in the way we think they should live, not the way they do live. The result is that we misrepresent the wild environment, thus purporting and instilling an anthropomorphic and unrealistic view of "nature" to the public. The legacy of this is that few people realize that in the "real world" life is hard and freedom has its price. Most animal young die slow and probably painful deaths. That predation is

everywhere; disease, parasites, and parasitism is rampant. Debilitating competition for food and sexual partners is prevalent, and malnourishment common.

All too frequently these issues are glossed over or ignored by zoo and aquarium exhibitory and interpretive materials. The cumulative result is a deeply ingrained and skewed public perception of the natural world. We have all, for example, heard people say that the Great White Shark is not the blood-thirsty killer portrayed in the movie "Jaws," which may be true, but neither is it a "sensitive and caring" top predator culling only the sick or weak. Indeed from the perspective of the hapless seal or sea lion the blood-thirsty killer version might, indeed, be the more accurate version. This skewed public perception is typical of the anthropomorphic way in which most of the public now views animals. In turn, this anthropomorphism is one of the largest obstacles to clear thinking with regard to biology and conservation, and to zoos and aquariums achieving their goals, whatever they may be (Robinson, 1992). We have created one of our own biggest problems, and this point cannot be over-emphasized.

There is no doubt that animals can and do powerfully evoke emotion and a response from the public which can be transformed into public pressure for action of one kind or another. Animal rights movements are products of zoos, aquariums and oceanariums having done too good of a job in partially and selectively enlightening the public about a handful of sexy megavertebrates, to the exclusion of the majority of living organisms, habitats and conservation in general. Living proof that a little knowledge is a dangerous thing, the public now threatens our very existence. Further evidence of this skewed public biological ignorance exists in their selective concern for only those animals above an arbitrary phylogenetic level, with little or no concern for habitats or the invertebrates as a whole. This ironic twist of fate, that through our, albeit limited success we now threaten our own ability to achieve our goals is disturbing. This is particularly true with regard to zoos.

But are zoos and aquariums responding appropriately? Zoos have frequently and as a whole, chosen to be characterized as modern day Noah's arks, where threatened species can be saved until such time as the waters of destruction have receded. And indeed ex situ breeding efforts to save Golden Lion Tamarins, Guam rails, and Hawaiian Geese, among others, are tributes to zoos ingenuity and dedication, and act as a focus to promulgate public concern. However, there is no way that the countless species now at risk --and those other multitudes soon to be at risk, can be saved by SSPs, in vitro fertilization, or cryopreservation (Robinson, 1992). The numbers just don't add up.

The question now facing Aquariums into the 21st century is whether to follow the path worn by zoos to this point, or whether to place emphasis in other areas. If, in fact there are too many endangered species for effective zoo vertebrate SSP programs to succeed it may well be impossible with fragmented fish populations and obscure non-charismatic fish species. I would suggest to you, for your consideration that now we must be as adaptive as the organisms we display, and possibly more so. Certainly, we must respond more quickly to pressures brought by public response, than our zoo cousins have in the past.

Growing anti-zoo sentiment basically centers on the issues of freedom vs. captivity and the effect captivity has on captive animals. It is based, thanks to us to some degree, on the public's misconceptions and misinterpretations of animals in the wild, as well as anthropomorphism, and on basic and simple ignorance. It necessarily follows then that our aquarium goals into the 21st century should be to combat these misconceptions and anthropomorphic trends by attempting to elevate the public's "biological literacy" as

Michael Robinson so eloquently puts it, so that they can better deal with the complex problems of today and tomorrow (Robinson, 1991).

Certainly it is a mistake to assume that the global problems of deforestation, habitat destruction, the over-exploitation of living resources and the biodiversity crisis are due solely to ignorance, and that educational enlightenment will, in and of itself, solve the problem (Wilson, 1988). Poverty, greed, and life and death necessity related to the pressures of population explosion and limited productivity are and will be the true causal entities. But educational enlightenment is our only hope for introducing sound public decision making into the next century. Because these globally acting forces threaten our habitats, a more holistic educational approach is mandated. Aquariums have a tremendous potential for biological education because they tap a fundamental human need to relate to other life on earth, and because they can be far more effective than zoos at depicting complex eco-systems, if done well. Ecologically oriented, truly realistic exhibits which demonstrate and in some cases duplicate ecosystems and the interdependent links between the environment and the living things within are required. Active research and holistic educational programs can no longer be optional or set aside for more convenient times (Robinson, 1989). In general, aquariums must become credible, biogeographical resource centers for regions, both now and into the future--if we are to succeed.

Similarly, gone are the days when research institutions can be sequestered behind the halls of academia, and gone, as well, are times when elevating general public awareness was a secondary consideration to institutions of higher learning and government agencies. Public outreach programs are now top priorities and have direct bearing on an institution's survivability. Under these conditions, and given the new mandates for credible scientific involvement, it is apparent today that "Scientific diving" and "diving for public aquarium activities" are now synonymous and reflect the commonalities of purpose, which exist today between public aquariums and the more classical "scientific institutions" of today.

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STABLE ISOTOPE ECOLOGY OF ALASKAN SOCKEYE SALMON LAKES

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Stable isotope ratios of carbon and nitrogen (expressed as $\delta^{13}C$ and $\delta^{15}N$) are used in food web studies to trace sources of these elements when sources differ in their natural stable isotope abundance. In the case of Alaskan sockeye salmon nursery lakes, two sources of nitrogen exist which can be distinguished by $\delta^{15}N$, (1) marine-derived nitrogen from adult salmon carcasses, and (2) nitrogen derived from atmospheric N_2 . $\delta^{15}N$ data suggest that the proportion of marine nitrogen in sockeye salmon lake biota varies markedly with size of escapement. Measurements of $\delta^{13}C$ in combination with $\delta^{15}N$, when used to assess the contribution of three carbon sources to food webs, (1) adult salmon, (2) carbon derived from atmospheric CO_2 , and (3) CO_2 released during decomposition (respiration) of detritus, suggest shifts in the feeding habits of fishes in sockeye salmon lakes over space and time.

INTRODUCTION

Large numbers of anadromous sockeye salmon (*Oncorhynchus nerka*) return to spawn and die in high-latitude large-lake ecosystems that are typically oligotrophic. Because adult salmon cease feeding before entering freshwater, their bodies consist of elements that were acquired in the marine environment. Thus, nutrients released from the decomposition of spawned out-salmon are of marine origin. The N (nitrogen) delivered to a major Alaskan sockeye salmon lake can be important when compared to background levels (Fig. 1).

Stable isotope chemistry of N sources

In nature, pools of given elements can acquire distinctive isotopic signatures due to processes that concentrate one stable isotope over another. Such is the case with marine NO_3-N which is enriched in ^{15}N compared to atmospheric N (Wada 1991). The marine N isotopic signature of salmon is conserved during remineralization and uptake by primary producers (Fig. 2) enabling $\delta^{15}N$ to be used as a proxy to quantify marine-derived N and by extension other nutrients within Pacific salmon freshwater ecosystems (Kline et al. 1990, 1993, Kline 1991).

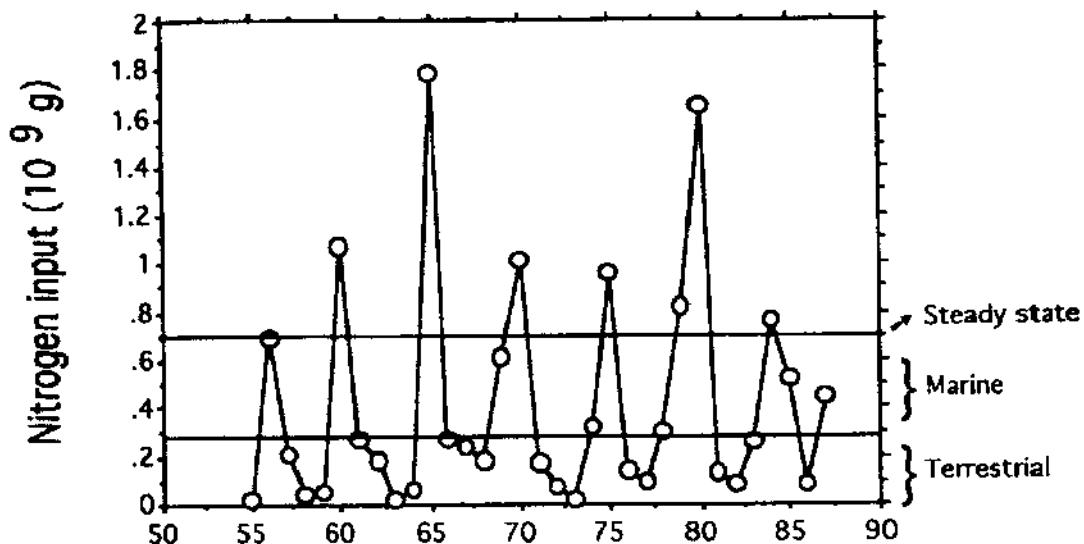


Figure 1. Mass balance of nitrogen for Iliamna Lake suggesting significance of marine-derived nitrogen versus terrestrial nitrogen with respect to the cyclic nature of salmon runs. Steady state input determined as described in text is compared to the mean marine nitrogen input from 1955 to 1987.

The isotope mixing model was based on comparing primary producers in habitats with and without anadromous salmon and verified by comparing $\delta^{15}\text{N}$ at higher trophic levels (Kline et al. 1990, 1993, Kline 1991).

Variation in marine N

Iliamna Lake and other Bristol Bay, Alaska area lakes collectively are the nursery grounds for the world's largest sockeye salmon fishery. Iliamna Lake is also the largest lake in Alaska (length ~ 100 km, maximum depth ~ 300m) with sockeye salmon runs that normally vary from ~ 1 to ~ 10 million (Eggers and Rogers 1987).

The stable isotope technique for determining MDN was tested by comparing $\delta^{15}\text{N}$ estimated values (Fig. 3) with MDN based on the mass-balance approach (Kline et al. 1993). The mass balance approach suggest that on average, salmon provide > 50% of the Iliamna Lake's N but that only peak runs supply more N than atmospherically-derived terrestrial N sources. This prediction by the mass balance approach matched the

MDN based on $\delta^{15}\text{N}$ (Fig. 3) because the peak year, 1984, of 10.4 million sockeye supplied > 50% of the N in food webs of offspring of that year's cohort (Fig. 3).

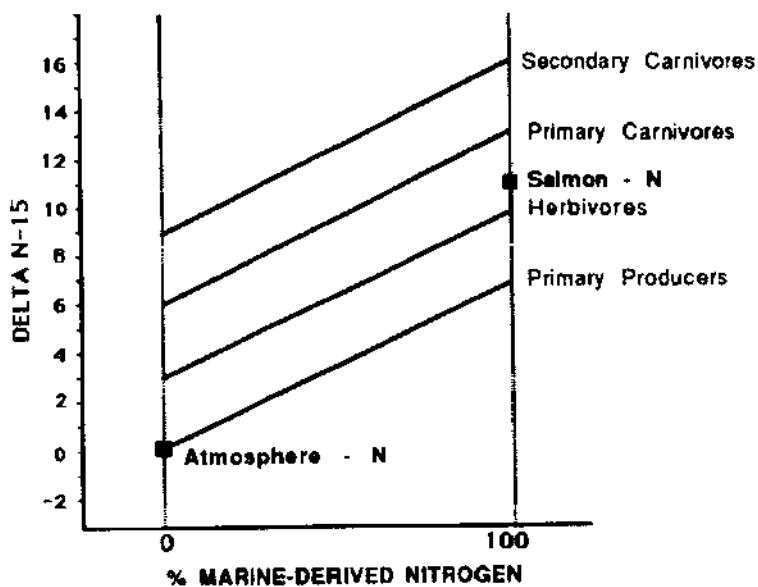


Figure 2. The mixing model used to estimate MDN from $\delta^{15}\text{N}$. The enrichment of ^{15}N in salmon relative to the ambient environment is indicated by the solid squares. Because algae selectively use ^{14}N when DIN is replete, the relative difference in $\delta^{15}\text{N}$ of MDN in algae is less than the difference in $\delta^{15}\text{N}$ between salmon and atmospheric N₂. The parallel mixing lines indicate the 3.4 per mil trophic enrichment in $\delta^{15}\text{N}$.

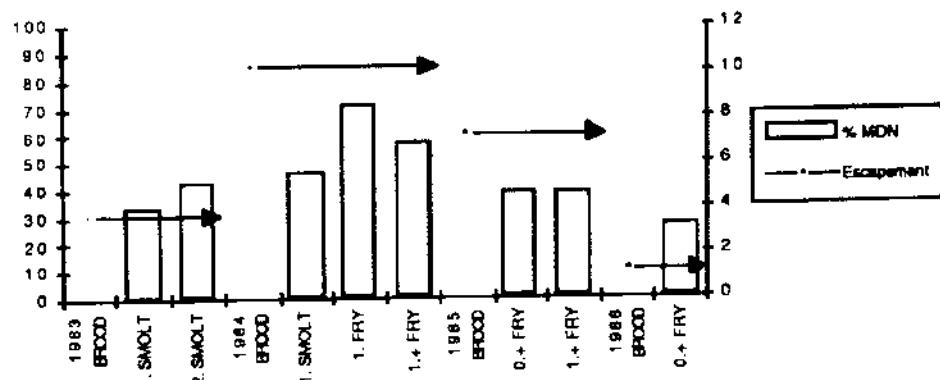


Figure 3. MDN variation in response to cycles in size of the spawning population based on $\delta^{15}\text{N}$ of their progeny. The left scale is % MDN (which varied from ~30 to ~70%) and right scale is escapement (which ranged from 1.2 to 10.4 million). The arrows suggest the carryover effect of escapement number into progeny show as bars underneath. Residual N pools may explain why offspring from the 1983 and 1985 year classes have similar MDN although escapement was different.

MDN of sockeye fry rearing in Karluk Lake, Kodiak Island based on $\delta^{15}\text{N}$ was 70-90% (Table 1). The high MDN in Karluk Lake food webs may be due to the small watershed within a mountainous terrain. Karluk Lake fry have had slight increase in MDN corresponding to an increase escapement in later years.

Table 1. Percent marine nitrogen derived from adult salmon in Karluk Lake sockeye fry as determined by ^{15}N content. Karluk Lake fry, distinguished by year of sampling, show relation of marine nitrogen to recent escapements.

Brood years (escapement)	Sample date	Age of fry	Percent marine nitrogen
1983-5 (< 0.5 million)	Summer 1986	0+, 1+, 2+	71
1985-7 (0.5 to 1 million)	Summer 1988	0+, 1+, 2+	91
1986-8 (0.5 to 1 million)	Summer 1989	0+, 1+, 2+	91

Stable isotope chemistry of C sources

Primary production in sockeye salmon lakes takes place in littoral community by benthic algae (periphyton) and in the limnetic community by phytoplankton. In Iliamna Lake, these two groups of primary producers have distinctive $\delta^{13}\text{C}$ which can be used in conjunction with $\delta^{15}\text{N}$ to estimate contribution of limnetic, littoral production (which collectively are autochthonous sources) and marine C and N in adult salmon, their gametes and developing alevins (which collectively are an allochthonous source) in lake biota (Kline 1991, Kline et al. 1993). This dual isotope approach suggested that remineralized N in autochthonous food webs is the primary pathway for utilization of MDN in Iliamna Lake. Thus stable isotopes indicated through what pathways as well as how much MDN occurred in lake biota.

Applications

Stable isotope chemistry of juvenile sockeye salmon at fry and smolt stages (Fig. 4) suggest significant variation in the relative role of MDN among Alaskan sockeye salmon systems. Two systems, Karluk and Red lakes (Kodiak Is.) have smolts with high $\delta^{15}\text{N}$ values that are inconsistent with the stable isotope mixing model (Fig. 2). Furthermore, the $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ of smolts from Karluk and Red lakes and the expected $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ from a diet of salmon eggs and fry overlap, suggesting cannibalism (Fig. 4). These potentially cannibalistic smolts were large (> 100 mm) in comparison to those from the other systems compared in this study (Kline and Goering 1993). Poor growing conditions, suggested by smolts <80mm, exist in lakes with both high and low MDN, suggesting that other factors, especially glacial conditions which limits light penetration and thus productivity (Koenings and Burkett 1987), control growth rate.

The Karluk Lake sockeye smolts with $\delta^{15}\text{N}$ values suggestive of cannibalism were considerably different from fry values obtained the previous summer. The shift in $\delta^{15}\text{N}$ between the fry and smolt stages of took place between late summer (August-September) and May (Fig. 5). Concomitant differences in weight also suggest that juvenile sockeye put on substantial growth during this period (Fig. 5 and Table 2). In

comparison Iliamna Lake fry and smolts overlapped in size range and had consistent isotopic values, thus there was no evidence of a change in diet there.

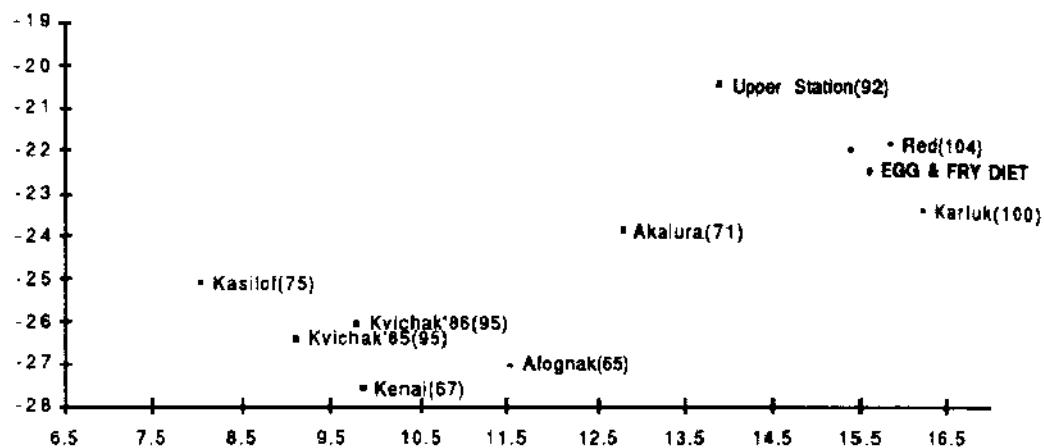


Figure 4. Comparisons of sockeye smolt $\delta^{15}\text{N}$ (horizontal axis) and $\delta^{13}\text{C}$ (vertical axis) values from 8 different Alaskan lake systems. Unlabeled point is Karluk 1989. Other points are from 1990 unless indicated otherwise. Forks length given in parentheses (From Kline and Goering 1993).

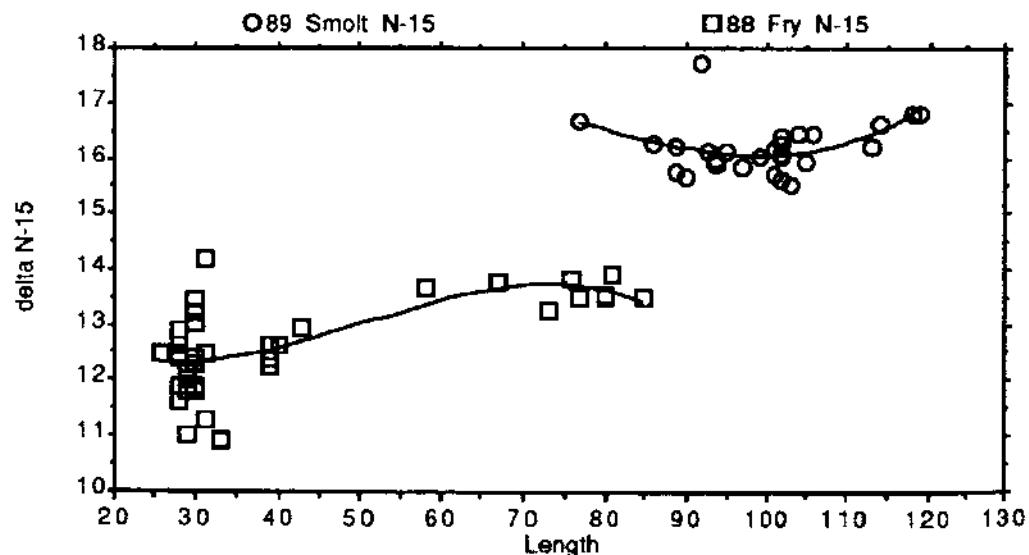


Figure 5. Shift in $\delta^{15}\text{N}$ suggesting change in diet between fry (circles) and smolt (squares) stages of Karluk Lake juvenile sockeye salmon.

Even more enigmatic is that fry in Karluk Lake grow slowly, requiring 2 to 3 of freshwater residence to attain smolting size compared to the typical 1 to 2 years of other systems. This fact is difficult to reconcile with the large size of Karluk lake smolts unless something unusual takes place. Further sampling of Karluk and Red lake sockeye fry

and smolts suggest the same pattern. The consistency in these systems suggests a system-wide attribute that is distinctive when compared to other sockeye nurseries.

Table 2. $\delta^{15}\text{N}$ of fry collected from Karluk Lake in Aug 1990, October 1991 and fry from Red Lake collected in October 1991 compared with smolts collected from the Karluk in May 1990 and 1991 and the Red River in May 1991. Data from Kline and Goering 1993

Fry year	$\delta^{15}\text{N}$	length	weight	year	$\delta^{15}\text{N}$	Smolt		weight
						length	weight	
Karluk Lake								
90	12.5	44.0		0.65		91	16.3	109.0
91	14.5	47.2		0.35		92	16.8	99.1
Red Lake								
91	14.9	53.6		0.57		91	16.6	102.5
								6.13

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MARINE RESEARCH AT THE SMITHSONIAN INSTITUTION

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The Smithsonian Institution fulfills a variety of museum and research functions designed to enhance our nation's scientific and educational experience. The Institution is the world's largest museum/research organization with ongoing national and international projects in conservation issues and environmental research. Several hundred Smithsonian curators, research scientists and staff, as well as visiting scientific collaborators are involved in underwater biological research that focuses on the biodiversity and systematics of species, coral reef ecology, conservation projects and the global change dilemma. Smithsonian research centers and field stations are strategically located in Panama, Florida, Washington DC, Chesapeake Bay, Aldabra, and Belize. A brief history of the Smithsonian Institution's marine research efforts and scientific diving program evolution points to an increase in focused effort in advancing the science and practice of scientific diving. Preparations for the 8th International Coral Reef Symposium at the Smithsonian Tropical Research Institute in Panama are in an advanced stage, an effort that should highlight scientific diving on a worldwide basis.

SHARK-HUMAN INTERACTION IN THE EASTERN NORTH PACIFIC: AN UPDATE AND ANALYSIS

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We report on over 80 confirmed attacks by sharks, the majority of these involving the white shark, (*Carcharodon carcharias*), on humans in the eastern North Pacific since 1926 (first reported attack for this geographic area). We update and describe the approximately 25 shark-human incidents that have occurred since the publication by Lea and Miller (1985) in *Memoirs of the Southern California Academy of Sciences*. The majority of recent attacks (last 15 years) have occurred at the surface, near shore, and in the vicinity of pinniped colonies or river mouths. Attacks have involved surfers, swimmers, scuba divers, hookah divers, and kayakers. To date, wind surfers have not been implicated in an attack. Typical attack scenarios suggest that adult white sharks confuse their victim for a pinniped (mistaken identity), the normal item of prey. Territoriality as a factor is also considered. The extremely low fatality rate of shark attack victims in this geographic area compared to other areas of the world, and the question of white sharks as man eaters will be discussed.

COASTAL UPWELLING OFF THE CENTRAL FLORIDA ATLANTIC COAST: COLD NEAR-SHORE WATERS DURING SUMMER MONTHS SURPRISE MANY DIVERS

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Three 5-month time series recorded simultaneously from study sites located at 45 km intervals along the inner continental shelf of Florida's central Atlantic coast are used to investigate spatial and temporal characteristics of the region's summer upwelling. Results tend to support historical findings that upwelling along the inner shelf occurs as discrete events lasting on the order of several days to about three weeks during which time water temperatures may decrease 8°-12°C from the seasonal norm of 28°-30°C. The upwelling-induced temperature anomalies also exhibit patchiness over spatial scales on the order of tens of kilometers. Incorporation of local meteorological data indicated that, at best, wind stress played a supplementary role in producing the observed upwelling.

INTRODUCTION

Coastal upwelling, the intrusion of cold, deep, nutrient-rich water onto the continental shelf, is a relatively common seasonal phenomenon along the eastern margins of the Atlantic and Pacific Oceans. Upwelling in these regions usually occurs throughout the year as a result of semi-permanent mid-ocean high pressure centers which cause wind-driven, seaward-directed Ekman transport of the near-surface layers. The term is most familiar in the context of its association with high coastal productivity; i.e. high plankton productivity due to the increased nutrients in the upwelled water which supports commercially important fisheries. Some of the world's most productive fishing grounds exist along those areas off the west coasts of the Americas and Africa where upwelling is most pronounced.

Though upwelling results in good fishing, scuba diving during upwelling conditions can be very uncomfortable. Often, surface water is colder during summer than in winter, prompting divers to wear thick wetsuits or even drysuits to ward off the cold. Also, upwelling can cause substantial changes in water clarity due to changes in population densities of phyto- and zooplankton.

Though more often associated with the west coasts of continents, upwelling is not limited to these environments. The Atlantic coast of Florida is one of the relatively few east-coast regions which experiences well-defined and recurring seasonal upwelling. Upwelling has been documented as far back as 1944 when Green reported anomalously low surf temperatures off central Florida during the months of June, July and August. Taylor and Stewart (1957) conducted a more comprehensive study using a larger

temperature data base and mean monthly winds. Their measurements showed that the thermal effects of coastal upwelling were most apparent during mid summer months and confined to the coastline between 27°-30°N. They postulated that seasonal wind stress acting on shelf waters was the dominant force driving the observed upwelling. Lemming (1979) combined current and wind data with temperature data to focus on the across-shelf aspect of upwelling from the coast to the shelf break off Cape Canaveral. His results supported the hypothesis of upwelling as a response to local wind forcing. Studies by Smith (1981, 1982), however, indicated that wind stress probably plays a secondary or supporting role in driving coastal upwelling in this region. His results showed inner shelf near-bottom temperatures were not significantly correlated with either the longshore or the cross-shore component of the wind stress.

The purpose of this paper is primarily to document the temporal and spatial characteristics of upwelling over the inner continental shelf off the central Florida Atlantic coast using temperature data recorded simultaneously from three study sites. The study, also, re-examines the role that wind stress might be playing in initiating or terminating upwelling in this region.

DATA AND METHODS

Three film-recording thermographs (Environmental Devices Corporation, Type 109), accurate to $\pm 0.2^{\circ}\text{C}$, were positioned just above the bottom at study sites located along the 10 m isobath over the inner continental shelf. Stations were spaced approximately 45 km apart between latitudes 26°55'N and 27°45'N (Figure 1). Water temperatures were recorded bihourly from May 24 through October 31, 1983. The three temperature records were plotted versus time providing information on temperature fluctuations over time scales ranging from days to months. Comparisons of temperature variations between sites allowed characterizations over spatial scales on the order of tens of kilometers.

Wind speeds and directions, recorded hourly at the Vero Beach Municipal Airport approximately 10 km southwest of the northernmost study site, were obtained for the same time period. As the airport is about 6 km inland, wind speeds were increased to better approximate over-water conditions (Hsu, 1981). Wind data were converted to wind stress vectors by the method described by Wu (1969) for moderate wind speeds. Wind stress vectors were then decomposed into longshore (170°-350°) and cross-shore components for comparison with temperature records. Only the longshore components were used in this study. Due to the broadening of the continental shelf in the northward direction in this region, the longshore orientation utilized in the analysis is a compromise between the 160°-340° orientation of the coastline and the nearly north-south orientation of the 100 m isobath. Before comparison with temperature records, wind stress was smoothed to remove the seabreeze and other high-frequency fluctuations using an exponential filter that only integrated past wind stress events. Thus, the filter emphasized the most recent wind stress but incorporated to a lesser extent winds recorded earlier.

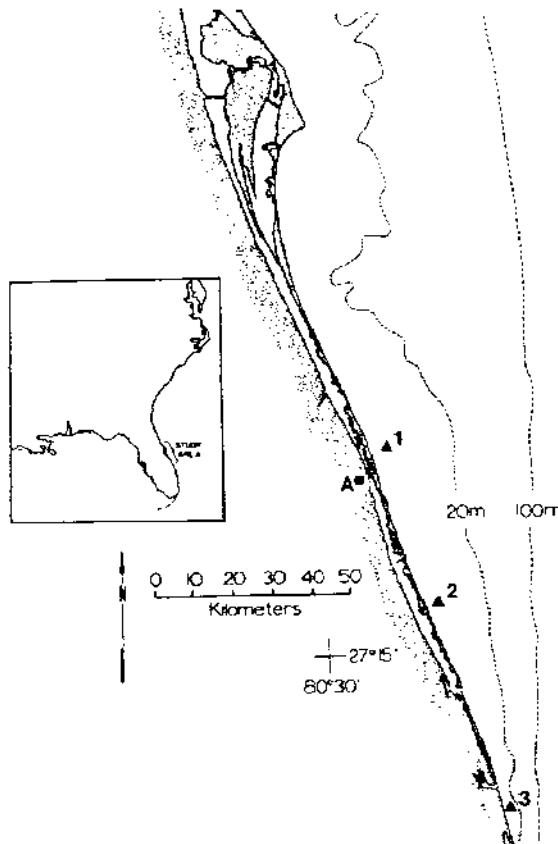


Figure 1. Study area on the Atlantic shelf of central Florida. Triangles represent locations of thermographs, the square shows the location of the Vero Beach Municipal Airport, and hatched lines indicate 20m and 100m isobaths.

RESULTS

A composite of temperatures recorded at the three study sites is shown in Figure 2. Upwelling was most apparent in the temperature record from the northernmost study site (Station 1, Fig. 2a). The dominant feature in the plot is the upwelling event occurring from August 8-21 during which time water temperatures were 5-7°C below the seasonal norm of 28°-30°C. During the 2 week event there were occasions when water temperature increased briefly, probably reflecting a temporary seaward retreating of cold water. Two periods of relatively low temperatures near the beginning of the record and another during the third week of September are probable indications of upwelling.

The record from Station 2 (Fig. 2b) shows a similar but somewhat more erratic pattern when compared to Station 1. The mid-August event is apparent as well as the short-lived decreases observed near the beginning of the record and during the third week of September. However, there are several distinct differences between the two records. The mid-August event that dominates the top plot is not as prominent in the record from Station 2. Temperatures returned to seasonal highs several days earlier at Station 2. Also, there were indications of upwelling or the initiation of upwelling during several time periods at Station 2 that do not appear in the more northern record; namely, during the first and fourth weeks of July when temperatures decreased 3-4°C for 4-5 days.

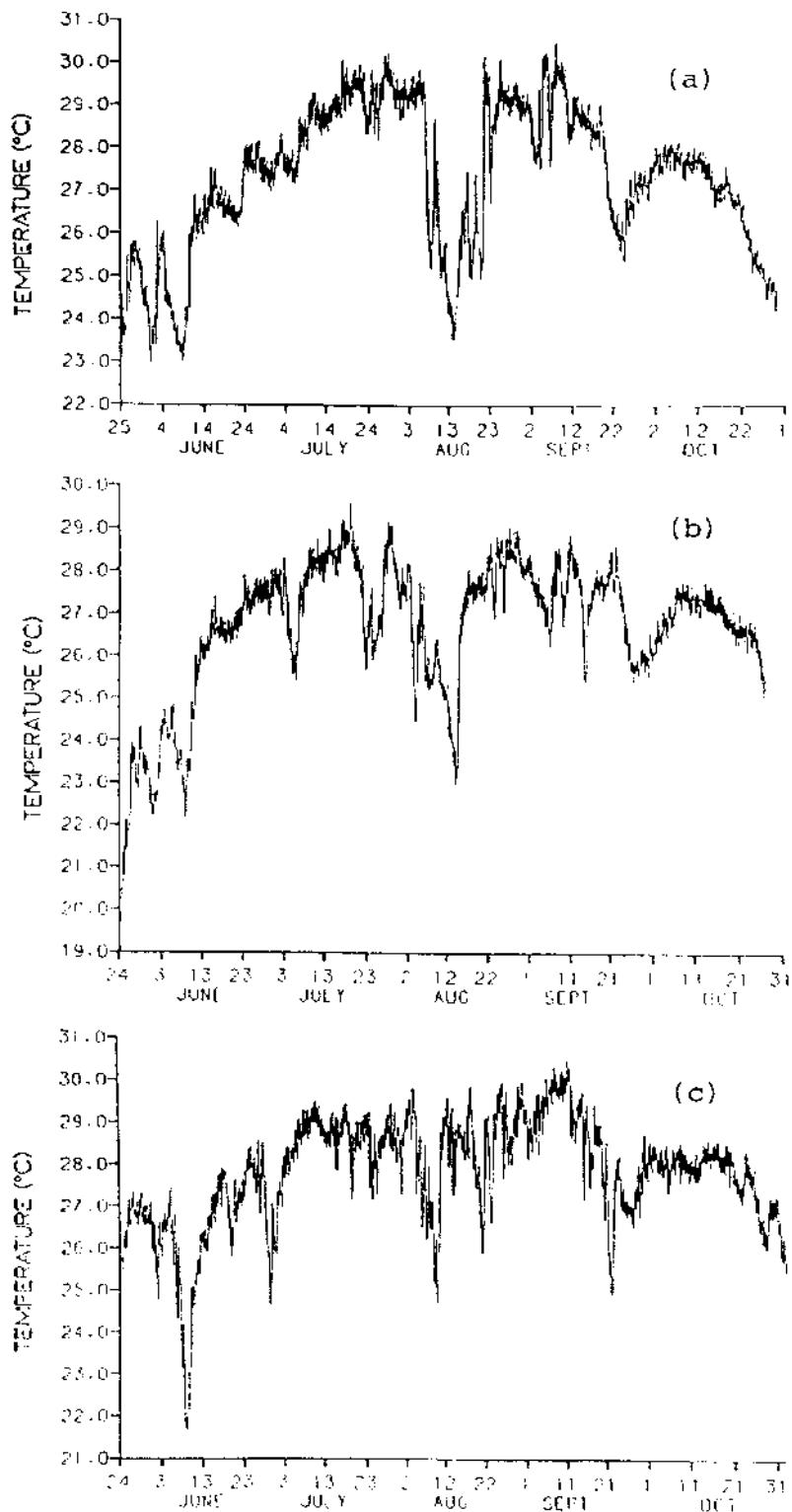


Figure 2. Time-plots of bihourly, near-bottom temperatures (in °C) recorded at Station 1 (a), Station 2 (b) and Station 3 (c), May 24 to October 31, 1983.

The temperature record from the southernmost study site (Station 3; Fig. 2c) reveals a somewhat different pattern than those observed at the more northern locations. Mid-August temperatures can be characterized by short-lived decreases of 2-5°C lasting only 3-4 days (possibly indicating the beginning of upwelling) followed by a return to temperatures approaching seasonal highs. The strongest indication of upwelling occurred during the second week of June when temperatures decreased from over 27°C to less than 22°C. A decrease in temperatures was observed at the other two study sites during this same time period but it was not as dramatic.

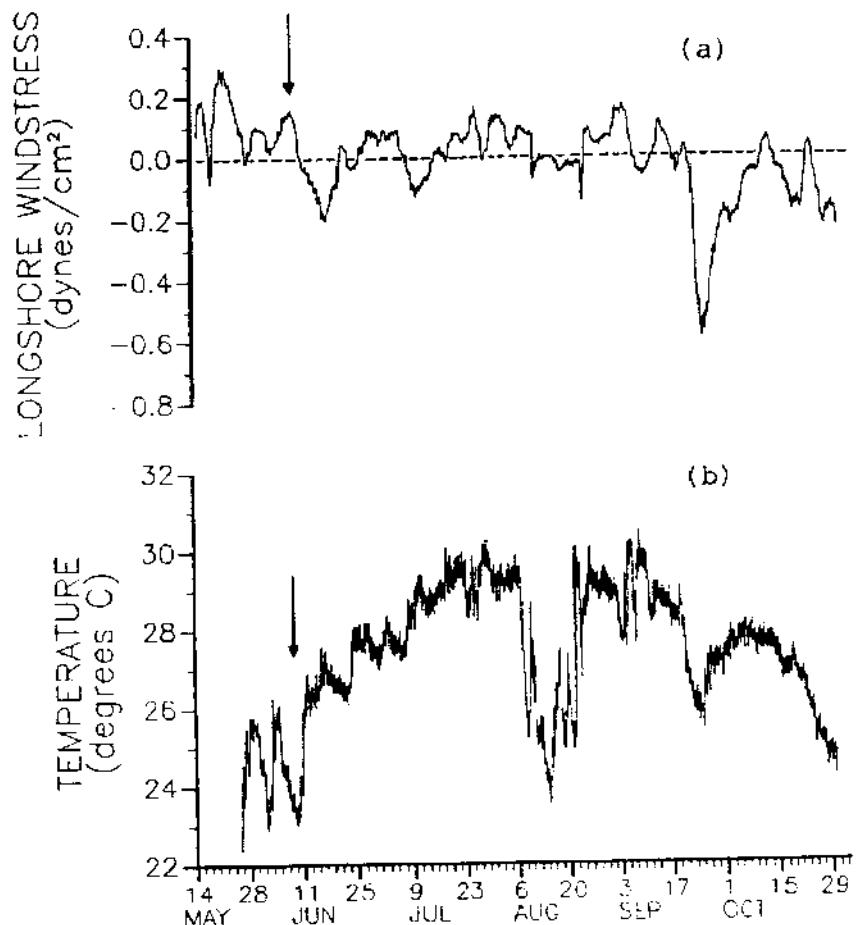


Figure 3. Composite time-plots of exponentially-filtered, longshore wind stress, in dynes/cm², (a) and near-bottom temperatures, in °C, (b) recorded May 24 to October 31, 1983. Positive values in the top plot indicate upwelling-favorable winds.

A composite of the exponentially-filtered, longshore component of wind stress plotted against time and the near-bottom water temperatures recorded at Station 1 are shown in Figs. 3a and 3b, respectively. Positive values in the upper plot indicate longshore wind stress to the north which is upwelling favorable. Results show a recurrence of upwelling-favorable wind stress roughly every two weeks throughout the study period. However, only one of the inner-shelf upwelling episodes recorded nearby corresponds in time to a period of upwelling-favorable wind stress (arrows). The pronounced 2-week event recorded in mid-August occurred when wind stress is very

weak and out of the northern quadrant; clearly a situation unlikely to produce upwelling via Ekman transport. Also, the upwelling episode recorded during the third week of September at all three study sites corresponded in time with strong winds from the north; again, wind conditions very unfavorable for upwelling.

DISCUSSION

The time series recorded at the three study sites show temporal upwelling patterns similar to those observed historically from this region (Smith; 1981, unpubl. data). The major event recorded at Station 1 in mid-August, when intruding water decreased temperatures 5°-6° from a seasonal norm of 28°-30° for approximately two weeks, is typical. The more temporary episodes recorded at all three sites is common. The weaker indications of upwelling observed at the two more southerly study sites may stem from their being on the southern extreme of the geographical range of documented upwelling off the east coast of Florida.

Upwelling events in this region are not only ephemeral but appear to be spatially patchy; that is, the occurrence of upwelling at one location is not necessarily indicative of upwelling at another location only a few tens of kilometers away. This point is best exemplified by the onset of upwelling twice during the month of July at Station 2 while there was little indication of significant temperature changes at sites only 45 km to the north and south during the same time period. Alternately, the temperature records indicate that a given episode can be widespread geographically, as indicated by the mid-August event that affected all three study sites; however, none of the sites experienced the same degree of upwelling.

Studies by Smith (1981, 1982, 1983) have shown that at a given latitude the entire shelf may not be involved in shoreward excursions of cool water. "Tongues" of cool water may only reach the mid-shelf or outer-shelf regions, especially further north, where the shelf becomes significantly broader. As the present study was confined to the nearshore areas of the inner-shelf, there were probably intrusions of cool water onto the outer- or mid-shelf areas during the study period that would not have been recorded by our thermographs. In addition, Smith's studies have shown a highly stratified water column across the entire shelf during times of a fully developed upwelling episode. Surface temperatures may be at or near seasonal highs of 28°-30° during those times while a strong thermocline exists in the mid to lower levels.

The idea of wind stress as a primary mechanism for driving the observed upwelling during the study period is not supported by the data. This finding is in contrast with results from some studies conducted in the same geographical area (Green, 1944; Taylor and Stewart, 1957; Lemming, 1979). However, Smith (1981) showed coherence values computed between wind stress and bottom temperatures to be generally well below the 95% confidence limit, particularly over the longer time scales usually associated with meteorological forcing. Results described here indicate that only one of the observed upwelling intrusions corresponded in time with upwelling-favorable wind stress and several events, notably the pronounced mid-August episode, occurred under wind conditions unfavorable to upwelling. It is noteworthy, however, that wind stress was upwelling-favorable at aperiodic intervals for approximately half of the study period and it is likely that wind plays a supporting role in driving the observed upwellings.

An alternate mechanism for producing upwelling in this region was postulated by

Smith (1981, 1982). He suggested that the local upwelling may be a response to meandering of the Florida Current. According to this hypothesis, during those times when the Florida Current meanders to the west and contacts the continental shelf, local geostrophy temporarily deteriorates leaving a shoreward-directed pressure gradient in the benthic boundary layer. Onshore movement near the bottom then advects cooler water onto the shelf. The hypothesis is supported by several lines of evidence. Meandering of the Florida Current streamlines has been documented using satellite imagery (Vukovich et.al., 1979) and by drogue measurements (Chew and Berberian, 1970; Richardson et.al., 1969). These meanders characteristically have wavelengths of 100-200 km. An amplitude of 5 km was reported for a meander in the Straits of Florida (Chew and Berberian, 1970). It is not clear how the meanders are generated, but low-frequency variations in flow over time scales of 1-2 weeks have been correlated with wind stress (Dåing et.al., 1977). Studies have shown a statistically significant relationship between longshore currents and temperature; as longshore motion increased, water temperature decreased within 1-3 days (Smith, 1981).

Biological studies have shown that upwelling off the Florida coast not only has a profound effect on the local hydrography, but influences the distribution and abundance of the biota as well. Green (1979) described differences in phytoplankton across the shelf at times before and after the onset of upwelling. Others (Atkinson et.al., 1978; Paffenhöfer, 1983) have shown high chlorophyll and dense phyto- and zooplankton populations resulting from shoreward transport of nutrients at near-bottom levels during Gulf Stream intrusions onto the Florida shelf. Yoder (1983), working over the southeastern U.S. continental shelf between Cape Canaveral and South Carolina, estimated that as much as 90% of the region's outer shelf primary productivity occurred during upwelling and, thus, upwelling is the dominant process affecting primary productivity at this location. Furthermore, the ecological impacts of upwelling are probably enhanced by the role it plays as a crossshelf exchange mechanism. Dissolved and suspended material (e.g. nutrients, plankton and possibly larvae of commercially important species) can be transported the entire width of the continental shelf.

Regardless of the effects, the data reported here and results from previous studies clearly indicate that upwelling over the inner continental shelf in this region occurs as discrete events lasting on the order of several days to approximately three weeks. During these events water temperatures typically decrease, at least temporarily, to values more characteristic of late spring or early winter conditions. The upwelling-induced temperature anomalies are limited not only temporally, but spatially as well, with little or no apparent pattern. There is no clear indication that upwelling events occur simultaneously at two or three locations consistently or that they move in a northerly or southerly direction. The available data from this study do not support the concept that wind stress plays a major role in driving upwelling in this region, although it is likely that wind stress plays a supplementary role in these events.

ACKNOWLEDGEMENTS

I thank Ms. Elizabeth Smith and Dr. Ned P. Smith for collecting the temperature data. Special acknowledgement to Dr. Smith for his help with data analysis and manuscript suggestions.

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BEHAVIORAL AND CHEMICAL ASPECTS OF BLACK BAND DISEASE OF CORALS: AN *IN SITU* FIELD AND LABORATORY STUDY

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*Black band disease of corals consists of a dense microbial community dominated by the cyanobacterium ("bluegreen alga") Phormidium coralliticum. The community, which is present as a line or band, migrates across the surface of infected scleractinian coral colonies, completely degrading coral tissue and leaving behind bare coral skeleton. Studies were performed on chemical microenvironments present within black band and their relation to behavioral patterns exhibited by microorganisms associated with the band. Vertical profiles of oxygen and sulfide within the top 500 micrometers of freshly collected intact band, measured using microelectrodes, demonstrated that the base of the band, near coral tissue, is both anoxic and contains sulfide. Oxygen microelectrodes were also used underwater to investigate vertical gradients of oxygen in black band in the natural environment. The *in situ* measurements corroborated the laboratory study, and showed that strong oxygen gradients were present throughout the band. The chemical dynamics and biological interactions within black band are similar to those reported from a wide variety of attached microbial communities found in diverse aquatic environments.*

INTRODUCTION

Black band disease of corals was first reported on reefs in Belize by Antonius (1973). Subsequently, black band has been observed throughout reefs of the Caribbean (Garrett and Ducklow, 1975; Rutzler et al., 1983; Edmunds, 1991), the Indo-Pacific (Antonius, 1985) and the Red Sea (Antonius, 1988). It is thought that the disease is contributing to an observed overall degradation of coral reefs (Williams and Bunckley-Williams, 1990; Porter and Meier, 1992; Peters, 1993). The disease is characterized by a dense band, 1 mm to 4 cm wide and <1 mm thick, which can be seen between apparently healthy coral tissue and freshly exposed, pure white coral skeleton. The band is composed of a microbial consortium dominated by a gliding, filamentous cyanobacterium, originally described as Oscillatoria submembranaceae by Antonius (1973), and

redescribed as Phormidium coralliticum by Rutzler and Santavy (1983). The band appears dark due to the cyanobacterial photosynthetic accessory pigment phycoerythrin. The band also contains numerous heterotrophic bacteria (Garrett and Ducklow, 1975), fungi (Ramos-Flores, 1983), and the sulfide-oxidizing filamentous bacterium Beggiatoa (Rutzler et al., 1983).

In the disease process, black band migrates across the surface of coral colonies, completely degrading coral tissue. Rates of movement have been observed up to 2 cm/day (Rutzler et al., 1983), which is highly destructive in that many scleractinian corals grow at rates of approximately 1 to 2 cm in circumference per year (Hudson, 1981). Typically, the disease is active only during the warmer months of the year when water temperature is at or above 25°C (Rutzler et al., 1983). The disease occurs on scleractinian corals, most commonly species of Montastraea, Diploria and Colpophyllia. Many, but not all, scleractinian coral are affected (see Antonius, 1981 and Rutzler et al., 1983 for details about susceptible colonies). Death of an entire colony can occur in one active season for small colonies (less than one meter), or after repeated infection in successive seasons (Richardson, personal observation).

It has been suggested (Rutzler and Santavy, 1983) that the cause of coral mortality is via cyanobacterial toxins, however this has never been tested experimentally. An alternate hypothesis is that the chemical environment of black band, i.e. anoxic, sulfidic conditions at the base of black band where the band contacts the coral, is responsible for coral death. Preliminary laboratory work by Richardson (1992) demonstrated anoxia at depths below 500 µm into the band, measured using oxygen microelectrodes on intact black band sampled using an underwater coring device. Indirect evidence that sulfide is present in the band microcosm consists of observations of the sulfide-oxidizing bacterium Beggiatoa sp. within black band (Rutzler et al., 1983). At times, dense populations of Beggiatoa are present on the surface of well-developed black band (Richardson, 1992). Demonstration that the cause of coral death is by chemical microenvironments produced by the microbial consortium present in black band requires, as a first step, direct measurement of the chemicals on a microscale coupled with an investigation into the microbiology of the consortium.

We report here results of laboratory measurements of oxygen and sulfide microenvironments present in freshly collected, intact black band (measured using oxygen and sulfide microelectrodes), and field experiments on both oxygen microenvironments in in situ black band (measured using in situ microelectrodes) and in situ behavioral patterns of the black band community.

MATERIALS AND METHODS

The in situ study was performed on Grecian Rocks, ca. 3 miles offshore from Key Largo in the Key Largo National Marine Sanctuary (now part of the Florida Keys National Marine Sanctuary) during July of 1992. The site is dominated by large (many >2 m diameter) colonies of Montastraea annularis morphotype 3 (Knowlton et al., 1992), with more than 50% of the colonies infected with black band disease at this particular site. Water depth on the reef ranges from zero (when colonies of Acropora palmata are exposed at low tide) to 10 meters. Water depth at the study site was 2.7 m (high tide).

Water temperature was determined by a data logging Thermomentor (Ryan, Inc.) permanently installed by the Key Largo National Marine Sanctuary approximately 25 m from the study site, and at a similar depth. Underwater light levels were measured using a

diver operated Biospherical Instruments Inc. irradiance meter (Model QSI-140), which measures photosynthetically active radiation (PAR) from 400 to 700 nm. Laboratory light levels were determined using a Biospherical Instruments QSL-100 averaging quantum irradiance meter, which also measures PAR.

In situ oxygen dynamics of the black band community were investigated using oxygen-sensitive microelectrodes of the style designed by Revsbech and Ward (1983), modified by R.G. Carlton. Our probes were constructed (by R.G. Carlton) with thicker tip diameter (ca. 150 μm) to reduce chance of breakage if contact was made with the coral skeleton. In addition, a stronger adhesive (Torr Seal, Varian Associates) was used to seal probe components. Additional details of microelectrode use and construction are described in Revsbech and Jørgensen (1986) and Carlton and Wetzel (1987). During use the cathode was polarized at -0.75 Volts relative to the anode. The subnanoampere-level output signal, which is a linear function of oxygen concentration, was displayed on a Kiethley 480 picoammeter. The probe was electrically connected to the polarizing circuitry and picoammeter (both contained in a submersible acrylic pressure housing) by a 3 m long cable. A two point calibration of the probe was made before and after making the in situ measurements by alternately immersing the probe in air-equilibrated (21% O₂) or anoxic (N₂ sparged) sea water.

Accurate positioning of a microprobe can only be accomplished with a micromanipulator. For the in situ measurements a modified microscope stage micrometer was used, which costs much less than a laboratory micromanipulator and was easier to maintain after exposure to salt water. The micrometer/manipulator was mounted on a lattice of 13-mm diameter aluminum rods which were anchored ca. 1 m in the sand adjacent to the subject coral. In a laboratory situation, probes can be accurately positioned at the liquid-solid interface with the aid of fiber optics or magnifying devices, which cannot be used by a diver wearing a facemask. In this study we detected the interface (i.e. surface of black band) by watching for a characteristic output signal change which occurs upon contact. This was accomplished by moving the probe toward the band in increments of ca. 50 μm . Under quiescent conditions the diffusive boundary layer (DBL) can be several mm thick around solid immersed objects (Vogel, 1981). When approaching from outside the interface, this method first identifies the surface of the DBL (Revsbech, 1989). At our study site the water depth was ca. 2.7 m, the active black band was at a depth of 1.7 m, and sea state was running ca. 1 m. [These conditions dictated that we perform the experiment as rapidly as possible for diving safety. This was the last day of our field program, and we had been waiting unsuccessfully for conditions to improve for the in situ study.] The high turbulence and velocity of the surge (>1 m/sec) were probably sufficient to reduce thickness of the DBL to <50 μm (estimate based on unpublished data of R. Carlton). Therefore, our spatial resolution in terms of detecting the black band/water interface was, at best, $\pm 25 \mu\text{m}$. After locating the black band/water interface, the probe was advanced in 100 μm increments. The visual contrast between the bright glass probe and the darkly pigmented microbial community made it possible to verify that the probe was inside the band. However, without being able to gauge the thickness of the band in situ, we opted to avoid breaking the probe by inserting it to a maximum depth of 400 μm .

Laboratory measurements of both oxygen and sulfide microprofiles were made from cores taken from the same *M. annularis* colony on which we conducted the in situ study. One inch cores were taken, which contained black band, exposed coral skeleton, and intact coral tissue, by using a pneumatic drill (attached to the first stage of a SCUBA cylinder with a high pressure hose) equipped with a hole saw. The remaining hole in the

coral colony was plugged with modeling clay to prevent infection. Experimental cores were immediately taken to Key Largo and placed in a 3 gallon aquarium with flow-through sea water. Light (natural sunlight) intensity was modified to that measured *in situ* by neutral density screens. The oxygen microelectrodes were calibrated in the same manner detailed above for the *in situ* study. The sulfide microelectrodes were calibrated using a set of different concentrations of sodium sulfide (NA2-9H2O) made up in anoxic (N2 sparged) seawater, which were in turn analyzed for sulfide concentration using a modified Pachmayr assay (see Richardson and Castenholz, 1987) to generate a standard curve. Calibration standards ranged from 0 to 2 mM sulfide. Both oxygen and sulfide microelectrodes were inserted by micromanipulators. Simultaneous, continual oxygen, sulfide, and light measurements were made and recorded on Kipp and Zonen chart recorders. Light was measured using the Biospherical Instruments QSL-100 sensor.

Underwater photography was carried out with a Nikonos 5 equipped with a 1:1 macrolens, and a Sea & Sea Motormarine II using both a 35 mm and a 1:2 macrolens.

RESULTS AND DISCUSSION

In situ microprofiles of oxygen concentration were measured in an intact section of black band growing on a large, otherwise healthy, colony of *Montastraea annularis*, morphotype 3 (Knowlton et al., 1992) as shown in Figure 1. The band ranged from <1 mm to 1 cm from front to back, and was <1.0 mm thick. Measurements were made near the active front (by intact coral tissue), middle, and back (by freshly exposed coral skeleton) of the band. Oxygen profiles at the three band sites are shown in Figure 2A. The front and back measurements were made ca. 1 mm from the actual edges of the band. A second O2 microprofile, measured 4 mm from the initial one, also 1 mm from the front of the band, was measured as a duplicate and agreed closely with the initial profile (data not shown). During the experiment, *in situ* light was 7.2×10^{-16} quanta/cm²/sec.

At each of the *in situ* microsites oxygen concentration at the black band/water interface exceeded that of the bulk water phase, which was 194 μ M. The highest observed oxygen concentration in the band, 315 μ M, occurred at the surface of the back edge (Figure 2A). At all band sites, oxygen concentration decreased with depth. The deepest (middle of band) measured profile showed that anoxic conditions existed at the base of the band. Although the oxygen concentration gradients at the front and back of the band had similar slopes between 0 and 200 microns, our data are not sufficient to conclude whether the front or back of the band are as strongly depleted in oxygen at their bases. [All laboratory experiments, however, showed anoxia at depth; see below.] Due to the sea conditions, we opted for limited depth in most of the measurements to avoid electrode breakage.

The laboratory experiments on chemical microenvironments present in black band on freshly collected, intact cores were conducted in much more detail due to the fact that we didn't have to contend with strong surge and minimal time. Figure 2B shows the front edge oxygen and sulfide profiles of a core collected from the same colony shown in Figure 1, but at a different site on the active band. Light intensity was 8×10^{-16} quanta/cm²/sec during the experiment (adjusted to approximate the *in situ* level by shielding the experiment with two layers of neutral density screen). The oxygen profile revealed a surface maximum of 500 μ M. Oxygen penetrated the band to a depth of 500 μ m, below which conditions were anoxic. The laboratory cores did not show the same variation in oxygen levels as were measured *in situ*. While the middle profiles had a similar ~i.e. relatively lowest) oxygen penetration, the front edge was often as oxygenated as the back (Carlton and Richardson, unpublished data).



Figure 1. *In situ* oxygen microelectrode study. A. Study coral with microelectrode apparatus. B. Photomacrograph (2:1) showing oxygen electrode with tip penetrating band.

The higher oxygen concentration at the surface of the core as opposed to the surface of *in situ* black band is probably due to the relatively lower turbulence in the aquarium, which allows accumulation of oxygen. The aquarium water was kept in motion by the flow-through system, but was obviously much less dynamic than that present in the turbulent conditions *in situ*. The high turbulence and surge present when we carried out the *in situ* study were not rare for this reef, however, they are hydrodynamically difficult to duplicate in the laboratory. Conversely, there are many days when the reef is calm, and one would expect surface black band oxygen levels to increase. Therefore, the surface oxygen levels in black band both *in situ* and in the cores must be considered as examples of a range of possible oxygen distributions controlled by an interaction between photosynthesizing *Phormidium corallyticum* and turbulent flow of oxygen away from the surface of the band.

The variation in oxygen profiles across the width of the band (Figure 2A) may be explained by a number of scenarios. There may be a higher biological demand for oxygen at the front and middle of the band, where, presumably, coral tissue degradation is actively occurring and heterotrophic oxygen demand, fueled by higher nutrient supply, is much greater. Or, there may be a true higher photosynthetic rate at the back of the band. Both the *in situ* and laboratory (data not shown) black band profiles revealed relatively higher oxygen levels associated with the back edge. This pattern may be related to higher light near the exposed coral skeleton, reflected from and through the calcium carbonate, which could potentially support a faster rate of oxygenic photosynthesis. There has been much work on the photic responses of cyanobacteria (Castenholz, 1982), and it has been demonstrated that natural populations of cyanobacteria position themselves at a light intensity which is optimal for support of maximal photosynthesis (Garcia-Pichel and Castenholz, 1990). Cyanobacterial strategies to maintain a preferred light regime include phototaxis and avoidance of very high light (which suppresses photosynthesis) by photophobic responses (Castenholz, 1982).

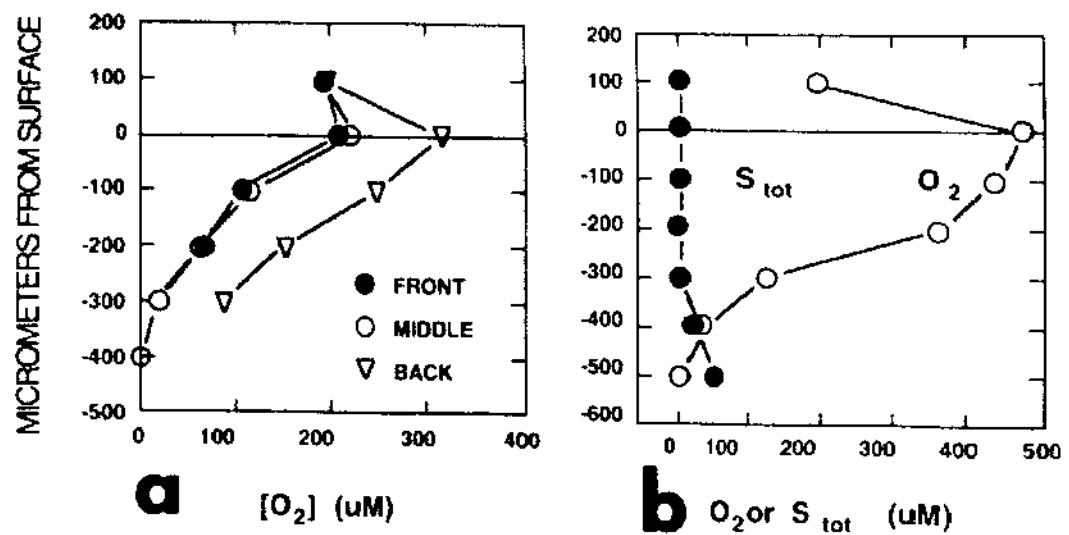


Figure 2. Oxygen and sulfide distributions in black band on *M. annularis*. A. Oxygen microprofiles, measured *in situ*. B. Oxygen and sulfide microprofiles, measured in a core.

The cyanobacterial light responses operating in microbial mat communities might control both black band migration patterns, and the differences in in situ oxygen across the band. If the cyanobacteria present in black band are migrating to maintain a light regime to support optimal photosynthesis, and optimal photosynthesis is revealed as highest concentrations of oxygen found at the back edge of the band, it would be expected that they remain at the high light (back of band) environment during daylight hours and not migrate across coral colonies. Such a pattern is suggested by the oxygen profiles. Conversely, if the population is avoiding high light and migrating away from the reflective coral skeleton, one would expect photosynthetic rate (thus oxygen levels) to be suppressed near the back of the band, which is not seen in the oxygen data. In the latter case, oxygen profiles would be more likely to be due to microbial interactions.

In a previous study (August, 1991), *in situ* patterns of the horizontal migration of black band in terms of light intensity and known cyanobacterial photic responses were measured on 6 coral heads with active black band (Richardson, unpublished data). A total of 536 measurements were made over a 100 hour period. The results of this study showed that none of the cyanobacterial photic responses are controlling the migration of black band across coral surfaces, thus the oxygen profiles are not the result of movement of *P. coralliticum* to optimize photosynthesis. These results suggest that control of oxygen dynamics within black band is apparently more complicated than in microbial mats, where virtually all known cyanobacterial motility patterns are ultimately controlled by light.

Sulfide was also measured in the laboratory cores. Figure 2B shows that sulfide was absent from the surface of the band to a depth of 300 μm . While some oxygen was present at this depth, it decreased rapidly to 0 by 400 μm . Sulfide increased with depth to a maximum measured value of 80 μM at a depth of 500 μm in the band (the deepest measurement taken due to potential electrode breakage). The direct measurement of sulfide by the sulfide-sensitive microelectrodes confirmed the presence of sulfide and anoxia within the black band microcosm, suggested by the previous observations of dense populations of sulfide- oxidizing bacteria. Figure 3 shows two macrophotographs (taken in August, 1991) of the vertical migration of a population of *Beggiatoa* sp., a gliding, sulfide-oxidizing bacterium which positions itself via negative aerotaxis (Jørgensen and Revsbech, 1983) at an oxygen/sulfide interface to optimize oxidation of sulfide as an energy yielding metabolic mode. Figure 3A shows the surface of black band at 8:00 am. The white surface is due to highly refractile granules of elemental sulfur present in cells of *Beggiatoa* as a result of intracellular sulfide oxidation. Figure 3B shows the same part of the band at 3:15 pm the same day. As light increased (and oxygen accumulated), *P. coralliticum* replaced *Beggiatoa* as the surface microbe. Note that black band has moved across the coral polyps.

The oxygen and sulfide microprofiles measured in this study are consistent with observations from other types of microbial mat communities such as epipelagic periphyton on lake sediments (Carlton and Wetzel, 1987), intertidal mud flats (Bebout et al., 1987; Pinckney and Zingmark, 1991), and mats found in sulfide- rich hot spring outflows (Richardson and Castenholz, 1987). Characteristics seen almost universally in photosynthesizing mat communities include oxygen supersaturation near the surface (where the rate of photosynthesis is maximal), a nearly linear oxygen concentration gradient just below the maximum, and low or zero oxygen concentration at the base of the community. The oxygen and sulfide profiles together are indicative of the respiratory oxygen demand extant in the lower strata of the black band community, as well as the chemical reduction of oxygen by sulfide present near the base of the band. The

observation of movement of Beggiatoa to the surface of the band during darkness is also quite similar to vertical migrations in sulfide-rich microbial mats, which includes both migration of the microorganisms and vertical migrations of the oxygen/sulfide interface. Such profiles are controlled by diel changes in surface oxygenic photosynthesis coupled with bacterial sulfidogenesis at depth, (Jørgensen et al., 1979), and have been shown to control vertical migrations of cyanobacteria (Richardson and Castenholz, 1987) as well as Beggiatoa (Jørgensen and Revsbech, 1983).

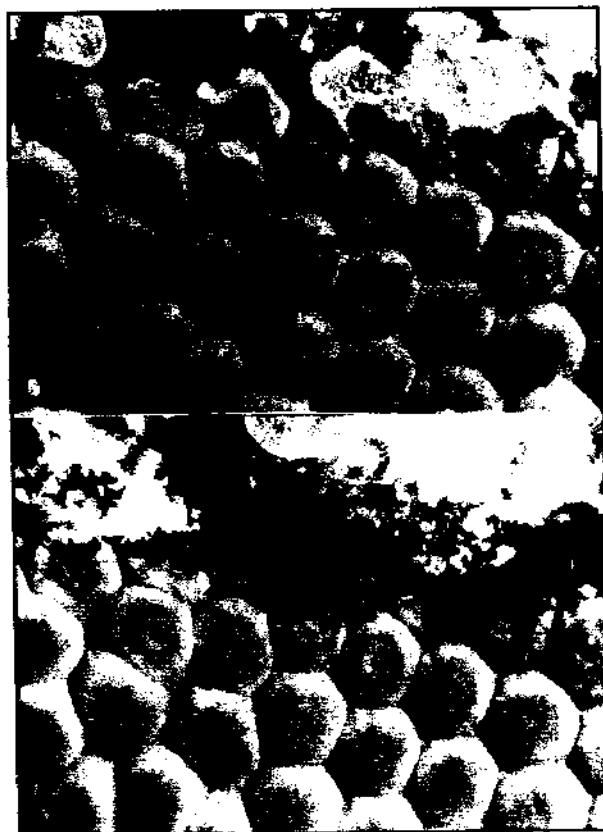


Figure 3. *In situ* vertical migration of Beggiatoa in black band on M. carvernosa. A. Beggiatoa on surface at 8:00 am. B. Phormidium on surface at 3:15 pm. Nail diameter is 9 mm.

Most microbial mat communities are present on sediment or accumulated mat material undergoing diagenesis, both of which serve as carbon sources to the surface, photosynthetically active layer. The black band community is the first case we know of a microbial mat community present on an animal, and actively killing the animal. The high concentrations of sulfide, plus anoxia, present in black band support the hypothesis that coral mortality associated with black band disease is related to chemical microenvironments. Sulfide is an inhibitor of aerobic respiratory electron transport, acting similar to cyanide as a respiratory poison. It is highly unlikely that coral tissue can survive exposure to anoxic conditions with 80 μ M sulfide. Thus, as black band moves over coral, it exposes coral tissue to a toxic chemical microenvironment which may very

well be the specific mechanism of death of the coral. Subsequent to coral death, the degradation of coral tissue may serve as a source of both organic carbon and nutrients to the black band community, thus ensuring a nutrient source in the nutrient poor environment of coral reefs.

ACKNOWLEDGEMENTS

We would like to thank Katharine Dedek, Kevin Kuta, and Chris Borne for assistance in the field, and Greg McFall for the photographs of *Beggiatoa*. This research was conducted by grants from the National Oceanographic and Atmospheric Administration's National Undersea Research Program, NSF Grant BSR-9107546 administered by the University of North Carolina at Wilmington, under research permit KLNMS-1-92 from the Key Largo National Marine Sanctuary.

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STUDY PLANNING, TESTING, AND REALITY: PLATFORM REMOVAL AND ASSOCIATED BIOTA

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About 80 structures are removed annually by use of explosive charges placed 5 m below the seafloor inside major support elements. While commercial and sport fishing and the discard of shrimp bycatch are known to decrease reef fish stocks in the GOM, there is no doubt that the necessary use of explosives during platform removals also kills fish. Using preliminary data and firsthand experience from 1991, Minerals Management Service (MMS) designed a formal study to examine the severity of explosive removals on fish stocks when compared with the effects of commercial/recreational fishing and bycatch discard. In 1992, MMS signed an Interagency Agreement with the National Marine Fisheries Service (NMFS) to perform the investigation. The study required that fish mortalities associated with nine explosive removals in less than 40 m of water depth be investigated. The first shakedown cruise, in late 1992, showed that numerous adjustments to diving procedures and equipment were needed. After design simplification and diving practice, work performed during an early 1993 cruise indicated that the study objectives could be met with the new procedures and equipment. The applicability of the study plan and methodology to an actual platform removal is discussed.

INTRODUCTION

Major production platforms are large complex structures supported by up to 16 legs of steel that rise upward through the entire water column. Platforms are set in place by driving steel pilings through the legs deep into the seafloor. Below the surface the structure is further strengthened by horizontal and diagonal steel-pipe bracing. Working machinery and personnel sit above the water supported by a steel network that is intentionally overbuilt and remarkably secure (Gallaway and Lewbel, 1982).

The underwater area of platforms varies with depth since a platform in deeper water has a typically wider base. Gallaway and Lewbel (1982) estimated that a multi-leg platform standing in approximately 45 m of water provides 3-4 acres of hard surface substrate. Given that there are at least 2,300 platforms in Federal waters on the OCS in the northwestern Gulf, then about 8,000 acres of hard substrate are provided by the underwater supports of these platforms. Additional hard substrate is created on the seafloor under each platform as a cutting pile of rock chips accumulate during the drilling of wells. This surface is augmented by barnacle shells and shell hash that slough off upper support members, accumulating in layers up to 3 m thick (Driessen, 1985).

Estimations of the increase of hard surface substrate contributed by platforms to the northwestern Gulf area are as high as 28 percent (Stanley et al., 1991). Stanley et al. (1991), using dual beam hydroacoustics, estimated the number of fish at well over 10,000 around and under a platform in 33 m of water.

Studies of fish assemblages under offshore oil/gas platforms in the north-central Gulf were performed by Continental Shelf Associates (1982), Gallaway (1981), Scarborough-Bull (1989), and Sonnier et al. (1976). Many species of desirable fish in the north-central Gulf of Mexico are found only at natural hard/live bottom areas or at offshore oil/gas platforms. These species include numerous species of groupers and snappers. For species such as these, availability of natural habitat is thought to be the limiting factor to abundance, with the presence of platforms allowing for population expansion and an increased fishery (Linton, 1988). Gallaway (1985) estimated that oil/gas platforms in the northwestern Gulf of Mexico constituted 11 percent of the available snapper fishery. These observations indicate that the multiple use and importance of oil/gas platforms to the fishery of the northwestern Gulf should be considered in scenarios of fisheries management.

Offshore platforms are not intended to be artificial reefs or permanent structures. The requirement that platforms be removed within one year after cessation of production originates from legal and regulatory mandates (USDOI, 1987). Platform removals are dynamic, complex, and hazardous salvage operations. They require the expense and coordination of several major companies and numerous personnel who handle inherently large numbers of machinery and huge pieces of equipment. Once the production wells are plugged and abandoned according to regulations, all machinery and upper platform levels are cut apart and salvaged. What remains is the lowest level, about 3 m above the water, still firmly attached to the hollow support legs with their interior anchor pilings and free-standing well conductors that protrude through the floor grating.

The platform removal season extends from May through October with most of the actual platform removals occurring during July, August, and September. At least 100 platforms per year are removed from the Gulf of Mexico. About 80 percent of these removals use explosive charges placed 5 m below the seafloor inside the hollow pillars to sever the well conductors, platform anchor pilings, and support legs (USDOI, 1987). First the conductors are severed, pulled out, and salvaged. Then the pilings and support legs are severed and the remaining subsea structure plus the lowest above-water level is pulled free from the seafloor and salvaged in one piece.

The Gulf of Mexico Fishery Management Council (FMC) remains very concerned over the declining stocks of reef fish, especially red snapper, in the Gulf of Mexico (Goodyear and Phares, 1990; Hoogland, D. personal comm., 1993). Prior to work presented at the AAUS annual conference in 1992, information about fish kills during removals came from examination of post-detonation floating fish only (Caillouet et al., 1986; Duronslet, 1986; Fontaine, 1986; Klima et al., 1988; Ross et al., 1990). Attempts to examine fish that may have sunk to the seafloor as well as those that floated to the sea surface after detonation(s) had not previously been made. Conclusions from Scarborough-Bull and Kendall (1992) explained that estimations of species richness or abundance of total fish killed during an explosive platform removal should not be made from examination of only those fish that float to the surface.

Having reported in 1992, on fish kills associated with explosive removals examined in 1991, the U.S. Department of the Interior, MMS, Gulf of Mexico Region then entered into a Interagency Agreement with the National Oceanographic and Atmospheric Administration (NOAA)/NMFS to design a formal study to examine the

severity of explosive removals on fish stocks when compared with the effects of commercial/recreational fishing and bycatch discard. Negotiations concerning the study design lasted six months during which time both agencies settled on objectives and methodologies. The award was funded by MMS to NMFS laboratories at Galveston and Miami in June of 1992. Galveston personnel perform data collection after which Miami personnel perform data analyses.

From the time of the award it required an additional five months for NMFS to finalize a vessel contract by which time no further removals would occur during 1992. Although some work has been performed, the rapidity at which the study progresses is disappointing. At the present date two shake-down cruises have been completed, two deep-water ROV observations attempted, and one actual explosive removal studied. Comparison of the following description of the methods to be employed and results represent an example of the reality of the inherent difficulty of field work on this scale.

METHODS

Most platform removals occur in the north-central Gulf in water depths less than 40 m. The project plan involves two phases both of which will take place in the north-central Gulf. Phase One consists of the construction and testing of equipment and methods described below (mark-recapture, in-water diver census, lift nets, remotely operated vehicle (ROV) and diver transects) at three platforms in five to 40 m of water depth. In addition, estimation of fish community structure by ROV will be conducted at a site deeper than 40 m that is also scheduled for explosive removal. All collection materials will be in place before the derrick barges and tug boats arrive on-site. Phase two will address six platform removals in the five to 40 m depth range.

Nine petroleum platforms in the Central Gulf Outer Continental Shelf (OCS) planning area that are scheduled for explosive removal will be studied. Prior to the detonation of charges, an intense mark and release fishing effort will be conducted by five personnel, each fishing 15 hours a day. Resident fishes will be captured (hook and line) tagged and released at the platform for three days prior to the detonation of charges. The release of a minimum of 500 tagged fish per day for three days will be targeted. The initial density and composition of fishes at the platform can be estimated using the ratio of marked to unmarked dead fish collected following the detonation of charges.

Video documentation by Phantom ROV during Phase One will be used as an additional methodology to estimate fish population sizes. The ROV will be leased on an as-needed basis and will be operated by NMFS technicians. The ROV will be deployed at four random sites around each platform. The census will take place in a vertical cylinder from the surface to the bottom. Bohnsack and Bannerot (1986) developed a fish census technique using SCUBA divers which will be modified slightly for the ROV. Visibility permitting, the camera will scan a 360° arc of five m radius for at least 15 min at each site around the platform. Fishes will be counted and identified to genus or species and lengths will be estimated as possible. The ROV documentation is the only method scheduled for examination of the deep water platform.

To complement the ROV estimate of fish population sizes, divers will conduct in-water census beneath the platform to examine waters not accessible to the ROV. Divers will employ the Bohnsack and Bannerot (1986) census technique described above, in which divers scan a 360° arc of five m radius for at least 15 min at each chosen site. Four non-overlapping sites will be sampled beneath each platform.

Three primary sampling techniques are proposed for the collection of dead or stunned finfish after demolition. Some dead or stunned fish species are likely to float to the surface of the water immediately following detonation of charges. All fish regardless of species, will be collected from two Zodiacs, each with a two-person team using telescoping dip nets. Sampling will begin immediately after the contractor and demolition supervisor declare that it is all clear. No restriction to the distance from the platform that fish can be collected will be in effect because the Zodiacs may have to follow fish drifting down-current. Each individual fish will be identified to species if possible. Each fish of commercial or recreational importance (grouper, snapper, trout, ling, tuna, blue runner, triggerfish, mackerel, red drum, black drum, sheepshead, Atlantic spadefish) will be measured and weighed. The remaining fishes will be counted and weighed by species. Fish age will be estimated using published information relating fish size to age. All samples will be processed on the deck of the primary support vessel and discarded on-site.

Other fishes are likely to settle to the bottom after the explosions. Equal numbers of lift nets with purse lines (each covering 16 sq m) will be deployed within 100 m of the platform. Twenty-six lift nets will be deployed within a 0-50 m distance from the platform, 13 placed randomly upcurrent and 13 placed randomly downcurrent. Thirteen more lift nets will be placed randomly within a 50-100 m distance from the platform on the downcurrent side. These nets will be designed so that deployment and retrieval can be accomplished without the use of divers. Dead or stunned fish will be allowed to sink to the bottom for 30 min following the detonations. Then retrieval of the lift nets will begin using the secondary support vessel. Processing will be the same as described above.

Directly beneath the platform lift nets with purse lines (each covering 9 sq m) will be placed to cover approximately 80 percent of the bottom. Each net will be marked and tagged for later reference to position. Dead or stunned fish will be allowed to sink to the bottom for 30 min following the detonations. Then retrieval of the lift nets will begin using divers and lift bags. The divers will inflate lift bags to aid their moving the nets from under the still standing platform to the primary support vessel. The nets will be recovered by winch to the deck and all dead fish removed and processed as described above.

Divers will also census sinking fishes along transects radiating from the platform. Eight transect lines (100 m long) will be deployed, one each from the four corners of the platform and from the midpoints between corners. Six transects will be sampled from 0-50 m, three on the downcurrent side of the platform and three additional transects chosen at random. In addition, all three transects on the downcurrent side of the platform will be continued out an extra 50 m to their end. Fish will be collected and bagged by two-man diver teams every 5 m along the transects. Collections will extend out two m on either side of the transects.

Fish mortalities will be calculated for each 5 m transect increment. These numbers added to the information gathered from dead fish collected from under the platform and from floating fish will be used to estimate the total mortality of fishes that occurred during the platform abandonment operation. The ROV will be employed in advance of the divers to estimate the amount of dead fish along the transects and to compare with the diver census method.

Shake down cruises to test the lift nets, transects, and the ROV took place on December 7-11, 1992, January 7-9, 1993, and March 30 - April 1, 1993. Attempts to survey fishes at a deep water site with the ROV occurred during the first and third cruises. Methods as similar as possible to those described above were used during an

actual explosive removal operation from July 12-20, 1993. The platform removed was an unmanned four-leg, two well-conductor structure that was emplaced in 1981.

RESULTS

The need for technical changes in methodology or gear is inherent in a pioneer study such as this. During the first shake down cruise technical difficulties encountered with the ROV included an inoperative compass and depth gauge which prevented determination of the location of the ROV in relation to the platform during dives. Diver in-water surveys showed that most fish were concentrated near the vertical pilings on the up-current side of the platform. Visibility was estimated at 20 m in the upper water column and at less than 2 m on the bottom at 66 m. During practice deployment of the 100 m transects at a platform in 20 m of water, the bottom visibility was zero. This reduced efficiency and slowed deployment to an unacceptable time. Deployment of open water, purse lift nets was partially successful despite problems with the davit, winch system, and large airplane tires chained to the vessel side for protection. Divers reported that the purse lines were tangled and could not be engaged. Diver deployment of frame lift nets directly under the platform was successful although it was decided that a size smaller than 3 sq m would be easier to handle.

The second shake down cruise did not involve use of the ROV. During transect deployment some stakes which held the transect in place were erroneously inserted through some of the mesh collecting bags. However, divers successfully secured both ends of the transect lines and swam them as they would during an actual removal. Due to zero visibility, divers carried a buddy line between them with a float line attached to the buddy line. A dive flag was secured to the float line at the surface. A boat operator and safety diver monitored the progress of divers by following the float in a Zodiac. Divers unsnapped a mesh bag from the transect, mimicked collecting fish while swimming 5 m to the next stop where they exchanged bags on the line. The purse nets were replaced by a simpler open water frame net which worked well. Divers reported no tangling and several nets were deployed and retrieved successfully a number of times. Deployment time of the nets beneath the platform was reduced by replacing a rope loop in the bridle with a steel ring which made it easier for divers to attach air bags. The pursing device was improved with the placement of soft, rubber clips around the edges of the net. These clips held the net covers open and were easily detached to allow pursing when the nets were retrieved. Modifications to the davit and winch system included a locking device to prevent unwanted movement of the davit and a larger block to accommodate lines and fasteners. Addition of a new lifting bridle to the Zodiacs made for easier deployment and retrieval of both boat and motor.

The third shake down cruise was an attempt to conduct an ROV survey. The survey was hampered by strong currents and building seas. The ROV made little headway against the current and was extremely difficult to steer. The ROV became tangled in the platform on two occasions. During those incidents, the ROV was brought alongside the vessel, decked, and the umbilical cable removed and capped. This end of the cable was returned to the water and pulled through the platform using the opposite end of the cable. Additional attempts to perform surveys at the platform using the ROV were unsuccessful, although divers performed a number of in-water surveys without problems. Continued use of the ROV during this study is in question.

Demolition and removal of Conoco's Ship Shoal 158 Platform C (SS-158-C) took place on July 19, 1993. The barge and tugs arrived at the platform on the afternoon of July 18. The primary support vessel arrived on site July 13 and the 500 hours of fishing began. This rather tedious effort proved to be nearly worthless. Although in-water diver census revealed numerous fish species of various year-classes, the fish were not biting. However, the 500 hr effort was completed with fewer than 100 fish tagged and released. Deployment of 48 open water lift nets, eight transects, and eight purse nets under the platform were completed before the winch system self-destructed on July 16. A decision was made to steam to port and make the necessary repairs as all work (except another day's fishing) was completed. Once in port two of the NMFS divers, hired on a temporary basis, quit the job. The vessel returned to SS-158-C sometime late during the night of July 16 and fishing recommenced. Although both the NMFS Field Party Chief and the present author had attended a lengthy meeting with all companies involved to discuss logistics and coordination before removal, several events occurred that were unexpected.

The Global Marine DB3 derrick barge is an enormous structure 60 x 30 m in dimensions and dwarfed by its own 700 ton lift crane at one end. Placement of the anchors for this barge may take up to 10 hours and discussion of the anchor chains and possible entanglement with lift nets and transects had been discussed and dismissed since the anchors are set at least 1000 m away from the barge and the chains brought taunt. Unknown was the fact that the barge would sweep the bottom on the side of the platform from which it would work with steel cables prior to anchoring to ensure non-entanglement. Numerous lift nets and several transects had to be cut from the cables. When detonation took place and fish floated to the surface workers on the barge began to retrieve larger fishes. It required persuasive radio communication to receive back some, but not all, of the fish that were taken. When the demolition took place the four legs, anchor pilings, and the two conductors were all detonated at 0.9 sec intervals. This caused most of the fouling material such as barnacles to fall from the structure into the purse nets beneath the platform. In turn this made it impossible to lift the nets from below the platform. They were simply too heavy for the patched davit and winch system. Instead, divers were sent with tagged mesh bags to retrieve dead fish from the nets as best they could. Swimming transects that had remained intact worked quite well. However, during retrieval of one line with attached bags containing many dead fish, the support vessel cut the line with its propeller. The secondary support vessel arrived on July 18th as planned. The NMFS transferred two personnel to that vessel to retrieve the lift nets placed around the platform in open water. Fish in those nets were processed on that vessel and bagged. All fish and nets were transferred to the primary support vessel and the second vessel released. Bagged fish were inspected and identifications verified. The primary support vessel then remained at least 1000 m from the platform with no divers in the water as the barge moved back to the platform and continued the removal. Categories of dead fish, e.g. surface floating, under platform, from lift net number 38, etc., were kept separately and eventually processed and discarded before the primary vessel headed for port.

CONCLUSIONS

At the time of this writing the author has not received a copy of the data for the removal of SS-158-C from NMFS. The data gathered during the removal is incomplete but may contain interesting information once it is analyzed. As NMFS personnel had not previously been involved in an investigation of an explosive removal at the time of

demolition, it was a great learning experience that had been difficult to explain. Certain facets of both the removal operation and study design were not well understood until after the NMFS Party Chief/Principal Investigator could see for himself. Many more fish were killed than was expected and a greater number of fish sank to the bottom than floated to the surface. The NMFS divers learned how to handle dead fish on the bottom. If not properly grasped underwater, a dead fish will simply slip out of your hand. At least one person thought at first that this indicated the fish were still alive.

As Project Manager for this study the author plans to call a meeting to discuss suggested contract modifications for future removal investigations. It is possible that there will be less emphasis on fishing and more on bottom retrieval of dead fish and expanded use of divers for in-water surveys. That may decrease a major cost of vessel time. The ROV question will have to be resolved and the davit and winch system redesigned and built. It will likely be over-built to handle the unexpected offshore situation just as the platforms themselves are over-built. The transects will remain in the study but perhaps be deployed and traversed at the same time after demolition.

The study will continue until nine explosive platform removals have been thoroughly investigated. The first was the worst.

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BRIDGING THE EXPERIENCE GAP: TECHNIQUES FOR REDUCING THE STRESS OF ZERO VISIBILITY TRAINING

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Zero visibility diving may be the most stressful activity that the vast majority of divers ever experience. When zero visibility conditions are encountered, even seasoned clear water divers may find themselves stressed to the point that they must terminate the dive. Some training techniques useful in preparing divers for the zero visibility environment emphasize composure, others have more emphasis on problem solving abilities while still employing stress. Techniques are available which will aid in the training process while reducing the stress element. Designed to be conducted with supervision in a shallow pool, the Zero Visibility Maze and associated techniques, offer divers an introduction to the sightless diving environment with a minimum of stress. Unlike an obstacle course, there are no entrapments, entanglements, restrictions or time limits (other than air availability). The divers must solve only minor problems of direction and orientation, while completing a series of objectives. This approach allows divers new to the sightless or extremely limited visibility diving environment time to become acclimatized and aids in developing confidence, tactile and orientation skills.

INTRODUCTION

At some point on the experience curve, divers usually become comfortable with their abilities under a variety of diving conditions. As this happens, the diver may lose contact with the fact that certain skills or conditions were difficult and stressful when first experienced.

As divers become more and more comfortable with specific tasks and conditions, the gulf of understanding widens until they can no longer comprehend another persons apprehension of a given situation. This experience gap produces a variety of reactions. From the view of the inexperienced diver, someone with the ability to function, or in some cases thrive, under conditions they find barely tolerable may be looked upon with awe. The diver with a mastery of certain situations may look on someone less comfortable and wonder: "Will this person ever be able to handle this, or am I going to have to go in and recover the body?"

Both views are flawed, blinded by the experience gap that neither can perceive because of their relative position. The new diver doubts they will ever possess "what it takes"; the experienced diver sees only the gulf between abilities and can not understand why.

This is one of the challenges of instruction; to bridge the experience gap. To take a person with "minimal diving abilities" (a relative term) and turn him or her into a diver capable of handling almost any situation smoothly, efficiently and safely. This challenge can be formidable under the best circumstances, but the topic here concerns less than optimal conditions.

Zero visibility diving may be the most stressful activity that the vast majority of divers ever experience. It removes the sense most people use to perceive the world and replaces it with a wet blackness where imaginary monsters lurk, waiting and hungry. All those fears of the things that go bump in the night have an avenue into the conscious mind. Without orientation aids, direction becomes only up or down, all other bearing is lost in the murk. Reason can be forced out and the unprepared may find themselves rapidly approaching panic.

Working in zero visibility conditions require divers to rely on senses other than sight for primary information. They must learn to use tactile information and develop the ability to picture in their minds what they feel with their hands and bodies. In many cases the physical structure of the dive site can provide divers the cues necessary to keep track of their position. On sites where physical structure alone is inadequate, the use of a guide line or other introduced navigational aid may be necessary. However, under field conditions, experience has demonstrated that the availability of structure or guide lines will not insure that divers remain oriented. This is especially true for less experienced divers. To aid in the development of these skills there are a variety of approaches which may be utilized.

Some training techniques emphasize composure by placing divers in stressful situations. An example of such a training exercise is the "Regulator Stress Course" employed by Julius Wiggins of the National Academy Of Police Diving, Miami, FL. Other techniques such as the "Zero Visibility Obstacle Course" (Sellers, Scharf 1990) have more emphasis on problem solving abilities while still employing stress. Both of these courses can be effective tools in the training of divers for the zero visibility environment. However, these techniques may require divers to demonstrate composure levels which many do not possess due to limited experience. To reduce the experience gap, other less stressful methods may be employed, thereby allowing divers to ease into zero visibility diving.

The concept of the Zero Visibility Maze and the exercises associated with it were developed for use with the East Carolina University (ECU) program of Maritime History and Nautical Archeology. The Maritime Program attracts participants covering the entire spectrum of diver certification, but most have never experienced zero visibility diving. As a result, the majority of the divers coming into the program do not have the composure or orientation skills required to perform tasks that would otherwise be routine.

Brad Rodgers, Underwater Archeologist with ECU stresses the similarities of the proper movement pattern for zero visibility diving to the slow, controlled movements of Ti Chi. This concept appears to be one of the most difficult for divers new to this environment to grasp. Almost without fail, divers inexperienced in zero visibility conditions will move too rapidly and with such heavy handedness that they can be a hazard to themselves and anyone or anything in the water with them.

Slow, deliberate movements must be stressed. As well as paying greater attention to the tactile signals being received from the entire body. A diver experienced with zero visibility conditions, in tune with their body, can identify certain objects as readily with other body parts as they can with their hands. It is as if the entire body becomes an

antenna. This too is a concept that does not come easily to those new to the sightless environment. Couple this inattention with movements too rapid for the conditions and it is no wonder that inexperienced divers becomes disoriented.

Working with the ECU Maritime Program in the field has reinforced the ideas that: To accomplish a specific task under zero visibility conditions, it is essential that the divers know where they are on the dive site and: That the divers require the ability to identify specific items by touch. The use of the Zero Visibility Maze during training can go a long way toward providing divers the background needed to later maximize the time spent working under blackout conditions.

The Training Process

Begin the process of acclimatization/desensitization by blacking out the divers' mask and having it donned in a staging area. While limiting vision may be accomplished by tying something over the mask, a more realistic effect is achieved if a mask with an opaque skirt is modified. Spray painting the faceplate inside and out with several coats of paint will reduce the light coming in considerably; or by removing the faceplate and replacing it with a material such as rigid gray PVC, an effect of total blackness may be achieved. Painting and replacing the faceplate require the dedication of equipment for zero visibility training, an alternate method is to blackout the divers personal mask by covering the faceplate with duct tape. This method will produce the desired effect, but it tends to leave a sticky residue which may not be appreciated.

After vision has been blacked out, have the participants assemble their diving equipment unassisted. This process should be observed to assure it is done correctly. The objective is to insure equipment familiarity and enhance diver confidence. If a mistake is made, inform the person of the mistake and have it corrected. A variation here is to tell the individual that there is a mistake in the way they have assembled their equipment, but not to identify the mistake. The diver must then locate and correct the mistake.

When the divers have correctly assembled their equipment, they are ready for the next task. Give the divers a tactile puzzle to solve. This can be as simple as a series of nuts and washers on a bolt, to be removed and replaced in the same order; or as complex as a childs' shape puzzle.

The Playschool shape puzzle is a wonderful challenge under blackout conditions. It consists of 18 different shapes to be placed into a box with 18 matching holes. This puzzle may be completed in less than five minutes (usually by parents of small children) or may be perceived as totally baffling. A hint about the Playschool puzzle; the shapes have a tactile design present on each piece that corresponds to the side of the puzzle on which it belongs.

In addition to the shape puzzle, the divers should be put through a series of item identification exercises both in and out of the water. By handling a variety of common every day articles and items that are project specific, the divers can develop a mental picture of objects they may encounter in the field. The divers may be asked to identify every day articles based on life experience. With more unusual project specific objects, the identifications should be made prior to the divers handling of the items.

Items should be as varied as possible and should include objects which could produce minor physical injury if mishandled. The objective here is not to injure the divers, but to make them aware of the potential for cuts, scrapes or punctures. The

concept of grasping with a light touch is reinforced by the manipulation of items such as hooks, frayed wire, metal with rough edges, etcetera. The divers should be cautioned before they are given these items to handle. After the divers have completed the item identification orientation they are ready to progress to the Zero Visibility Maze.

Unlike an obstacle course or other higher stress exercise, the Zero Visibility Maze does not require one on one supervision. Spot checks of the divers involved in the drill are usually all that are necessary. For most circumstances, four to six divers are easily supervised by one safety diver. This reduction in supervision requirements translates into larger numbers of participants in a given time period.

A primary purpose of the maze exercise is to allow divers the time to develop and improve personal movement and orientation skills. Therefore, direct contact with the divers by the supervisors should be kept to a minimum. The supervising divers should be instructed to limit their contact with the participants to situations that would be considered a threat of injury, or when an individual is being so heavy handed that they are destroying the maze structure.

As with any underwater activity, the divers and supervisors should be briefed on predetermined emergency and recall signals. When the exercise is conducted in a shallow pool, personnel overseeing the activity are outfitted in snorkeling gear to facilitate ease of movement.

The design of the maze need not be overly complicated, but it is important that the structure be stable. If it is applicable, design the Maze to be similar in structure to what the divers will experience in the field. For example, if a grid will be used on an upcoming dive site, the pattern of the grid should be duplicated as closely as possible. This preconditioning allows the divers to become accustomed with "the feel" of the structure they will encounter.

Set up of a Zero Visibility Maze does not entail a major investment, though the heavy handedness of many divers require the use of taught lines and rigid or semi-rigid materials. Items common to many pools (for example; aluminum benches) may be assembled to provide a recognizable structure. With this established, a series of guide lines overlaid to provide an orientation aid is all that is necessary for the basic maze structure.

For inexperienced divers to receive the maximum benefit from this exercise, the maze should contain easily identifiable landmarks. Features such as distinctive corners, knots, direction indicators, and the use of a variety of construction materials will provide attentive divers the cues necessary to quickly become and stay oriented. Objects similar to those the divers have handled on the surface should be placed at various points throughout the maze. These objects are used for item identification, location exercises as well as orientation aids.

The divers enter the maze by way of decent/ascent lines. The number of entrances, while exercise specific, should reflect what the divers will encounter during field work. They are required to use these lines for ingress or egress.

The divers may be assigned any number of tasks as part of the maze exercise, but the first assignment should always be to explore the maze and become oriented.

Examples of diver tasks may include:

Have the divers draw a map of the Maze before visually inspecting it.

Have the divers list on a slate the objects they encounter.

Have the divers retrieve specific objects from specific locations.

The Grope. Have the divers identify other divers they encounter during the

exercise.

Show the divers a drawing of the maze and have them follow a specific path from entry to exit.

Place the divers in contact with the Maze at an unknown point and have them locate a specific object or exit point.

Have the divers assemble or disassemble portions of the maze.

Assignments are limited only by imagination, but it is suggested that the progression be from the simple to the more complex. The tasks should reflect what the divers will be expected to accomplish in the field.

CONCLUSION

The Zero Visibility Maze and related techniques were conceived to address specific problems encountered while training inexperienced divers for blackwater and brownwater conditions. The techniques discussed here, while designed for controlled zero visibility sites such as those associated Underwater Archeology, have application to any diver training where zero visibility is a concern. As with any other stressful activity, experience and training under zero visibility conditions are the keys to mastery. No training program is perfect and all training programs should constantly evolve if the needs of the individual diver are to be met. It should be emphasized that the training techniques discussed here, while stressing the development of individual skills, do not profess the elimination of safe diving practices, but are a useful training aid for preparing divers for the zero visibility environment.

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THERMAL PROTECTION REQUIREMENTS FOR THE DIVERS HANDS

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In water temperatures below 10°C (50°F) the skin temperature of the hands may determine the exposure limit of a dive. With state of the art passive thermal protection systems, dive profiles exist in which the diver can lose partial or full function of their hands long before experiencing any signs of hypothermia. The diver, at the same time, may also be exposed to the risk of nonfreezing cold injuries of the hands. Non-freezing cold injuries involves tissue damage resulting from prolonged exposure to "frigid" 0 to 10°C (32-50°F) water and serious injuries can result. The injuries can involve damage to blood vessels, tissue and nerves. Performance loss and non-freezing cold injury thresholds are a function of skin temperature of the hands and time. The diver requires fully functional hands from the standpoints of safety and work performance. When planning a dive the diver needs to be aware of the performance loss potential and the risk of nonfreezing cold injury. This paper will explore these problems and ways to minimizing performance loss, and the avoidance of non-freezing cold injuries.

DIVE VISUALIZER:A SYSTEM FOR USING REAL-TIME, THREE-DIMENSIONAL COMPUTER ANIMATIONS TO EXPLAIN DIVE COMPUTERS

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The day is very near when a dive computer will be a staple item in every diver's kit. As a result, perhaps the most serious issue surrounding dive computers today is the question of user competence. Given the disappointing results of recent studies, it appears that vanishingly few divers understand how their computers actually work (Huggins 1991, Hill 1990, and Gilliam 1991). Blind reliance on computers has been documented in numerous case studies of decompression illness (DCI) (Huggins 1991, Overlock 1988, Overlock 1991). Given the high level of literacy that most divers possess, it is unlikely that these failings are simply the result of an inability to understand an owner's manual. It is far more likely that a poor grasp of decompression theory is to blame. Better tools for explaining dive computers and decompression theory are clearly necessary.

This article addresses that need by introducing decompression visualization, a visual tool for explaining how dive computers work. Using state-of-the-art three-dimensional graphics and advanced animation techniques, this unique tool presents decompression concepts using easy to understand, interactive graphical displays.¹ Even advanced concepts in decompression theory can be explained in a matter of minutes.

In this paper, we'll use decompression visualization tools to clearly explain a number of advanced decompression theories. We start with a very simple example which shows why deep dives are shorter than shallow dives, and which introduces the reader to our visual notation. Then we'll move on to another simple example showing why repetitive dives are theoretically less safe than single dives, explaining what we refer to as the "ratcheting effect" of multiple repetitive dives.² Finally, we'll explain the

¹The figures presented in this paper are mere snapshots of the real-time animations produced by the author's decompression visualization system. The system used to create these visualizations performs in real-time and full 24-bit color, supports multiple decompression models, gas switching, gas limits, and varying ascent and descent rates. Unfortunately, the visualizations which can be presented here are limited by the restrictions of the monochrome printed page.

²This can serve as an explanation of the AAUS Guidelines For Use of Dive Computers, rule number 13: "Multiple deep dives require special consideration." These guidelines were presented at the

often poorly understood reasons behind the common rule "always dive your deepest dive first."³ By taking a single two-level dive as an example and visualizing it first as a "deepest first" dive and then as a "deepest last" or "reverse profile" dive, we graphically show one reason why reverse profiles carry a higher theoretical risk of DCI.

These examples show the potential of three dimensional computer illustrations to explain even advanced concepts in an engaging, intuitive manner. Fully understanding decompression theory requires an intuitive grasp of applied mathematics, the ability to interpret very large datasets, and a basic understanding of applied physiology. Decompression visualization tools lessen the need to understand the mathematics and greatly reduce the difficulty of interpreting extremely large datasets. For these reasons and others, we believe that decompression visualization is an important tool that will become common among model creators and model users alike.

Applying Decompression Visualization: A Simple Example

A question I have seen stump more than one experienced diving instructor is "Why are deep dives so short and yet result in low repetitive letter groups, while shallow dives can be so much longer?" In traditional diving curricula, the teaching emphasis is on how to use a particular set of dive tables, and not on how to understand (and therefore make informed decisions about) the decompression theory underlying the models. This particular example probably has not stumped the informed reader: the answer is simply that deeper dives are controlled by faster compartments which offgas quickly, resulting in short dives and short offgassing times, while shallower dives are controlled by slower compartments, resulting in longer dives and longer offgassing times.

This explanation is correct, but it is hardly easy to understand. The explanation assumes quite a bit of background knowledge, including at least an understanding of exponential rise and decay, half times, compartments, and Haldanean decompression theory in general. It is unlikely that the average diving student will walk away from this explanation with an intuitive grasp of the answer. Instead, it seems far more likely that she will instead receive the strong impression that decompression theory is difficult, and that further questions will be answered in as dense a manner as was the first one. This is, we believe, a fair assessment of the current state of the average instructional situation.

However, it need not be so. Haldanean decompression theory is in fact not very complicated, and with appropriate tools, any instructor can give clear, concise explanations of decompression theory that will really stick with their student. Let's take a look at how an instructor might answer this same question if she had access to the tools we present in this paper.

1988 AAUS Dive Computer Workshop, and can be found in M.A. Lang, R.W. Hamilton (Eds.): *Proceedings of AAUS Dive Computer Workshop*. 1989, p216.

³This rule is also rule number 12 of the aforementioned AAUS Guidelines For Use of Dive Computers: "Repetitive and multi-level diving procedures should start the dive, or series of dives, at the maximum planned depth, followed by subsequent dives of shallower exposures."

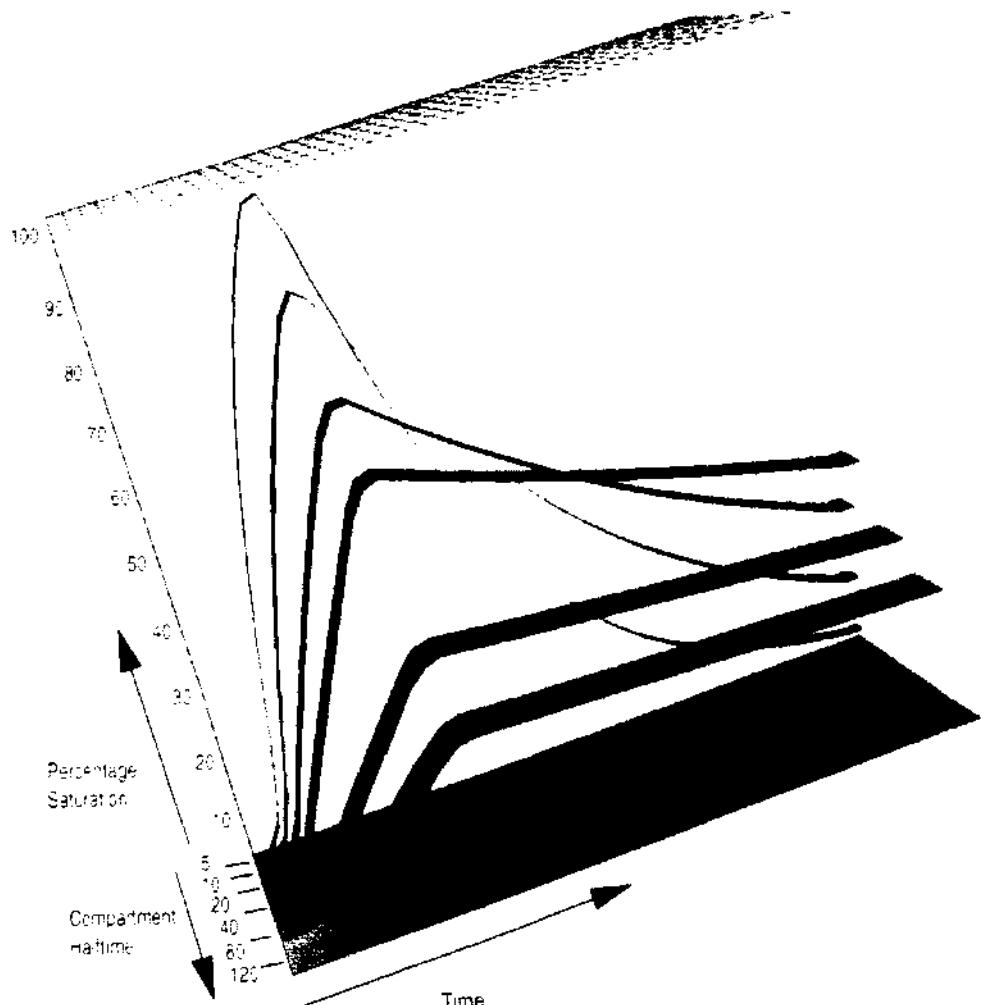


Figure 1: Single dive to 130fsw (39m) for 10min, followed by a one hour surface interval

To explain the concepts, our instructor might simply perform a quick visualization of a sample dive profile. Let us choose 130 fsw (39 meters) as our target depth, with a bottom time of 10 minutes, followed by a surface interval of 60 minutes. Figure 1 presents the resulting compartment visualization, using the 1957 US Navy decompression model as our underlying model.

At this point a few words of explanation are in order on the visual notation we use in these visualizations. You'll notice that there are six *compartment ribbons* floating in space. These ribbons depict the continuous compartment loadings for each of the six compartments in the US Navy model. Their halftimes are noted along the lower left edge of the chart. You'll notice that the widths of the ribbons are proportional to their halftimes, and that their darkness and elevation are proportional to their inert gas loading; darker and higher ribbon segments are more saturated than lighter and lower ribbon segments.

Time progresses from left to right along the horizontal dimension, and the vertical dimension is the percentage loading of the compartments, capped by a mesh "ceiling" indicating the no-decompression limit. Finally, the floor of the visualization is the two-dimensional projection of the ribbons: it's the "shadow" cast by the compartment ribbons, and its lightness or darkness corresponds exactly with the shading of the ribbon segment directly above it.

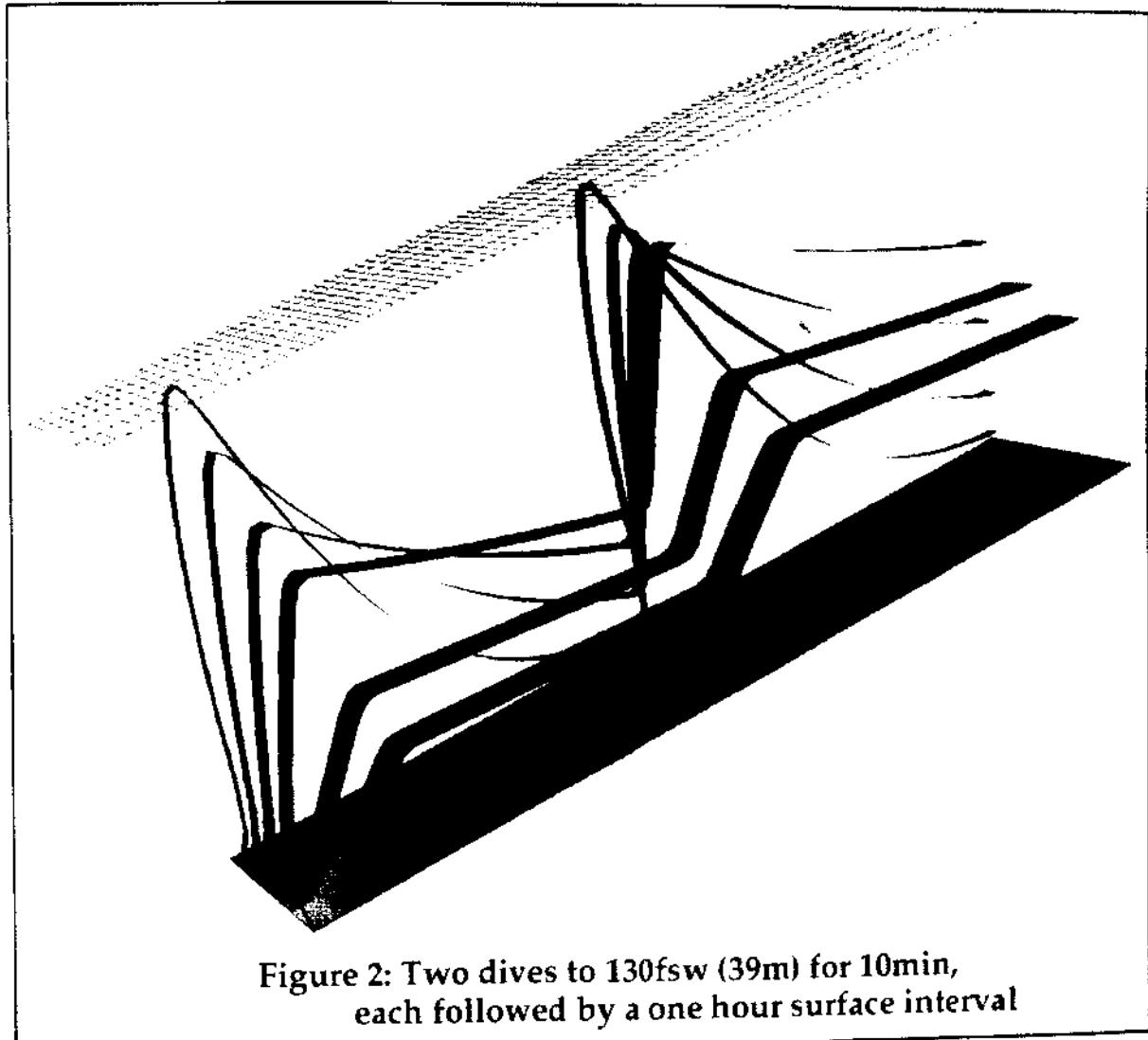
You'll notice that all the major features required for an understanding of the model are presented in this single picture. First, we have the ribbons themselves; these graphical representations show in three dimensions the important parameters of every Haldanean model: compartment halftime, compartment loading, and the change in compartment loading over time as a result of the dive profile. The no-decompression limits are graphically portrayed as the mesh "ceiling" above the ribbons, while the time progresses from left to right, in the same left-to-right fashion as you are currently reading this text. The "hot spots" are easily placed in context by looking for the dark projections of ribbons onto the "floor" of the visualization.

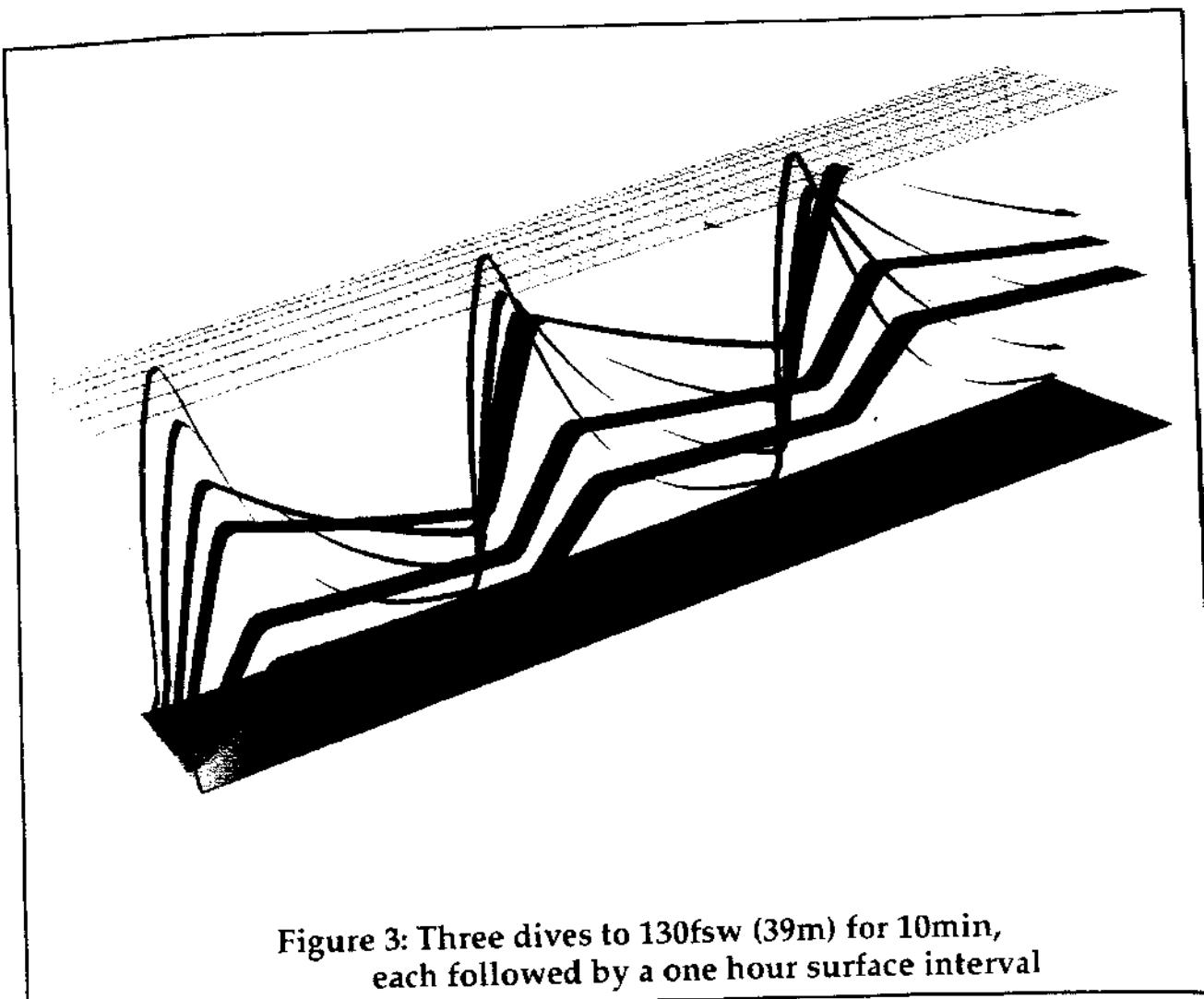
How close we came to the model's no-decompression limits at all points during the dive simulation is grasped intuitively because of the nature of the visual presentation: very little explanation of this point is necessary when the decompression visualization is used as the teaching tool. Watching the progression of compartment loading and unloading over time gives students an intuitive understanding of "slow" vs. "fast" compartments in dive computer models, an understanding which is important to have but very difficult to gain from most conventional teaching tools.

It is also important to note that in the online version of these presentations, not only is full 24-bit color and shading used, but the real-time three-dimensional nature of the underlying graphics system⁴ is used to full advantage. As a learning tool, it is important that the student have the ability to interact with the model. Being able to directly manipulate the data by "grabbing it" with the mouse gives students a feeling of control over a very difficult subject, and makes the learning experience more enjoyable.

In addition, rotating and zooming in and out on the data allows the instructor to highlight specific regions of interest, thereby focusing the student's attention on exactly the area of the dive simulation which the instructor wishes to discuss. These are not trivial advantages when the instructor and student have limited time in which to

⁴These simulations are performed on Silicon Graphics workstations using the OpenGL graphics library. Additional simulations have also been performed using IRIS Explorer 2.0, a data visualization tool which ships with every Silicon Graphics workstation.





interact: efficiency, interactivity, and customized attention are key ingredients of high quality education and can make for a lasting learning experience.

Now let's move on to a more complex subject: repetitive diving.

Second Example: Why Repetitive Dives Require Special Attention

Repetitive diving is commonly cited as a contributing factor in DCI events (Dovenbarger, 1991) yet few people understand why. This confusion is not surprising: why should dives done the day before affect the next day's diving, especially when many tables say you are completely clear in as little as six (6) hours after your last dive, no matter how strenuous? In fact, some computers turn off overnight after many profiles, and must be restarted for the next day of diving. With tables and computers giving one message and dive guidelines from such agencies as DAN and the AAUS giving very different messages, where is the truth?

Let's apply decompression visualization to this question by visualizing a repetitive dive sequence. What we seek to illustrate are trends that appear only after several dives are simulated. Figure 2 presents a visualization of the profile used in our previous example, but this time repeated twice in a row: 130fsw (39msw) for 10 minutes, then a one hour surface interval, followed by another dive to 130fsw (39msw) for 10 minutes, and another one hour surface interval. Note that at the end of our first dive (Figure 1), none of our compartments were completely offgassed: no compartment was back to its pre-dive condition. After the second dive (Figure 2), we begin to see that the slower (wider) compartments are "ratcheting" upwards: because they never dropped back to their former level, they are starting the next dive slightly more loaded (higher), and increasing from there. Thus, at the end of the second dive, we see that most compartments are higher than at the end of the first dive.

Let's repeat that sequence a third time and see the trends. Figure 3 presents the results of three identical dives to 130fsw (39msw) for 10 minutes, each followed by a one hour surface interval. The upwards trend in the slower compartments is now pronounced, but what has happened to the faster (narrower) compartments? The faster compartments (5-, 10-, and 20-minute halftimes) are, for practical purposes, unaffected by the repetitive dives. They are in fact fast enough that the one-hour surface interval unloads them enough that they do not come into play: they do not limit our repetitive diving. This is a graphical demonstration of the somewhat advanced decompression theory concept that fast compartments do not control repetitive diving.

This point made, let's reinforce which compartments do in fact control repetitive diving by looking at one more iteration of the same profile. Figure 4 presents four repetitions of our 130fsw (39msw) dive. Viewed from a slightly different perspective, it becomes quite clear that the slower compartments have "ratcheted" upwards to a point where they clearly control the future dive sequences which this diver may make. In fact, at the end of the fourth dive (noted as point A on Figure 4), the diver has come just to the no-decompression limits of the 40-minute compartment, and to repeat the dive one more time would be to enter a decompression situation.

This is an important concept for students to grasp: repetitive dive profiles tend to be controlled by the slower compartments, since for most reasonable surface intervals of one hour or more, the faster compartments effectively offgas enough to permit almost infinite repetitions of most dive sequences. An additional insight which can be pointed out to the student with very little additional instructional effort is that were the diver to enter decompression by repeating this dive profile one more time, the slow offgassing of the controlling 40-minute compartment, as evidenced by its very shallow slope on our graphs, would indicate that the time spent decompressing would be quite long.

Finally, it is a simple matter to extend the surface intervals in the simulation while the students watch. By doing this, students immediately see that with appropriately longer surface intervals, the 40-minute compartment will no longer control the dive series. This can be reversed, of course, by shortening the surface intervals, with the eventual result that faster compartments than the 40-minute compartment eventually begin to control the dive series. These two experiments reinforce for the student the effect that longer surface intervals can have on the theoretical loadings of the decompression model.

Now let's apply decompression visualization to dive profile planning by exploring exactly why reverse profiles are so taboo.

A Final Example: Why Reverse Profiles are Discouraged

"Reverse profiles" have come under fire in recent years because they have resulted in much higher than expected incidences of DCI. A reverse profile is a multi-level dive or a series of dives with the deepest portion at the end. To most students, it isn't at all obvious just why these profiles are associated with increased risk of DCI. Aren't you spending the same amount of time underwater either way? Aren't you inhaling just the same amount of nitrogen on both dives? There are just too many variables and too many numbers for most people to "see" what is happening.

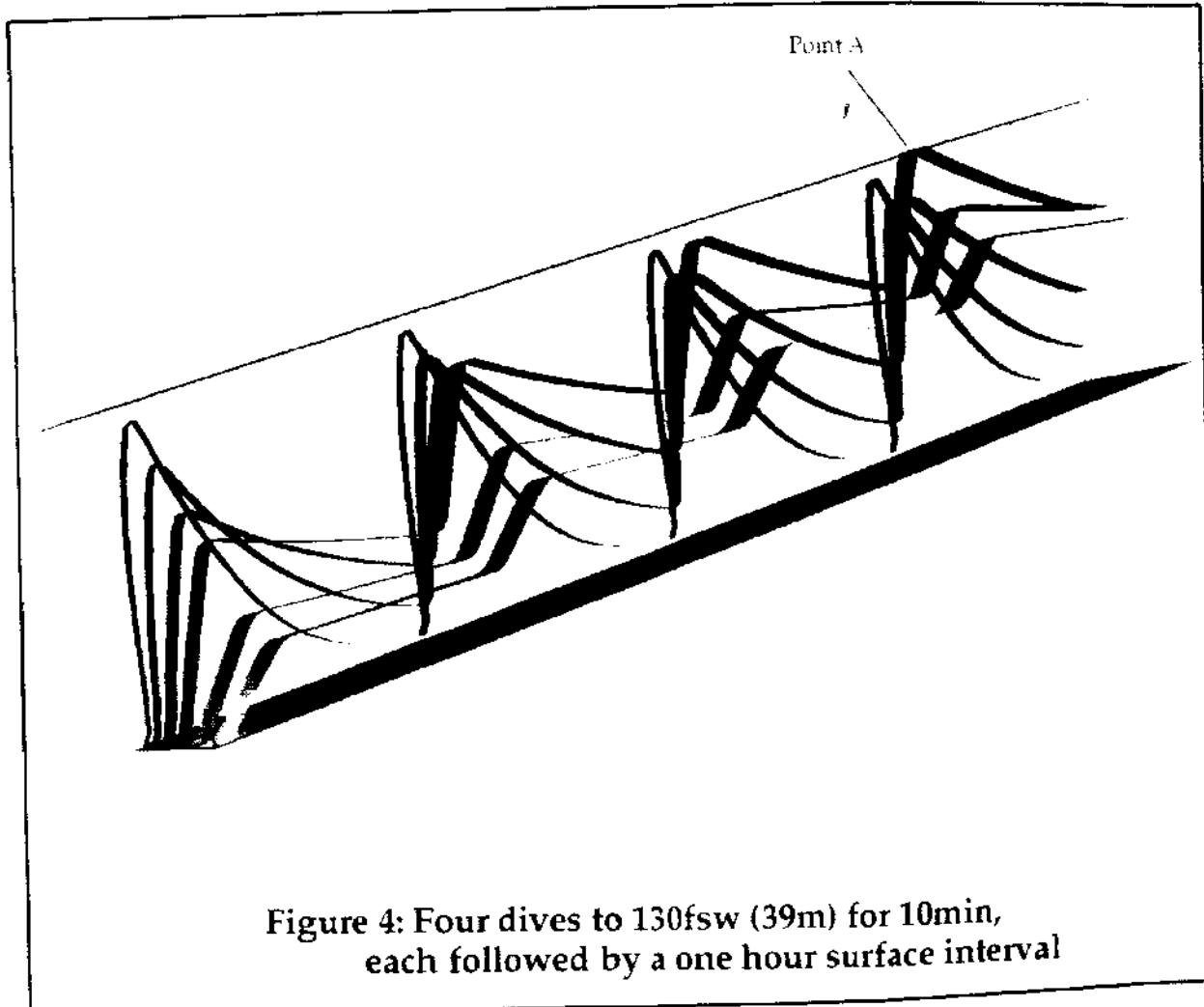
To see what's going on, we can use decompression visualization tools to turn the reams of numbers the simulators generate into a single, intuitive, three-dimensional image. We start by choosing a "deepest first" profile, then look at the exact same profile "reversed." Our sample multi-level profile is quite simple, but fairly representative of a possible recreational diver's multi-level profile: 130fsw (39msw) for 10 minutes followed by 60fsw (18msw) for 30 minutes.

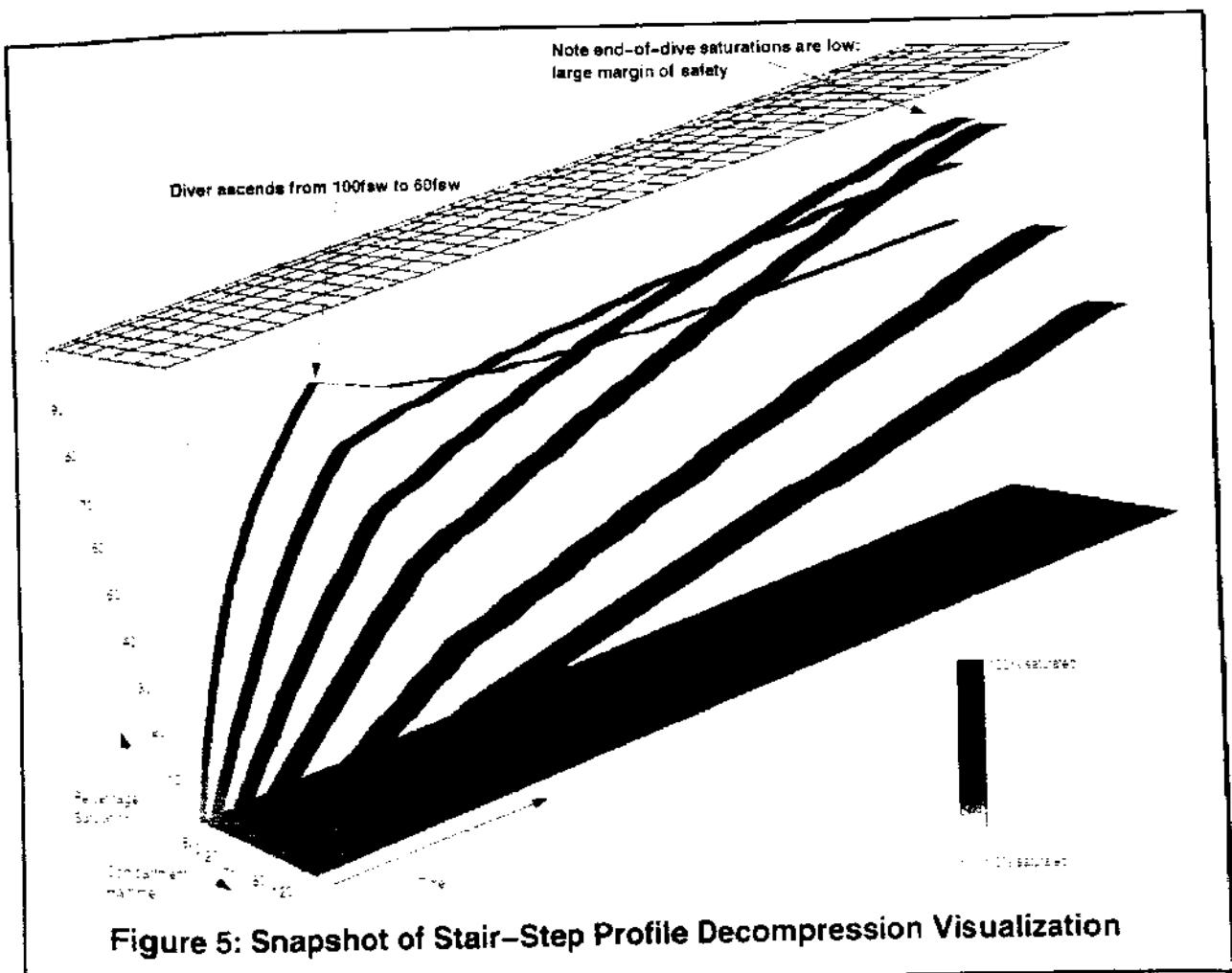
Figure 5 presents a decompression visualization of that profile, again using the 1957 US Navy model. Numerically speaking, the deepest-first profile yields a peak compartment saturation of 84%, representing a worst-case theoretical safety margin of 16%. Should the dive need to be aborted at the moment of peak saturation, that 16% theoretical safety margin suggests that a safe ascent could easily be performed.

Graphically speaking, it's obvious that this dive stays a fair distance away from the model limits. Most importantly, the trend of the compression and decompression is clear: the deep segment quickly loads the fastest compartments, but they drop out of the picture as the diver spends time at 60fsw (18msw). The exponential curve of on- and off-gassing is apparent even to the untrained eye, as is the rapid saturation of the 5 minute compartment during the 30 minute stay at 60fsw (18msw). Measured by maximum inert gas loading incurred, this is clearly a relatively safe decompression.

The second dive is the same profile with the two segments reversed: 60fsw (18msw) for 30 minutes first, followed by 100fsw (30msw) for 10 minutes. Figure 6 presents the visualization of the reversed profile. Compare the two visualizations briefly before continuing, and you'll immediately see that the two profiles have very different results on the theoretical model loading.

Numerically speaking, the reverse profile pushes very close to the model limits. The peak compartment saturation is 97%, yielding a safety margin of only 3%. This is a staggering difference for such a slight reordering of the profile: after all, both profiles have the same down time, same maximum depth -- even the same average depth. Why, then, is there such a big difference between the two profiles?





Here's where the two visualizations are worth more than thousands of numbers. You can immediately *see* why the reverse profile dive is worse. The reverse profile does the wrong thing -- it loads up all the compartments during the first segment of the dive. When the deeper segment is tacked on, the faster compartments⁵ are already "primed" and hence rapidly approach their limits. The fast compartments thus get an undesirable "head start" on their loading. On the other hand, the first profile loads the two fastest compartments early on, and then the shallower segment unloads them during the rest of the dive.

Thus with the aid of our three-dimensional visualization, we can see that the rule of "deepest first" is really a means to force us to move from depths controlled by faster compartments to depths controlled by shallower compartments. What this points out is the need for a truly understandable explanation of decompression, in which the underlying reasons for today's poorly understood "dive planning rules" become obvious and explicit. With this kind of tool, each individual can understand why these "rules of thumb" exist, in a way that is so understandable that months or even years down the road, the diver will remember intuitively why those rules exist. The more senses are engaged in the learning process, the more is learned; the visual learning promoted by these tools is long lasting, and the core concepts we are teaching are well remembered.

Caveats

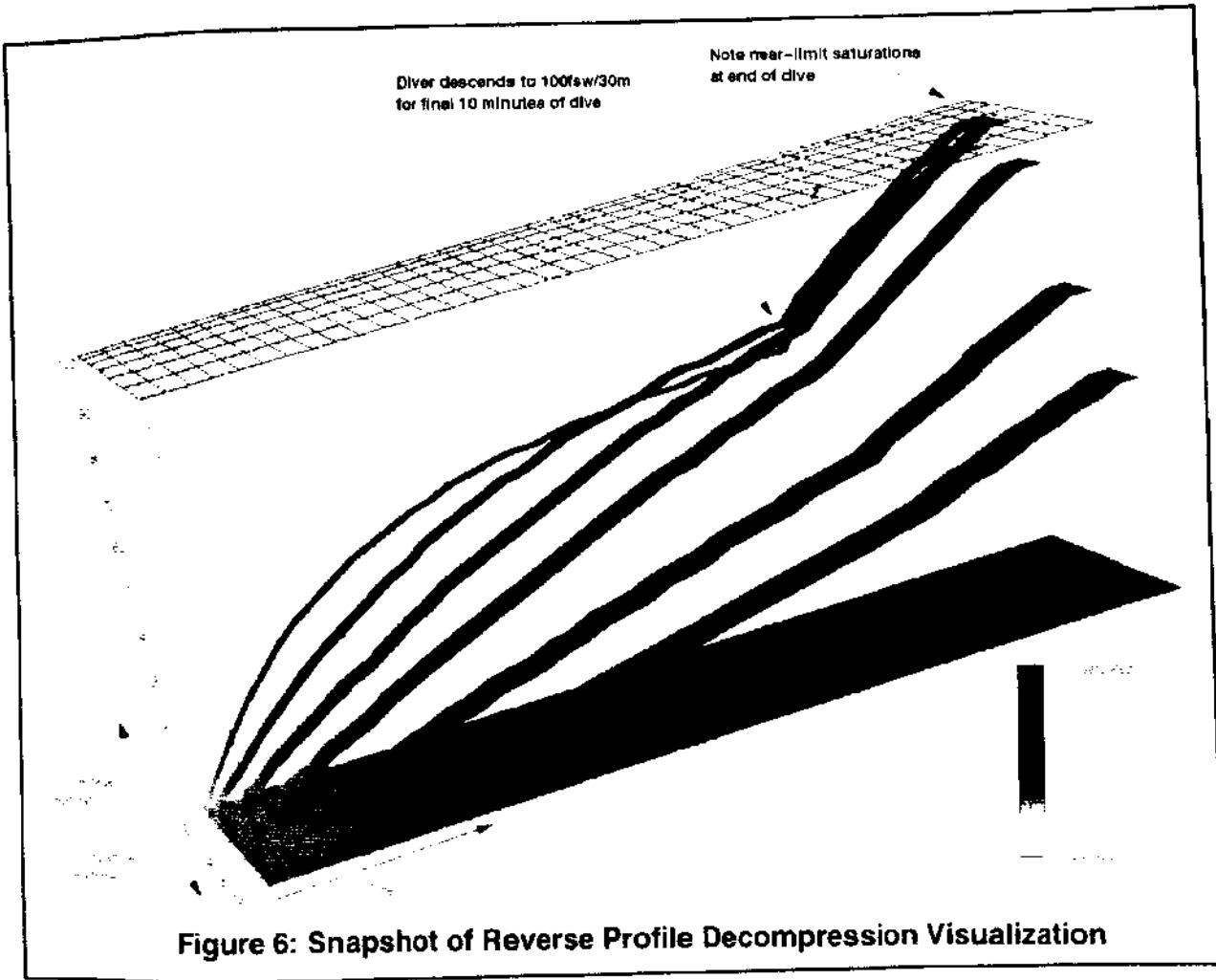
Perhaps more so with visualizations than with any other tool, it is important not to remind the student that the visualization at hand is *not* a complete explanation of the phenomenon in question. No matter how many dimensions we may use, a single visualization does not tell all. Every decompression model is necessarily a drastic and purely theoretical oversimplification of the very complex human body. No student should be encouraged to place absolute faith in the results of any computer program, no matter how compelling or intuitive. A human body cannot be adequately described by a few simple equations. Many additional factors complicate the picture, including at least ascent rate, exertion, hydration level, PFO, and individual susceptibility. Decompression visualization and dive computers are tools, they are not replacements for sound judgment.

CONCLUSIONS

Diver education needs to take a step forward so that users can more fully understand the decompression models to which they entrust their lives. *Decompression visualization* is that leap. Presenting the decompression data from simulated dive profiles in a visual three-dimensional format allows users to quickly grasp the model's behavior and predict future behavior. The result is a quantum leap: from laborious interpretation of reams of numeric data to intuitive visualizations of the model's behavior.

These decompression visualizations allow students to visualize thousands of numbers in a single picture or sequence of animations. The bottom line is that these

⁵A "faster" compartment is one with a shorter half-time, thus its loading or saturation changes faster.



visualizations draw on the brain's innate image processing power to make all this data analysis become intuitive. The human brain has an astonishing amount of processing power dedicated to the perception and interpretation of color, three-dimensional information. As a result, the brain finds it relatively easy to quickly track the saturations and the behavior of each compartment in one of these dive visualizations. Comparison of different decompression simulations becomes as simple as holding up two sheets of paper and eyeballing the trends. How many thousands of numbers and words would it have required to adequately explain what these images presented in a few square inches? And, perhaps most importantly, which is most easily understood and remembered

Decompression visualizations such as those presented in this paper will certainly be a part of the future of decompression theory instruction.

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MONITORING FRINGING CORAL REEFS FOR HUMAN IMPACTS

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The Whitsunday Islands are the largest group of continental islands inside the Great Barrier Reef, with highly diverse fringing coral reefs. Their biology is still poorly understood. The reefs are heavily utilised by commercial tourism, yachts, fishermen and divers. Paradoxically, the desire to present the prettiest coral reefs to visiting divers focuses anchor damage onto the richest reefs. This chronic stress may inhibit the recovery of the reef from severe natural disturbances, and result in lowered biological diversity. This is cause for concern by the Great Barrier Reef Marine Park Authority.

The Fringing Reef Monitoring Program has been established to provide objective scientific information on human impacts to the reefs of the Whitsunday Islands National and Marine Parks for use by district administrators in management. Sampling with photographic quadrats allows efficient use of u/w field time, and creates a permanent record which can be analysed to quantify cover of benthic species, etc. Comparisons between intact and affected reef areas will determine whether biological changes to these communities have occurred.

Preliminary reconnaissance suggests that some species are very susceptible to anchor damage, while others may be favoured. Ongoing monitoring of impacted areas will allow administrators to gauge the effectiveness of management techniques such as moorings, area closures, etc.

INTRODUCTION

The Whitsunday Islands are the largest group of continental islands inside the Great Barrier Reef (Figure 1), and contain extensive areas of fringing coral reef characterised by high biological diversity, and containing numerous hard and soft coral species, as well as many other invertebrate organisms and fishes. The biology of these reefs is still poorly understood, and many species (particularly soft corals) have yet to be described. For instance, two new species of Scleractinia as yet undescribed, have recently been discovered along a stretch of mainland coast scheduled for a major resort development (Stokes, 1990).

Present and projected development for tourism is likely to impact these reefs by accelerating nutrient enrichment (eutrophification) and sediment loading of nearshore and island waters, as well as by more direct physical effects such as anchor damage. These stresses are already evident in the Whitsundays and have been identified as causes for concern by the Great Barrier Reef Marine Park Authority (GBRMPA) (Baldwin, 1990).

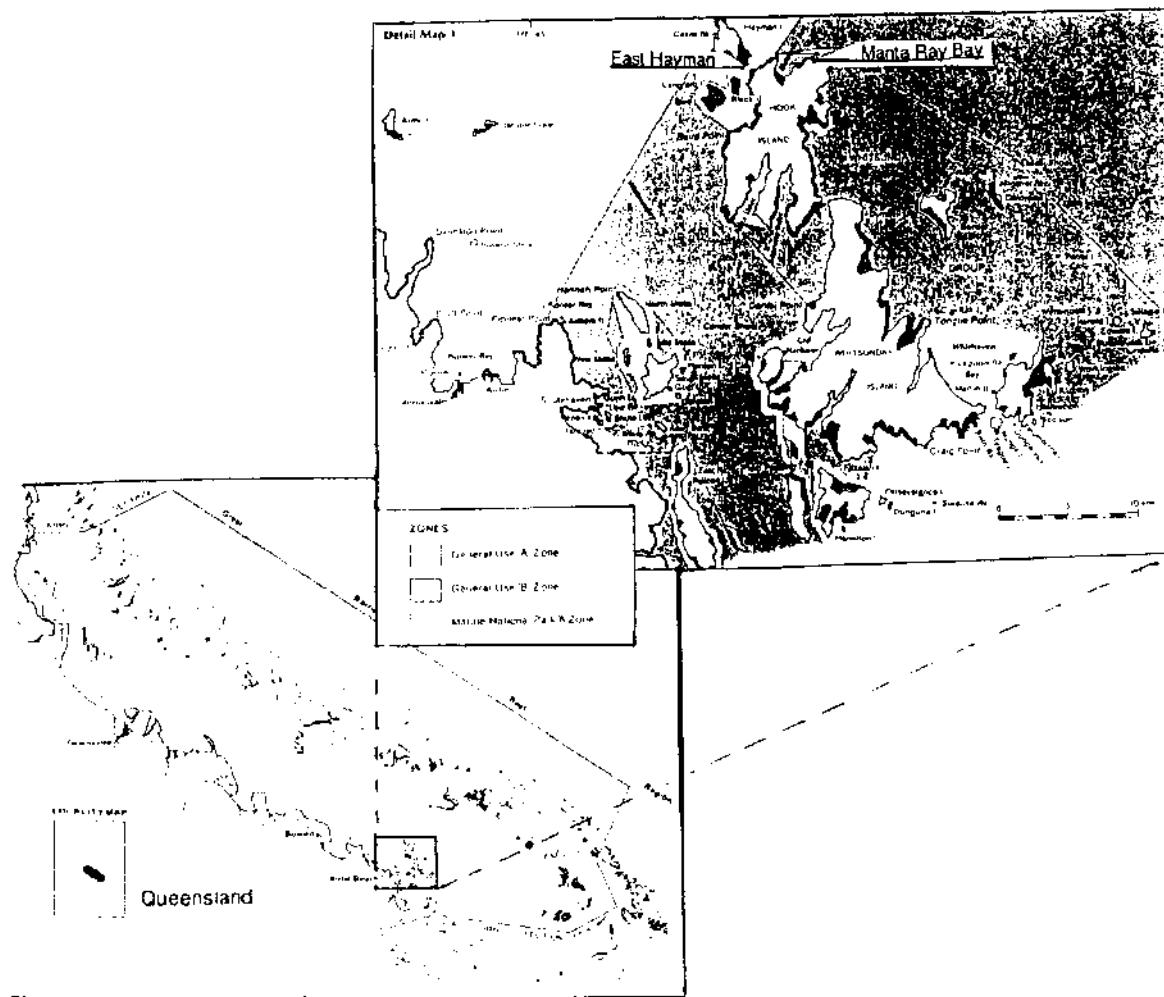


Figure 1. Whitsunday Islands, Australia.

Paradoxically, the desire to present the prettiest coral reefs to visiting snorkellers and scuba divers results in the damage being focused on the richest reefs. The limited extent of aesthetically and biologically rich fringing reefs results in locally very heavy usage, with attendant inadvertant destruction. The complex of fringing reefs between Hayman and Hook islands, the best developed system of reefs in the Whitsundays, and arguably unique among the reefs of the Great Barrier Reef, already show considerable damage. The area is heavily utilised by commercial tourism operators, bareboat charter operations and private yachts, fishermen and divers.

In a 1990 survey, Harriott and Fisk reported visible damage in all of the coves they visited along the north end of Hook Island. In one, (Luncheon Bay), they found damaged corals in 40 (22.2%) of the 180 1M2 quadrats inspected. Because of the large tidal range in the Whitsundays (>3 M), small boats used as tenders often run aground at low tide, causing additional damage to the reef tops, as well as the faces. While coral damage might seem primarily a cosmetic concern, the sheer numbers of reef users make it a significant impact. In the Whitsundays, approximately 50 commercial tour vessels of all sizes are supplemented by 130 "bareboat" charter yachts. One estimate is that the bareboats are responsible for about 3,000 anchorings/ year. (Mr. Norm van't Hoff, personal communication).

It is believed that chronic anthropogenic stresses such as these may inhibit the recovery of reefs from severe natural disturbances such as storm waves and freshwater inundation, resulting in lowered biological diversity and changes to community composition (Kinsey, 1987). These stresses are already evident in the Whitsundays and have been identified as causes for concern by GBRMPA (Baldwin and McGinnity, 1987). Unfortunately, the present unfettered approach to tourism and other development treats environmental concerns as an afterthought, if at all. Little or no baseline data is available for these reefs. Clearly, if the original composition of a reef community is unknown, the extent of human impacts cannot be gauged. Early responses of the biota to stress are likely to be overlooked until degradation is severe and obvious. The loss of species from the reefs may never even be known.

METHODS

Methods for estimating the extent of anchor damage were adapted from those of Harriott and Fisk. A transect or grid of 1M2 sample points is laid down, and evaluated visually for percent and type of coral cover, presence and type of any coral damage, depth and substrate type. Percent categories recorded are <10, 10-50, 50-90 and >90 % cover. Cover type is identified as hard, soft or mixed coral species. Coral damage is reported as fragments, missing pieces, overturned heads and abraded surfaces. The latter is most difficult to ascribe to any specific damage event; although this is the form that chain damage to massive forms (e.g. *Porites*) usually takes. These techniques are easy to teach to the volunteers involved in the monitoring program, who generally have little background in field sampling. The results provide a very general overview of community type and the extent of impact, primarily suitable for identification of areas where more in study is needed. However, there are 127 principal anchorages listed in a popular cruising guide to the Whitsundays; most have some fringing reef (Colfelt, 1970). Very few have been studied at all. Applying even this simple assessment to all of them is still a formidable task.

Photographic quadrats are being used to quantify % cover of benthic species, bare substrate, damaged areas, etc. Photoquadrats allow efficient use of u/w field time, and create a permanent objective record which can be analysed at leisure. Comparisons between the benthic fauna of intact and affected reef areas will help to determine whether the impacts of heavy usage (i.e. anchoring, small boat and diver damage) have caused any change in community composition, or loss of biodiversity.

Photoquadrat methods are derived from those reported in Thompson (1980 and 1982). A Nikonos V with a 35mm lens and SB 102 strobe is used to photograph the reef from a distance of 1M, recording an area of approximately 1M2. A transect is run up the

reef face from the sand/ reef interface to the beginning of the reef flat (generally from a depth of 25'-30' to 3'-6'). Quadrats are evenly spread between the sand and reef top. Often, isolated coral bommies (large *Porites* heads) are found down the sand slope slightly deeper than the continuous reef edge; these are included in the transect if the sand/ reef interface is much shallower than 25'-30'. Reeftops are covered with a 100M transect, with quadrats spaced every 5M, at a constant depth (2M in the following cases).

The resulting photoquadrats are projected onto a 67cm x 100cm screen marked with 50 randomly placed dots. Each dot covered by a given organism represents 2% cover of the organism on that slide. Organisms are identified to the closest taxonomic level possible from the photo (generally genus). Percent cover data is subjected to an arc sin transformation prior to analysis for variation.

The primary drawback of photoquadrats is that they only sample the epibenthos, and may under-represent cryptic forms such as sponges, etc., which could indicate ecological changes on impacted reefs. Their main advantage is that they record a permanent record of everything occurring within the quadrat, and can be evaluated for parameters not initially envisioned. For example, they can easily be re-reviewed using Harriott and Fisk's anchor damage criteria, and added to that data base.

RESULTS AND DISCUSSION

Preliminary reconnaissance suggests that species such as staghorn forms of *Acropora* may be tolerant of, or even favoured by chronic disturbance, while plate and table forms of *Acropora*, and especially the thin laminar *Montipora* plates, are very susceptible to the kinds of damage done by anchors and chains.

Photoquadrat transects of two fringing reef sites in close proximity have been selected to illustrate the type of data which may be derived, and its implications. A heavily damaged site was compared with a relatively pristine reef edge. Manta Ray Bay is a cove on the north end of Hook Island with abundant coral growth. It has been heavily used both as an anchorage and a dive site for the past decade. It has recently been designated closed to anchoring, and moorings are slated to be installed.

Fisk and Harriott observed that: "The bays most susceptible to anchor damage are those with gentle reef slopes and plentiful delicate staghorn corals in <3m of water at low tide, . . . Since this survey has been carried out, we have been told that the reef at Manta Ray Bay has this type of reef structure, and hence may be more susceptible to damage than the other bays on northern Hook Island."

The comparison site was selected by a local charter skipper, Mr. Norm van't Hoff, who has been cruising in the Whitsundays for the past fourteen years. It was on the East edge of the fringing reef of Hayman Island, across the channel from Hook island, about 4 km from Manta Ray Bay. Because of currents in the channel and lack of good anchorage, the East Hayman site is little visited. Mr. van't Hoff, who has been active in the effort to have moorings installed in sensitive areas for a number of years, considered it representative of what Manta Ray Bay had looked like in earlier years.

Both sites were visited over a two day period in late January 1993, at the end of the Christmas holidays, a major boating period. Considerable fresh damage was obvious in Manta Ray Bay, and several yachts and a dive boat were anchored in the cove during our visit. Seas had been calm for a number of months prior to the sampling period. The most recent cyclone to pass through the area, "Joy," occurred in December, 1990. It has

been over twenty years since the last major cyclone: People still talk about the damage wrought by "Ada" in 1970.

Two transects were photographed at each site. One was run vertically along the reef face from the sand interface (approximately 10M depth) to the reefstop (approx 2M), and a second along the reefstop along the 2M depth contour. Visually, the reef faces appeared comparable; however, the reefstops were more heterogenous. The Manta Ray Bay reefstop shoals gradually into the cove and terminates at rocky cliffs surrounding a pocket beach, while the East Hayman reefstop is part of a large shelf reef which projects several hundred meters out into the channel beyond Hayman Island. This reefstop is subject to more current, and lacks the sand patches found inshore at Manta Ray Bay.

Visually, the differences between the reef faces were as follows: The East Hayman face had good cover of plate forming *Acropora* spp. and *Montipora aequituberculata*; additional encrusting and massive forms filled in the spaces, with little bare substrate. At Manta Ray Bay, plate corals were noticeably absent, and although there were massive and encrusting corals present, many appeared abraded or otherwise stressed, with large bare patches. The reefstop at East Hayman shifted gradually from the plates of the edge to more representation of staghorn forms of *Acropora* and other species, while the Manta Ray Bay reefstop was composed of dense stands of staghorn *Acropora*, primarily *A. formosa*, with very few plates.

These qualitative observations were supported quantitatively by the phototransect results (Table 1), which also provided several supporting insights not visually obvious. For instance, it is commonly held that zooxanthellate soft corals (Alcyonaria) may "Take over" a reef damaged by cyclone or other physical damage. However, they were not especially more abundant at Manta Ray Bay than at the less disturbed East Hayman site. Visually, it sometimes appears that the hydrozoan "Fire Coral" *Millipora*, often found in high current areas, is particularly abundant on damaged reefstops. This was not found to be the case. On the other hand, the clonal Zoanthid *Palythoa* sp., a "Pavement Coral" considered indicative of stressed habitat, represented 4.25% cover of the Reef edge at Manta Ray Bay, a transect which had over twice the bare substrate (56%) of any other transect. *Palythoa* did not occur in any other transect.

Table 1: Abundance of Major Coral Taxa by Site and Transect.

SITE	Massive	Monti	Plate	Stag	Total Ac	Mill	Sof	Palytho	Bare
M Ray face	20.38	2.00	0.62	1.50	2.12	0.00	9.38	4.25	55.88
E. Hay. face	10.80	34.20	17.40	0.40	17.80	8.00	7.00	0.00	21.00
E. Hay. top	3.60	28.40	24.40	10.00	34.40	0.00	9.00	0.00	24.00
M Raytop	6.32	0.00	9.37	51.16	60.53	3.05	9.79	0.00	20.32

Massive forms (*Porites* spp., *Goniopora* sp., *Favia* spp., and *Leptosinisa* sp.), which one might expect to be more resistant to anchor and chain damage, account for 20% cover in the same transect, and 10% or less in the others. Conversely, *Montipora* spp. (almost entirely the thin plate-like *M. aequituberculata*), the most abundant species of the East Hayman reef (approx 30% cover), was virtually absent at Manta Ray Bay. Plate forming species of *Acropora* represented about 20% cover in both of the East Hayman transects, yet were almost absent (<1%) on the Manta Ray Bay reef face. The Manta Ray Bay reefstop, which had the greatest abundance of *Acropora* (60% cover) and of corals as

a whole (80%), had just under 10% cover of *Acropora* plates. Every individual plate showed obvious damage in the form of missing pieces and surface abrasion. One plate encountered in the transect had been recently overturned. (Living tissue was still present on the former top surface). These trends are shown graphically as (Figure 2). For ease of interpretation, data from the two adjacent, and more similar, East Hayman transects has been averaged.

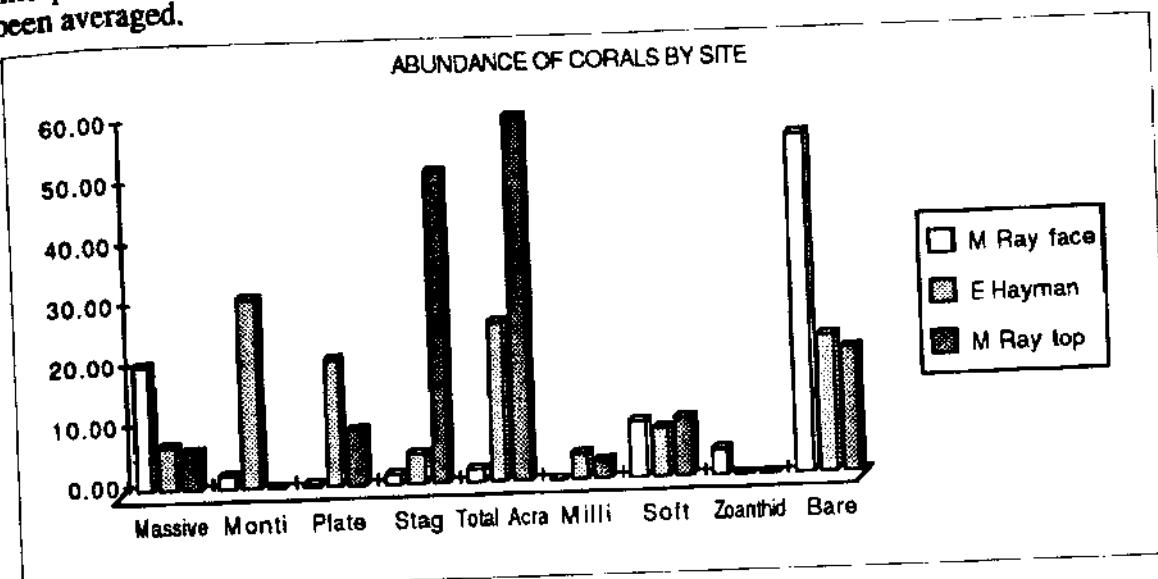


Figure 2. Abundance of major coral taxa by site and transect.

The high coral cover of the Manta Ray bay reeftop was primarily due to the 60% cover of staghorn forming *Acropora* spp. These species readily continue to grow from fragments, even on sand bottoms, providing that they are not buried. Hence, they may actually be propagated by breakage. They are among the fastest growing of reef corals, at rates of up to 20 cm per year. (Dr. Jaime Oliver, personal communication). They often occur in large thickets in suitable back reef habitat. It is tempting to characterise the thickets in Manta Ray Bay as "Hedges." In many spots, the tips have been broken off in a neat line, presumably from anchor chains raking over the beds. Of the 19 reeftop quadrats, recent damage (living fragments, clean white breaks in the stumps) was visible in 13, or 81% of the sample.

The staghorn *Acropora* often show blunt and misshapen tips, where previous damage has healed, but they are seldom uprooted entirely, or killed. Some plates also show healing after pieces have been broken from their rims. Many plates showed abrasion damage on their tops, and some had smaller soft or hard corals growing on them. Abrasion can provide bare substrate for another coral to colonise, and possibly overgrow the stressed plate. The more common form of damage occurs when an anchor or chain simply overturns an entire plate. A number of recently overturned plates were encountered in preliminary reconnaissance on the Manta Ray Bay reeftop. Some overturned plates, less recent but not especially old looking, were larger than any still living in the cove.

"... there may have been long term changes to the reef structure as a result of chronic damage over long periods. It is possible that repeated anchor damage has changed the community to favour the types of coral that can resist or adapt to physical

damage. For example, surviving corals might include large massive species that can survive the impact, or species which fragment naturally and are capable of successfully regenerating from small fragments. Other species might be excluded over time if persistent impacts occur. It would be impossible to determine whether such changes have occurred without a long term study and baseline data." (Emphasis added.)

The greater vulnerability of plate forming *Acropora* to the chronic physical stress created by heavy human usage may favour the staghorn species. The loss of the larger plates, and the microhabitat they create, may alter community composition toward an even greater dominance of the staghorn *Acropora*, at the expense of other species of the reef community. Further characterisation of the fringing reefs of the Whitsundays will help to establish whether this is occurring. In the meantime, it is clear that a system of moorings is imperative to reduce the ongoing impact on already stressed reefs.

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IMPROVED METHODS FOR VISUAL AND PHOTOGRAPHIC BENTHIC SURVEYS

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As funding for field work becomes more competitive, it is increasingly important to produce cost effective quantitative results using calibrated methods. Much effort is devoted to visual and photographic surveys which are routinely employed to determine the density and distribution of animals or other organisms, the type and abundance of biological ground cover, and geological features near or on the ocean bottom. A number of techniques have been developed for estimating the scale of the study site and the size of individual specimens so that the observations can produce quantitative data for numerical analyses and comparative studies.

This paper will review some of the more commonly employed methods and will present recently-developed, laser-based approaches to quantify visual and photographic images. The techniques to be discussed involve the projection of small light spots as fiducials onto the surface to be surveyed, so that the observer or camera can record absolute scale information. Demonstrated applications and proposed methods will be described.

INTRODUCTION

Visual and photographic surveys are commonly used to quantitatively measure the abundance and diversity of benthic biota. A number of studies have shown that photographic methods mitigate some of the major biases associated with traditional physical sampling, i.e., traps, trawls, grabs (Tusting, 1986; Bortone et al., 1992; Barry and Baxter, 1993). For some studies, photography complements mechanical sampling by establishing undisturbed spatial relationships prior to disruption by the sampling method (Emory et al., 1965; Ewing et al., 1967).

A wide variety of methods and techniques have been developed to allow repeatable and comparative studies to be performed. All depend on the establishment of at least one metric scale or dimension on the image to provide a basis for analysis and calculations. The addition of small, inexpensive lasers to these visual/photographic

systems can greatly improve the measurement accuracy by providing one or more absolute dimension. For video and film recording, the scale information can be permanently recorded as small bright spots directly on every frame recorded.

TRADITIONAL QUANTITATIVE METHODS

Metric Scale or Grid

The simplest and most direct method is to introduce a scale directly into the scene to be recorded (Owen et al., 1967; Maney et al., 1980; Cassidy, 1991). This can be accomplished by laying a ruler or a frame-like grid on the bottom prior to photographing it. For hard-bottom environments, small flags or other markers can be permanently attached or embedded to denote the boundaries of the study area (Gittings et al., 1990). To further define the boundaries, high-visibility lines are often strung between markers. This approach is accurate and effective for shallow-water sites that will be visited on a repetitive basis. It does require the study site to be carefully mapped and marked prior to the survey work. For random sampling, transects, or other situations where advance delineation of the site is impractical, other methods must be used.

Photogrammetry

Photogrammetry is the science of making measurements using photographic techniques (Wolf, 1983; Newton, 1984). Many of the methods described in this paper can be considered to be photogrammetric techniques. However, the term is usually used to describe a special version of stereo-photography in which measurements are made in three dimensions. A precisely mounted and calibrated pair of cameras record two overlapping images of the same scene under controlled geometric conditions. The images are later processed with a highly-specialized image processor to provide precision measurements from the recorded images. Although this type of photogrammetry is a well-established technique for underwater inspection and measurement, it has only occasionally been used for benthic-survey work because of its complexity and cost (Schuldt et al., 1967).

Most photogrammetric work has been done with film-type cameras, and most commercially available equipment uses 35-mm-format cameras. Recently a video-based system has been developed for undersea photogrammetry (Turner et al., 1991). Although the resolution and accuracy of this system is lower than that obtainable from film recording cameras, the images can be processed on line and the measurements performed in nearly real time.

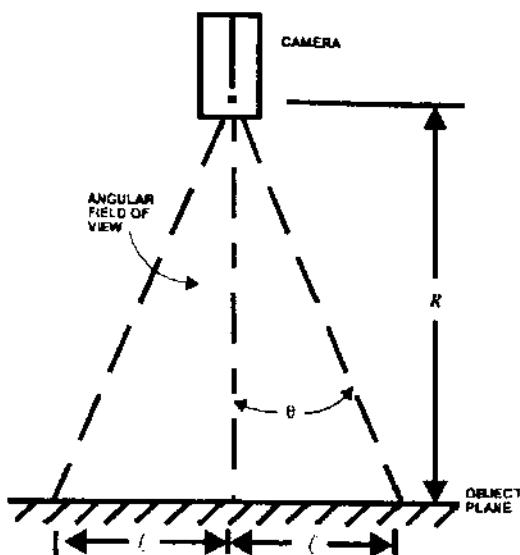


FIGURE 1

Relationships between the camera's angular field of view, range and field of view at the object plane.

Predetermined Field of View

If the angular field of view of a camera and the geometry between scene and camera are known, the size of the photographed area can be calculated and the size of the object within the field of view can be estimated. A simple example is shown in Figure 1 where the camera is viewing a surface normal to the camera's axis at a range of R meters. The angular field of view is determined by the focal length of the camera f and the format size of the film or image sensor (in the case of a video camera) x . The angular field of view can be directly measured by photographing objects of known size at known distances from the camera. Alternatively, it can be calculated from Equation 1 for most cases of interest (Ray, 1984).

$$\text{Angular field of view} = 2\theta = 2 \tan^{-1} \left(\frac{x}{2f} \right) \quad (1)$$

where:

θ = the half-angle as shown in Figure 1

x = the format dimension in mm, and

f = the focal length of the camera lens

For a rectangular format, there are three ways of measuring the size of format: horizontally, vertically, and diagonally. For the common 35-mm camera film, the format is 36 mm horizontally, 24 mm vertically, and therefore, 43 mm diagonally. For video cameras, the diagonal format (diameter of the image sensor) is usually specified and the width-to-height ratio is 4 to 3. The focal length used in Equation 1 is the effective focal length when photographing objects in water. The effective focal length of a lens in water is longer than in air by a factor of 1.33 (assuming a flat view port). For example, a 28-mm lens becomes a $1.33 \times 28 = 37$ -mm, focal-length lens when used in water.

With reference to the geometry of Figure 1, if the range R and the angular fields of view are known, then the dimensions of the photographed area can easily be calculated:

$$\left. \begin{array}{l} \text{Vertical field of view} = 2l_v = 2R \tan \theta_v \\ \text{Horizontal field of view} = 2l_h = 2R \tan \theta_h \end{array} \right\} \quad (2)$$

where θ_v = the half angle of the vertical angle of view, and

θ_h = the half angle of the horizontal angle of view

The size of feature or animals near the object plane, within the field of view, can be estimated by direct scaling with respect to the size of the field of view. This simple and effective approach to quantitative photography has been used since the 1940s. Early systems used a weight to trigger the camera when it made contact with the bottom (Ewing and Vine, 1946; Owen et al., 1967). Harold Edgerton, the pioneer in underwater photography and acoustics, used a sonar system to trigger his camera a predetermined distance above the bottom (Edgerton, 1967). More recently a mechanical frame has been used to accurately position the camera above the bottom prior to photographing it (Cassidy, 1991).

Perspective Grid Approach

If the camera axis is not positioned perpendicular to the scene as in Figure 1, the transformation between the object plane and the image plane is no longer a simple linear one, but is one requiring the solution of equations containing trigonometric functions. Traditionally, the method of obtaining quantitative information from an image produced by a camera aimed forward and downward involves the mathematical

generation of a perspective grid that is superimposed over the image to allow metric scaling of the scene photographed (Wakefield and Genin, 1987).

Scientists using the 35-mm forward-looking cameras on the manned submersible ALVIN are provided perspective grids to assist in scaling and size determination from the photographs (D. Foster, personal communications). This method requires that a number of parameters of the camera and its platform be known: the angular field of view of the camera, its height-above-bottom, and the angle of the camera's axis with respect to the surface photographed (Rosman and Boland, 1986). In many cases one or more of these variables is unknown, making quantitative analysis impossible (Rice and Collins, 1985).

Visual and Photographic Surveys

Surveys and censuses are routinely performed by divers or vehicles swimming above the seafloor and observing organisms within a fixed field of view. A number of techniques for obtaining transect data and analyzing it have been developed (Chayes et al., 1984; Boland and Lewbel, 1986; Bortone et al., 1986, 1989; Maney, et al., 1990; Linquist et al., 1992; Barry and Baxter, 1993; Michalopoulos, et al., 1993). Absolute measurement of area of coverage requires a knowledge of the observer's or camera's height-above-bottom. This distance is usually based on an educated estimate rather than an actual distance measurement.

Another visual technique, the point method, requires that a scuba-equipped diver occupy a position on the bottom and slowly turn to scan a circular region of the bottom recording the number of individual fish observed by species during a fixed time interval. The chosen radius is usually 5.6 meters to provide a sample area equal to $\pi r^2 = 100$ m². Fish size is also estimated during these censuses (Bortone et al., 1989, 1992). The size of the circle is estimated using a known-length line deployed on the bottom as a visual reference.

The simple, two-laser range finder (to be discussed in a following section) would allow a direct measurement of range for applications such as these. Laser-based altimeters would facilitate flying an ROV a known distance above the bottom and make it easier to measure uncontrolled variations in altitude during a transect. The basic size-measurement system (to be described) can provide each frame of photographic data with an absolute metric scale so that quantitative analyses can be performed on individual images. The size calibration information also allows variable magnification to be applied to the images so that the scale of each photograph can be normalized. This facilitates the production of mosaics (Steeves and Shafer, 1991) and is being used with computer-assisted image processing (MacDonald, et al., 1992).

LASER-ASSISTED MEASUREMENTS

Camera/Laser Range Finder

A single laser can be used with a camera to obtain an accurate and simple range finder as shown in Figure 2. For this case, the camera's axis must be nearly perpendicular to the surface photographed. The laser must be mounted rigidly with respect to the camera's axis so that the following geometric parameters are known:

y = offset of the laser with respect to the camera's axis, and
 R_0 = range at which the laser beam crosses the camera's axis.

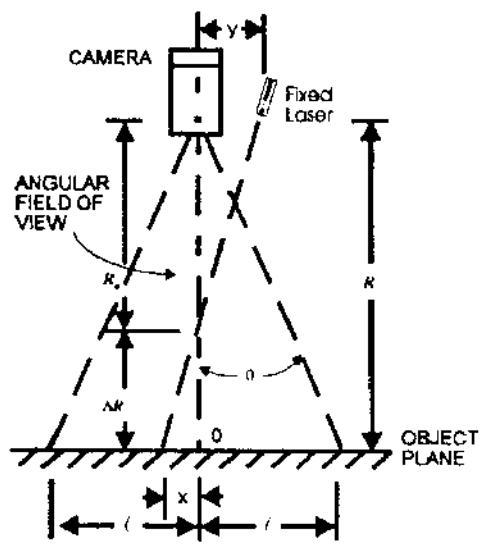


FIGURE 2

The use of a camera and laser to provide a simple range finder.

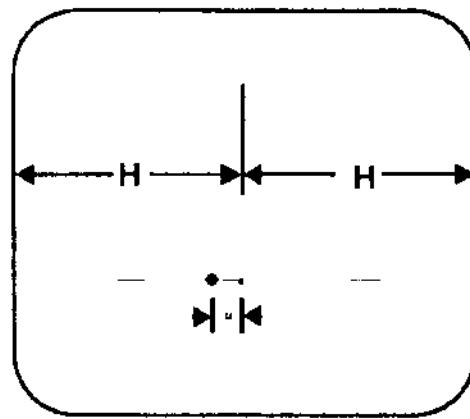


FIGURE 3

The image produced by the camera/laser combination of Figure 2.

In addition, the angular field of view 2θ of the camera must be determined (calculated from Equation 1 or measured). From the geometry of Figure 2:

$$R = R_0 + \Delta R \quad (3)$$

$$\Delta R = R_0 \left(\frac{x}{y} \right) \quad (4)$$

$$l = R \tan \theta \quad (5)$$

For convenience, we define a dimensionless parameter K:

K = ratio between the displacement of the laser beam from the point 0 at the object plane and the half width of the camera's field of view, or

$$K = x/l \quad (6)$$

The image produced by the camera and laser positioned as indicated in Figure 2 will be as shown in Figure 3, with the light spot displaced to the left of the center of the image. Because of the idealized geometry, the transformation between the object plane and the image plane is again a simple linear transformation. For this special case, K is also equal to the ratio between the displacement of the laser-generated spot and the half width of the image or

$$K = u/H \quad (7)$$

Combining equations 5, 6, and 7:

$$x = (u/H) R \tan \theta, \text{ and} \quad (8)$$

solving equations 3, 4, and 8 for R :

$$R = \frac{R_o}{\left[1 - \frac{R_o}{y} \left(\frac{u}{H} \right) \tan \theta \right]} \quad (9)$$

The ratio u/H is obtained from the image and the other parameters are obtained from the camera/laser geometry. This method is applicable to video, film, or computer produced images.

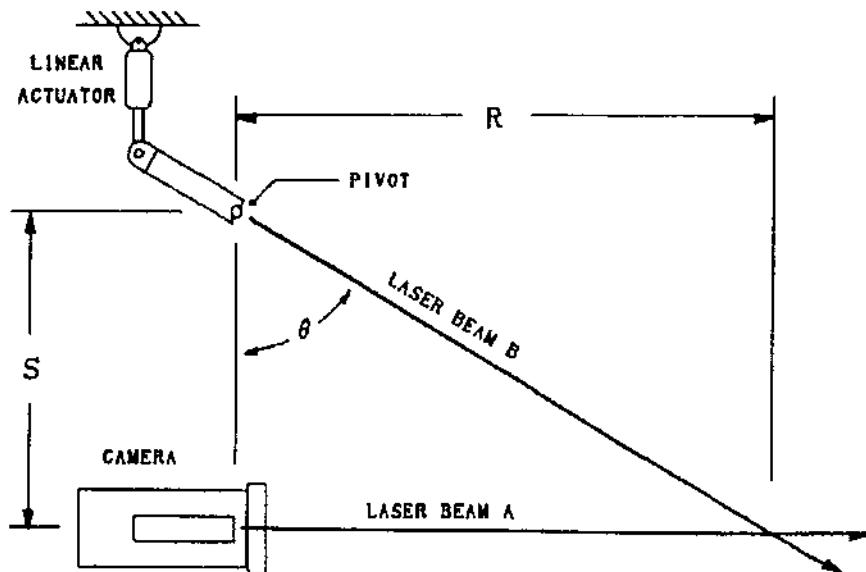
Two-Laser Range Finder

A similar system for determining range using a pair of lasers is shown in Figure 4 (Tusting, 1990).

In this case, one of the lasers is mounted parallel to the camera (or other viewing system), and the second laser is mounted so that it can be pivoted and the angle θ recorded. The moveable laser is adjusted until the two light spots coincide, and the range is calculated from the equation:

$$R = S \tan \theta \quad (10)$$

This method of measuring range differs from the one described previously in two ways: it is applicable to a situation where the viewing system is a person rather than a camera, and the precise location of the lasers with respect to the camera is not critical.



$$R = S \cdot \tan \theta$$

FIGURE 4

An application of lasers and triangulation to measuring the range from a camera to a target.

Two-Laser Sizer

One of the earliest applications of lasers to underwater measurements involved a pair of parallel lasers to obtain direct size information (Tusting, 1986; Auster et al., 1989; Tusting and Davis, 1993). This method has been widely accepted and recently adopted as a training aid to help divers estimate the size of fish *in situ* (McFall et al., 1992). The parallel-laser sizer provides a geometric and mathematical basis for much of the following discussion of quantitative photography as it relates to improved survey methods. Therefore, the basic mathematics will be presented in some detail.

The simplest geometry involves positioning a camera perpendicular to the surface to be photographed as in Figure 5. Two lasers are attached to the camera with their axis parallel to and equidistant from the axis of the camera (Tusting, et al., 1989). Two parameters concerning the camera/laser geometry are assumed to be known or measurable:

θ_A = the half angle of the horizontal angle of view, and

x = the spacing between the lasers and the camera's axis.

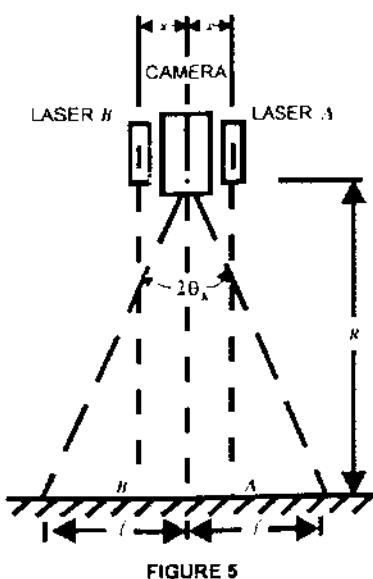


FIGURE 5

A two-laser photographic measurement system

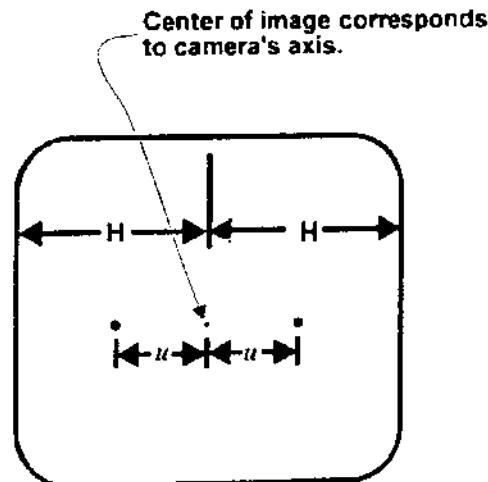


FIGURE 6

The image obtained from the camera/laser system of Figure 5.

In addition, the image format (or ratio of the horizontal to vertical fields) is assumed to be known. We define a ratio K as the fraction of the horizontal field of view spanned by the pair of laser beams as indicated in Figure 5 or:

$$K = \frac{x}{l} \quad (11)$$

Also from Figure 5, it is determined that

$$\tan \theta_k = l/R \quad (12)$$

Combining Equations 11 and 12:

$$R = \frac{x}{K \tan \theta_k} \quad (13)$$

Determining the range R allows the size of the horizontal field of view to be calculated from Equation 12 and also the area viewed since the format is known. For this simple case, the ratio K can be obtained directly from the corresponding image because the transformation between the object plane and the image plane is a simple, linear transformation (refer to Figure 6):

$$K = u/H \quad (14)$$

The dimensions of u and H are arbitrary—*inches or cm for an image on a video screen or photographic print or more conveniently, pixels, if an image analysis system is being used*. The laser dots also allow objects to be directly scaled from the image in both the vertical and horizontal directions. This is true even if the camera's angular field of view is not known—for example, if the camera is equipped with a variable zoom lens.

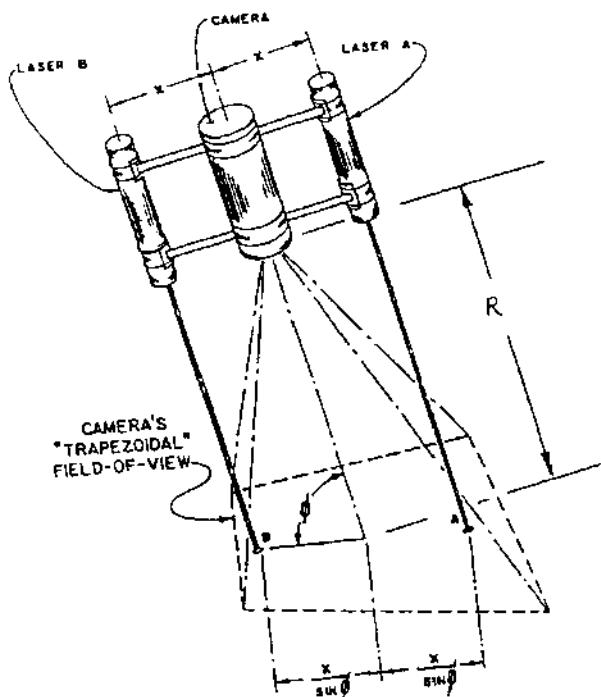


FIGURE 7

A two-laser size measurement system with a perspective view of the bottom

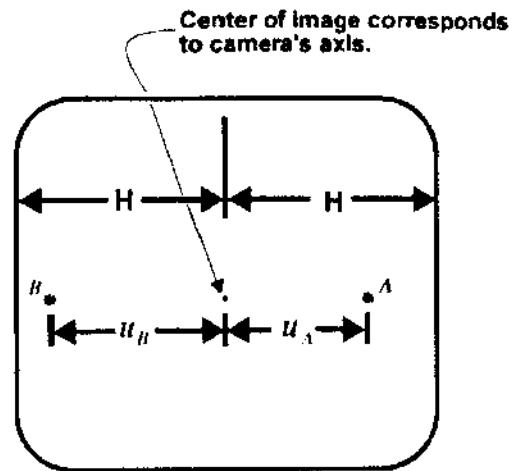


FIGURE 8

The image observed with the camera/laser system of Figure 7. As a result of the nonorthogonality of the camera's axis, the two laser spots are not offset equal distances from the center of the image.

Consider now a more complicated situation, one in which the camera/laser system is tilted fore and aft by an angle Φ . This configuration provides a simple perspective view of the bottom as shown in Figure 7. In this case, the transformation between the object plane and the image plane is no longer a linear transformation. The mathematics is complex and is presented in detail in a readily available paper (Wakefield and Genin, 1987). In this paper, it is shown that a complete dimensional analysis can be performed if the tilt angle Φ and the range R are known. The paper develops the Canadian or perspective grid method of analyzing the image and performing size measurements from the grid generated.

The image corresponding to the perspective view is shown in Figure 8. The rectangular-shaped image corresponds to a trapezoidal-shaped region of the bottom. The light spot marked A results from the intersection of laser beam A with the bottom and similarly for the light spot marked B. The distances u_A and u_B are not equal as in the case of the perpendicular viewing case. In fact, the difference in these two offsets is what allows the range and tilt angle Φ to be calculated (Davis and Tusting, 1991). The equations are:

$$R = \frac{xH}{2 \tan \theta_b} \left[\frac{1}{u_A} - \frac{1}{u_B} \right] \quad (15)$$

and

$$\tan(90 - \phi) = \frac{H}{2 \tan \theta_h} \left[\frac{1}{u_A} - \frac{1}{u_B} \right] \quad (16)$$

where the various parameters are defined in Figures 7 and 8. A number of simplifying assumptions are made to obtain Equations 15 and 16. For a more general discussion, refer to the paper by Davis and Tusting.

Four-Laser Measurement System

Adding a pair of orthogonally mounted lasers as in Figure 9 allows measurements to be made when the camera's axis is tilted both horizontally and vertically. A discussion of this method and the corresponding equations for range and tilt angles are presented elsewhere (Davis and Tusting, 1991). A comprehensive derivation of the mathematics for this and other laser measurement systems will be published soon.

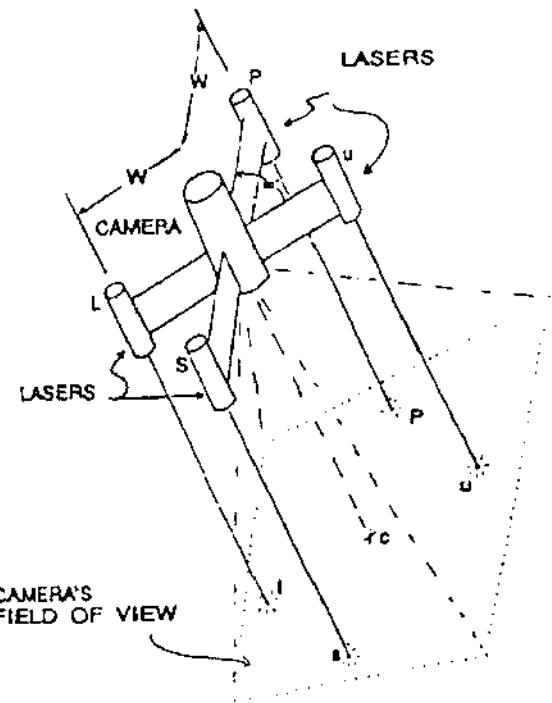


FIGURE 9

Four-laser size measurement system which allows the camera to be tilted in both the horizontal and vertical directions.

The system shown in Figure 9 allows the range to be calculated from two equations—one using the displacements of the upper and lower laser projections, and the second using the displacement of the port and starboard laser projections. A comparison between the two calculations provides an estimate of the measurement accuracy.

DISCUSSION

Lasers can be employed to assist in improving the accuracy of underwater benthic surveys. They can be used in a variety of geometries to measure and record range and distance. Two or more parallel lasers can be directly used to quantify the images obtained from downward looking cameras. Advantages include:

- The measurements are repeatable and calibrated by simple geometry.
- The fiducial marks are visually apparent to the observer at the time the observations are made.
- The calibration data is recorded directly on every image, facilitating later analysis.
- The method is conceptually simple and easy to interpret.

For perspective photography, laser calibrations allow a direct and simple measure of range and tilt so that calculations and perspective grids can be generated on individual frames even if the geometric parameters are changing from frame to frame. For photographic transects made with rapid-cycling, film-recording cameras, the calibration data can be used to correct for uncontrolled variations in height-above-bottom, allowing better mosaics to be constructed with less effort.

ACKNOWLEDGMENTS

The authors would like to thank Annell Nelson for her care in typing the manuscript, preparing illustrations, and correcting the text. This work was supported by internal funds of the Harbor Branch Oceanographic Institution, Inc., Monterey Bay Aquarium Research Institute, and a grant from the Atlantic Foundation. This is HBOI Contribution No. 961.

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ROCKS AND FISHES: SUBMERSIBLE OBSERVATIONS IN A SUBMARINE CANYON

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A multi-disciplinary study to assess the importance of small-scale refugia to species of large rockfishes in deep water was undertaken recently in Monterey Bay. Isolated rock habitat at depths suitable for rockfishes in Soquel Canyon (up to 300 m) was identified using fine-scale bathymetry and sidescan sonar imaging, verified with visual observations from a manned submersible, mapped and quantified. Species composition, size, and habitat specificity (type and depth) of fishes associated with these features were estimated using a video camera and parallel laser system along transects made from a submersible. High numbers of large (up to 1 m) rockfishes were locally associated with rock ledges, small caves, crevices, and overhangs. Two distinct assemblages of rockfishes were obvious from clustering analysis; small species were associated with mud and cobble substrata of low relief, while larger species were found under ledges or near large structures on vertical walls, ridges and boulder fields. Relative abundance and size of the largest species were higher and larger, respectively, when estimated from submersible surveys than from partyboat catch records from adjacent areas. We suggest that discontinuous rock outcrops of high relief are inaccessible fishing areas and thereby provide natural refuge for commercially and recreationally important fishes in Soquel Canyon.

INTRODUCTION

Rockfishes (Sebastodes sp.) are heavily exploited in recreational and commercial fisheries along the west coast of the United States. These fisheries in California are worth over \$1 billion, considering all associated industry, with recreational value far exceeding that of the commercial fisheries (Lenarz 1987). Rockfishes have been harvested

continuously in California with various types of gear (e.g., hook and line, gill net, and trawl) since the early 1900's. Historically, rockfish landings have been especially high in Monterey (Phillips 1939).

Many species of rockfishes are slow-growing, long-lived, and older at maturity. Because of these life history characteristics, as well as patchy distributions and likely residentiality, local stocks of rockfishes are particularly vulnerable to over fishing. Indeed, decline in abundance and size of large species of rockfishes has been noted in recent recreational catches landed in the Monterey area. As local stocks within the bay become depleted, the Monterey fleet has expanded its range to greater distances from port.

Commercial and recreational fisheries can affect the sustainability of coastal rockfish populations by changing their distribution, abundance, and diversity. Accurate assessment of these factors is critical for effective management, protection, and restoration of this resource. Because California is considering the use of marine harvest refugia, and is in the process of designating four marine ecological reserves, it is important to identify and describe the extent of naturally-occurring refugia.

There is little information on the distribution, abundance, and other ecological characteristics of mature rockfishes associated with deep-water rocky habitat in the Monterey Bay area. This type of habitat is beyond SCUBA capabilities, and the rocky substrata prohibit accurate estimates of fish abundance using conventional trawl surveys. Despite this lack of knowledge, deep-water rocky features are important rockfish habitats. Using the submersible *Delta* for two exploratory dives in 1991, we observed high numbers of mature rockfishes and lingcod (*Ophiodon elongatus*) associated with an isolated rocky outcropping surrounded by a field of mud along the side of a submarine canyon in Monterey Bay. Our observations of species of large rockfishes in deepwater in the Monterey Bay submarine canyon system were somewhat surprising because they represent very low numbers in the hook and line fisheries in the area and are rarely observed during shallow water surveys by scuba divers. Geologists were likewise surprised at the incidence of such variable habitat in a canyon that was thought to be dormant and without the influence of erosion processes.

Our general objective is to assess rockfish resources and their potential habitat in the Monterey Bay submarine canyon system by combining geophysical techniques and submersible observations. Our working hypothesis is that rocky outcrops along the headward sides of these canyons serve as refugia for deep-dwelling rockfishes. We are evaluating rockfish species composition, abundance, and species-specific size distribution within heavily-fished and lightly-fished areas of the same depth and habitat. Because most of the shallow and more accessible rocky areas in Monterey Bay have been heavily fished continuously for most of this century, fishes in these areas expectedly would be fewer in number, smaller, and not yet reproductively active when compared with less accessible areas. Exposed rocky habitat in relatively deep water outside the depth range of recreational fishing could serve as spawning and birthing grounds for larger, and perhaps older individuals of some species, and may produce a significant number of recruits.

METHODOLOGY

This represents preliminary findings during the first year of a multi-year study supported by NOAA National Underseas Research Program (NURP), and conducted by

fisheries biologists from NOAA, National Marine Fisheries Service's Pacific Fisheries Environmental Group (PFEG), Moss Landing Marine Laboratories (MLML), the California Department of Fish and Game (CDFG), and the Marine Science Institute at University of California, Santa Barbara, and geologists from the U.S. Geological Survey (USGS) and MLML.

Our approach to assess rockfish assemblages and their habitat in inaccessible, lightly fished areas included three methodologies performed as three phases of the 1992 pilot study. First we conducted a survey of the bathymetry in our Soquel Canyon study site using a 3.5 kHz echo sounder integrated with Global Positioning System (GPS) navigational data along track lines. From this survey we produced a fairly fine-scale bathymetric map of the area (Fig. 1), with depth contours at only 20 m intervals. We identified areas of high relief and potential slumping, and used this map to select our submersible dive sites.

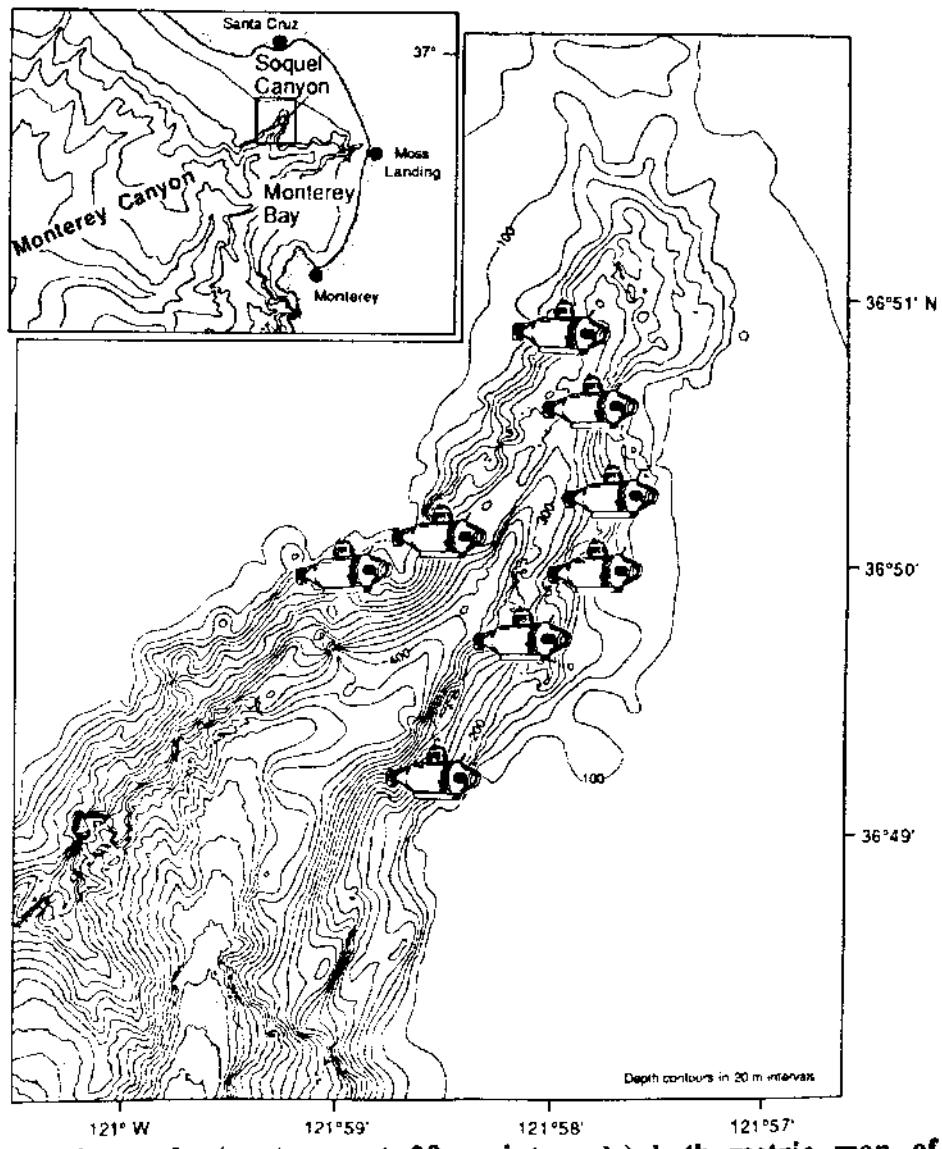


Figure 1. Fine-scale (contours at 20 m intervals) bathymetric map of Soquel Canyon and sites of submersible dives.

During the second phase of our study, eight sites (Fig. 1) were evaluated as potential rockfish habitat using the *Delta* submersible in water from 80 to 305 m. at the head of the canyon. The *Delta* is a relatively small submersible, accommodating one scientific observer and one pilot, has a maximum operating depth of about 350 m, and a cruise speed of 1.5 knots. An acoustic track-point system on board the support vessel monitors the underwater location of the submersible. Most of the dives were made during daylight to avoid potential bias due to diel activity patterns of the fishes. Each dive was documented continuously with a high-8 mm video camera and associated lights that were externally mounted to the starboard side of the submersible; the scientific observer verbally annotated each video tape. A hand-held high-8 mm video camera and voice recorder were used as back-up data recorders. Divers de-briefed themselves after their dives, including transcribing observations on fishes and habitat from audio and video tapes into a computerized database on board the support vessel.

To quantify fish abundance and habitat use, belt transects (e.g., Pearcy et al. 1989, Stein et al. 1993) of 10-min duration were conducted 1-2 m from the bottom at 0.4-0.9 knots. In spite of recognized biases of visual transect techniques (i.e., effects of observer and lights, resighting mobile fishes, variable width of transect and field of view), belt transects are probably the most appropriate survey method considering the sedentary behavior of demersal rockfishes and the heterogeneity of their environment. Transects were purposely of short duration to maintain constant depth within the rock habitat at each station. All fishes in front of the viewing port on the starboard side of the submersible were identified (when possible) and counted.

Two parallel lasers were mounted on either side of the external video camera at a fixed distance of 39.5 cm apart (Fig. 2). The projected reference spots on the video tape, and also visible *in situ* to the observer, were critical in estimating the size of fishes and distance traveled along a transect. We made measurements by comparing the size of a fish or habitat feature to the known spacing of the two bright laser spots when the object was nearly perpendicular to the camera and lasers (Davis and Tusting 1991). We estimated the length of each transect, independent of submersible speed and bottom currents, by tracking the spacing of the two laser spots along the transect's path. The video tapes were used to supplement *in situ* observations of the scientist and provide independent estimates of habitat coverage and fish size.

Submersible dives also were made by marine geologists at six sites. Relief, habitat type, size and depth range of features were described; these field descriptions assisted the biologists in planning dives at each site and for post-cruise assessment of habitat. Microhabitat of the dominant fish species was characterized at each site. Substrata type included various combinations of mud, cobble, boulders, and rock ridge. Species-specific abundance at each site was standardized by distance covered during a transect.

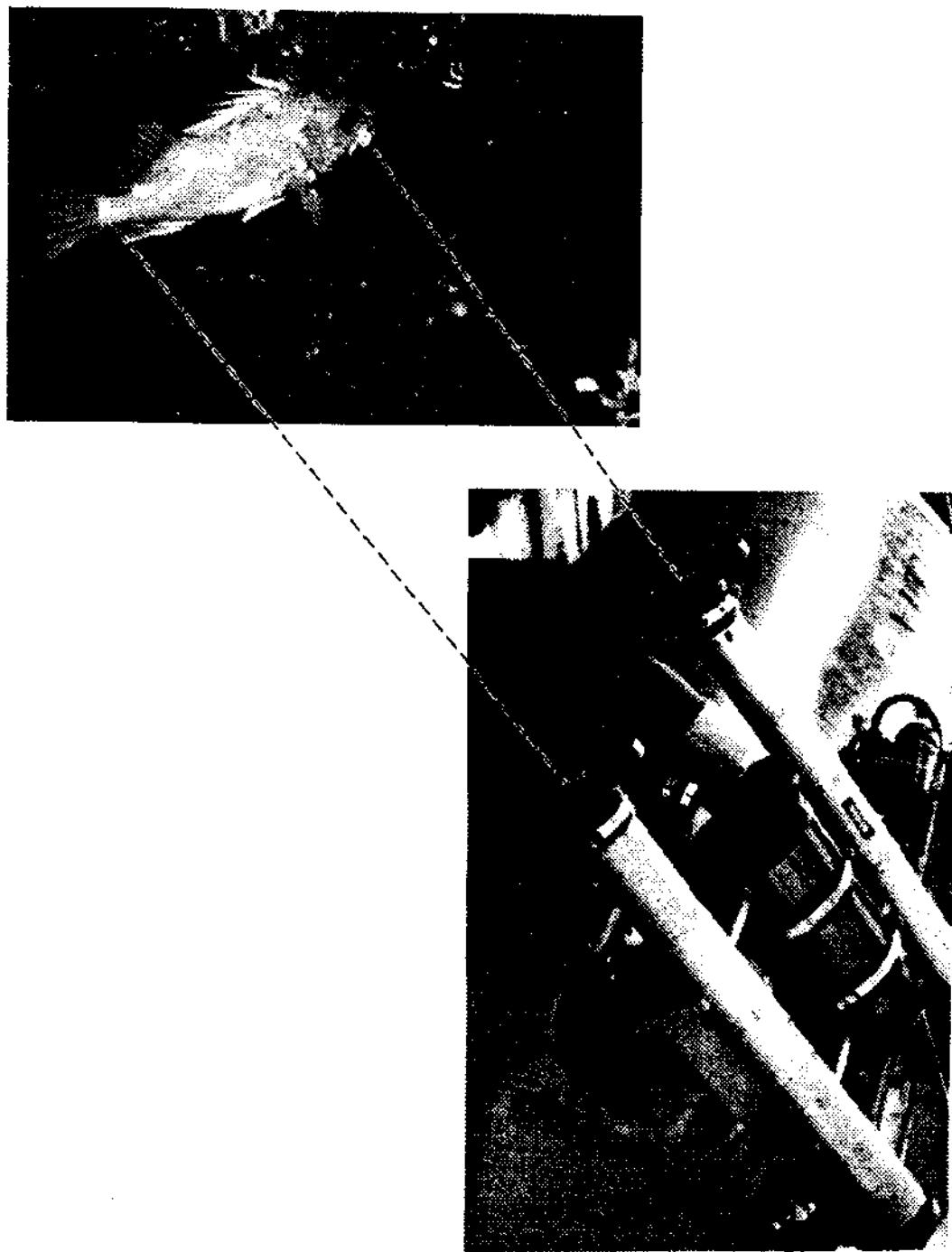


Figure 2. Composite demonstrating (a) the parallel lasers mounted to an underwater camera and used to estimate fish size and distance travelled along transects, and (b) an *in situ* video print of a greenspotted rockfish (*Sebastodes chlorostictus*) at interface of boulder and mud substratum (laser spots are evident).

During the third phase, which was concurrent with the submersible operations, we conducted a sidescan sonar survey of Soquel Canyon. Four nights were spent surveying the north end of the canyon, particularly over 80-300 m of water. Side scan sonar is the perfect method for differentiating blocks of hard substrata, which appear dark, from surrounding soft bottom sediments because of their greatly different reflection characteristics (Fig. 3). The sonographs along each track line of the survey were combined with navigational plots from differential Global Positioning System (GPS) to form a mosaic of the canyon walls. When plotted on regional bathymetry, the side scan targets are identified as areas of positive or negative relief. We are using these maps or mosaics to quantify the amount of rocky outcrops available to rockfishes. Our interpretations of the sonographs were verified by observations made from the *Delta* submersible.

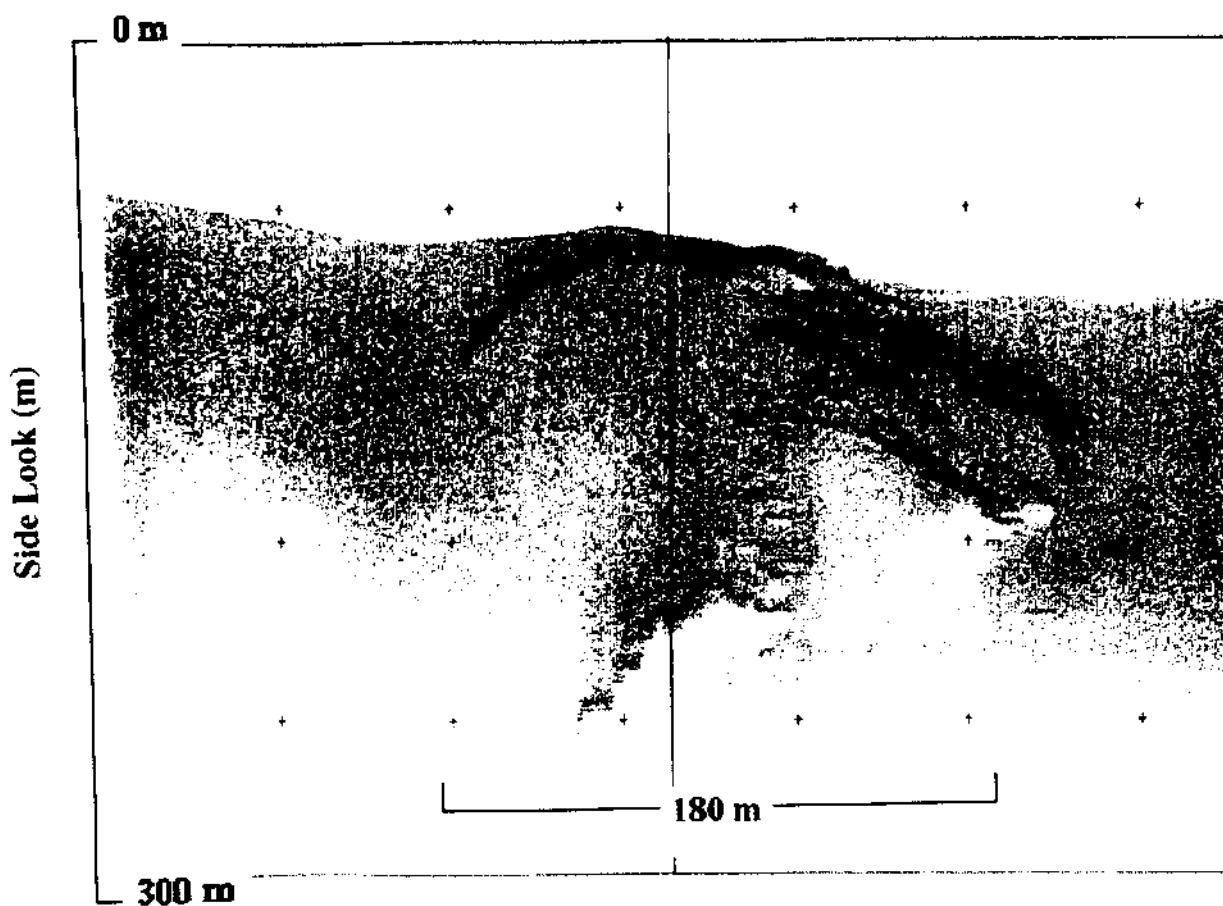


Figure 3. Side scan sonograph of outcrop on wall of Soquel Canyon. Strong acoustic reflectors are from exposed bedding faces, white areas are shadows behind faces, and gray areas are the result of non-reflective muds. These beds, primarily sandstone and mudstone, compose the rockfish habitat. These interpretations were verified by direct observations from submersible.

RESULTS AND CONCLUSIONS

From the geophysical surveys and observations made during seven geological dives at six sites, Soquel Canyon is characterized by extensive erosion, with sharp, steep relief in much of the headward part. This particular geomorphology is thought to have been created by marine and river erosion during the last low-stand of sea level (H. G. Greene, USGS, Menlo Park, CA, pers. comm.). This canyon is made of many isolated rock outcrops that provide ideal shelter for large fishes.

Twenty-three biological dives were made at eight sites during the pilot study in Soquel Canyon. Five of these sites, in particular, have been verified as excellent rockfish habitat from visual observations using the submersible *Delta*. From post-cruise analysis of 42 10-min transects representing 7.1 km total distance covered, we observed 2,470 individual fishes from 37 species, of which over 2,000 from 21 species were rockfishes. The major rockfish habitat types in Soquel Canyon include vertical cliffs with joints, fractures, and overhangs, small and large ledges, talus slopes and boulder fields of exposed sandstone and mudstone interspersed with soft mud and crinoid fields.

Clustering analysis of all bottom types based on relative abundance of fish species indicates habitat partitioning. Small species, such as sharpchin (*S. zacentrus*), striptail (*S. saxicola*) and halfbanded rockfish (*S. semicinctus*), were associated with mud and cobble substrata of low relief. Mud-rock combinations were dominated by greenspotted rockfishes (*S. chlorostictus*; Fig. 2) and an assortment of larger species (e.g., bocaccio, *S. paucispinis*). The largest species up to 1 m in length, such as cowcod (*S. levis*) and yelloweye rockfish (*S. ruberrimus*), were closely associated with larger structures such as rock ledges, small caves, crevices, and overhangs. Many rockfishes of all sizes were associated with some structure, including boulders, cobble, invertebrates such as crinoids and sea anemones, debris, and simple shallow depressions in the mud. In seeking shelter near rock outcrops, large rockfishes may be excavating the friable semi-consolidated mudstone; this type of bioerosion could result in some of the geologic characteristics of the habitat.

From our pilot study, the preliminary conclusion is that heterogeneous, rocky habitat of high relief surrounded by soft mud in deep water of submarine canyons supports viable adult rockfish populations. These fishes are likely protected from excessive harvest because these habitat characteristics make them difficult to target. Indeed, species and size composition of rockfishes in the catch of the recreational fisheries in the Soquel Canyon area are very different from assemblages described from our submersible observations at similar depths and locations (Fig. 4). The more mobile species (e.g., chilipepper, *S. goodei*, and yellowtail rockfishes, *S. flavidus*) that tend to aggregate off the bottom may be more vulnerable to the hook-and-line fisheries, whereas the larger benthic species (cowcod, yelloweye rockfish, and lingcod) are relatively more abundant in the submersible observations.

Because of the many canyon systems in or near Monterey Bay, deep-water rocky habitats are accessible relatively close to heavily-fished areas, making comparisons among sites possible. From our fishery-independent estimates of rockfish abundance and habitat availability and characterization, we can evaluate the need for protecting populations of rockfishes on small-scale refugia. These areas could serve as sources of recruitment for fish populations being reduced by fishing, and identifying critical habitat for mature adults could prevent further declines in population numbers of some species.

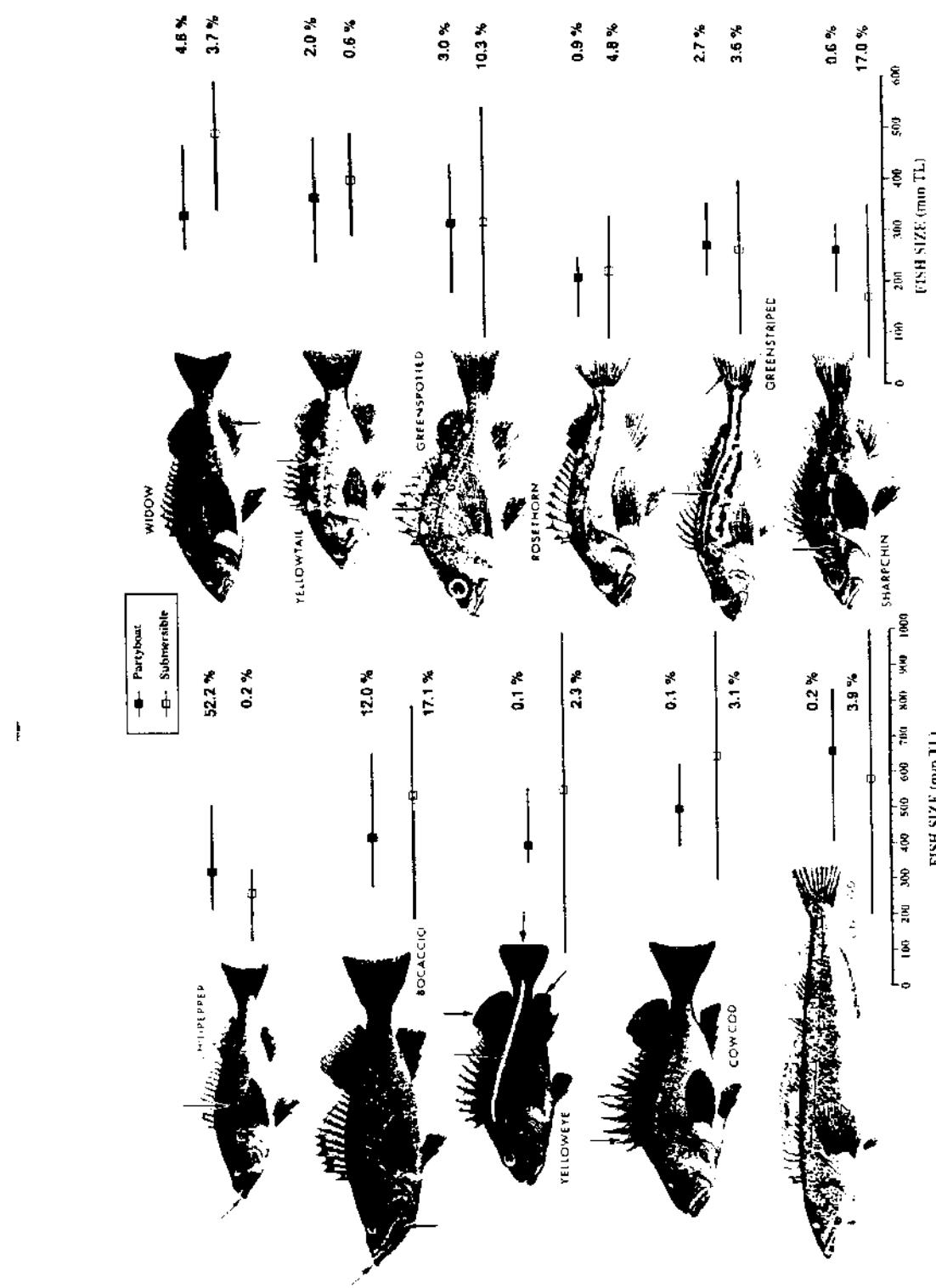


Figure 4. Many species of rockfishes are relatively more abundant and larger in Soquel Canyon than indicated in catch records. Percent abundance, mean and range of sizes are indicated for fishes caught by partyboat fleet and observed during submersible surveys at 90-209 m in Soquel Canyon. Illustrations are from Eschmeyer, Herald, and Hammann (1983).

Our observations demonstrate the utility of submersibles for evaluating fish populations that are closely associated with deep-water rocky areas and unavailable to other methods of evaluation. Manned submersibles provide the scientists with superior field of view to directly quantify abundance and size, and identify difficult species. The high resolution video camera produces a continuous record of observations and allows observers to review their rockfish identifications. Submersible techniques are ideal for surveying potential small-scale refugia without altering the population parameters being quantified.

FUTURE RESEARCH

We are continuing our study of submarine canyons in the Monterey area to compare fish assemblages in lightly- and heavily-fished habitats of similar type and depth. From our recent geophysical survey, we are identifying likely rockfish habitat along the south edge of Monterey submarine canyon and on isolated outcrops in the bay. These ledges have been accessible to substantial and continuous fishing pressure in Monterey Bay for several decades. Using transect techniques and the *Delta* submersible, we will survey fish populations, and compare species and size composition to those from catch records collected by CDFG observers on board Commercial Partyboat Fishing Vessels in the area.

We are currently assessing the likelihood that submarine canyons having structure similar to Soquel Canyon represent significant habitat for rockfishes. Geologic characterization of the headward parts of Monterey, Ascension, Ano Nuevo and Carmel Canyons (particularly from 100-350 m water depth) is in progress, with direct observations from a submersible planned for summer of 1994.

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