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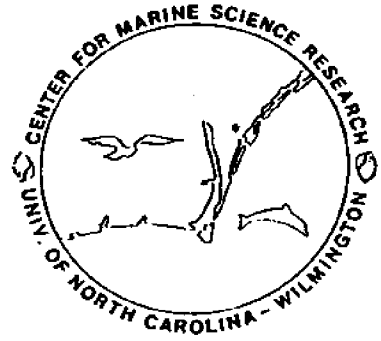

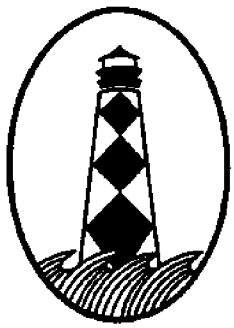

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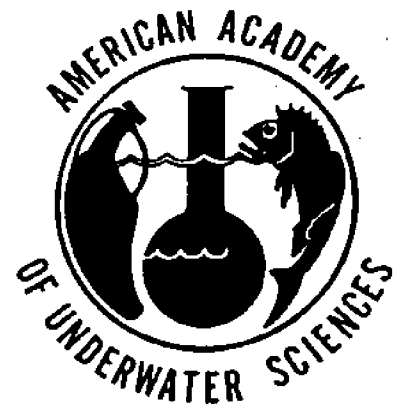

PROCEEDINGS OF THE

AMERICAN ACADEMY OF UNDERWATER SCIENCES
TWELFTH ANNUAL SCIENTIFIC DIVING SYMPOSIUM

September 24-27, 1992
University of North Carolina at Wilmington
Wilmington, North Carolina

LAWRENCE B. CAHOON
EDITOR

American Academy of Underwater Sciences
947 Newhall Street, Costa Mesa, California 92627 USA

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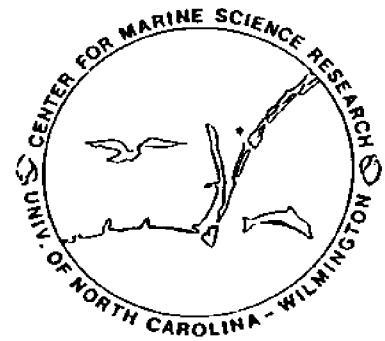

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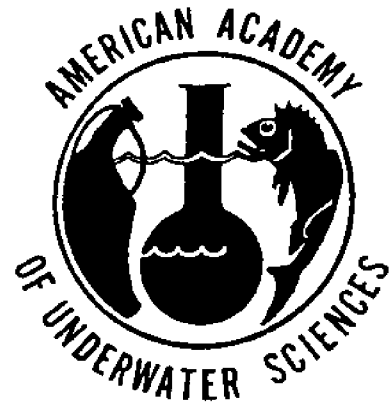

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Proceedings of the
American Academy of Underwater Sciences
Twelfth Annual Scientific Diving Symposium
"Diving for Science....1992"

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Cover illustration by Gloria Crowell.

Introduction

The *Proceedings* of *DIVING FOR SCIENCE....1992* contain 24 papers presented at the 12th Annual American Academy of Underwater Sciences Scientific Diving Symposium, September 24-27, 1992, at the University of North Carolina at Wilmington, Wilmington, North Carolina. The Academy sponsors these symposia to disseminate information and to stimulate discussion on the advancement of undersea science and technology. Diving safety is also an important research and operational focus of the Academy.

The American Academy of Underwater Sciences is recognized as an authority on scientific diving and undersea technology. As such, it has a responsibility to disseminate new information in a published format. The Academy's publications include the proceedings of the annual symposia, proceedings volumes on *Cold Water Diving* (1988), *Dive Computers* (1989), *Safe Ascents* (1990), *NITROX Diving* (1991), *Polar Diving* (1992), and *Repetitive Diving* (1992), technical manuals, diving standards, and a quarterly newsletter, the SLATE. The aforementioned publications are published by and available through the American Academy of Underwater Sciences, 947 Newhall Street, Costa Mesa, CA 92627. The *Diving for Science...1992 Proceedings* contain papers on diving, scientific results, research tools, archaeology, and physiology. Final assembly and camera-ready production of these *Proceedings* was performed by Michael Lang, Smithsonian Institution.

The Symposium was hosted by the Center for Marine Science Research at UNC Wilmington, with assistance from the NOAA National Undersea Research Center at UNC Wilmington. *Proceedings* editor was Lawrence B. Cahoon. Special thanks to reviewers for the *Proceedings* papers: Terry Withers, Bill Kirby-Smith, Steve Mastro, Doug Kesling, Rich Carpenter, Fritz Rodhe, Dave Dinsmore, George Simmons, Richard Laws, Steve Ross, Robert Diaz, John Ogden, George Sedberry, Richard Lawrence, Ivar Babb, Fritz Kapraun, Giselher Gust, Marsh Youngbluth, Steve Blair, Robert Vadas, and Tony Clare. Many thanks are owed to Dana Ward and Jeff Jolly in the Academic Computing Services office at UNC Wilmington for timely help. Michael Lang of the Smithsonian Institution provided critical assistance. We are grateful to UNC Wilmington for the use of their meeting facilities. We thank the Center for Marine Science Research for hospitality and support. We thank the University of North Carolina Sea Grant College Program for its support of this *Proceedings*.

Lawrence B. Cahoon
for the Organizing Committee

Contents

UNDERWATER ARCHAEOLOGY BY BRAILLE: SURVEY METHODOLOGY AND SITE CHARACTERIZATION MODELING IN A BLACKWATER ENVIRONMENT - A STUDY OF A SCUTTLED CONFEDERATE IRONCLAD, C.S.S. GEORGIA

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Advancement in the science and application of underwater remote-sensing instrumentation has provided the archaeologist with the tools to conduct research in a blackwater environment. This paper discusses the development, application, and methodological approach used to conduct an underwater survey and site characterization of a scuttled Confederate ironclad, the CSS Georgia, in a dynamic tidal and zero-visibility riverine environment. Remote-sensing data collected from this Civil War shipwreck have provided interpretative information to help reconstruct the structural and physical integrity of this important historic shipwreck as she lies in 18 m of water in Savannah Harbor, Georgia. Detailed methodological techniques and a zero-visibility archaeological site characterization model are presented. The model to be presented includes the reconstruction of the site through the use of marine remote-sensing instrumentation and the integration of these data to formulate the interpretative framework.

Introduction

Shipwrecks found in United States riverine and coastal environments are generally in waters that have varying degrees of underwater clarity. Many factors interact to reduce water clarity over these wrecks. These factors include turbidity from sediments from surface runoff; biological activity such as algal blooms fed by nutrients entering streams from agriculture, sewage treatment, dredging activities, and their associated discharges; and freshet transport of resuspended sediments and organic debris, to name but a few of these sources. Suffice to say, the prevailing condition most of the year in major rivers and the coastal littoral is poor-to-zero underwater clarity. Depending upon the particular observer these low visibility conditions are typically termed "blackwater" or "zero visibility."

The conduct of archaeological research using standard survey mapping and excavation procedures is difficult if not next to impossible in the most extreme cases of zero visibility. This is particularly so for the wreck site of the CSS Georgia, a Confederate ironclad sunk in the Savannah River (Georgia) in December 1864 (ORN Series I, Volume XVI:482). Located 11 miles above the river bar off Fort Jackson (Fig. 1), the site is constantly covered by fresh-to-brackish, silty water rich in organics. Mixed by 6-10 foot tides, the suspended particles are constantly entrained by currents in the water column (U. S. Army Corps of Engineers, 1982). In addition to poor visibility, water depth, currents, and shipping traffic

make this a difficult site at which to use divers. These conditions have hampered the use of visual recording techniques and fostered the use of instrumental techniques in the survey mapping and characterization of the CSS Georgia site. These techniques in turn have been embedded in a research methodology and organized to provide data and analysis for several levels of inquiry about the vessel and its context.

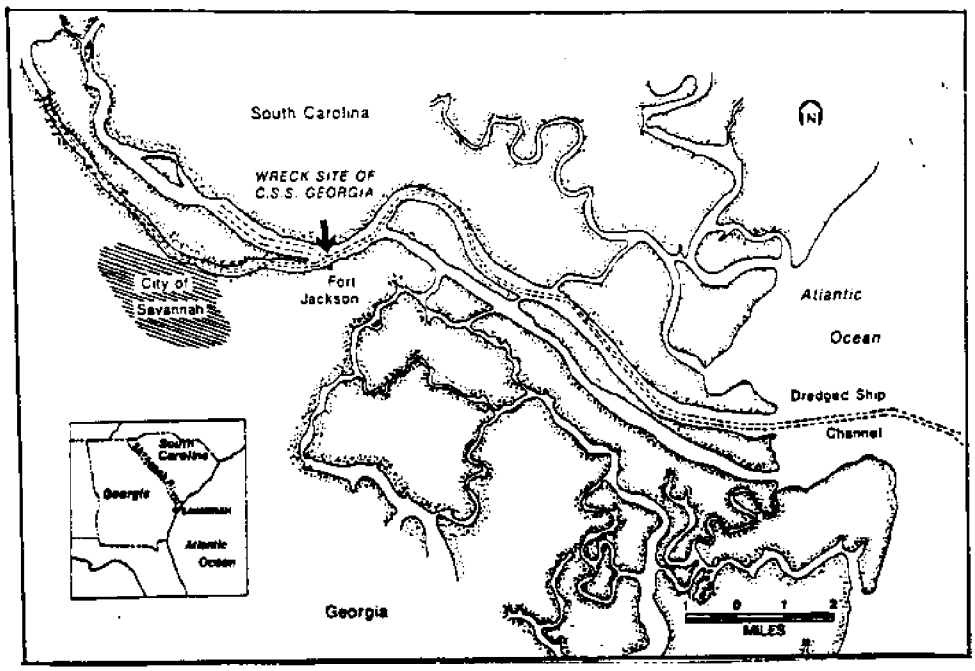

Figure 1. Location map.

Methodology is most correctly defined as the "study of method." It is not the simple explication of a technique or techniques used in the study of an archaeological problem (Pelto, 1970). It is "logic-in-use" involved in selecting particular observational techniques, assessing their yield of data, and relating these data to theoretical propositions. In the case of the archaeological study of the CSS Georgia we have attempted to gain, through a wide range of primary observations, data for a series of generalizations about the vessel and its historical period. To make these observations required the use of instrumental techniques and a subsequent enhancement of their data by graphic and digital means.

Objectives

Our first objective was to characterize the wrecksite of the CSS Georgia in as much physical detail as possible (Fig. 2) within the restrictions placed on us by its zero visibility environment. Other objectives were to:

a) relate these instrumental data on the wreck to historical data on the dimensions and general form of the vessel when in service during the War Between the States;

b) assess the current distribution and orientation of the wreckage; and

c) develop alternate models for the visual display and analysis of instrumental data so as to accomplish best the preceding objectives and improve the rigor and specificity of our methodology for zero visibility archaeological research.

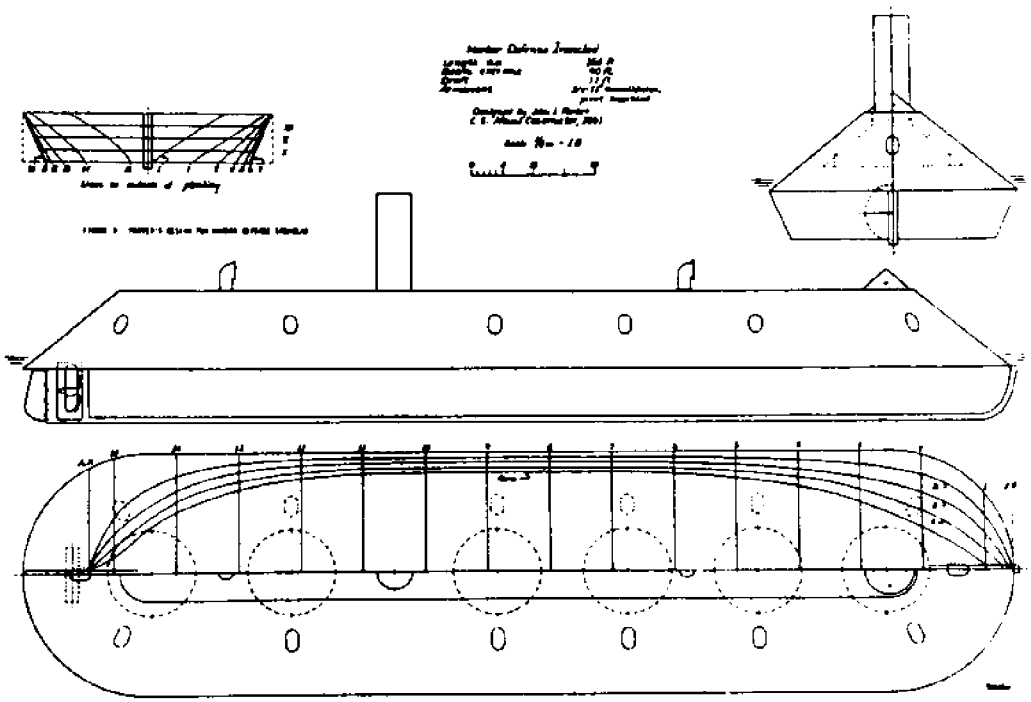

Figure 2. Harbor Defense Ironclad (after Porter, 1861)

Background of the vessel

In November 1864, General William T. Sherman began his "infamous" march to the sea from Atlanta to Savannah. Soon after Fort McAllister was captured by Union troops on December 13, the city of Savannah was pressed on two sides by Sherman and on another by a Union naval blockade. At this time, the Savannah Harbor Defense Squadron consisted of eleven armed vessels–seven gunboats and four ironclads. One of these was the CSS Georgia (Fig. 3). During the final hours of the siege of Savannah, the CSS Georgia was towed to a defensive position across from Fort Jackson to defend the river channel below the city. She trained her batteries against the Union naval advance. Her broadside, facing east, was fitted with two 9-inch Dahlgren smoothbores and two 32-pounder rifles. She also had one 32-pounder mounted in the extremity of the vessel. On her spardeck was a 24-pound howitzer.

Figure 3. Engraving of CSS GEORGIA published in Harpers Monthly, 1863.

Early on December 20, 1864, Sherman's troops captured Fort Jackson (Baker *et al.*, 1981), and the Confederates, to avoid its capture, quickly scuttled the CSS Georgia by opening all of the seacocks. Today, she rests in about 55 feet of black, silty water--remarkably preserved, but broken in her superstructure by harbor dredging activities performed since her sinking. She has proven as formidable in her resistance to destruction by these modern foes as she was in the past.

Built in 1862, her construction, interestingly, was accomplished through funds raised by members of the Ladies' Gunboat Association. These women of Savannah, Augusta, and other Georgia communities contributed over $115,000 for her construction. The need for such a vessel was intensified during the height of the women's solicitation of funds when, in March 1862, the news reached Savannah of the engagement of the USS Monitor and the CSS Virginia (better known as the USS Merrimac). This battle proved to be the turning point in the development of naval warfare in Savannah, just as the battle and the war, in general, proved a turning point in the history of naval warfare all over the world. It pointed out that the most effective defense was the ironclad ship, and the most effective offense was the rifled gun. In this atmosphere of excitement and expectation over ironclads, the CSS Georgia was born (Garrison and Anuskiewicz, 1988:74).

The CSS Georgia was essentially a steam-powered floating battery--a barge-type structure roofed over with wood at an inclined angle and then covered with railroad iron cladding. Such ironclads were, according to various historical accounts, also described as "floating forts." One observer of the CSS Georgia called it "an ironplated monster a la Merrimac" (ORN Series I, Volume XVI).

The CSS Georgia is an enigma because of the discrepancies in her construction details. These discrepancies are directly related to the condition in which she was built during the war. The CSS Georgia remains an enigma and a major source of historical and archaeological data on the "War between the States." As detailed architectural knowledge does not exist for the CSS Georgia, her reconstruction necessarily proceeds on thin ground. The vessel had no keel, was unstable in the water, was too heavy and cumbersome to float without the aid of her engines or to maneuver under her own power (Kollack, 1950), first planned as an ironclad "gunboat," but she was actually used as a floating battery. These criteria present a number of possibilities for consideration in a realistic reconstruction. The CSS Georgia floated for 20 months on the Savannah River, moored near Elba Island where, if the situation required, she could bring her broadside to bear on either channel of the river (Nordoff, 1863). In December 1864, when General Sherman was approaching the city of Savannah on his famous march, the CSS Georgia's fate was decided: as per orders from Commander Hunter, because of her lack of sufficient motive power, she was to be scuttled if Union forces reached Savannah (ORN Series I, Volume XVI:482). On December 20, 1864, the city of Savannah was evacuated and the CSS Georgia was scuttled, making her resting spot for the next century and more on the bed of the Savannah River.

She was considered a "failure" by some contributors and termed a "mud tub" and a "marine abortion" by others (Lawrence, 1961; Still, 1971; Kollack, 1950; Scharf, 1887). Although imperfect and oft times disappointing vessels, such ironclads by their simple presence prevented many a Union thrust at Southern ports (Johnson and Buel, 1962).

Instrumental imaging and enhancement techniques as used on the CSS GEORGIA

Because of the difficulty of mapping the site with conventional underwater archaeology techniques, a variety of remote-sensing methods was applied. These included video, magnetometer survey, tight-beam bathymetric survey, and side-scan sonar surveys. The video proved unable to see through the thick sediment load of the river and was abandoned. The side-scan sonar produced informative images and will be discussed later. The magnetometer and fathometer produced large amounts of data; ten thousand bits of data per run were typical. To image and process such quantities, Amdahl and Prime computers at Texas A&M University were employed, and the final images were plotted on Versatec and Printronix plotters. We looked at three ways of displaying the instrumental database. The magnetometric data were plotted as a curved line contour map only. The bathymetric

data were plotted as 1) curved line contour maps, 2) color contour maps, and 3) three-dimensional grid element contour displays, which could also be used to produce stereoscopic views.

The side-scan sonar data were converted to gray scale by film recording and image enhancement. The combination of these techniques with diver verification of salient features allowed a definition of the nature and extent of the wreck site. This information will serve as an essential part of the planning process for the future evaluation and disposition of the vessel. Plans to enlarge the Savannah River navigational channel will eventually necessitate the removal of the vessel.

A number of features in the aerial view of the river and the city of Savannah to orient successive images should be noted. The river flows from right to left, the main channels being the south or lower one on the left and the north or upper one on the right. The northern shore is South Carolina and the southern shore is Georgia. The wreck is located off Fort Jackson in the middle of the river at the limits of the juncture of the main dredged channel and the channel dredged for the Back River. The angle of intersection of the lines of the back and main channels can be used to orient the other graphics.

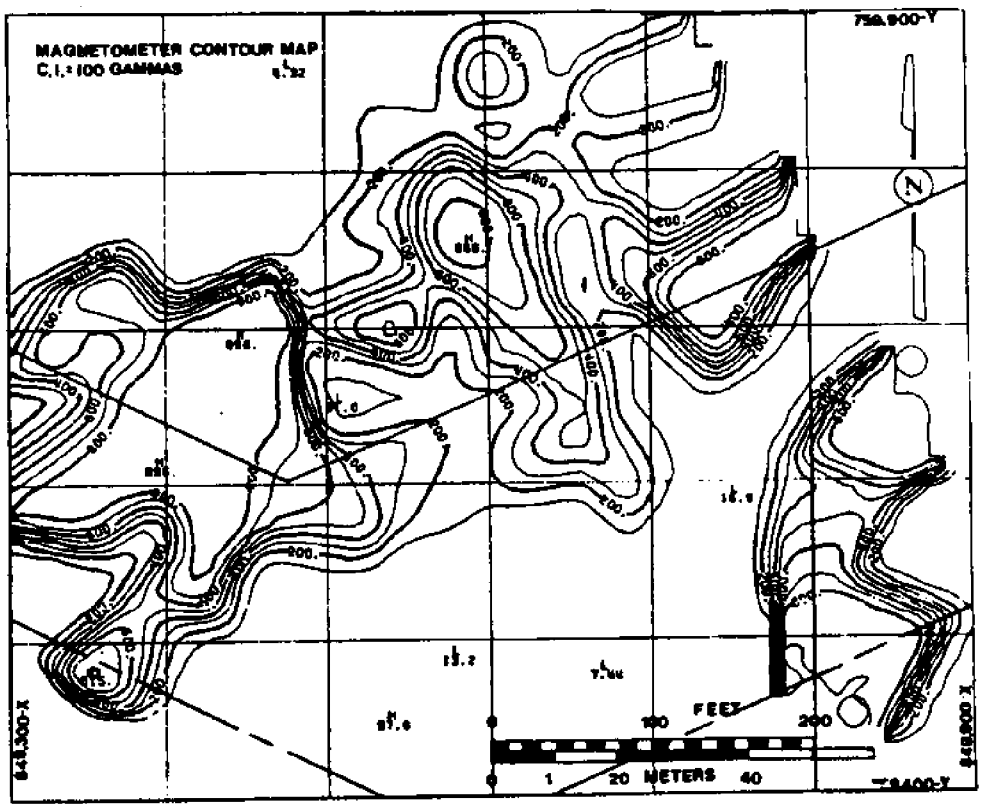

Figure 4. CSS GEORGIA wreck site magnetometer contour map.

A. Magnetometric Survey Data

The first computer graphic is a planimetric presentation of magnetic anomalies at the wreck site (Fig. 4). The contour interval is 100 gammas; each square of the superimposed grid is equal to 100 feet. The anomaly on the lower right is due to a modern anchor that was later recovered; the anomaly on the lower left is the chain and anchor to the wreck buoy marking the site. The other contours record an intense magnetic concentration to the north of the main channel at the point of intersection with the back channel and to the east of that intersection. This information does serve to approximate the location of the wreck; but because the CSS Georgia was an ironclad and thus an object of intense magnetism, it is difficult to obtain detail using this technique. Data for this image were obtained by passing over the site, and taking magnetometer readings in coordination with navigational fixes to

locate positions where the readings were made. These data were then run through a program that performed trigonometry and positioning of readings. Contour maps were produced using a "Conrec" program subroutine (Reid, 1980). This magnetic contouring technique has been used for some years (Breiner and MacNaughton, 1965), and more complex graphics of magnetometric data have been produced (Arnold and Clausen, 1975a; Breiner, 1973, 1975; Upham, 1979; Anuskiewicz, 1985, 1989).

B. Bathymetric Survey Data

1. Curved Line Contour Maps

Fig. 5 illustrates the development of our knowledge as the computer-processed database was expanded. For these maps the same bathymetrically generated database was imaged as a regular smooth line contour map (Fig. 5), with a contour interval of one foot. However, to the uninitiated, contour maps are often difficult to read; even to the experienced, subtle features may escape notice. We also felt an even more dynamic presentation was needed to image features for analysis and use as an orientation model for divers working on the site.

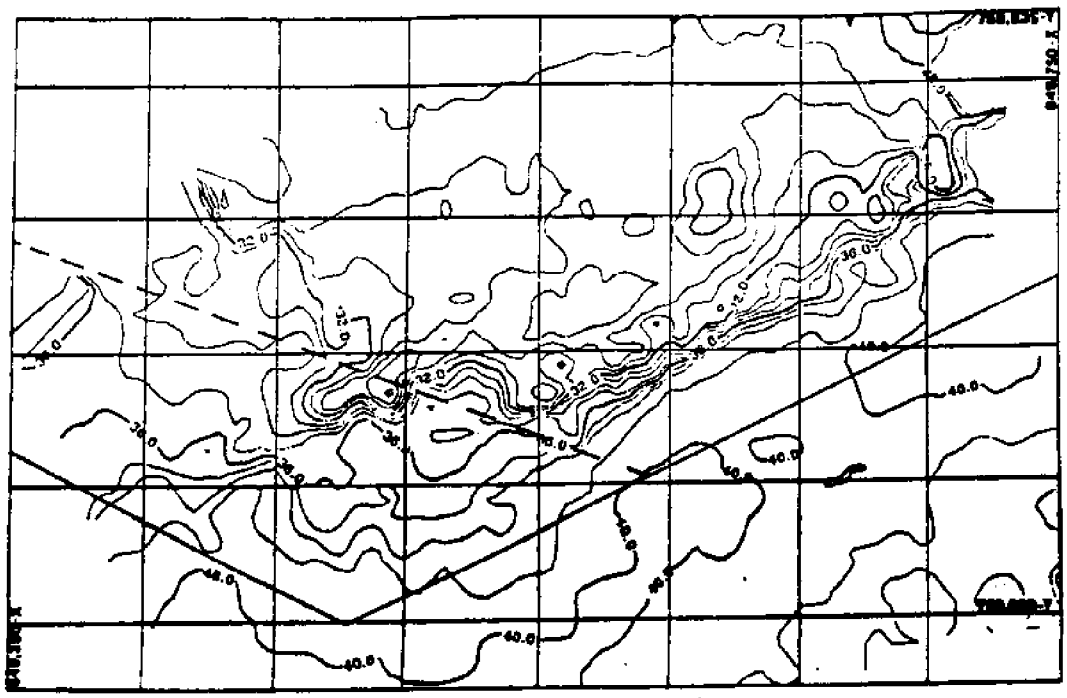

Figure 5. Computer generated bathymetric contour map of the CSS GEORGIA. Contour interval = 1 ft.; grid spacing = 50 ft.

2. Color Contour Maps

The use of color in graphics imaging has the advantages of being visually appealing as well as adding contrast to specific features or areas of interest. By manipulation of a color palette available with many CAD-CAM (computer-aided design-camera) systems, one can assign various hues to a specific parameter, which in our example was relief elevation in feet. In Fig. 5 we can highlight the major area of wreckage and debris scatter down on or onto the adjacent channel slope.

C. 3-d Grid Element Contour Display

Three-dimensional graphics (Fig. 6) translate the data into images that approximate a perspective or isometric view of the actual terrain. In Fig. 6, the CSS Georgia wreck is in the center of the image, looking downstream, with the dredged channel on the right and the undredged river bottom on the left. A second dredged channel comes into the main channel from the left foreground of the image.

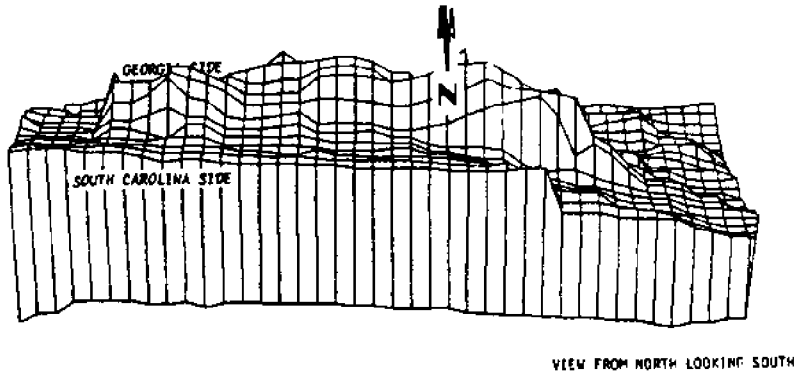

Figure 6. 3-D view of the wreck site looking south.

Three-dimensional graphics programs often allow the user to select the viewing angle, offering the opportunity to "swim" around the site and to study the terrain from any position. Fig. 7 represents the same data base used in Fig. 6, rotated over 90 degrees. In graphics, we speak in terms of x, y, and z axes, with x and y often being directional, such as meters north or east of an origin. The z axis may represent height, as in bathymetric surveys, or some other variable such as magnetism. Both contour and isometric views comprise lines having either constant x values or constant y values, but the isometric view has had contour lines added, lines of constant z value. These contour lines follow two-foot intervals and were generated by a single change to the program used to create Fig. 6.

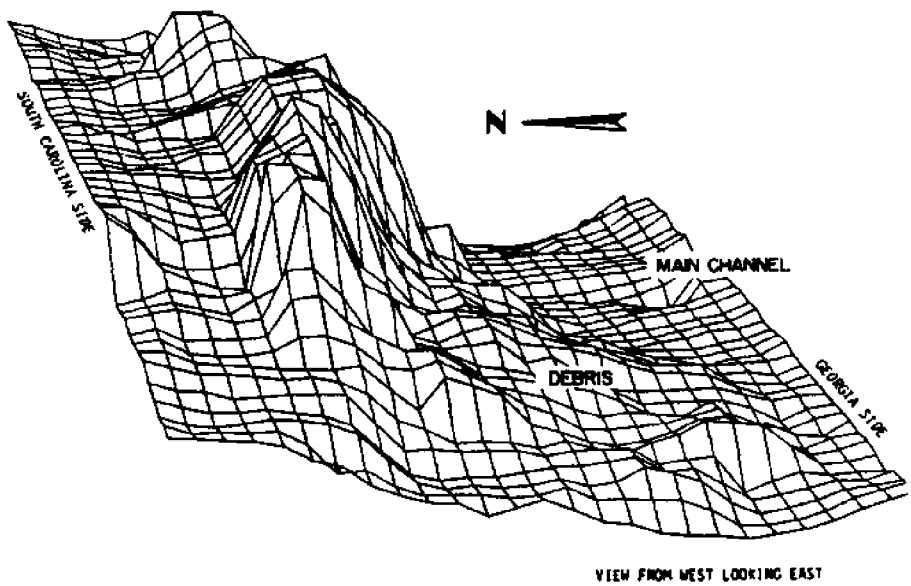

Figure 7. 3-D view of the wreck site looking east.

D. Side Scan Sonar Survey Data

Figs. 8, 9, and 10 are sonographs taken with two different instruments. The difference in the instruments was the frequency of the transmitted pulse, which was 100 or 500 kHz (kiloHertz). Resolution in sonar images is related to the pulse length and frequency. The higher the frequency (hence shorter pulse length), the smaller the object that can be resolved.

The first image (Fig. 8) is an unenhanced 100-kHz sonograph of the wrecksite. The track of the vessel is shown and can be related to previous graphics. Two large pieces of wreckage can be seen with a

gap between them. To examine this view further, a photographic enlargement was made and printed as a positive to reverse the light and dark gray tones. This next view (Fig. 9) shows the enhanced sonograph with areas of no signal return shown as shadow or dark features. This treatment of the sonar image clearly improves the amount and the conceptualization of the detail seen.

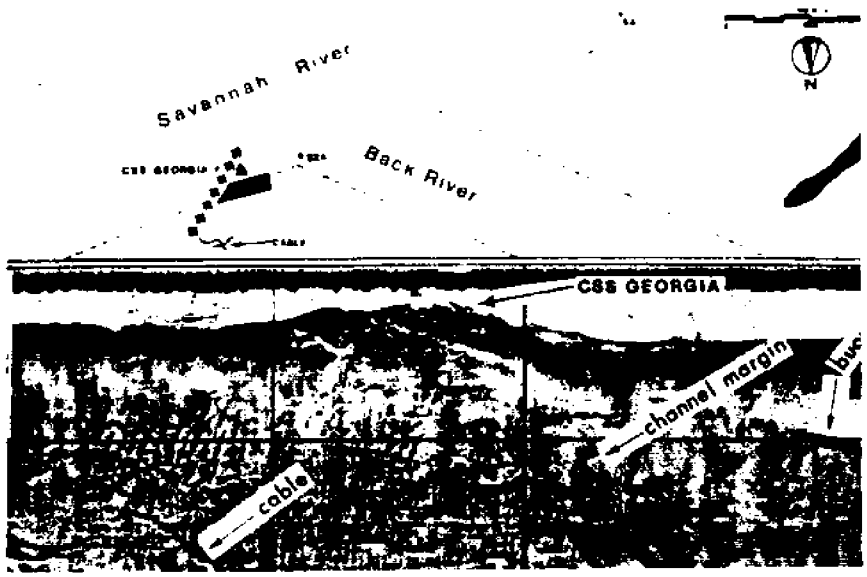

Figure 8. 100 kHz side scan sonar view of the CSS GEORGIA.

Figure 9. Black and white reverse color enhanced sonograph of Figure 8.

The last sonograph (Fig. 10) is a gray-tone enlargement of a 500-kHz image with no reversal of the standard negative presentation. The broken condition of the exposed casemate is dramatically shown in the 500-kHz sonograph of the wreck. Removal attempts in 1866 by blasting reportedly raised 80 tons

of armor from the casemate (Reports of the Chief of Engineers, 1872) and probably produced the condition observed in this view.

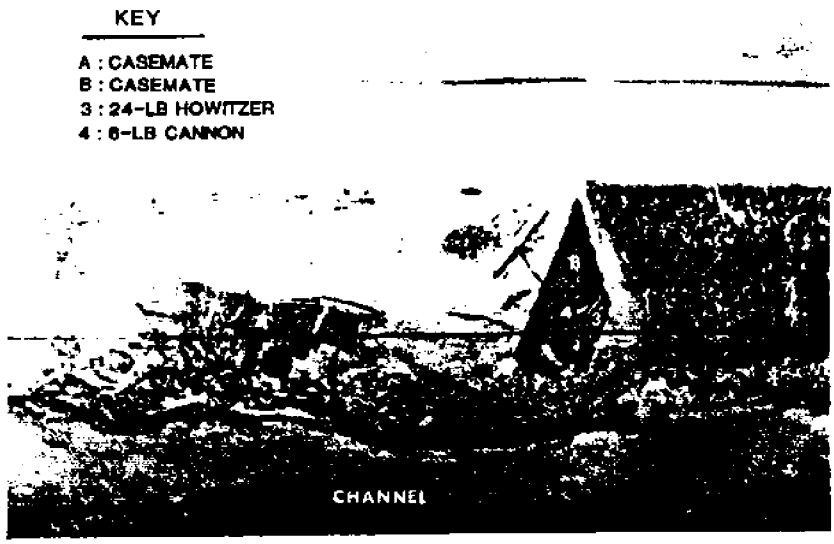

Figure 10. 500 kHz side scan sonar view of the CSS GEORGIA.

Limited diver hands-on correlation of the mapping studies yielded precise locations of major structural features as well as locations of artifacts such as ordnance and ship's gear. All artifacts removed for study were archaeologically mapped prior to recovery. No excavation was done except in the case of ordnance. One hundred shallow-buried Brooks rifled projectiles were excavated by hand to remove them from the main shipping channel of the Savannah River.

Summary

By using the variety of instrumental techniques discussed in this paper we have been able to characterize the zero-visibility wrecksite in some detail, and we have largely accomplished our research objectives.

Our hope to relate the instrumental data to historical data on the CSS Georgia dimensions and general form has proven successful to the extent that we can evaluate more confidently conflicting reports on her length and breadth (Dictionary of American Naval Fighting Ships, 1963; Reports of the Chief of Engineers, 1872; Schomette, 1973; Scott 1862) that the vessel dimensions varied as much as 110 feet in length (150-260) and 10 feet in breadth (50-60). The shape and size of the casemate sections agree very well with the two published drawings of the vessel and with most contemporaneous reports (cf. Garrison and Anuskiewicz 1988). Other details on the vessel such as hull shape and layout of internal decks must await further study such as trial excavations.

The objective to assess the distribution of wreckage was fully met. The magnetometric, bathymetric, and sonar data all converge to give a reliable estimate, in areal terms, as to the extent of the wreckage. The vessel's orientation still remains somewhat of a mystery, as no key indicators such as funnel, pilot house, rudder, or propeller have been identified. The location of the recovered portside 32-lb rifled gun as seen in the data does support an orientation with bow upstream. This interpretation relies heavily on the original deployment of this gun in the vessel's battery and may not be reflective of the late-1864 placement of the reduced number (6 versus the original 10) of guns known for that time (Garrison and Anuskiewicz, 1988).

Our attempt to model the wrecksite instrumentally has been met with mixed success. Naturally, we would like to determine a realistic and accurate likeness of the vessel as it exists and to retrodict her form as she existed in 1864. This we have not done. Computer graphics and image enhancement have filled in large gaps in our understanding of the site; however, as we have pointed out, equally large gaps in archaeological detail remain.

Computer graphics have been used for some time in archaeology (Breiner and Coe, 1972; Arnold, 1974, 1975, 1976, 1979, 1982; Arnold and Clausen, 1975a and b; Kaplan and Coe, 1976; Weymouth, 1976; Weymouth and Nickel, 1977; Frankel, 1980; Garrison and Anuskiewicz 1988; Anuskiewicz, 1989). Arnold has used computer graphics to good effect, imaging the results of several shipwreck surveys in the Gulf of Mexico. Weymouth has done a series of magnetometer surveys and refined techniques for surveying as well as imaging the results of the surveys. He employs SYMAP (Dougenik and Sheehan, 1979), a program from Harvard's Laboratory for Computer Graphics and Spatial Analysis, which uses alphanumeric symbols to define distinctions between contours as shades of grey, lightness and darkness. Harvard is using a three-dimensional program, ASPEX (Hanson, 1980), which can use the same data used by SYMAP to generate images in three-dimensions. SYMAP and ASPEX are user-friendly programs and require no background in computer science to use. Thanks to such pioneering work, computer graphics have become a frequently used interpretive tool.

Our enthusiasm must be tempered by an awareness of the potential perils posed by computer models. There is a tendency to regard an image as the image. That a representation is computer-generated, on glossy paper, with elaborate fonts, in lovely colors can lead us to suspend our critical faculties and lend the image an air of authority it may not merit. When looking at them we should reserve our judgment and study them carefully.

Baker and Garrison (1983) have pointed out many of the underlying problems arising from less-than-real or inaccurate representations of archaeological features from instrumental data. To discuss them in depth here would diverge too far from the central aim of this paper. Factors such as data density, weighting, windows of influence, and grid size are key considerations in the proper modeling of instrumental data (Baker and Garrison, 1983; Barnes, 1973).

The work on the CSS Georgia has been a laboratory for the evaluation of several of these methods. The results have been positive but not perfect. The present study has demonstrated these techniques to be powerful aids to survey methodology in the blackwater environment. These conclusions have even more weight when one considers the dynamic field of computer graphics today. Coupled with the availability of new graphics software and enhancement algorithms, significant improvement in the imaging of buried and black water archaeology sites is at hand. A recent example of this is the impressive imaging of archaeological magnetic data by Scollar *et al.* (1986). These techniques give the nautical and terrestrial archaeologist alike new conceptual tools for developing interpretative frameworks for instrumental data.

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VISUAL CENSUS AS A MEANS TO ESTIMATE STANDING BIOMASS, LENGTH, AND GROWTH IN FISHES

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A point-count visual census technique was used, in situ, to estimate population size as well as length of individual fish during the summer months off the Canarian Islands of El Hierro in 1989 and Fuerteventura in 1990. These data were used to determine fish standing biomass to assess and monitor the environmental health of the inshore habitat. The standing biomass for parrotfish, Sparisoma cretense, off El Hierro (an island as yet undeveloped by the tourist industry) was 399 - 409 g/100m². The more heavily developed and populated island of Fuerteventura had a standing biomass of only 209 - 228 g/100m² for this same species. The length-frequency data indicated parrotfish growth in 1989 may have been an artifact owing to the ability of in situ observers to determine fish length precisely, especially in larger fishes. However, an increase in length was also noted for rainbow wrasse, Coris julis, during the same five week period. This suggests that it is possible for observers to determine growth, in situ, when the length classes are small and/or the number of fish observed is large enough to account for increased variability in the data set caused by in situ observation.

Introduction

Visual census techniques have recently gained acceptance in preference over surface tended techniques in gathering community and life history data on fishes dwelling at inshore, demersal biotopes (see reviews by Barans and Bortone, 1983; Harmelin-Vivien *et al.*, 1985; Bortone and Kimmel, 1991). This preference is based on several features. For example, visual census techniques are non-disruptive to the habitat (Bardach, 1959). They permit repeat assessments of the community without

removal or handling of the organisms (Bortone and Kimmel, 1991). In addition, they are generally less selective than most other sampling techniques (Brock, 1954).

Visual assessment techniques are not, however, without their own inherent problems and limitations. These techniques are usually employed in situ, requiring participants to use SCUBA. Depth and observation time are, therefore, often limiting factors in their implementation. These limiting factors can be somewhat relieved by using mixed gases with SCUBA, remotely operated vehicles (ROV's), or submersibles equipped with video recorders but then there is a concomitant increase in expense and technical skill with their deployment.

Important to this discussion, however, are some of the problems underlying the quality, type of data, and utility of data collected by visual census techniques. Virtually every researcher employing a visual survey technique has used different sample design parameters with regard to area, amount of sample time, and survey protocol (Bortone and Kimmel, 1991). This has made effective comparisons between studies by different authors nearly impossible. Many studies have gathered fish data with regard to the number of individuals and their size. While it is known that participants can practice this ability and develop skill at making accurate assessments as to these parameters (Bell *et al.*, 1985; DeMartini *et al.*, 1989) there still remain questions regarding the accuracy and precision of these data in establishing population and community parameters (Greene and Alevizon, 1989).

We were afforded an opportunity to conduct visual assessments on the inshore fish fauna off the islands of El Hierro and Fuerteventura, respectively, in the Canary Islands during the summers of 1989 and 1990. The oceanographic conditions and spatial isolation from a continental land mass has led to the formation of a unique faunal assemblage that is in danger of being overexploited due to a rapidly developing tourist industry. A detailed analysis of the total fish fauna off El Hierro (Bortone *et al.*, 1991) indicated the parrotfish, Sparisoma cretense ("Vieja" in Spanish), is one of the more abundant species specific to the inshore fish fauna. This species is also recognized as being economically important to the local fishing community (Perez, 1979; Guzman, 1982). Unlike most other parrotfish species this species is gonochoristic and displays no evidence of sexual inversion in the Canary Islands (Gonzalez, 1990). Nothing is apparently known about other aspects of the reproductive behavior or territoriality of the vieja. Our initial study hinted that the changes in fish length in the parrotfish population during the sampling period may have been due to growth. As indicated above, visual surveys have attained recognition as being an important and perhaps "improved" method for gathering life history and population parameters of inshore fish species. In the present study we present standing biomass mass estimates for parrotfish which can be used to compare the relative productivity and fishery conditions among the Canary Islands. In addition, we present a more careful investigation into the data obtained on fish length from visual surveys and their utility in determining in situ growth in another species. The data presented for this study are part of an ongoing assessment of the fishes and fishery resources of the Canary Islands (Bortone *et al.*, 1991).

Materials and Methods

Description of the Study Area:

The Canary Islands are composed of seven inhabited islands located 100-450 km off the northwest coast of Africa. The inshore substrate is composed principally of basalt, sand, or basalt and sand; the variation in substrate being associated with average wind and direction as well as proximity to the Continent. Two islands were surveyed in this study. El Hierro, the smallest and southwesternmost island, is the most remote and least commercially developed of the inhabited islands and has an inshore substrate composed principally of basalt. Fuerteventura, the second largest island located only 100 km off the African coast, has an inshore substrate of basalt sand. See Yanes (1984) and Bacallado *et al.* (1989) for a more complete description of the Islands.

Survey Method:

We employed a point-count visual survey technique described by Bortone *et al.* (1989) in which a diver using SCUBA occupied the center of a circle having a radius of 5.6 m and, turning slowly, recorded the number of individuals by species for a 5 min time interval. In addition, length (total length, TL) was estimated for each fish to the nearest 1 cm for fish less than 10 cm TL and to the nearest 5 cm for fish larger than 10 cm TL.

Biomass was estimated by calculating weight from estimated length as suggested by Brock and Norris (1989) using the length-weight relationships for the parrotfish determined by Perez (1979) and Gonzalez *et al.* (1986). Additionally, an examination of length-frequency was conducted using length-frequency/sample date information from histograms constructed with the CoHort graphics package (CoHort Software, Berkeley, California, USA).

To examine the potential for growth occurring among parrotfish, fish were grouped into class intervals of 5 cm according to color type. These types included: Red - mature females, bright yellow and red with a grey shoulder patch; Mottled - probably immature females, blotched pink and grey in no discernible pattern; Dot - mature males, grey with a distinct humeral spot; and Grey - probably immature males, grey but lacking a humeral spot. The potential for growth was also examined for the rainbow wrasse, Coris julis ("Doncella" in Spanish) for fish less than 10 cm TL in 1 cm size class intervals. Five time intervals, each approximately one week in duration, were established to relate fish length to time. These time intervals began on 7 July and ended on 15 August in 1989 off El Hierro and in 1990 off Fuerteventura for parrotfish and in 1990 off Fuerteventura for rainbow wrasse. The analysis on length-time herein was done by examining modal length rather than mean length because the length-frequency distributions were often multimodal and the variance for length was high (Bortone *et al.*, 1991).

Results

Standing biomass:

In a previous study that determined the standing biomass of parrotfish off El Hierro, Bortone *et al.* (1991) noted that the average total length of 7001 parrotfish observed in 1045 visual surveys was 15.79 cm. Using the equations of Perez (1979), where $W = 0.0135\ L^{3.05}$ based on 435 specimens (W = weight in g, L = total length in cm), it was determined that the average standing biomass for parrotfish during the summer of 1989 off El Hierro was 409.08 g/100m^2. A slightly lower value of 398.74 g/100m^2 was calculated using the length-weight regression equation of Gonzalez *et al.* (1986), where $W = 0.0135\ L^{3.0222}$ based on 1235 specimens. In comparison, during the summer of 1990, off Fuerteventura, we observed 1259 parrotfish in 907 samples having an average of 21.84 cm TL. The standing biomass of parrotfish during the summer of 1990 off Fuerteventura was 228.00 g/100m^2 and 209.27 g/100m^2 when using the equations of Perez (1979) and Gonzalez *et al.* (1986), respectively.

Growth:

Bortone *et al.* (1991) indicated a statistically significant trend for the population of certain color types of parrotfish to increase in length with time (*i.e.*, week in which sample was recorded). An examination of this relationship using histograms (Figs. 1 - 4) reconfirms this observation during 1989 off El Hierro for the color types Red and Dot. The Red parrotfish showed a 5 cm increase in length from a mode of 12.5 cm TL (class median) to 17.5 cm TL during the 5 week study period. Dot also showed a 5 cm increase in length from 17.5 cm TL to 22.5 cm TL during the same time period. The other color types, Mottled and Grey, did not display any obvious increase of fish length with time.

The data on fish length, which were gathered by visual census in 1989, indicated that growth may have been observed. Examination of a similarly collected data set from an identical time period presents an opportunity to test this hypothesis. However, none of the color types displayed obvious shifts in modal fish length with time (Figs. 5 - 8). The modal peak for Red was consistently 22.5 cm TL

Figures 1 - 4. Length-frequency histograms with regard to week of sample for parrotfish from El Hierro during 1989.

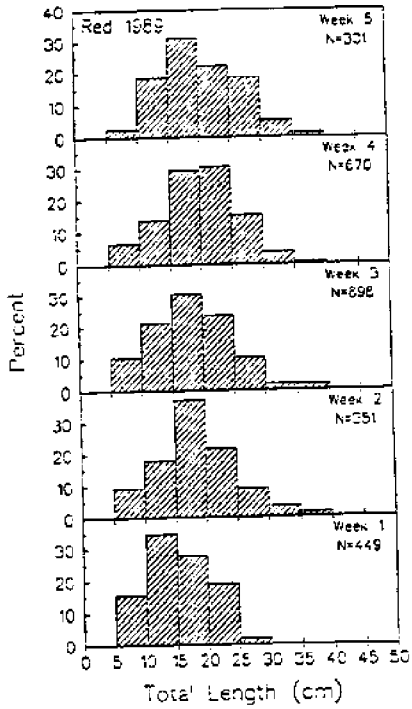

Fig. 1. Red color-type.

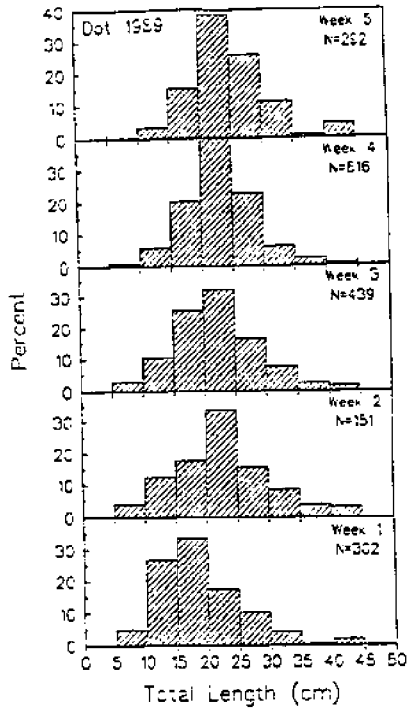

Fig. 2. Dot color-type.

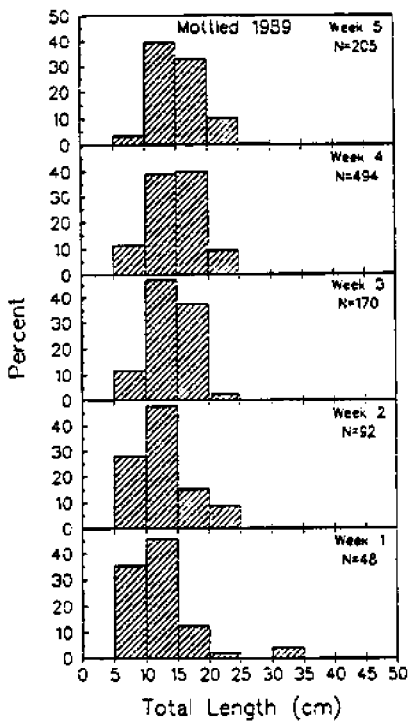

Fig. 3. Mottled color-type.

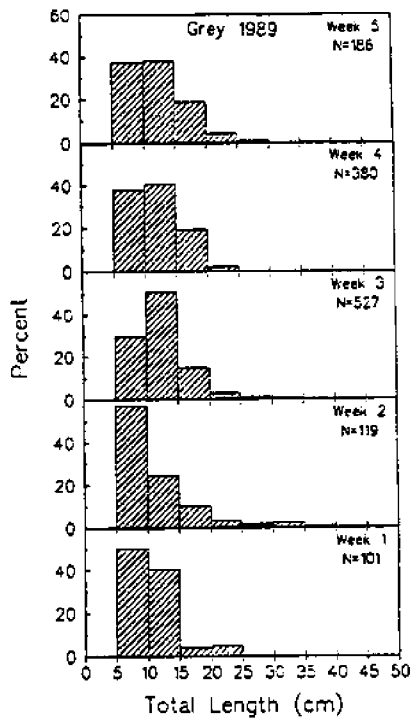

Fig. 4. Grey color-type.

Figures 5 - 8. Length-frequency histograms with regard to week of sample for parrotfish from Fuerteventura during 1990.

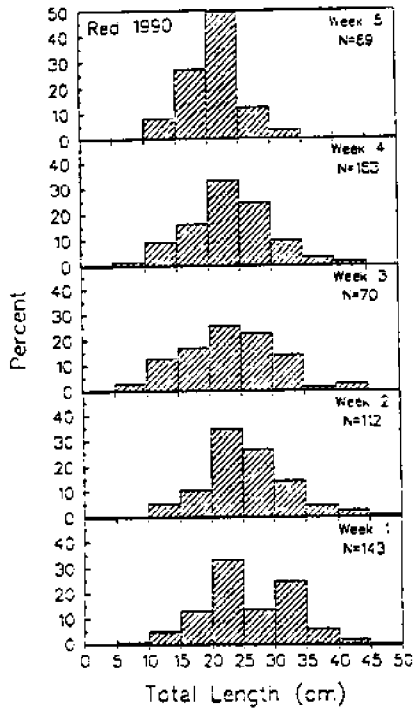

Fig. 5. Red color-type.

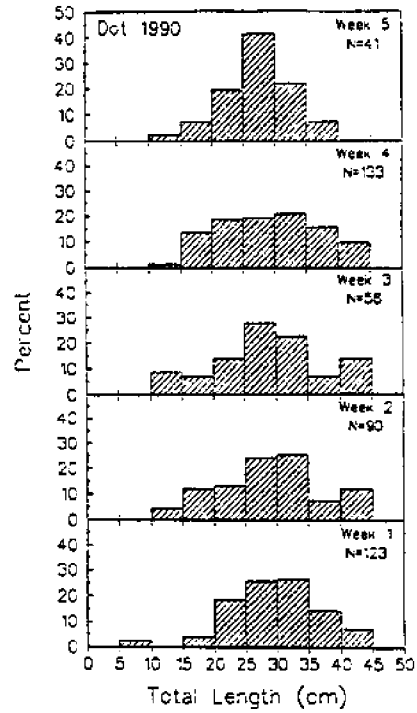

Fig. 6. Dot color-type.

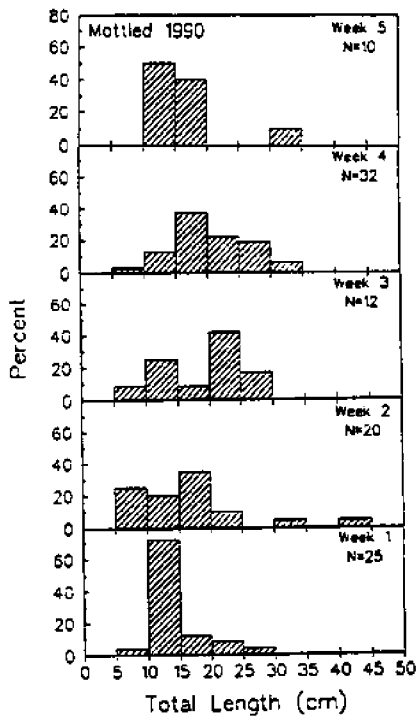

Fig. 7. Mottled color-type.

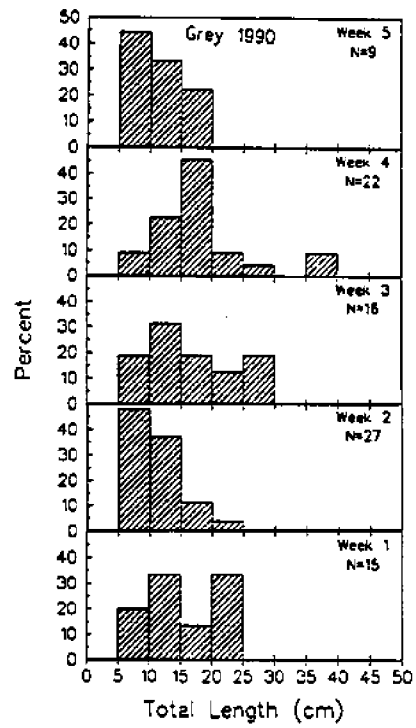

Fig. 8. Grey color-type.

throughout the study while modal length for Dot and Grey were varied and showed no distinct trends with time. A possible exception may occur for Mottled. The population of this color type showed a tendency toward increasing from 12.5 to 17.5 cm TL for the first four weeks but then reverted to 12.5 cm TL for the last week of observation.

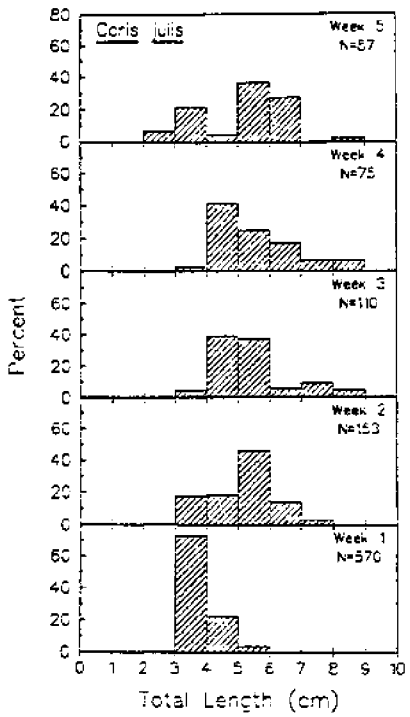

Fig. 9. Length - frequency histogram with regard to week of sample for rainbow wrasse, Coris julis, from Fuerteventura during 1990.

To determine further if fish growth could be observed with visual census we examined the length-frequency/time relationship for rainbow wrasses. As the rainbow wrasse were generally quite small in the study area we examined the length-frequency/time relationship to the nearest 1 cm. An inspection of this relationship (Fig. 9) indicates a distinct shift in length from 3.5 cm TL in week 1 to 5.5 cm TL by week 5. The length-frequency histogram for week 5 also indicates the presence of an additional peak at 3.5 cm which may be indicative of recruitment of a new group of juveniles to the population.

To determine if this may have occurred, we present a length versus number-of-fish-per-sample histogram for each week (Fig. 10). There was a definite tendency for the number of individuals in the population, as measured in units of number-of-fish-per-sample, to decrease dramatically between weeks 1 and 4. During week 5 there was an increase in the number of fish per sample which may also be indicative of recruitment.

Discussion

The standing biomass parameters indicate the utility that such data may have in describing the relative environmental condition or "health" of an area. A comparison of the population data for parrotfish indicates a substantial difference between the two islands with regard to the number, average size, and standing biomass of this species. These differences reflect an anticipated difference relative to the degree of perturbations in the surrounding waters of each island. Further studies may make use of these parameters as it becomes more necessary to carefully monitor the biological fitness or condition of this and other parts of the world.

The initial study by Bortone *et al.* (1991) indicated the potential for determining growth among parrotfish by gathering data on fish length with visual census techniques. Reexamination of this tendency does not lend support to this idea, however. Our hypothesis, that visual census can be useful in determining growth among parrotfish must be rejected, based on the evidence presented herein. It should be noted, however, that parrotfish growth is rapid during the first few years of life (17 cm during the first year, 9 cm during the second year, and 6 cm in the third; according to Perez, 1979). Given this rather rapid growth rate it is entirely possible to recognize growth visually in a 5 week sampling period during summer months in which the growth rate is usually greatest.

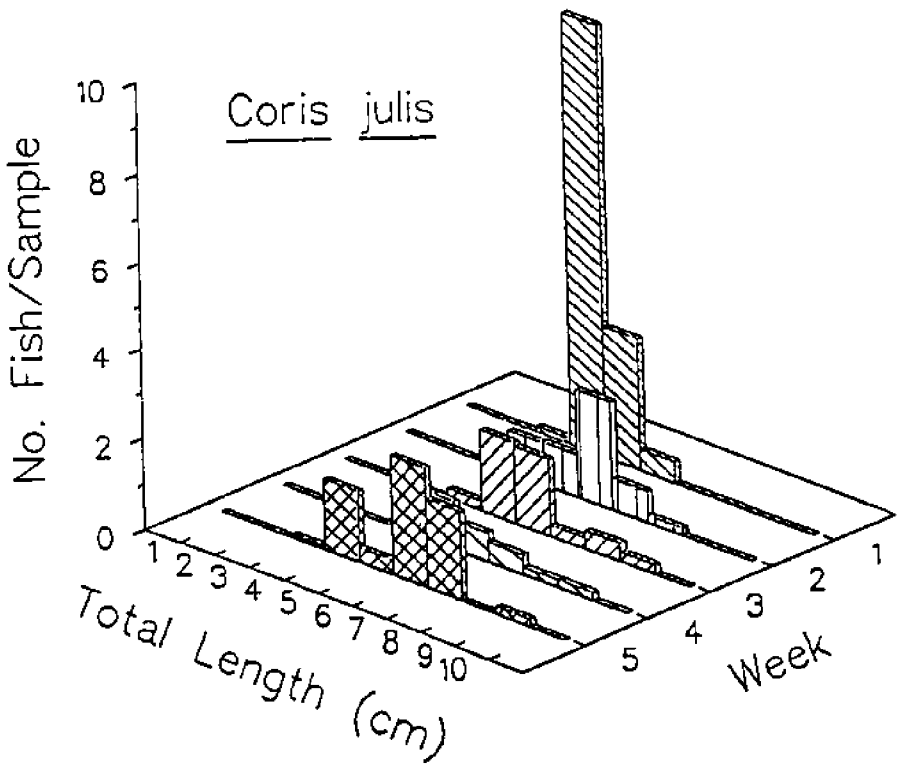

Figure 10. Length versus number-of-fish-per-sample histogram with regard to week for rainbow wrasse, Coris julis, from Fuerteventurra during 1990.

One explanation for our failure to observe growth using visual census in 1990, other than the fact that growth may not have occurred or the observers cannot accurately assign fish to 5 cm length intervals, is that the numbers of parrotfish off Fuerteventura were fewer. A total of 1259 fish (1.39 fish/sample) were observed as opposed to 7001 (6.70 fish/sample) during the same time interval off El Hierro in 1989. Only 597 Red and 455 Dot parrotfish were observed in 1990 compared to 2669 and 1800 fish of these color types in 1989, respectively. It may be that the visual technique requires a great deal more data than a typical length-frequency/growth analysis due to a higher variance in length parameters imparted by in situ observation.

We support this statement by noting that growth seems to be observable, in situ, among rainbow wrasses. These fish are small and growth is potentially more apparent in percent change in length per time interval. In addition, we were able to obtain length data on 995 rainbow wrasses during the study (nearly twice the number for any of the parrotfish color types in 1990). This could indicate that this number was adequate to observe growth if we assume that the increased length among rainbow wrasses were, in fact, due to growth.

It is intriguing to consider the potential utility that visual census data could have to assess recruitment. In addition, the rapid decline in the number of rainbow wrasses observed per sample may be indicative of high predation on these juveniles. The data presented for the rainbow wrasse indicate several avenues of research worthy of pursuit.

One must be cautious in interpreting these observations, however. Many aspects of fish behavior could have contributed to the appearance of growth, such as size- and sex-specific migration by the fish. Simultaneous procurement of specimens coupled with extensive visual assessments should provide the data to validate the implication that visual surveys can provide growth information on fishes.

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ENVIRONMENTAL SAMPLING TOOLS DESIGNED FOR USE ON A LOW COST REMOTELY OPERATED VEHICLE (LCROV)

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One of the tools most frequently utilized by the National Undersea Research Center at the University of North Carolina at Wilmington (NURC/UNCW) is a low cost remotely operated vehicle (LCROV or ROV). In the past, the primary functions of this ROV have been visual reconnaissance and data sampling via electronic sensors. Due to the development of the devices discussed in this paper, the ROV now has the additional capability of obtaining and storing multiple samples of seawater and some types of seafloor. Two water samplers and two seafloor samplers have been designed and fabricated to operate as an extension of an existing ROV mounted manipulator arm. The devices are controlled by one or more of the existing manipulator motors. Each of these tools are intended to be part of a growing resource pool of interchangeable ROV-based instruments for use by the Center in its support of undersea research.

Introduction

Exploration in an alien environment, whether under the sea or on some distant planet, can be greatly enhanced by the use of remotely operated or autonomous robotic devices. Such devices allow scientists to obtain information, collect environmental samples, and perform tasks at sites where extended human presence is difficult, dangerous, or impossible. An example of this type of device is the remotely operated vehicle (ROV) used by the National Undersea Research Center at the University of North Carolina at Wilmington (NURC/UNCW) in its support of undersea research. As the result of a joint venture between the Mars Mission Research Center at North Carolina State University (MMRC/NCSU) and NURC/UNCW, four research tools were recently developed for use with this ROV system. These devices will allow the ROV to accomplish tasks that previously required some other system. These new research tools are the focus of this paper.

The ROV, a Deep Ocean Engineering Super Phantom II, was purchased by the Center in 1987. This highly portable vehicle has since been used over 300 hours in support of more than 25 different science missions. During this time, the principal function of the ROV has been to collect scientific information using video cameras and electronic environmental sensors. Through the use of a simple claw with two-

degree-of-freedom positioning, limited environmental sampling has been possible. Due to the technical needs of the Center-funded science community, NURC/UNCW personnel decided to develop a selection of interchangeable tools that would allow the ROV to collect a variety of physical samples. With the ROV and these new tools, a scientist could simultaneously view a research site, measure a variety of in situ physical properties, and collect multiple samples of the environment in question.

The technical expertise that made this tool development possible was found primarily in a group of students and faculty at the North Carolina State University (NCSU) component of the Mars Mission Research Center (MMRC). After initial discussions between representatives of the two research centers, a decision was made by NURC/UNCW to fund the design and fabrication of four prototype sampling tools by a group of sixteen NCSU undergraduate students and their faculty advisors.

Design Requirements

The students were given a list of constraints including:

- Each sampler must attach to the existing manipulator arm in place of the existing simple claw.
- Each sampler must be actuated only by existing manipulator motors.
- The samplers must operate at depths of up to 1000 ft.
- The tools must be durable. Reliability of the devices during multiple operations at sea must be insured.
- Maintenance and repair must be easily accomplished in the field with standard components.
- Drag on the ROV system should be kept to a minimum by limiting the size of the device(s).
- Each water sampler must collect six separate 100 ml water samples, each on the command of the operator.
- Each seafloor sampler must gather three separate 100 cc sand or mud samples, each on the command of the operator.
- Each seafloor sample must be obtained from the top 5 cm of sand or mud.
- Once collection is complete, sample containers should be sealed to prevent contamination.
- The fabrication costs of each sampler, including materials and machining, should be under $1000.
- Each device is to have a target in-water weight of 3 lb. The water sampler has an allowed tolerance of 0.25 lb. The bottom sampler has an allowed tolerance of 0.5 lb. Similar weights for each device allow for the quick exchange of tools without a required alteration of ROV trim.

Additional desired characteristics included the visual confirmation of sample capture, limited requirements on the operator, and the ease of sample removal at the surface.

Based upon these requirements and desired characteristics, two student design teams for the water sampler and two student design teams for the seafloor sampler were assembled. These teams underwent a series of design reviews attended by their faculty advisors and NURC/UNCW personnel. During these reviews, the design basis of each team was critiqued and potential problem areas were discussed.

Following this review process, each design team performed structural analysis testing, completed fabrication of the sampler prototypes, documented their designs, presented a seminar on operational procedures, and assisted in the integration and testing of each device at NURC/UNCW. All four teams were successful in their efforts. The following section of this paper demonstrates two solutions to each design problem. Experience gained during field tests of these devices during the upcoming NURC/UNCW operational season will prove valuable in the future development of additional environmental sampling tools.

Results

LCPD Water Sampler:

The LCPD water sampler (Fig. 1a,1b) incorporates six separate syringe mechanisms into one turntable structure. Each of these sample containers is fitted with a check valve and a drain plug. The actuator originally intended to rotate the manipulator about its longitudinal axis is now used to select the syringe to be operated. The actuator designed to control the operation of the claw pulls the selected piston. The movement of this piston creates a pressure differential that opens the check valve and draws water into the sample chamber. Multiple seals are used throughout each syringe to ensure that each sample remains uncontaminated.

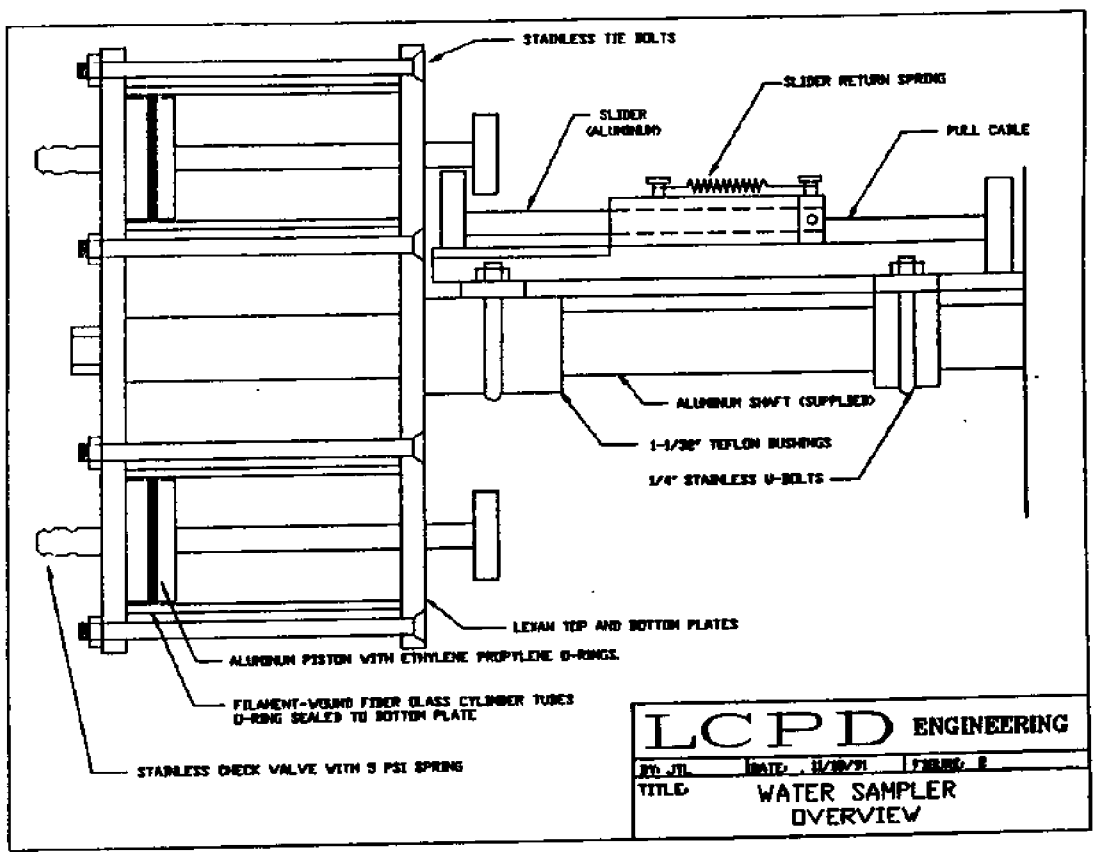

Figure 1a. LCPD Water Sampler (side view)

The turntable body consists of six wound fiberglass tubes sandwiched between two circular plates of lexan. The apparatus is held together by stainless bolts spaced around each cylinder. The rest of the structure is aluminum with the exceptions of Teflon bushings, one stainless steel spring, and ethylene

propylene o-rings. One stainless steel 5 psi check valve and screwtop drainplug are located at the end of each cylinder. High density syntactic foam is used to fulfill the in-water weight requirements.

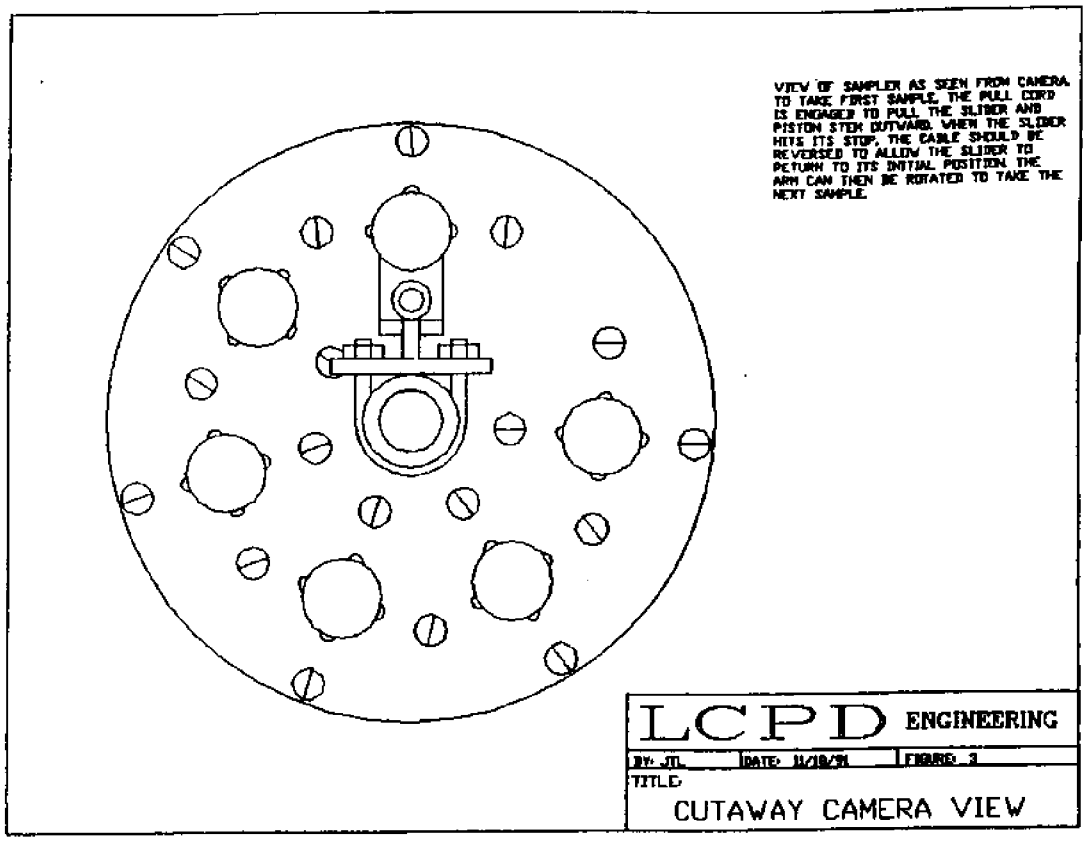

Figure 1b. LCPD Water Sampler (camera view)

Once the ROV is on site, water collection is a simple process. The operator, relying on video or a measured rotation time period, selects a cylinder by rotating the tool. On the command of the operator the piston is fully retracted. As a result, the check valve opens and water fills the chamber. The operator must then allow the sliding actuator to return to the ready position. This process is continued until up to six samples have been taken. After recovery of the ROV, the samples are retrieved by removing each drain plug and manually depressing each piston head. A distilled water rinse of the syringes is all that is required to prepare the device for reuse.

NVLA Water Sampler:

The NVLA water sampling tool (Fig. 2a,2b) consists of a long cylinder containing a multistage plunger. This combination of plunger and cylinder form six sample chambers. When one or more of these chambers is located in the open portion of the cylinder, circulation holes allow free flow between that chamber and the surrounding water. As the plunger assembly is moved, these chambers shift into the closed area of the cylinder. Since this area has no circulation holes, the samples are confined until removal on the surface. The plunger is moved by a drive mechanism similar to that of a caulking gun. The plunger shaft fits through a hole in one end of a stainless steel lever. When the claw pull motor is activated, a cable pulls on the free end of this lever. The lever first rotates and then locks on the shaft. Continued operation of the motor slides the plunger until one chamber is moved from the open section to the closed section of the cylinder. Release of the cable allows a spring to return the lever to its original position. This mechanism ensures the capture of a single sample per activation without the need for visual confirmation. To allow removal of the samples, six drain plugs are located in a bar attached to the outside of the closed end of the cylinder.

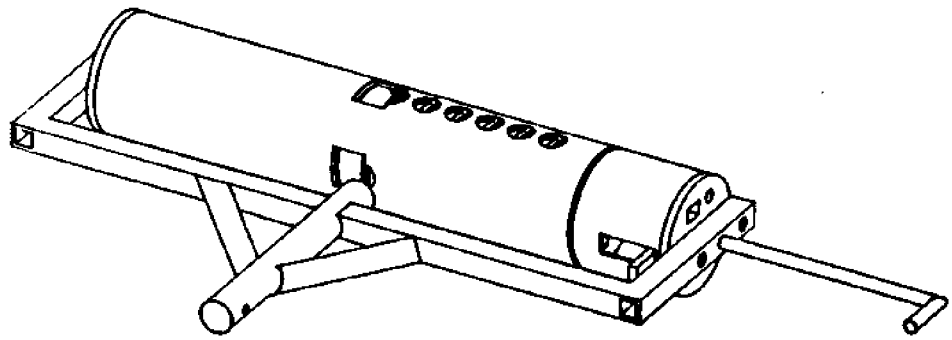

Figure 2a. NVLA Water Sampler.

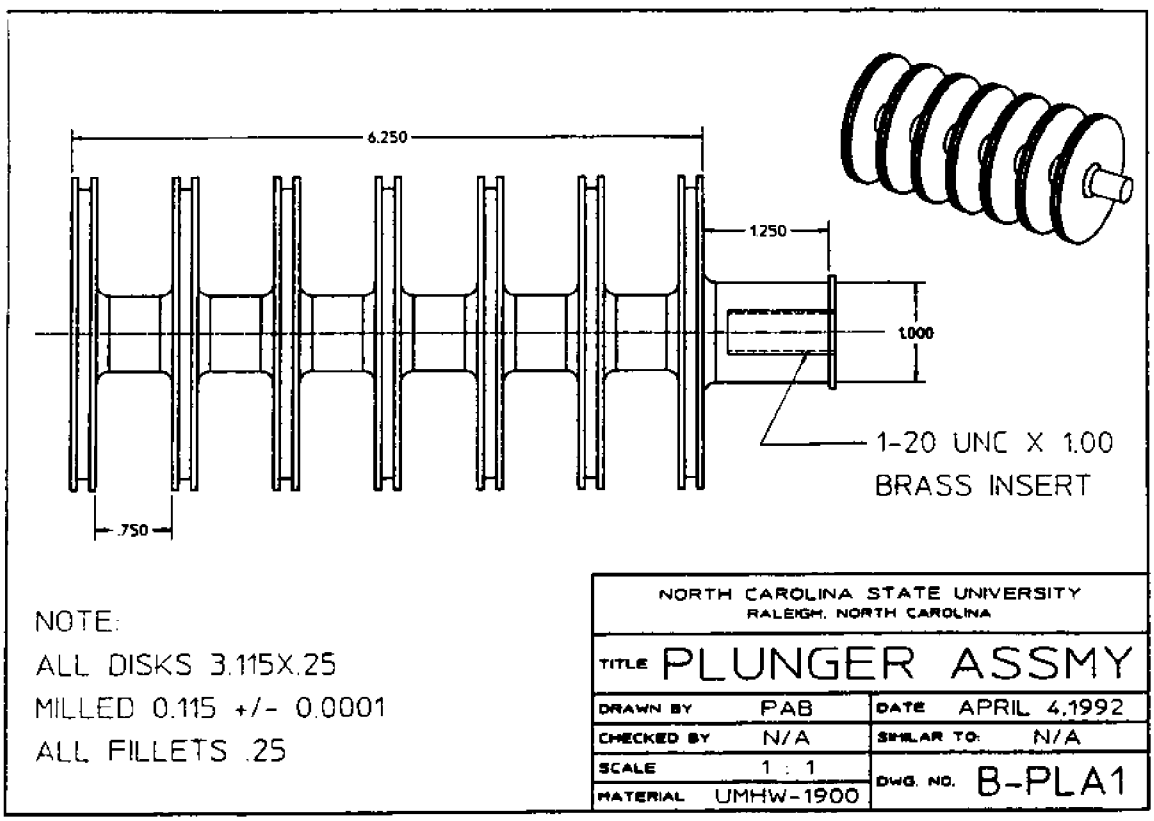

Figure 2b. NVLA Water Sampler Plunger Assembly.

Both the cylinder and the plunger are machined from low friction 1900 UHMW polymer. Silicon O-rings are used to separate the sample chambers. The incrementing device, illustrated in Fig. 4, consists of a stainless steel push rod, spring, and lever arm encased in a UHMW cylinder.

This tool is prepared for operation by sliding the plunger so that all chambers are in the open section of the cylinder. Once on site, the operator actuates the plunger using the claw pull motor. This process is repeated until up to six samples have been collected. Once the ROV is on the surface, the samples can be recovered with a syringe through the use of drain plugs fitted into the side of the cylinder.

Tri-Scoop 1000 Seafloor Sampler:

The Tri-Scoop 1000 seafloor sampler (Fig. 3) is a disk structure with three cups equally spaced around its perimeter. These cups have spring loaded lids that close automatically after a sample is taken. The manipulator elevation control motor lowers and raises the sampler relative to the ocean floor. After the disk is placed on the ocean floor, a rotation of the device, coupled with a short sideways thrust of the ROV motors, collects a sample, seals the storage container, and brings the next cup into position.

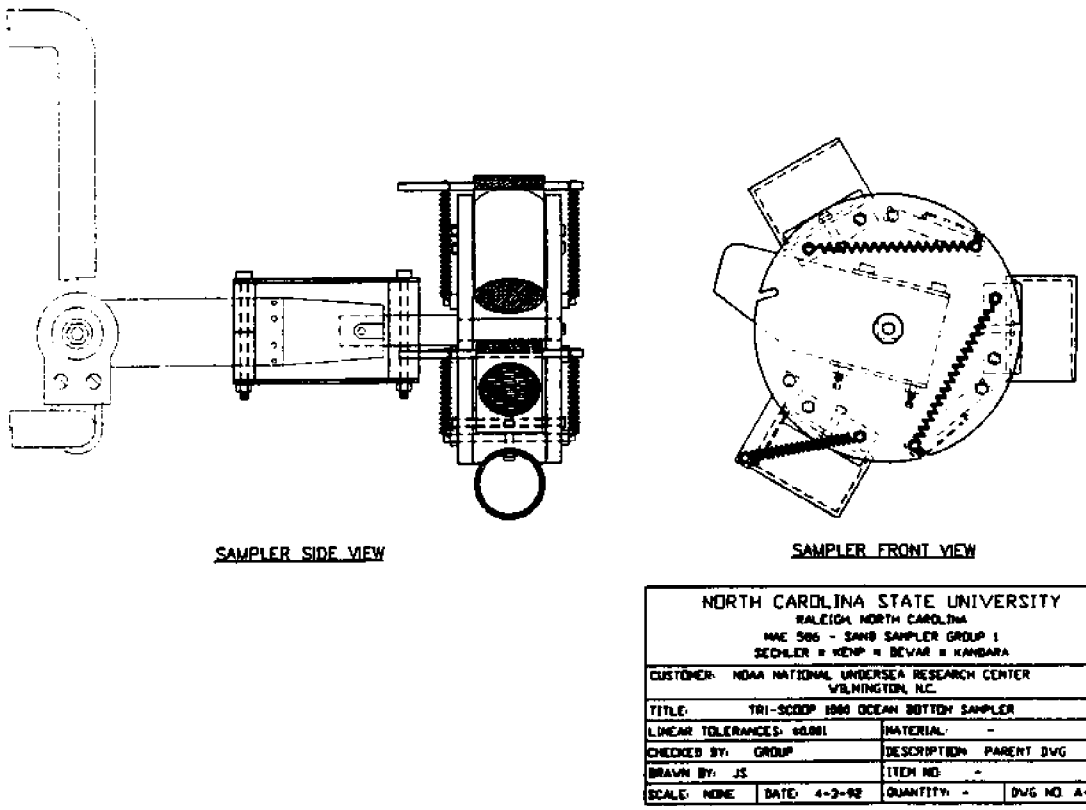

Figure 3. Tri-scoop 1000 Ocean Bottom Sampler

The device is composed of two lexan discs that are supported through their centers by an aluminum shaft. The exposed end of this shaft fits into the manipulator rotation motor. Between the discs, equally spaced around the perimeter, are three UHMW mounting blocks. Each mounting block holds a 180 cc aluminum cylindrical cup. Each container has an aluminum lid that pivots about a stainless steel shaft. An aluminum O-ring seat, fitted with a nitrile O-ring, is bolted to each lid. The mouth of each of the aluminum containers is chamfered for a water tight seal between the lid, O-ring, and container. Each lid is held in position with two stainless steel extension springs. A UHMW static trigger arm, used for closing the container lids, is clamped to the manipulator rotation motor.

When preparing this tool for use, the operator opens all three of the spring loaded container lids. The tool is then positioned so that the first container is ready to enter the bottom. After the ROV is positioned on the ocean floor, the sampler is lowered with the elevation motor until the bottom edge of the tool touches the seafloor. To take a sample, the operator activates the manipulator motor necessary to rotate the sampler disk. This rotation causes the first container to rotate downward and scoop a sample of the bottom. During the final portion of the rotation, the extended end of the lid/trigger rod comes in contact with the fixed lever. The rod end slides along the surface of the lever,

forcing the lid to rotate. At a critical point, the springs take over and snap the lid shut. The operator then stops the rotation of the tool and the device is ready to take another sample. This process can be completed three times. Once on the surface, the samples can be removed by manually opening the spring loaded lids.

NCSU-2 Sand Sampling Tool:

The NCSU-2 Seafloor Sampler (Fig. 4) is similar to the Tri-Scoop 1000 in that they both have three cups that scoop the sand or mud and then seal until manual retrieval on the surface. The fundamental difference is that instead of rotating the entire tool, this NCSU-2 tool relies on a sprocket driven conveyer belt to drive the sample cups into the bottom. This belt is driven by a system of two sprockets sandwiched between lexan plates and one pulley. The pulley, fixed to the axle of the upper sprocket, is rotated by the manipulator claw motor pull cable. This action causes the belt and cup assembly to rotate. The lower cup enters the seafloor, collects a sample, and exits the soft bottom. Additional rotation allows the torsional spring to close and seal the lid.

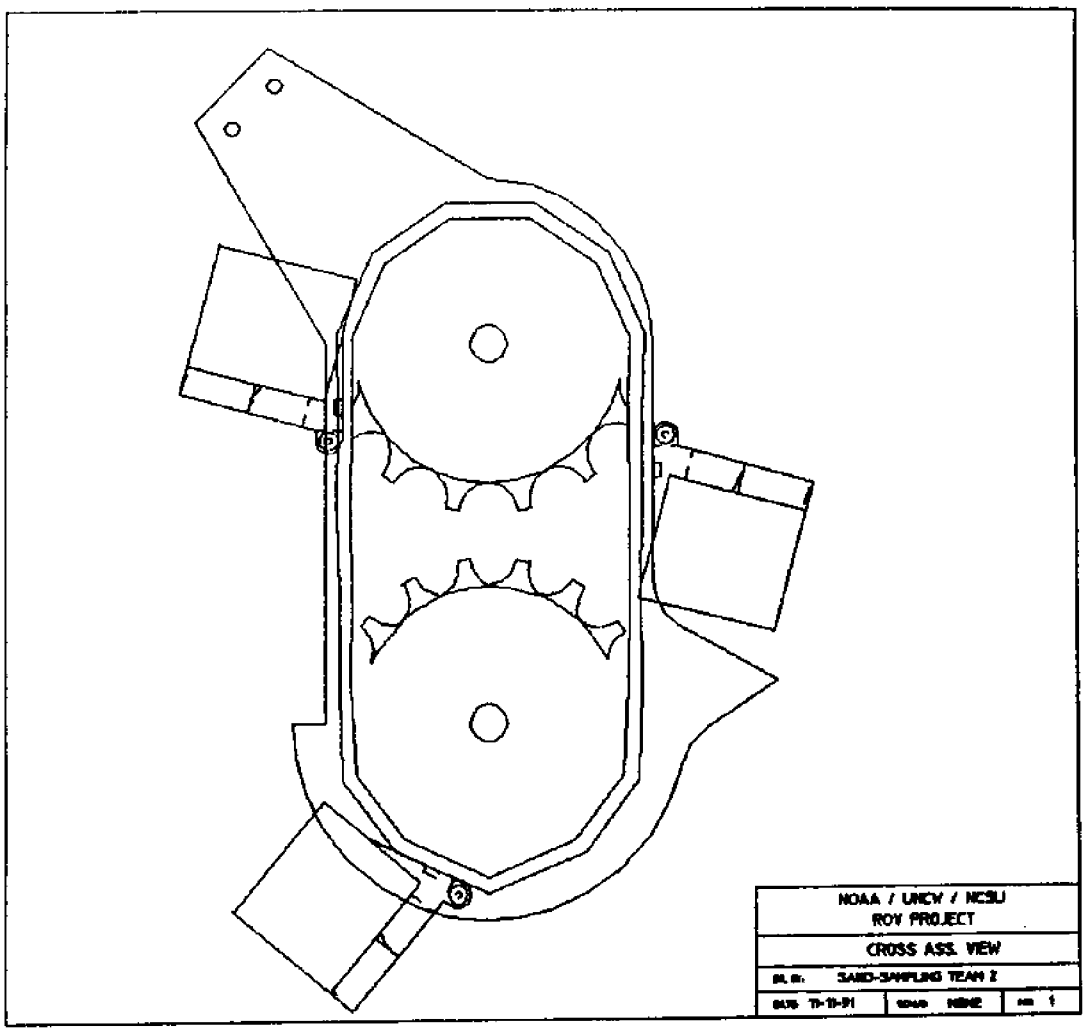

Figure 4. NCSU-2 Ocean Bottom Sampler

The sampling cups are 125 cc lexan containers screwed into an aluminum adapter. This adapter is rigidly attached to the acetal plastic and stainless steel belt. This belt rides on nylon sprockets housed between two lexan panels. The aluminum container lids are controlled by tracks in the two side panels. These tracks are designed to keep the lids in an open position until the sample is taken and to allow a

torsional spring to close the lid when sampling is complete. A two-inch diameter nylon pulley is employed to transmit the torque generated by the pull cable to the sprocket. The sprockets rotate on two stainless steel shafts.

To prepare the sampler for operation, the drive pulley is reversed manually until the doors on all three containers are open. The belt is rotated forward until just before the first sample cup reaches the bottom of the conveyer belt loop. In order to take a sample, the ROV is first placed on the bottom. The operator then rotates the conveyer using the claw pull motor. This action causes the first sample cup to collect a sample of the soft bottom and seal itself against contamination. Once the lid on the sample jar is closed and the second jar is moved into position, the rotation is halted. This process may be completed up to three times. Removal of the ocean floor samples is achieved on the surface by either manually opening the lid or disconnecting the jar from the adapter.

Conclusions

A group of undergraduate students successfully designed, fabricated and tested four tools that allow the NURC/UNCW ROV to take multiple samples of seawater and seafloor in a design effort funded by NURC/UNCW. All samplers function as anticipated, are simple to operate, and are easy to maintain. As a result of this project, the students received a valuable educational experience in engineering design and project management. The sponsoring institution received custom designed research tools that will expand the capabilities of a proven undersea research system. Results of this project indicate that an inter-university collaborative effort can provide benefits to all parties involved.

Acknowledgements

The National Undersea Research Center at the University of North Carolina at Wilmington is one of six university-based research centers sponsored by the National Oceanic and Atmospheric Administration (NOAA). The philosophy of the Center is to support environmental research programs with relevant high tech diving systems. The Center currently has access to virtually all of the region's marine ecosystems through a unique combination of in-house expertise and equipment and leased systems. The Center currently has expertise in leased submersibles, the underwater laboratory AQUARIUS, air and enriched air (NITROX) diving support, manned submersibles, and unmanned remotely operated vehicles.

The Mars Mission Research Center, co-located at North Carolina State University and North Carolina A&T University, is one of nine engineering research centers sponsored by the National Aeronautics and Astronautics Administration (NASA). Its mission includes the development of basic technologies that may be used both for space exploration and the education of students in aerospace engineering. Emphasis is placed on mission planning, hypersonic aerodynamics, composite materials, and structural dynamics and control.

PRELIMINARY INVESTIGATION: PLATFORM REMOVAL AND ASSOCIATED BIOTA

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Platforms are long-term, high profile structures of pilings and conductors that pass from beneath the seafloor to above the sea surface. They are built to remain stable and safe for a minimum of 20 years. Since the majority of GOM platforms are hundreds of miles from natural reefs, they function as de facto artificial reefs and have increased habitat by 28 percent. Sessile animals settle and develop on the subsea surfaces and depth limited and reef-related fishes locate to a platform and remain structurally faithful. Complex communities evolve and eventually over 10,000 individual fish may affiliate with a single platform. Federal law mandates that the entire structure be removed within a year after hydrocarbon production ceases. The use of explosives 16 ft below the seafloor is the only reliable method of piling and conductor severance. Quantitative surveys and photo documentation were performed before and after explosive severance at three removal sites to evaluate effects on the platform communities. Preliminary data indicate that death is related to distance from seafloor and explosive mechanics; the first detonation(s) kill(s) the most fish; and total fish killed cannot be estimated from those that float. There is little discernable effect on motile invertebrates able to maintain contact with the platform surface during detonation.

Introduction

There are approximately 3,700 oil and gas production platforms in Federal waters of the Gulf of Mexico. 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). Platforms provide habitat of several depth zones as well as a surface-shallow water zone. 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 150 ft. 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 north-western Gulf, about 8,000 acres of hard substrate are provided by the underwater supports of these platforms. Additional hard substrate is created on the sea floor under each platform as rock chips accumulate during the drilling of wells. This "cuttings pile" is augmented by mollusk shells that slough off upper support members, accumulating in layers up to 10 ft. 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 100 ft 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 include numerous species of groupers and snappers. For species such as these, availability of natural habitat is thought to be the factor limiting abundance, with the presence of platforms allowing for population expansion and an increased fishery. Gallaway (1985) estimated that oil/gas platforms in the northwestern Gulf of Mexico supported 11 percent of the available snapper fishery. Since 1980, the number of commercial species landed from the northwestern Gulf of Mexico has increased from 26 to 82. The number of species from this area, valued at over a million dollars, has tripled. Again this increase in commercial landings and value has been attributed to increased habitat in the northwestern Gulf of Mexico from the presence of oil/gas platforms (Linton, 1988). 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 ocean and coastal 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 that 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 10 ft 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.

At least 100 platforms per year are removed from the Gulf of Mexico. About 80 percent of these removals use explosive charges placed 16 ft 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) is very concerned over the declining stocks of reef fish, especially red snapper (Goodyear and Phares, 1990). Recent assessment of the red snapper stock by the FMC resulted in restrictive harvest limits for recreational and commercial fisheries in the Gulf and an interest to examine critically other sources of mortality on reef fish species. A National Marine Fisheries Service (NMFS) study, conducted to evaluate the impact of explosive removals on endangered species, documented a large number of floating dead red snapper (Klima *et al.*, 1988). Although there is no doubt that the use of explosives during platform removals kills fish, anecdotal accounts of fish mortalities at removals range from "a few fish" to estimates in the thousands.

The U.S. Department of the Interior (USDOI), Minerals Management Service, Gulf of Mexico, desired to examine fish kills associated with explosive removals before designing and funding a formal study to examine the effect of removals on reef fishery stocks. Prior to the work presented in this paper, information about fish kills during removals came from examination of post-detonation floating fish only (Caillouet *et al.*, 1986; Duronslet, 1986; Fontaine, 1986; 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. Since reef fish are structure faithful and many species demersal, e.g., red snapper, it was believed that an examination of the bottom both pre- and post-detonation(s) was essential.

Methods

The platform removal season extends from May through October with most of the actual platform removals occurring during July, August, and September. During the 1991 season, two platforms were visited just before and during removal operations: platform number 8 in West Cameron Block 176 (WC-176) and platform C in East Cameron Block 65 (EC-65). A third structure, platform A in High Island Block 520 (HI-520), was visited just before final removal but about one month after detonation and salvage of 12 well conductors. Three MMS divers traveled to each of the removal operations. One diver concentrated on the fish community, one on the invertebrates, and the third assisted where needed.

Observations of the fish and biofouling communities at WC-176 and EC-65 was conducted by using a stationary visual census technique for fish (Bohnsack and Bannerot, 1986) and macrophotography for invertebrates and biofouling. Fish surveying was performed with the diver remaining stationary while listing and then counting the fish within a clear horizontal range of vision. Additional information noted during fish identification included depth, temperature, approximate size/age, and behavior. The macrophotography set-up consisted of a Nikonos IV-A underwater camera and 35 mm lens, an Oceanic Model 2000 underwater strobe, and a 1:2 extension tube complete with framer.

Observations of fish and invertebrates from the surface to a maximum depth of 130 ft were conducted at HI-520 just before and after severance of the eight pilings. All fish that floated to the surface after the conductors were removed (about one month prior to diving) had been retrieved, measured, and counted. These records were obtained from NMFS.

West Cameron 176 Platform No. 8:

Data concerning fish and invertebrate distributions, densities, and diversities were gathered during two dives made at this site on August 26, 1991, the day prior to the first detonations. During the first dive, a qualitative survey of fish was performed looking both inward and outward from all sides of the platform at 10 ft vertical intervals from the bottom to the surface. During the second dive, a concerted effort was made to survey fish within the bottom nepheloid layer inside and 3 ft outside the entire base of the platform.

Within 30 min after detonation of the single well conductor a dive was made to survey living fish and invertebrates as above. The number of dead fish per estimated square meter on the bottom was counted at seven random sites under the platform. Dead fish were recovered to the surface from these sites, from numerous other locations under the platform, and during sweep searches from all sides of the platform using a 20 ft line. As many fish as possible that floated to the surface were retrieved. All fish were identified to species, counted, measured, and internally examined.

Detonations of the support legs and anchor pilings occurred 12 hours after the conductor was salvaged. Within 30 min after detonations a dive was made to survey living fish and invertebrates as above. On the bottom, a brief external examination easily distinguished fish killed previously by the conductor detonation from recent deaths. Fresh-killed fish under the platform and out to about 20 ft were counted and most recovered to the surface. As many fish as possible that floated to the surface were retrieved. All fresh-killed fish were examined as above.

East Cameron 65 Platform C:

Due to an unexpected delay in the arrival of diving equipment, it was not possible to survey the biota associated with EC-65 prior to removal detonations on October 21, 1991. Within 30 min after detonations of the two well conductors a dive was made to survey fish and invertebrates as above. The number of dead fish per estimated square meter on the bottom was counted at seven random sites under the platform. Dead fish were recovered to the surface from these sites, from numerous other locations under the platform, and during sweep searches from all sides of the platform using a 20 ft line. Due to another unexpected lack of equipment the number of floating dead fish was estimated and as many as

possible were retrieved. All recovered fish were identified to species, counted, measured, and internally examined.

Detonations of the support legs and anchor pilings occurred 14 hours after the conductors were salvaged. Within 30 min after detonations a dive was made to survey living fish and invertebrates as above. On the bottom, a brief external examination easily distinguished fish killed previously by the conductor detonations from recent deaths. Fresh-killed fish under the platform and out to 20 ft were counted and most recovered to the surface. All fresh-killed fish were examined as above.

High Island 520 Platform A:

Detonations and salvage of conductors were performed on August 3, 1991. Approximately one month later data concerning fish and invertebrate density and diversity were gathered during one dive made at this site prior to detonations of the support legs and pilings. From the surface to a depth of 130 ft a qualitative survey was performed looking both inward and outward from all sides of the platform.

Within 30 min after detonations a dive was made to survey living fish and invertebrates from the surface to a depth of 130 ft. Any dead fish that remained on the platform were counted and the amount of attached invertebrates that had been shaken loose was estimated. Any fish that floated to the surface were retrieved.

Results and Discussion

West Cameron 176 Platform No. 8:

This platform was unmanned, in 58 ft of water, over 10 years old, supported by four major support legs, and contained one well conductor. Pre-detonation surveys indicated that at least a thousand fish were in direct and constant association with the platform and would be unlikely to travel even a short distance to another location. Several hundred pelagic fish were also associated with the platform at the time of the surveys. There was a variety of motile invertebrates with numerous gastropods laying eggs and recently laid egg-cases from many different species. In addition, there was an extensive mat of attached sessile invertebrates.

After the conductor detonation, fish floated to the surface within two minutes. Some of the fish were stunned and soon began to flop about the surface, but most were dead; however, no floating fish recovered their capability to swim and re-submerged. Internal examinations of surface recovered fish revealed that all swimbladders were vastly expanded and most had to be deflated to examine other organs. All internal organs exhibited some degree of hemorrhage and trauma.

The subsurface survey after the conductor detonation indicated that pelagic fishes may not be killed. Live Spanish mackerel were recorded at about 40 ft of depth before and after the conductor detonation 20-25 ft out from the platform. Although they appeared to be schooling more tightly as they circled the platform after the conductor detonation, there was no change in depth, swimming attitude, or response to divers. Other pelagics, such as blue runners, that may have been inside the platform closer to the conductor were observed to swim erratically and shimmy for several seconds as a fish will that is struck in the central nervous system. Fish located high in the water column such as blennies, belted sandbass, juvenile sergeant majors, and immature damselfish were still alive.

Dead fish littered the bottom under the platform after the conductor detonation. Internal examination of fish recovered from the bottom revealed that the viscera was completely traumatized with hemorrhagic livers, kidneys, and exploded organs. All swimbladders appeared to be ruptured in an expanded condition. Dead red snapper of at least three year classes were retrieved to the surface. The estimated meter square sampling and discussion between the commercial divers on site and the MMS divers agreed that at least five fish per square meter were dead on the bottom beneath the platform. Inside dimensions for WC-176 then indicated that at least 1,845 fish were dead on the

bottom from a 35 lb detonation of Composition B to sever a single well conductor. Overnight a school of about 40 Atlantic spadefish and one hammerhead shark were seen swimming about the platform. After the four pilings were detonated the following morning, two redfish were recovered from the bottom within 15 ft of the platform. The redfish were fresh kills as evidenced by the color of their gills, the clarity of their eyes, and the lack of sediment on their bodies. Internal examinations revealed that the organs had suffered extensive trauma and that the swimbladders had ruptured outward. The hammerhead shark was not recovered and all the spadefish that had moved in overnight either floated dead to the surface or were found dead on the bottom. The swimbladders of floating spadefish were expanded but intact. Other than the redfish and Atlantic spadefish, fewer than 10 fish either on the bottom or floating were thought to be fresh kills. Most of the newly floating fish were obviously killed the previous day and floated up due to the production of gas during decay. Many of the motile invertebrates and blennies lived through both the conductor and piling detonations. Some of the larger blennies were stunned and could be handled for a short time before they quickly swam away. The fact that blennies and motile invertebrates were not killed agrees with the findings of Young (1991) that marine organisms without swim bladders are highly resistant to explosive shock. About 60 percent of the total sessile invertebrates were knocked off the pilings. After the conductor detonation an area about 6 ft in radius around the base was littered with pieces of barnacles. After the pilings were detonated many fish that had been killed by the conductor blast remained on the bottom and were covered with chunks of barnacles and layers of sediment.

East Cameron 65 Platform C:

This platform was unmanned, in 70 ft of water, 6 years old, supported by four major support legs, and contained two well conductors. The two conductors were detonated at 0.9 sec intervals. After the conductor detonations fish floated to the surface within two minutes. Some of the fish were stunned and soon began to flop about the surface, but most were dead; however, no floating fish recovered their capability to swim and re-submerged. It is estimated that about 600 fish floated away before they could be recovered or examined. Internal examination of surface recovered fish revealed all had expanded but intact swimbladders.

Subsurface random retrieval of dead fish was performed the next day, 14 hr after conductor detonations. Comparison of necropsied dead fish recovered from the surface and bottom was difficult due to the 14 hours of decay before recovery of dead fish from beneath the platform. However, a number of specimens recovered from the bottom could be examined and exhibited internal conditions comparable to those seen during the WC-176 removal. The estimated meter square sampling and discussion between the MMS divers agreed that at least seven fish per square meter were dead on the bottom beneath the platform. Inside dimensions for EC-65 then indicated that at least 1,500 fish were dead on the bottom from two 40 lb detonations at 0.9 sec intervals of Composition B to sever two well conductors.

The subsurface survey after the conductor detonations at EC-65 also indicated that pelagic fishes may not be killed. Several cobia were recorded at about 30 ft of depth 14 hr after the conductor detonations swimming in and around the platform. It is unknown if they were associated with the platform before the detonations. They were not recovered or seen during subsequent dives. Fish located high in the water column such as blennies were still alive. It required a total of 14 separate detonations of a total of 670 pounds of Composition B to remove EC-65. Decaying fish floated to the surface intermittently during operations and had obviously been dead several days. Few fish and/or invertebrates were seen alive during the dive made after the final round of piling detonations.

High Island 520 Platform A:

This platform had been manned, was in over 200 ft of water, over 10 years old, supported by eight major support legs, and had contained 12 well conductors. The information from the NMFS concerning the fish that floated to the surface after conductor detonations about one month earlier revealed that this platform supported a diverse group of tropical fish. This is probably due to its distance from coastal influences (Gallaway and Lewbel, 1982).

Prior to the piling detonations a survey to 130 ft of depth indicated that except for blennies in less than 30 ft of water very few living fish were associated with the platform. A few small unidentified grouper were seen near the conductor collars. No motile and few sessile invertebrates were seen. After piling detonations no fish floated to the surface. Except for a few blennies seen during the post-detonation dive no other living fish were observed. Due to the depth no observations were taken of the bottom at any time.

From investigation of three explosive platform removals during 1991, it appears that the first detonation(s) kill(s) the most fish. An underwater explosion generates a direct shock pulse as a compression wave (Connor, 1990; Regalbuto, *et al.*, 1977). This direct shock strikes the water surface and reflects back into the water as a rarefaction decompression wave. The initial compression wave appears to concuss and mortally traumatize the internal organs of fish close to the subsea location of the explosion. In addition, the combination of the compression and decompression waves apparently creates sufficient pressure gradients to first pressurize gas in the swimbladder and then expand it to the point of outward rupture.

Fish that were higher in the water column and further from the direct explosive shock wave experienced varying degrees of concussion and internal trauma, but less pressurization of gas in the swimbladder. They were, however, closer to the decompression wave, which upon reflection from the surface caused expansion of the gas in the swimbladder and consequently they floated.

Comparison of estimates of floating fish and of the number of fish dead on the bottom strongly suggests that fewer fish float to the surface than sink after initial detonation(s). 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.

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OXYGEN SAFETY IN THE PRODUCTION OF ENRICHED AIR NITROX BREATHING MIXTURES

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The practice of enriched air ("NITROX") diving is expanding, and new systems are coming on line to produce the breathing mixtures. Enriched air diving involves use of gas mixtures in which some of the nitrogen in air is replaced with oxygen; the advantage is a reduced decompression obligation. Methods of acquiring enriched air mixtures include a continuous process developed by NOAA whereby oxygen is added to the input air stream of an oil-free compressor, mixing in the tank or a storage vessel by partial pressure, or purchasing pre-mixed gas from industrial gas suppliers. For safe mixing, air to be mixed with oxygen must be free of all liquid hydrocarbons. This is possible but difficult using an oil-lubricated compressor because filtration has to be of adequate design and well maintained; this process is not recommended because of the rigorous maintenance requirements. Oil-less or oil-free compressors are best for producing oil-free air; air meeting many breathing gas purity requirements may nevertheless have too much oil for mixing with 100% oxygen at high pressure. Using pure oxygen and nitrogen is not recommended because of the possibility of breathing pure nitrogen. The recommended way of acquiring enriched air mixes for small operations is to purchase prepared mixtures and pump them into scuba tanks using an oxygen-compatible booster pump. Diving equipment used with enriched air should be clean and lubricated with oxygen-compatible lubricant, but need not have special components if it has no possibility of coming in contact with high pressure oxygen; such equipment should be dedicated to that service and so labelled.

Introduction

The use of enriched air diving gas mixtures, variously called NITROX, enriched air NITROX, EA, EAN, EANx, oxy-nitrogen, or airox, has begun to expand from the well defined and controlled environment of scientific diving into the mainstream recreational diving community. Almost daily, new scuba retail shops are establishing enriched air fill stations and selling gas to presumably qualified "NITROX" divers.

Many professional retail operations have taken the necessary and costly steps to produce oxygen-enriched air mixtures safely and to prepare equipment correctly for enriched air service. However, a

significant number of misinformed retailers appear to be attempting to cut corners, with potentially catastrophic results. Any recreational diving or diving-related incident creates a long, lingering negative image about diving with the general public. This negative impression carries over into the scientific diving community and can effect program acceptance and funding. Our first objective is to make every effort to keep such incidents to a minimum; when they do happen we encourage the scientific diving community to ensure that correct information is disseminated.

In January 1992, the question of recreational enriched air diving was formally addressed at a workshop entitled "Evaluating enriched air ("NITROX") diving technology" (Hamilton, 1991). This was conceived by *aquaCorps Journal* and sponsored by the Scuba Diving Resource Group. Great progress was made at this meeting in terms of identifying and explaining enriched air NITROX issues, and several equipment manufacturers have responded by beginning to supply equipment suitable for enriched air diving. There are still some concerns, and the Workshop identified several areas needing work and set up a process to address these concerns. Recreational enriched air diving is moving ahead with enriched air technology, and, as just mentioned, it is in the best interests of the scientific diving community to monitor and where appropriate to assist this development process to help ensure that it follows a safety-oriented path.

This paper makes an effort to review for both the recreational and scientific diving communities the latest oxygen safety information and techniques.

The last few years in recreational diving may well be most remembered for the development and utilization of new techniques, including the use of enriched-air breathing mixtures by "average" divers, and particularly the dramatic development of "technical diving". This involves diving to depths beyond - perhaps well beyond - those considered acceptable for recreational divers, using special breathing mixtures, performing decompression stops, and depending on expensive and complex equipment in excess of that used by the typical diver. There is little doubt that many of the more useful and dependable technical diving techniques will in due course find their way into scientific diving (some already have), but technical diving is not the subject of this paper. It is important to retain the concept that enriched air NITROX diving is not technical diving.

A primer on enriched air ("NITROX") diving

The use of breathing gas mixtures other than air for diving operations is not a new concept (see for example, Hamilton et al., 1989). The U.S. Navy documents in its diving manual the use of oxygen-enriched breathing mixtures of oxygen and nitrogen in rebreathers as early as 1959, and commercial use may date back even further. Anyone who has been SCUBA diving has used a "mixed" gas; air is composed of about 78 % nitrogen, 20.9% oxygen, 0.9% argon, and traces of others. Of these gases oxygen is the only gas required to support human life, but it is also the one most toxic. The other major components of atmospheric air, those breathed by a diver in a gas mixture, are regarded as "inert"; biologically these serve as vehicle and diluent gas for the oxygen. They serve to limit its physiological effects, at high pressures especially, but also at atmospheric pressure over the long haul.

As a diver's breathing gas, air has a couple of shortcomings. In what may seem ironic for an essay on "NITROX" diving, the main problem with air is the nature of the diluent gas, nitrogen. Air causes narcosis when breathed at pressures of several atmospheres, is dense at high pressures, and its main component nitrogen is fairly soluble in body fluids and may be difficult to get rid of after any but rather short dives or exposures to pressure. Air also has a fixed oxygen percentage, which results in oxygen partial pressure, PO_2, being well below the optimal physiological limits on shallow dives, and it often may be too high on very deep air dives. Because of the unfavorable decompression aspects of nitrogen, it is desirable to have the oxygen fraction in the breathing mix as high as it can be without its causing adverse effects. This use of more nearly optimal - higher - oxygen fractions to reduce the nitrogen component and its effect on decompression is the basis of enriched air or "NITROX" diving.

Some rather attractive aspects of air as a breathing gas for diving are its ready availability and generally low cost.

The returns are still out on whether replacing some of the nitrogen in a breathing mix with oxygen will reduce narcosis. Some divers believe that it does, but this has not been measured objectively even though attempts have been made, and it has been shown experimentally that oxygen can exert narcotic effects in its own right.

So the objective is to reduce nitrogen, and the method is to increase the oxygen component, to "enrich" the mix. The consequence of this is that oxygen is toxic, and this toxicity can become a problem at high pressures. The physiological effects of elevated oxygen partial pressures can range from temporary lung irritation to an epileptic-like convulsion which can cause a diver to drown. Oxygen exposure management is therefore a major component of diving with mixes having elevated oxygen percentages; fortunately, for the range of mixtures and depths used in both scientific and recreational diving, this is a relatively easy task.

Custom mixed diver breathing gases are designed to overcome the inherent problems and limitations associated with compressed air diving. Enriched air diver breathing gas mixtures utilize a higher percentage by volume of oxygen and lower nitrogen than natural air.

Having a nitrogen fraction lower than air has the advantage of allowing longer no-stop dives, or conversely requiring less decompression time for dives requiring stops, than standard air dives of the same depth and duration. Because of oxygen toxicity limitations this prevails over the range of 40 to 130 fsw (feet of sea water pressure) and is most efficient in the range 60 to 100 fsw.

In order to take advantage of these decompression advantages it is necessary to have special decompression procedures ("tables") or to modify standard air tables appropriately. The principle of "Equivalent Air Depth" or EAD is a method of calculating a decompression based only on the nitrogen component of the mix in use, and relating it to an equivalent air table having the same nitrogen component (actually the same partial pressure of nitrogen). The result EAD is for a shallower depth and requires a shorter decompression obligation. The EAD principle is sound physiologically, and it has built in conservatism due to the requirement to select the next most conservative table when the equivalent depth does not come out exactly the same as the original depth. This same factor makes the method uneven in its conservatism, more efficient in some places and more conservative in others. Another aspect of EAD calculations is that they may suffer from any inherent unreliability of the parent tables.

Prepared enriched air "NITROX" tables have been developed by the National Oceanic and Atmospheric Administration and published in the NOAA Diving Manual (1979; 1991). These are based on the equivalent air depth principle applied to the U.S. Navy Standard Air Tables. The NOAA tables are defined for two specific enriched air mixes; the NOAA NITROX I and II tables use mixtures with 32 percent and 36 percent oxygen by volume, respectively.

It should be mentioned again, that because enriched air nitrox tables optimize the oxygen concentration of the mixture in order to minimize the nitrogen fraction that affects decompression, great care must be taken to stay within the maximum oxygen partial pressure exposure limits. The new NOAA (1991) manual also provides useful advice on managing oxygen exposure.

Mixtures with high oxygen fractions also create problems related to the "oxygen compatibility" of the equipment in contact with these mixtures, because of the possibility of an oxygen-related fire. This is the main theme of this paper, and incidentally was the prime motivation for the Enriched Air Workshop, much of which is covered in following sections (see also Mastro and Butler, 1990).

Fire Safety Issues Involved in Handling Oxygen

The concept of oxygen compatibility

High pressure oxygen is used safely every day by industry, aviation, and in medical applications. These industries have learned that the key to oxygen safety is the use of specially prepared and compatible equipment by trained personnel following specific procedures.

Oxygen compatibility is a relative term of great importance to anyone using high pressure oxygen. Oxygen is not a flammable gas or vapor such as acetylene or ether. It is a strong oxidizing agent (others are chlorine and nitrous oxide), and will combine chemically with compounds and fuels causing an exothermic chemical reaction. The rate at which this chemical reaction or "oxidation" takes place is very important; it may be slow or fast.

Slow oxidation of metals is known as corrosion or rusting. The deterioration or drying out of soft materials such as rubber, or in the case of SCUBA equipment, neoprene O-rings, is often the result of slow oxidation.

If the rate of oxidation is moderately fast, it is usually termed a "fire." An explosion is another example of high speed oxidation.

Another important concept is that **everything** will burn, or will oxidize to some degree when heated to a sufficiently high temperature in the presence of oxygen. A good example of this is an oxyacetylene cutting torch, with which steel a foot thick may be cut (actually burned) by simply heating the steel to above 1100 degrees and blowing a stream of high pressure oxygen on it.

The resistance of a material to oxidation may be used to define its "oxygen compatibility."

The concept of combustion

Chemical combustion requires three components - fuel, an oxidizer, and an ignition source of sufficient temperature to start the reaction. If any one of these three components is missing or available in insufficient quantities under a specific set of conditions, combustion cannot take place. This "fire triangle" becomes more complicated in high pressure air and oxygen systems, and dealing with it presents a special set of problems.

As oxygen pressure and therefore the number of molecules increases in the presence of a fuel, the temperature necessary to initiate combustion of the fuel - the "auto-ignition" point - decreases dramatically. In 3,000 psig oxygen systems, for example, the auto-ignition point of a given valve seat material may be only a fraction of the temperature required to set the material on fire in atmospheric air, if indeed it will burn in air at all. Many materials which will not readily burn in high pressure air conditions, even at very high temperatures, may literally explode in the presence of high pressure oxygen. The selection and use of the materials most resistant to auto-ignition in high pressure oxygen is critical to safely preparing equipment for enriched air NITROX service.

Fuel in breathing gas systems

The most likely source of fuel in compressed air systems is liquid oil. This oil contamination may be the result of improperly cleaned parts attached to the system or compressor lubrication oil which has been allowed to accumulate in the system. While the amounts of oil necessary to support the combustion process are dependent on many factors, some of the low-toxicity lubricating oils that are recommended by manufacturers for use in high pressure breathing gas compressors will ignite spontaneously if heated to their 300 to 450 degrees F auto-ignition temperature in the presence of oxygen. The amount of condensed hydrocarbon-based oil necessary to allow sustained combustion in a high pressure air system has been established experimentally at values as low as 20 milligrams of condensed oil per square foot of internal piping area, under certain conditions. The exact amount of contamination is more or less

proportional to the amount of air pumped by the compressor, adjusted for the quality and frequency of the filters and the maintenance of the compressor.

Other fuels worthy of consideration include component soft goods such as valve seats, regulator diaphragms, O-rings, and Teflon sealing tape that comes into direct contact with the air stream. Generally speaking, components designed and cleaned for oxygen service are acceptable for high pressure enriched air systems.

To minimize risk, lubricants used in gas exposed components such as valve threads should be rated for oxygen service. Ignition has occurred with silicone greases used in high pressure air systems. There is a popular misconception that silicones are not flammable; this is not the case. Silicones should be replaced with an oxygen compatible lubricant.

Sources of ignition

The real key to safe high pressure air, enriched air NITROX, and oxygen system operation is to keep temperatures well below those necessary to cause auto-ignition of even the least oxygen-compatible materials. In practice, this can be more difficult than it sounds. The most common source of high temperatures in gas systems is rapid local compression of the gas within the pressure compartments and piping system, a phenomenon known as "dieseling."

Rapid compression of a manifold with a working pressure of 3,000 psig can produce momentary gas temperatures in excess of 1,600 degrees F, a value well in excess of the 400 to 600 degree auto-ignition temperatures of the silicone lubricants that have been used in many SCUBA tank valves and regulators. In elevated oxygen conditions, these temperatures have produced diesel-type auto-ignition of, for example, Dow-Corning Silicone Compound #111.

Another source of high temperatures in gas systems is caused by the localized friction that occurs as gas moves through valves and orifices at sonic velocities. The use of slow opening "globe" style oxygen service valves will help to reduce gas velocities and the heat of compression. Due to their rapid opening characteristics, ball valves are not recommended for oxygen service at pressures greater than about 125 psig by the CGA, ASME-PVHO, and NFPA piping sub-committees.

A less likely cause of auto-ignition in SCUBA equipment, but one that is very likely in larger storage manifold systems, is particle impact. High gas velocities can pick up and carry metal particles and other debris downstream at hundreds of feet per second, and this can cause sparking and very high localized temperatures as the particles impact bumps and bends in the piping. This has real significance in oil-contaminated HP-air manifold systems where an oxygen circuit has been added to produce enriched air NITROX by the partial pressure method.

Guidelines for the Use of Oxygen in SCUBA Equipment

Most "off the shelf" SCUBA equipment is assembled at the factory using soft goods (O-rings, seats, gaskets, etc.) and lubricants that are considered to be "compatible" with air but not oxygen.

It is important to note that the only fires in SCUBA equipment known to the authors have occurred with pure oxygen (none that we know of with air or oxygen-enriched air). Other incidents have been reported anecdotally, but we lack reliable documentation of them. The 1992 enriched air workshop presented expert opinion that mixtures up to 50% could be used safely with ordinary materials and oxygen compatible lubricants, but called for research to confirm this opinion with data.

The need for oxygen cleaning

It is recommended that all system components having either direct contact with oxygen or the "indirect" possibility (*i.e.*, not normally in contact with oxygen, but that might be as a result of a

reasonably foreseeable malfunction) be cleaned to "oxygen service" standards. The same recommendation applies to enriched air mixtures above 40 percent oxygen by volume.

The cleaning process should remove particles and combustible contaminants to a value below that which might result in a fire under extreme conditions. These values are difficult to quantify, and for this reason it is best to evaluate systems that will be used in oxygen or enriched air services as being contaminated if any particulate, oil, or carbonaceous residue is detected visually or by using the cotton swab method.

Cleaning criteria for many oxygen and high pressure air systems are beyond the capability of the average user, and they may be prohibitively expensive if done by an outside oxygen cleaning firm or lab. There are, however, methods that can be used "in-house" by knowledgeable technicians that will produce acceptable results.

The Compressed Gas Association Pamphlet CGA G-4.1-1985, "Cleaning Equipment for Oxygen Service" is a basic introduction to various industry accepted oxygen service cleaning methods. Unfortunately, it does not adequately deal with specific step-by-step procedures, or provide insight as to some of the material and solvent incompatibility problems that might be encountered.

SCUBA gear designed for HP air service is usually not recommended or safe for oxygen service as supplied by the factory, primarily because of the soft goods and lubricants used. These parts can often be replaced, but before disassembling, cleaning, and replacing these parts it is best to contact the manufacturer to find out what is recommended. You may inadvertently effect performance and could void your warranty. Several manufacturers now offer equipment that has been factory prepared for HP oxygen or enriched air NITROX mixture service of up to 50 percent oxygen. It is best to dedicate oxygen compatible and cleaned equipment to oxygen or enriched air NITROX service only. It should be labelled prominently, to prevent both recontamination or inadvertently breathing the wrong gas.

Do not use air from oil lubricated compressed air sources as an "add" gas in the production of mixed gases, especially where the oxygen percentages are greater that 25 percent by volume. Modifying existing conventional oil-lubricated air compression systems for the production of enriched air NITROX is potentially dangerous and is not recommended by any company producing oil-lubricated compressors. Properly chosen and sized filters can produce clean air, but they require constant monitoring and maintenance to ensure continued production of air sufficiently clean to be used for making enriched air.

Make every effort to reduce gas compression rates and velocities in gas systems in an effort to minimize gas temperatures in the internal piping. This is a function of both design and operating technique.

It is good practice never to use the SCUBA tank or oxygen storage cylinder valves to control the flow of pure oxygen or even enriched air NITROX. This is done in many air cascade filling operations. The proper method to control high pressure oxygen flow is to use a pressure-regulated flow control system.

Bare steel or zinc electro-plated steel tanks are preferable for oxygen service applications for several reasons. Aluminum material is less "oxygen compatible" than steel and will burn vigorously if ignited in the presence of a high pressure oxygen atmosphere. Steel tanks require significantly higher temperatures to auto-ignite and will burn less vigorously if ignited. Bear in mind that these are relative terms of material oxygen compatibility. The likelihood that an HP SCUBA tank would be consumed in a high pressure oxygen-induced fire is remote.

Of greater significance to aluminum cylinders is the fact that oxygen compatible chlorofluorocarbon type lubricants, such as those made by Halocarbon Products Corp., and Fluorolube brand products made by Hooker Chemical, Inc., are specifically not recommended by their manufacturers for use with aluminum or magnesium alloy materials due to the possibility of chemical reaction and ignition under

certain conditions. The reason is that these products contain chlorine, and this can react with freshly machined aluminum. Additionally, these lubricants have just recently been shown to effect some oxygen compatible elastomers such as Viton-A, casting doubt on whether they should be recommended for use in combination with elastomers.

Perfluorinated polyether-based oils with Fleurotelomer thickeners are the most inert and probably the best overall class of lubricants for these oxygen-related applications. The DuPont Krytox 240 series products work well and are the best known, but they tend to lose some of their lubrication qualities at low temperatures. Christo-Lube MCG-111 lubricant manufactured by Lubrication Technology, Inc., has good low temperature characteristics, is safe in oxygen, and is comparatively low priced. Both materials are compatible with most common elastomers, and both are far superior to ordinary and/or silicone compound lubricants.

Primary Methods for Producing Enriched Air Breathing Mixtures

At the center of the enriched air NITROX issue is gas mixture quality assurance. There are two primary field techniques for preparing enriched air mixtures, the cascade (partial pressure) method and the NOAA enriched air NITROX continuous mixing system. These along with other techniques need proper care to ensure correct mixes and to avoid contamination that may lead to oxygen fires.

Partial-Pressure Cascade Mixing

The age-old technique of mixing by partial pressure, "cascading", can be employed using pure factory supplied gases (e.g., nitrogen and oxygen), which eliminates many of the trace contaminant issues associated with compressed air. The term "cascading" applies to filling from cylinders in a bank one at a time, starting with the cylinder having the lowest pressure and working up. This method conserves the higher pressure gases and makes it possible to work without a booster pump.

The use of pure nitrogen (or any pure inert gas) is strongly discouraged because of the possibility that someone will be given a pure inert gas to breathe; this obvious and preventable error has accounted for far more diving fatalities in commercial deep diving operations than several more publicized occurrences such as improper decompression.

More convenient and efficient is the practice of "enriching" oil-free compressed air with oxygen. In this technique pure oxygen is added to an empty or nearly empty storage cylinder or SCUBA tank, and clean high-pressure air is added to the oxygen, filling the cylinder to its pressure capacity at the desired oxygen concentration (according to calculations based on the gas laws). This technique requires good temperature stability and highly accuracy pressure measurement to be precise. If used with high pressure air produced from an "oil-lubricated" compressor this technique is inherently dangerous, because of the likely presence of lubricating oil contamination.

The NOAA mixer

The development of the NOAA continuous NITROX mixer in 1987 greatly simplified enriched air mixing. The system consists of an "oil-less" high pressure compressor, oxygen analyzers, and a mixing manifold designed to enrich compressor intake air to the desired oxygen fraction. The enriched air then passes to the compressor from which it goes past the analyzer into the storage cylinders or directly into SCUBA tanks. The amount of oxygen added to the intake stream is adjusted according to the composition of the output gas.

This continuous mixing technique requires the use of an oil-free or oil-less compressor to eliminate the possibility of compressor lubricating oils coming into contact with the oxygen-rich mixture. The potential combination of oil, high oxygen concentration, and adiabatic heat of compression represents a significant fire and explosion hazard, so oil-lubricated compressors must be avoided when using the NOAA continuous mixing process.

Compressor Considerations

The Debate over the need for "oil-free" air

Standard oil-lubricated high pressure air compressors that are installed, operated, and maintained in accordance with manufacturers' specifications can provide acceptable breathing air for SCUBA and other breathing apparatus under most breathing air quality standards. However, air that is **suitable for breathing** can nevertheless contain enough condensable hydrocarbons to contaminate a system, and this air may likewise not be acceptable for mixing with oxygen.

The requirement for an "oil-free" compressor for mixing enriched air has been a topic of discussion and debate for several years. Faced with significant economic constraints, many operations have naively chosen to modify their existing oil-lubricated compressor systems, ancillary equipment and storage to produce enriched air mixtures rather than to purchase oil-free compressors and associated equipment. The relative fire and explosion risks inherent in this practice range from very low to very likely, and are dependent on many interrelated factors. This practice works well in careful hands, but is not generally recommended.

How much oil is too much for safe enriched air mixing?

The compressed air breathing standards in primary use today in the United States have been set forth by the Compressed Gas Association. Generally speaking, SCUBA compressor systems are designed to produce output air that complies with grades D, E, or, F. The essential difference in these grades is with respect to carbon dioxide and carbon monoxide concentrations. All these grades permit a condensed hydrocarbon contamination value of 5 milligrams per cubic meter of compressor system discharge air. This standard is based on the physiological observation that defense mechanisms in the respiratory system can deal with non-toxic hydrocarbon contaminants at this level. However, from a fire safety standpoint, the presence of any oil in excess of the range of a few parts per million (ppm) has proven to be dangerous.

This is an antiquated aspect of the CGA standard and is representative of the limitations of old technology. The fact that this standard remains in effect creates confusion on the part of breathing air consumers and leads users to believe that analysis indicating oil contamination lower than this physiologically-based level must also be acceptable for use as an add gas in the production of enriched air mixtures. This is an **extremely dangerous assumption**.

The question therefore arises: Realistically, how much oil is of concern in compressed air systems intended to support enriched air production? The fact is, any amount of perceptible oil or carbonaceous material in the internal piping of a breathing air system should be of concern from an oxygen safety standpoint. A simple method of system evaluation is to disassemble a piping connection just beyond the compressor's final filtration system, after the compressor has been run for many hours. Using a swab, wipe the inside of the piping, being careful not to leave any swab materials in the piping. Any discoloration of the swab is a baseline indication of the presence of some contamination; this must be further evaluated as to composition and source before returning the system to oxygen-related service.

Oil vs. oil-free compressor system designs

For the purpose of this discussion, high pressure breathing air compressors may be divided into three basic design categories: (a) oil-lubricated, (b) oil-free, and (c) oil-less.

Most high pressure breathing air compression systems in use today are of an oil-lubricated design in which the oil is in direct contact with the compression cylinders and hence with the processed air. These systems rely on post-compression filtration of the product air to remove entrained compressor lubrication oil, atmospheric water vapor, and trace gas contaminants such as carbon monoxide. These

trace contaminants, particularly CO, may be present in the intake gas or can actually be produced by lubricating oil oxidation within the compressor itself under certain conditions.

Oil-free compressors are hybrid designs that use self-lubricating materials to eliminate the need for oil lubrication in the upper breathing air compression cylinder and valves. The lower crankcase is of a conventional oil splash or pressure feed type with a connecting rod arrangement that isolates the oil-lubricated crankcase from the oil-free upper compression head area. This type compressor still requires post-filtration systems to separate atmospheric water vapor, remove particles of the compressor seals or piston rings (usually polytetrafluoroethylene - Teflon - or carbon composite materials) self-lubricating materials, and to absorb or catalytically convert trace contaminants such as carbon monoxide that may be drawn into the compressor from the atmosphere.

Oil-less compressor designs have no oil in either the crankcase or the upper compression head. This design uses sealed bearing technology to eliminate the contamination risks associated with a conventional crankcase and liquid oil. Commercially available units are of relatively low volume output compared to the above designs. Oil-less compressors require the same filtration systems as the oil-free units.

Advantages and disadvantages of different compressor designs

For purposes of comparison, oil-free and oil-less compressors can be grouped together and compared with oil-lubricated compressors. Historically, oil-lubricated compressor designs have tended to be less expensive to purchase and maintain than oil-free compressor systems of similar capacity. Spare parts and local service representatives also tend to be more available and at lower cost because of the wider application of this type of compressor. Distinct disadvantages of oil-lubricated compressors is the real need for frequent preventive and interval maintenance, and the need to ensure proper filtration. In operations in hot and humid climates frequent filter changes are necessary to maintain discharge air quality. As this design is capable of producing carbon monoxide under certain operating conditions, oil-lubricated compressors used for breathing air should be equipped with a carbon monoxide catalytic converter and chemical indicators in addition to the standard filtration systems recommended by most manufacturers.

There are several disadvantages associated with oil-free and oil-less compressors. Because of the higher cost associated with these compressors (initial purchase, replacement parts, and service) they are not the first choice of most facilities requiring breathing air for SCUBA operations, and they are financially beyond the reach of most retail diving operations interested in promoting recreational enriched air NITROX diving.

The major advantage of oil-free designs is the absolute elimination of both oil contaminants and the possibility of producing carbon monoxide during the compression process. Particles from the piston ring and seal material must be removed by filtration. This type of design, specially prepared for enriched air NITROX service by the compressor manufacturer, is generally considered the only acceptable and safe method of producing oxygen-enriched air mixtures by the NOAA continuous blending method.

A viable alternative: Buy pre-mix from the factory

Prepared, analyzed, and certified breathing mixtures may be routinely purchased through specialty gas suppliers. For many low and some high volume enriched air NITROX diving operations, pre-mix has some distinct advantages when compared to the hardware and other costs associated with in-house mixing. Most commercial diving companies buy pre-mixed breathing gases (other than compressed air) from a specialty gas supplier in high pressure gas storage cylinders that are rented until returned to the supplier.

The costs associated with the purchase of pre-mixed breathing gases depends on such factors as the accuracy and analytical requirements of the mixture (enriched air is generally considered to have

production requirements of ±1 percentage unit of desired oxygen concentration; that is, ±1% of the total, not of the component), the amount of gas ordered, and the trucking distance from the specialty gas plant to the point where the gas will be transferred to dedicated enriched SCUBA tanks for use.

It may be helpful to research the costs and personnel time associated with buying pure gases and mixing, analyzing, and compressing it on site versus buying pre-mixed and certified breathing gas mixtures from a supplier and only having to deal with transferring it to SCUBA tanks. A distinct advantage of premix is that it has been analyzed for both major components and trace contaminant gases such as carbon monoxide down into the parts per million range and then certified for use under some standard by the manufacturer. Rarely can on-site analyses meet these criteria. A disadvantage of pre-mix is that more cylinders must be handled than when only oxygen has to be purchased. Further, pre-mix is generally not available in cylinders at pressures greater than 2400 PSIG. This presents a problem in diving situations where diver-carried SCUBA tanks are routinely charged to over 3,000 psig or more.

While not insignificant in cost, a pre-mix cascade and booster pump charging system is less expensive, more reliable, and produces better quality gas at the SCUBA tank than any other on-site mixing operation that we know of.

Cleaning Oil-Contaminated Systems

New high pressure air and oxygen system equipment is generally factory cleaned for oxygen service. If in doubt, when purchasing specify "items to be cleaned for oxygen service" or mention that the equipment is for high pressure breathing air service. Be careful to maintain the oxygen clean integrity of the components during construction and of the system thereafter.

When replacing an oil-lubricated compressor with an oil-free compressor all downstream system components must be cleaned. The cleaning process must remove particle and combustible material in contaminated manifolds, valves, piping, fittings, and flexible hose. These can be safely cleaned on-site by experienced personnel using a variety of techniques and equipment. Storage cylinders and regulators are difficult to clean adequately; these should be sent out to an oxygen cleaning service or replaced.

Most components that have formerly been used for the management and storage of oil-pumped compressed air can be cleaned and modified for enriched air NITROX service with proper care. However, they can easily be re-contaminated if again connected to an oil-lubricated compressor system.

Evolution of Enriched Air NITROX Mixing Within NOAA

On a somewhat historical note we want to document the development of enriched air mixing technology within NOAA. NOAA developed the techniques for using enriched air NITROX for open circuit SCUBA diving in the late 1970's. The initial mixture, identified as NOAA NITROX I, was a 32% oxygen mixture produced by mixing pure nitrogen and oxygen and compressing the mix with a booster pump; this was necessary because oil-free air was not readily available. All mixing system components, storage cylinders, and SCUBA equipment that came in contact with this gas mixture were cleaned and maintained to oxygen clean standards.

In 1986 the National Undersea Research Center at the University of North Carolina, Wilmington (NURC/UNCW), began an enriched air NITROX diving program under the guidance of NOAA's Dr. Morgan Wells. Initially the Center's mix was made by the established method using pure gases and a booster pump under oxygen-clean conditions. Technology advances had since developed "oil-less" high pressure air compressors, and soon enriched air was being produced by adding oxygen to oil-free air. Thus the term "enriched air NITROX" is a combination of the earlier "NITROX" and the more correct "enriched air."

NURC/UNCW still maintained all equipment that came into contact with high pressure enriched air in an oxygen-clean status. SCUBA tanks, valves, and first stages of SCUBA regulators were cleaned and rebuilt with soft goods and lubricants compatible with oxygen service. Because the Center used oil-free air exclusively in its diving systems, SCUBA regulators could be used with either air or enriched air with no risk of contamination to the regulator. However, enriched air SCUBA tanks were properly marked and dedicated to enriched air service.

In 1987-88 NURC/UNCW began using a second enriched air NITROX mixture of 36% oxygen. This mixture, known as NOAA NITROX II, was also mixed using the NOAA continuous mixing system. Because the Center was no longer using cascade mixing techniques and there was no longer a risk that SCUBA equipment would be exposed to 100% oxygen, the Center began to relax the stringent cleaning requirements of SCUBA equipment exposed to high pressure enriched air.

Today the only "NITROX" mixtures used by NURC/UNCW contain 32% or 36% oxygen and are produced by enriching air through an oil-free compressor. Through experience, investigation, and observation, the Center has developed a high degree of confidence in using standard SCUBA equipment with these two specific mixtures. Although a high cleaning standard for SCUBA equipment is still maintained, the Center no longer rebuilds a new off-the-shelf tank valves or regulators. However, during annual overhauls all equipment is re-lubricated using oxygen-compatible lubricants.

The Center also allows visiting diving scientists the option of using their own SCUBA regulators rather than Center-supplied equipment. Information is provided regarding the dangers of contaminants in the SCUBA first stage and where such contaminants may originate. Each diver makes an informed choice on whether her equipment has potentially been contaminated and therefore should not be used with enriched air mixtures.

Conclusions

The potential for fire in a high pressure gas system containing oxygen can only be minimized, never eliminated. SCUBA equipment can be cleaned and modified to include materials more compatible with oxygen by personnel otherwise expert in the servicing of such equipment, provided that they use the correct procedures and materials and oxygen-compatible lubricants. Diving equipment and compressor manufacturers are strongly encouraged to recognize and evaluate issues regarding oxygen compatibility and enriched air NITROX service, and to establish guidelines and internal policies. Diving equipment manufacturers should offer complete sets of equipment that can be dedicated to enriched air NITROX service, including tanks, regulators, gauges, and whips intended for this service. Further, they should establish cleaning facilities for their equipment to re-clean it annually or as needed. Safe enriched air NITROX SCUBA diving operations need close coordination with equipment manufacturers on matters of oxygen safety.

Conventional oil-lubricated compressors and filter systems produce output air with some hydrocarbon contamination. As a result, the use of air from oil-lubricated compressors as an add gas to and in contact with pure oxygen during the production of enriched air NITROX breathing mixtures is regarded as an unacceptable risk unless scrupulous and consistent maintenance can be ensured. The average retail dive shop owner does not have the technical expertise nor the financial resources necessary to maintain this type of high pressure air system at oil contamination levels acceptable for use in oxygen service. The use of oil-free or oil-less compressors is an acceptable and recommended method of making enriched air mixtures, but they are expensive to own and maintain.

Factory pre-mixed enriched air NITROX used with an oxygen clean transfilling system (booster pump) and diving equipment dedicated only to enriched air use is the best overall method of producing low volumes of enriched air NITROX for small operations or retail dispensing with adequate gas

quality assurances. Full oxygen cleaning is not necessary, but only oxygen compatible lubricants should be used.

The atmospheric entrainment and cascade methods are best suited to high volume enriched air applications, but additional safeguards must be added to the average air system to improve gas quality assurance and to reduce risk of oxygen-related fires.

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SAMPLING SMALL INVERTEBRATES AT THE SEDIMENT-WATER INTERFACE

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Small invertebrates associated with the sediment-water interface habitat were sampled at four sites in Onslow Bay, North Carolina from 1983 to 1989 by five methods: vertical plankton net tows, reentry trapping, emergence trapping, airlift sampling, and Ekman grab sampling. Comparisons of the assemblages captured show that each method samples a different assemblage of small invertebrates. Three more or less distinct assemblages of small invertebrates can be identified: a holozooplankton group distinguished by calanoid copepods, chaetognaths, and larvaceans; a demersal zooplankton group distinguished by cumaceans, gammarid amphipods, and mysids; and a meiofauna group distinguished by high proportions of nematodes and harpacticoid copepods. Several taxa are important fractions of more than one assemblage. Copepod nauplii appear to remain close to the bottom but do not enter or attach to the substrate, and are thus members of both the holozooplankton and demersal zooplankton, as are cyclopoid copepods, which do appear to associate with benthic substrates. Harpacticoid copepods are important members of all three groups. Individual species within these major taxa probably exhibit quite variable behaviors. Consequently, choice of methods used to sample these different assemblages is important.

Introduction

A numerically abundant and taxonomically diverse group of small invertebrates can be found in the sediment-water interface habitat in continental shelf waters. This group includes zooplankters that migrate downward as part of their diel vertical migration pattern, migratory demersal zooplankton that spend part of each day in direct contact with the bottom and part of each day in the plankton, larval forms that settle from the plankton onto benthic substrates, and interstitial meiofauna living in the sediments. These organisms range in length from approximately 0.1 mm, *e.g.*, nauplii, to tens of centimeters, *e.g.*, penaeid shrimps. They include herbivores and carnivores, and collectively support many economically important macrofauna.

A variety of specialized methods have been developed to sample small invertebrates in the interface habitat, since conventional plankton tows can not sample this habitat adequately. Demersal zooplankton have been sampled by emergence and reentry trapping, which take advantage of their migratory behavior (Youngbluth, 1982; Alldredge and King, 1985; Cahoon and Tronzo, 1988). Airlift samplers have also been used to sample demersal zooplankters (Stretch, 1985). Interstitial meiofauna have typically been sampled by sediment grabs (Coull *et al.*, 1982).

Each of these sampling methods has been shown to sample with significant biases. Emergence traps can be contaminated with holozooplankters (Youngbluth, 1982) or by crawling meiofauna (Robichaux *et al.*, 1981). Reentry traps can also be contaminated by crawling meiofauna and by larval forms entering the substrate (Robichaux *et al.*, 1981). Migratory forms may avoid traps, enter them preferentially, or migrate infrequently so that their actual abundance is underestimated by short trap deployments (Jacoby and Greenwood, 1988; Stretch, 1985). Airlift samplers may collect sediment-associated fauna that migrate infrequently more completely than the trapping methods, but may oversample zooplankters and meiofauna or undersample animals with escape responses. Grab samplers can not quantitatively sample animals in the water column, even those closely associated with the bottom. These sampling biases almost certainly produce incomplete, unrepresentative estimates of the actual taxonomic and numerical composition of the small invertebrate fauna of the interface habitat, particularly when only one method is used.

We have used each of these methods to study the taxonomic composition and abundance of the small invertebrates associated with several benthic communities in Onslow Bay, a portion of the North Carolina continental shelf, as part of our efforts to understand the trophic bases supporting the shelf's fisheries. We are particularly interested in the role of planktonic and soft-bottom food sources in supporting hard bottom-associated fishes. Consequently, we have sampled holozooplankton, demersal zooplankton, and meiofauna, and have often used two or more of the methods described above concurrently. Here we compare the assemblages sampled by different, concurrently used methods, and address two questions: How do the assemblages of small invertebrates sampled by plankton nets, emergence traps, reentry traps, airlift samplers, and sediment grabs differ taxonomically? What do the results of the different sampling approaches tell us about the distribution and behavior of the important small invertebrate taxa of the interface habitat?

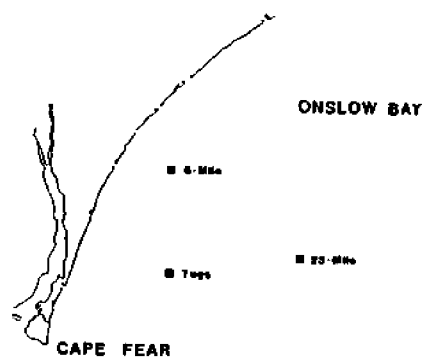

Fig. 1. Location of study sites in Onslow Bay, North Carolina, where small invertebrate collections were made, 1983-1989.

Methods and Materials

We sampled the small invertebrate fauna associated with the sediment-water interface habitat at several locations in Onslow Bay, the portion of the North Carolina continental shelf bounded by Capes Lookout and Fear and seaward by the western edge of the Gulf Stream. Data presented in this paper resulted from sampling at four locations in Onslow Bay (Fig. 1). Two of these sites were characterized

by open sandy bottom: the "6-mile" site, at a depth of 19 m, and the "Deep" site, at a depth of 41 m. The "23-mile" site is located at a natural limestone ledge, with depths ranging from 28-33 m, and was the most frequently sampled location. The "Tugs" site is an artificial reef consisting of two tugboats approximately 400 m apart. The sampling efforts described here were conducted from 1983 to 1989.

Most of the sampling employed emergence trapping and reentry trapping of demersal zooplankton. Emergence traps were conical nets of 95 µm mesh 1 m high with a steel ring sewed into the mouth to keep the net open and directly on the bottom during its deployment. A one liter polyethylene bottle with a funnel glued into its throat collected animals migrating upward in the trap. Reentry traps were polyethylene pans with snap-on lids, 21 cm x 21 cm, filled with 1.0 liter of cleaned sand, which animals entered from the plankton. Both kinds of traps were placed on the bottom by SCUBA divers, left overnight or, in some cases, during the day, and retrieved following closure of the traps on the bottom. We usually deployed ten reentry traps and six emergence traps simultaneously. Animals collected in the traps were concentrated on a 95 µm mesh sieve (after rinsing with fresh water to dislodge animals in reentry traps), fixed and stained in a seawater-5% formalin-Rose Bengal solution, and identified and counted with the aid of a dissecting microscope and appropriate taxonomic keys.

We collected zooplankton in the water column by towing a 105 µm mesh, 0.5 m diam zooplankton net vertically from the bottom. These collections were made at night during several of the overnight deployments of the emergence and reentry traps. The resulting zooplankton samples were fixed, stained, identified, and counted as above.

We used airlift samplers to collect small invertebrates associated with the substrate at two times and locations, in April, 1985, at the "Tugs" site and in August, 1989, at the "23-mile" site. The airlift device we used was a PVC pipe, 5 cm diam x 1.3 m length, with an air inlet approximately 40 cm from the lower end. We used a SCUBA tank hooked to the air line by a first stage SCUBA regulator to supply air. The airlift device created suction from air bubbles flowing out the top end of the pipe, which had a 105 µm mesh collection bag clamped on it to retain sediment and animals. Divers placed a fiberglass collar around an area (0.044 m^2) to be sampled, used the airlift to suction all the substrate inside the collar, replaced the sample collection bag, and moved the collar to another sampling spot to repeat the process.

We sampled small invertebrates in the sediments at the "23-mile" site in August, 1989, using an Ekman grab (15 cm x 15 cm) pushed to a depth of at least 6 cm by SCUBA divers. Sediment samples were placed in plastic bags, sealed, and returned to the surface, where they were fixed and stained as above. Grab samples were collected in triplicate every ten m along a transect running from the limestone ledge to a point 50 m from the ledge for a total of 18 samples. Animals were removed from the sediment samples by repeated washings with a hypersaline solution, concentrated on a sieve as above, and identified and counted using a dissecting microscope and appropriate taxonomic keys.

Results

The data sets resulting from our field sampling efforts at the various Onslow Bay sites in the period 1983-1989 did not represent a balanced set of methods vs. sites vs. sampling dates. A variety of constraints, including limited opportunity to sample, limited access to the offshore sites, and differences in the ease with which the different sampling methods could be used under field conditions contributed to the incomplete nature of the data sets we report here. Furthermore, the data from replicate samples were frequently not normally distributed. Therefore, we present estimates of percent similarity of the assemblages sampled by two or more methods, or of assemblages collected from two or more substrates or sequential periods from the same sampling location and date (Fig. 2). Exceptions to this pattern are comparisons of the assemblages sampled by reentry traps in 1983 vs. airlift and Ekman grabs in 1989 at the "23-mile" site, since this site was the most intensively studied of the four, and

comparisons of the assemblages sampled by airlift at the "Tugs" site in 1985 and at the "23-mile" site in 1989. Percent similarity values were calculated according to Brower and Zar (1977), using data from taxa that accounted for $\geq$ 1% of the total animals captured in at least one set of samples.

Percent similarities are generally highest for comparisons of the same sampling method at different sites or dates (Fig. 2). Thus, reentry trapping frequently yielded very similar assemblages of small invertebrates (up to 92% similarity), especially when samples collected from the same site on the same sampling trip are compared. However, reentry trap samples from different sites, *e.g.*, the "Deep" and "23-mile" or "Tugs" site are quite dissimilar, suggesting that reentry traps were sampling very different assemblages at these sites. Similarly, emergence trap samples are most similar for samples collected at more or less the same site and date, and are much less similar among different locations. Plankton samples and samples collected by airlift samplers are also more similar to other samples collected by the same methods than to other kinds of samples, although the percent similarities between samples collected by the same method are substantially lower for these two methods than for reentry trapping. Ekman grab samples were collected only once, precluding estimation of the consistency of the assemblage collected by this technique.

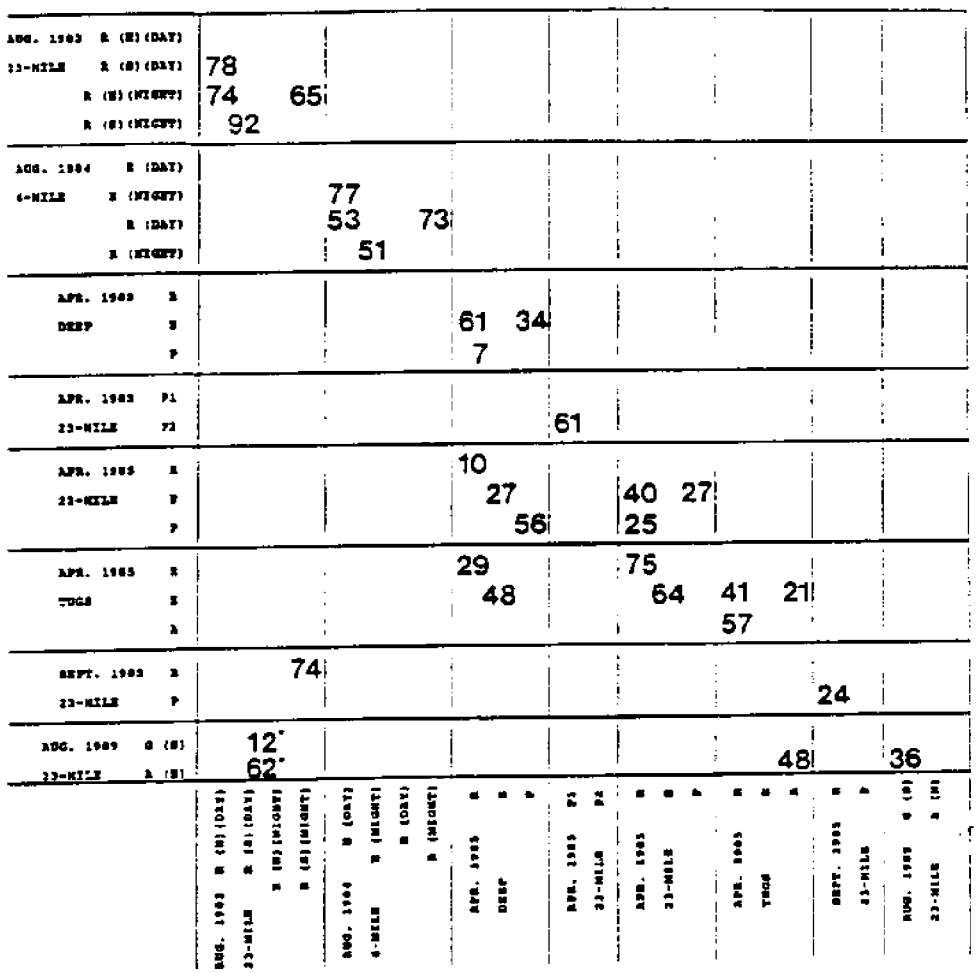

Fig. 2. Percent similarity values for assemblages of small invertebrates at Onslow Bay sites, 1983-1989. "R" = reentry trapping; "E" = emergence trapping; "P" = vertical plankton tows; "A"= airlift sampling; "G" = Ekman grab sampling; "(H)" = samples collected over hard substrate; "(S)" = samples collected over soft substrates; "(Night)" = samples collected at night; "(Day)" = samples collected during day (all unlabeled samples were collected at night).

Comparisons of percent similarity values for samples collected by different methods, even at the same site and date, show that each method is collecting a distinct assemblage of organisms. Assemblages collected by plankton tows are substantially different from assemblages collected by either reentry traps or emergence traps. Assemblages collected by Ekman grabs are also substantially different from those collected by other methods. Assemblages collected by reentry traps and airlift samplers are relatively more similar, as are assemblages collected by reentry traps and emergence traps.

We averaged the percent composition of the assemblages collected by each of the five sampling methods to facilitate overall comparisons among them, recognizing that averaging in this way obscures the lack of simultaneous comparisons in our field work (Table 1). Taxa that represented at least one percent of the total animals captured by any method are listed here. Differences among the sampling methods are again obvious. Reentry trap samples are dominated by harpacticoid copepods, cyclopoid copepods, and nematodes. Reentry traps also collect smaller, but important, numbers of larger invertebrates, *i.e.*, cumaceans, gammarid amphipods, mysids, and isopods. Emergence trap samples are dominated by copepod nauplii, harpacticoid, cyclopoid, and calanoid copepods, as well as noteworthy numbers of cumaceans, gammarid amphipods, larvaceans, and chaetognaths. Plankton samples are dominated by calanoid and cyclopoid copepods and larvaceans. Airlift samples are dominated heavily by harpacticoid copepods and nematodes. Grab samples are dominated heavily by nematodes and harpacticoid copepods. The latter two methods also collected an order of magnitude more total organisms than the other methods, but probably for different reasons, as we discuss below.

Table 1. Comparisons of small invertebrate taxa sampled by each of five collection methods. Data are weighted mean percent of total animals captured by each method. "R" = reentry trap, "E" = emergence trap, "P" = vertical plankton tows, "A" = airlift sampler, "G" = Ekman grab sampler. "n" is total number of samples used to generate data.

	R	E	P	A	G
Taxon n =	92	41	16	22	18
Copepod nauplii	0.04	0.36	0.05	<0.01	0
Harpacticoida	0.42	0.15	0.02	0.66	0.10
Cyclopoida	0.17	0.14	0.26	0.01	<0.01
Calanoida	0.01	0.14	0.31	<0.01	0
Cumacea	0.12	0.09	0	<0.01	0
Isopoda	0.01	<0.01	0	<0.01	0
Gammaridea	0.05	0.05	0	0.01	0
Ostracoda	0.01	<0.01	0.02	0.03	0.01
Mysidacea	0.04	<0.01	<0.01	0	0
Polychaeta	0.02	0.01	0.01	<0.01	<0.01
Nematoda	0.13	<0.01	<0.01	0.28	0.88
Chaetognatha	<0.01	0.03	0.03	0	0
Larvacea	<0.01	0.04	0.10	0	0
Mean # animals m^{-2}	15,860	19,970	16,675	149,000	159,000

Comparisons of the different sampling methods by taxon also illustrate differences among the assemblages caught by those sampling methods. Copepod nauplii, calanoid copepods, chaetognaths, and larvaceans were larger fractions of the assemblages captured in emergence traps and plankton tows than by other methods. Cumaceans, gammarid amphipods, and mysids were larger fractions of the assemblages caught in reentry and emergence traps than by other methods. Cyclopoid copepods were a much smaller fraction of the animals caught by airlift or grab sampling, but a similar number of them were caught by each method. Harpacticoid copepods, in contrast, were a major fraction of only the assemblages sampled by reentry traps and airlift samplers. Nematodes were also important members of the assemblages caught by reentry traps and airlift samplers, and dominated the assemblage sampled by Ekman grabs.

Discussion

We identify three distinct but somewhat overlapping assemblages of small invertebrates associated with the sediment-water interface that can be identified by comparisons of different sampling methods. We think that much of the overlap among assemblages may result from interspecific behavioral differences within the major taxa we sampled.

First, we identify an assemblage of animals that never leave the plankton to associate directly with the substrate, a group we term "holozooplankton", which includes copepod nauplii, calanoid copepods, chaetognaths, and larvaceans. Presumably zooplankters such as hydrozoan and scyphozoan medusae, salps, ctenophores and others that can be abundant in the zooplankton at times should also be included in this group. The diel migratory behaviors of these animals are likely to bring them into close proximity to the bottom, particularly in daylight, which can explain their occasional capture in reentry traps.

Another distinct assemblage includes taxa that reside in the sediments, termed interstitial meiofauna (Coull *et al.*, 1982). This assemblage includes very large numbers of nematodes and harpacticoid copepods. Capture of some of these animals by methods other than grab sampling implies that some of these species can swim and enter the water column at least occasionally (Palmer, 1988; G.T. Chandler, pers. comm.), or that a small percentage of the large numbers of these animals in the sediment enter traps on the bottom by crawling (Robichaux *et al.*, 1981). The numbers of harpacticoid copepods captured by each method imply that some species are likely to be partly or even wholly planktonic, as others have found (*e.g.*, Owre and Foyo, 1967). The large numbers of nematodes captured by reentry traps and airlift sampling implies that many of them must occur close to the sediment surface, where benthic microalgae, a likely food source, are concentrated (Cahoon *et al.*, 1990).

Previous work has focused on the third assemblage we identify here, the demersal zooplankton (Cahoon and Tronzo, 1988, submitted; Tronzo and Cahoon, 1989; Tronzo, 1989). We define this assemblage as those animals that associate closely with the sediment-water interface and routinely spend at least part of the time in the water. Anger and Valentin (1976) called this group the "hyperbenthos". Some of them, such as cumaceans, gammarid amphipods, and mysids, probably stay close to the sediment surface, which would account for their rare capture in nighttime plankton tows vs. their abundance in reentry and emergence trap samples. Others, such as harpacticoid and cyclopoid copepods, spend more time in or migrate farther up into the water column. In this case, further investigation is likely to show that some species are mostly or exclusively planktonic, while others are demersal.

The demersal group may also be defined to include forms that stay close to the bottom, even if they do not enter or attach to the substrate. Such forms may include copepod nauplii, which generally hatch from eggs in sacs carried by cyclopoid and harpacticoid copepods or laid singly to sink in the water by calanoid copepods.

We note the general absence of significant numbers of larvae of benthic invertebrates in our samples, with the exception of larval and juvenile polychaetes. A wide variety of larvae were captured in our studies, primarily by reentry traps, but rarely in numbers sufficient to account for 1% or more of the total captured (Tronzo, 1989). Nevertheless, we recognize that such larvae, which can be termed meroplankton, can be recognized as a distinct group whose collective life cycle includes periods as holozooplankton and as demersal and/or benthic forms.

None of the sampling methods we used in this study appears to sample all three assemblages we define above equally well. In part this owes to the limitations of each method, but also to the variability in the behavior of the animals themselves. Obviously, the choice of a sampling method or methods depends on the assemblage one wishes to sample. We consider vertical plankton tows to be a standard method for sampling the holozooplankton. However, we identify here some biases that may drive preferential selection of one method over others for sampling the assemblages that associate with the interface.

Grab sampling quite clearly is the method of choice for quantifying interstitial meiofauna. However, larger forms, such as cumaceans, gammarid amphipods, and mysids, which associate closely with the sediment and are likely to be important consumers in sediment communities, are not sampled well by this method. We speculate that these animals can actively avoid small grabs, especially when deployed by divers with the accompanying disturbance. Nelson (1979) collected amphipods with a ship-deployed sediment grab, but without establishing the quantitative accuracy of this method.

The majority of our airlift samples were collected over hard substrate at the "23-mile" site. The assemblage collected by this method may therefore represent the fauna resident on this kind of habitat, which includes a thin covering of sediment, or may represent bias in this collection method. We note, for example, the lack of the larger demersal forms in samples collected by airlift. The airlift collects planktonic animals from the water surrounding a partially enclosed area of substrate, so that serious overestimates of their abundance result. Thus, harpacticoid copepods may be overrepresented in airlift samples (Table 1) compared to other sampling methods.

Reentry and emergence trapping have been compared as tools for estimating the abundance of demersal zooplankton in previous studies (Alldredge and King, 1980, 1985; Cahoon and Tronzo, 1988). Reentry trapping appears to be a better method, in that emergence trapping captures more holozooplankters and fewer animals that remain close to the bottom. Reentry trapping, however, may be biased by the entry of some crawling harpacticoids and nematodes. However, given the abundance of these two taxa in the sediments, their presence in reentry traps may be indicative of their availability to demersal feeders, such as sciaenid fishes and penaeid shrimps. Thus, we prefer reentry trapping as a method for sampling demersal zooplankton.

Several of the comparisons between assemblages captured by the same methods at the same but in different locations, *i.e.*, in April, 1985, at the "Deep", "23-mile" and "Tugs" sites, showed very low similarities even when the same sampling method was used. These low similarities reflect major taxonomic differences among the demersal zooplankton assemblages associated with these sites. The demersal zooplankton at the "Deep" site were dominated by cumaceans, gammarid amphipods, and mysids, unlike the other two sites, which were dominated by harpacticoid and cyclopoid copepods (Tronzo, 1989; Cahoon and Tronzo, in press). We attribute this difference to the lack of planktivorous fishes at the "Deep" site compared to the two other, reef-associated sites. If airlifts and Ekman grabs undersample these larger demersal forms, the biomass and importance of demersal zooplankton sampled by these methods at sites where they dominate may be seriously underestimated.

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CHARACTERISTICS OF BENTHIC MICROALGAE FROM THE NORTH CAROLINA OUTER CONTINENTAL SHELF AND SLOPE: PRELIMINARY RESULTS

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Sediment samples were collected from seven sites on the outer continental shelf and slope off North Carolina in October, 1991, using a remotely operated vehicle (ROV). Sampling depths ranged from 45 to 222 m. Light and chlorophyll a data indicated that significant populations of productive benthic microalgae may extend from the continental shelf down the upper slope off North Carolina to depths of at least 63 m. Chlorophyll a detected at deeper depths probably represents inactive forms of microalgae. Examination of bulk sediment samples reveals a variety of pennate diatoms and an unidentified colonial chrysophyte. The ratio of fucoxanthin:chl a in sediment samples at 63 m suggests either photoacclimation to low light flux dominated by short wavelength light or abundance of unusually fucoxanthin-rich taxa. Benthic microalgae populations in upper slope habitats may be important food for slope consumers and may be important sources of organic carbon flux to the deep sea.

Introduction

Benthic microalgae are important primary producers in Onslow Bay, the portion of the North Carolina continental shelf bounded by Capes Lookout and Fear and the Gulf Stream. Cahoon *et al.* (1990) showed that benthic microalgal biomass, measured as chlorophyll a, exceeded integrated phytoplankton biomass across the entire shelf, accounting for as much as 80% of the chlorophyll a in Onslow Bay. Measurements of benthic microalgal production at inner and mid shelf stations showed that benthic microalgal production was nearly equal to integrated phytoplankton production in these shelf waters (Cahoon and Cooke, 1992). Examination of sediment samples revealed that the benthic microflora at inner and mid shelf stations was dominated by pennate diatoms, particularly monoraphic and biraphic forms, principally of the genera Achnanthes, Amphora, Cocconeis, Delphineis, Diploneis, Navicula, and Nitzschia (Laws and Cahoon, 1992; Cahoon and Laws, submitted).

Cahoon *et al.* (1990) also reported substantial chlorophyll a concentrations at depths beyond the shelf break (approximately 55 m) in Onslow Bay, down to depths of 285 m. Chlorophyll a concentrations sampled at six depths below 100 m averaged 0.45 μg chl a/g sediment, values similar to those found for mid- and outer-shelf depths. However, chlorophyll a concentrations in the shelf break

region were significantly lower than these values, which was attributed to physical disturbance at the shelf break and the coarse nature of sediments there (Cahoon *et al.*, 1990).

Cahoon and Cooke (1989) incubated sediments with ^{14}C-labeled bicarbonate solutions in situ at a 285 m station on the N.C. slope to determine if chlorophyll a previously detected at this depth by Cahoon *et al.* (1990) represented productive microalgae. A subsequent set of incubations in the laboratory using ^{14}C-labeled amino acids and sugars was conducted to determine if heterotrophic nutrition might support viable microalgae in these sediments. Neither autotrophic nor heterotrophic production was detected, leading to the conclusion that the viable chlorophyll a signal at the deepest slope sites resulted from resting stages of microalgae. Light flux at 285 m was up to six orders of magnitude less than surface incident radiation, well below the minimal light levels (0.1% surface incident radiation) thought to be capable of supporting autotrophic production (Falkowski, 1988). Incubations of sediment samples at low light levels (10-100 $\mu E\ m^{-2}\ s^{-1}$) in nutrient medium yielded cultures of four genera of planktonic diatoms, confirming that resting forms of microalgae occurred in these sediments.

Measurements of benthic microalgal biomass and production in another continental shelf ecosystem, Stellwagen Bank in Massachusetts Bay, showed that benthic microalgae flourished at depths below the 1% light level. Significant benthic microalgal production was measured at places and times at Stellwagen Bank when average integrated light flux to the bottom did not exceed 5-6 $\mu E\ m^{-2}\ s^{-1}$. Light levels in Onslow Bay at the stations visited by Cahoon and Cooke (in press) were usually much higher than 1% of surface incident radiation, and often approached 100 $\mu E\ m^{-2}\ s^{-1}$. Thus, productive benthic microalgae are likely to extend beyond the shelf break in Onslow Bay, even if their depth range does not approach 285 m.

This paper reports preliminary results of an effort to sample and describe a series of microhabitats from the outer shelf and slope of Onslow Bay and the characteristics of benthic microalgae associated with them.

Methods and Materials

Sampling was conducted at seven sites along an onshore-offshore transect across the shelf break in Onslow Bay, at depths of 45, 63, 96, 131, 157, 194, and 222 m (Fig. 1). The 45 m site was at or near the shelf break, and the other six sites were on the continental slope.

A SuperPhantom II remotely operated vehicle (ROV) deployed from a 45' vessel was used to collect sediment samples at each site. The ROV was equipped with a closing scoop device and a video camera positioned to observe the scoop. Samples were collected with the scoop from undisturbed areas of the sediment surface and returned immediately to the support vessel, where twelve sediment cores, 1 cm diam, were removed from each sample. Three of these were fixed with Lugol's solution, and the remainder were frozen. A bulk subsample of sediment was also collected and fixed with Lugol's solution.

Casts of a SeaBird CTD with a 4 pi quantum sensor were made at each sampling location. Variables measured included temperature, conductivity, salinity, PAR flux, and depth.

Six sediment subsamples from each location were analyzed for viable chlorophyll a according to Whitney and Darley (1979). This method employs extraction overnight at 4° C in 90% acetone, followed by partitioning of the extract with hexane and spectrophotometric measurement of absorption at 663 nm in the hexane phase. This method largely eliminates chlorophyll degradation products that are common in sediment samples, and which would otherwise yield spuriously high estimates of benthic microalgal biomass.

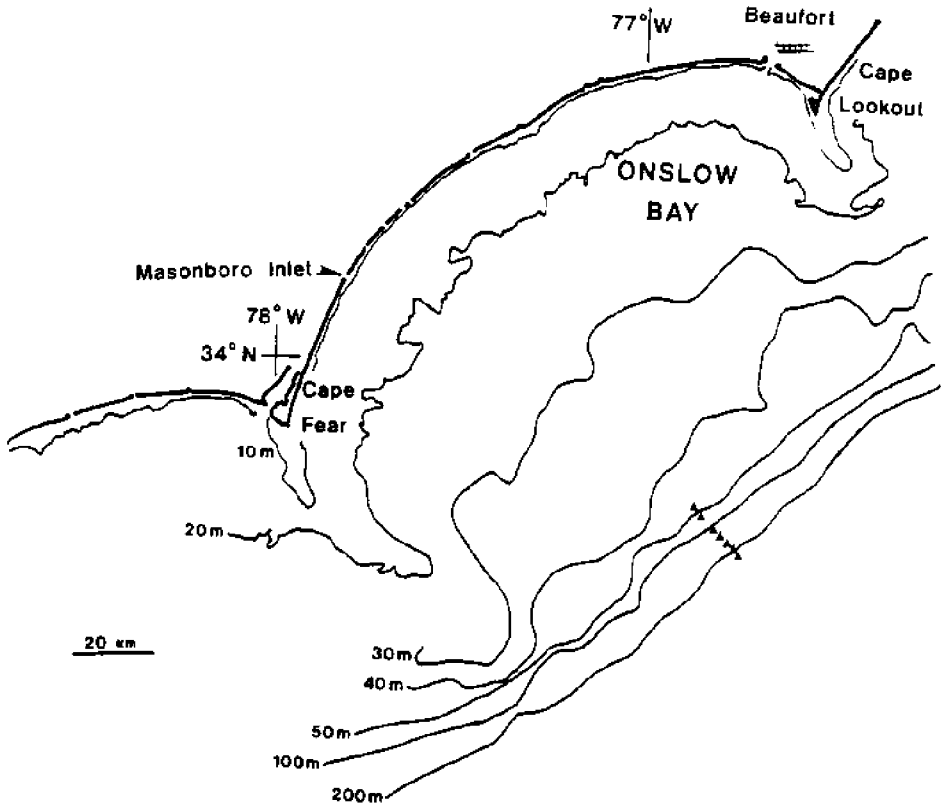

Fig. 1. Map of the North Carolina continental shelf and slope showing study sites sampled by remotely operated vehicles in October, 1991. Site depths are 45, 63, 96, 131, 157, 194 and 222 m.

Up to five sediment subsamples from the three shallowest sites were extracted as above for HPLC analysis of the microalgal pigments present. A 1 ml aliquot of a 90% acetone pigment extract was filtered (0.22 µm nylon membrane), mixed with 0.3 ml of an ion pairing reagent, and injected into an ISCO gradient HPLC system, following the protocol of Klein and Sournia (1987). Pigment peaks were identified by comparison to Klein and Sournia's (1987) results and to chromatograms from pure cultures of various algae with known pigment composition. Peaks corresponding to fucoxanthin and chlorophyll a were identified, and peak areas for these pigments were quantified using ISCO's ChemResearch software.

Sediment samples fixed with Lugol's solution were prepared for diatom identification and counts in the following manner. After measuring the volume of each sample, they were placed in 400 ml beakers and treated with hydrogen peroxide and nitric acid solutions to remove organic matter, followed by acidification with hydrochloric acid to remove carbonates. Sediment subsamples were then mounted on 22 mm^2 coverslips according to the settling technique described by Laws (1983). Coverslips were mounted on microscope slides with Hyrax mounting medium (RI 1.71). Identifications and counts of diatoms were done on an Olympus BH-2 compound microscope using differential interference contrast illumination at 1,250 X. The results reported here are based on examination of two samples (slides) from each of the three shallowest stations. Valve counts to determine relative species abundances were done according to the ribbon count procedure outlined in Laws (1983). At least 500 valves were counted from each sample.

Results

The seven sites presented gradients of sediment composition, bottom temperature, and light flux to the bottom (Table 1). The shelf break site at 45m had very coarse siliceous sediments that included carbonate shell fragments and pieces of old coralline algae. Sediments at the 63 and 96 m sites had finer grained siliceous sediments that included fine shell hash. Sediments from the 131 m site and deeper were more olive-green/black in color, reflecting the presence of characteristic slope minerals, including glauconite and phosphorite, and reduced contents of siliceous sands. Bottom temperatures remained close to surface temperatures (approximately 26° C) at the shallowest three sites, but then declined rapidly with depth. Light flux to the bottom declined exponentially with depth.

Table 1. Environmental data for N.C. slope sites in October, 1991.

Depth (m)	Bottom Temp. (° C)	PAR flux ($\mu E\ m^{-2}\ sec^{-1}$)
45	25.8	46
63	24.0	11.2
96	20.6	1.44
131	17.7	0.28
157	16.1	0.10
194	14.6	0.03
222	*	*

* - CTD cast to this depth prevented by strong current.

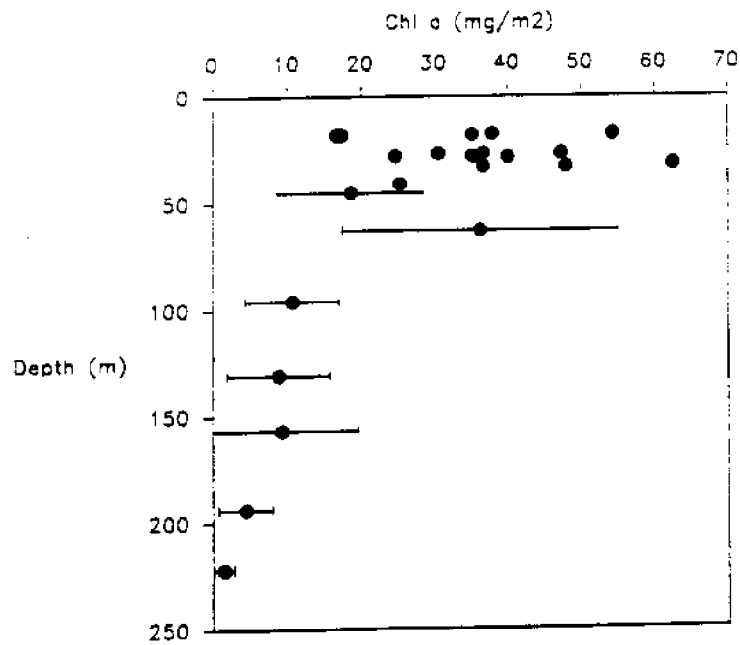

Fig. 2. Distribution of benthic microalgal biomass measured as mg chl a m^{-2} vs. depth for North Carolina shelf and slope samples. Dots with error bars (1 s.d.) are for samples collected by remotely operated vehicle in Oct., 1991 in this study. Single dots are data from N.C. shelf sites reported by Cahoon and Cooke (1992).

Benthic microalgal biomass as chl a was found to be highest at the 63 m site, and was comparable to chl a values for shelf sediments found in previous studies (Fig. 2). Chl a values at the shelf break (45 m) site were lower, a pattern observed previously (Cahoon *et al.*, 1990). Chl a concentrations in sediments at 96 m and below averaged 10 mg m^{-2} or less and were barely detectable at the 222 m site.

Analyses of the fucoxanthin and chl a contents of pigment extracts from sediment samples by HPLC was possible only for the 63 m site, owing to the sensitivity limits of the detector used. The ratio of fucoxanthin:chl a was quite high for these samples compared to ratios found for samples from other locations receiving higher light fluxes (Fig. 3).

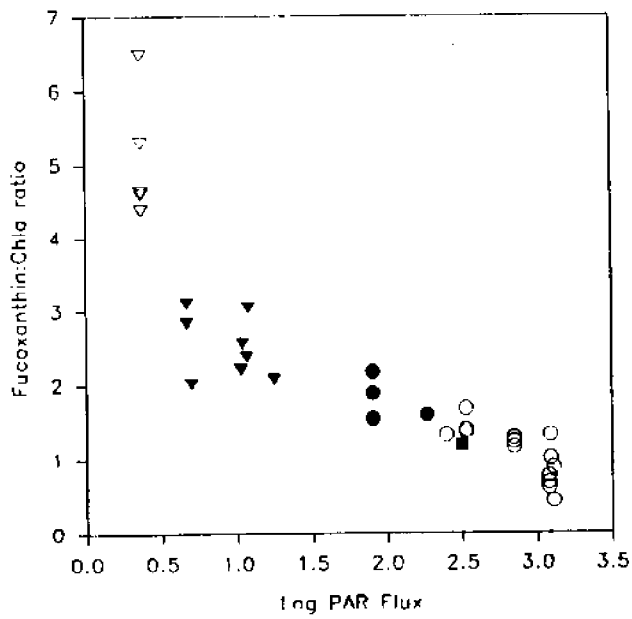

Fig. 3. Response of fucoxanthin:chl a ratio to PAR flux at various shelf and slope sites. Open triangles = N.C. slope site (63 m, this study), closed triangles = Stellwagen Bank (Cahoon *et al.*,submitted), closed circles = N.C. shelf sites, open circles = carbonate and sand sites in Florida Keys, closed square = siliceous sand site at Cedar Key, Florida. Fucoxanthin:chl a data are ratios of peak areas obtained from HPLC analyses of sediment samples.

Observations of cleaned bulk sediment samples revealed the presence of various diatoms, mostly pennate forms, in all samples. Diatom valves were most common in the two shallowest samples, but their abundance drops markedly at the 131 m site. Valves of planktonic taxa were extremely rare in all samples. Diatom valves were extremely rare in samples from the deepest site (222 m) and consisted only of fragments or partially dissolved valves. Significant numbers of a colonial chrysophyte were also observed in sediment from the 63 and 96 m site.

The microfloral assemblages at the 45 and 63 m sites were relatively well developed and were similar to assemblages we studied at a 35 m site in Onslow Bay (Cahoon and Laws, submitted). The diatom flora at the 45 and 63 m sites was dominated by benthic epipsammic and epipelic taxa including Amphora (12%), Cocconeis (28%), Diploneis (11%), Navicula (22%), and Nitzschia (6%). The flora was relatively diverse with a minimum of 50 taxa recorded in one sample from the 63 m site. The only planktonic taxon recorded in these two samples was rare Paralia sulcata. Rare broken and dissolved valves of Coscinodiscus and Actinocyclus were recorded at the 131 and 157 m sites.

Discussion

Light flux to the bottom appears to be adequate to support viable benthic microalgae at shallow slope depths. Light fluxes measured at the 63 m site were as much as twice as high as light fluxes observed to support significant microalgal growth at Stellwagen Bank (approximately 5 $\mu E\ m^{-2}\ s^{-1}$, Cahoon *et al.*, submitted; Table 1). Estimates of light flux to the bottom using a surface incident flux of 2200 $\mu E\ m^{-2}\ s^{-1}$ and light data from Table 1 to calculate a value of the light extinction coefficient, k, predict that 5 $\mu E\ m^{-2}\ s^{-1}$ may reach depths of 80 m in slope waters with this clarity. The 0.1% light level calculated by Falkowski (1988) to be the theoretical limit for autotrophy corresponds to a depth of 90 m in N.C. slope waters. However, Palmisano *et al.* (1985) reported production by benthic diatoms in Antarctic waters at light levels down to 0.6 $\mu E\ m^{-2}\ s^{-1}$, which corresponds to a depth of approximately 110 m. Thus, viable autotrophic microalgae might extend below the shelf break off North Carolina.

The chl a data similarly support the conclusion that productive benthic microalgae extend to shallow slope depths. Chl a values at the 96 m site and deeper are significantly lower than those observed at the 63 m site, and suggest either a small autotrophic population or the presence of inactive microalgal forms, such as spores or cysts, which can retain pigment but do not conduct photosynthesis. The latter interpretation is consistent with the observations of Cahoon and Cooke (1989) from a much deeper site.

There are two interpretations of the chl a data from the 63 m site as an indicator of microalgal biomass. First, phytoplankton resident at depth for some time are known to exhibit "shade adaptation", a condition in which chl a content of cells increases (Falkowski, 1981). In this situation measures of chl a would overestimate actual microalgal biomass. However, we favor a second interpretation, based on the quality of light reaching this site in these waters (Type II oceanic waters, Jerlov, 1970). At the 63 m site over 99% of the light reaching the bottom is composed of wavelengths between 400 and 550 nm, which are usually most efficiently absorbed by accessory pigments, rather than chlorophylls (Parsons *et al.*, 1984). Thus, increases in fucoxanthin:chl a ratio may be adaptive for the shorter wavelength light field predominating at 63 m (Rowan, 1989). We observed a high ratio of fucoxanthin:chl a in samples from the 63 m site, as this explanation suggests. Therefore, chl a concentrations in the sediment samples from 63 m might be a measure of microalgal biomass reasonably comparable to values from shallower shelf samples.

The high fucoxanthin:chl a ratios measured at the 63 m site may also be explained in two ways. High fucoxanthin content may be a physiological "option" for deep-dwelling microalgae, a response to dominance of the PAR spectrum at low light fluxes by blue light. Alternately high fucoxanthin:chl a ratios may reflect dominance of the microalgal assemblage at 63 m by taxa with characteristically high fucoxanthin contents. We are pursuing both hypotheses in laboratory experiments with microalgal cultures under controlled light conditions.

The area of the N.C. slope that appears capable of supporting significant benthic microalgal production is not large in comparison to the extent of the N.C. continental shelf (Fig. 1). However, benthic microalgae growing near the top of the continental slope may provide a food source for slope consumers that is much more concentrated and accessible than other sources, such as phytoplankton or benthic microalgae transported from shallower shelf areas. It is also likely that benthic microalgae on the upper slope make a proportionately greater contribution to the flux of organic material into the deep sea than do microalgae on the shelf, since downslope transport is more likely. The absence of planktonic diatoms from these upper slope sediment samples is particularly intriguing, considering their abundance in slope waters from this area of the western North Atlantic (Marshall, 1982), and their abundance in mid-slope (250-1000 m) sediments from an area off Cape Hatteras we studied previously (Laws and Cahoon, 1992). Continued investigation of the productivity, physiology, taxonomic composition, and distribution of benthic microalgae in continental slope habitats is therefore necessary.

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EMERGENCY BREATHING SYSTEM FOR HELIUM-OXYGEN DIVING TO 300 FEET OF SEAWATER

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SCUBA diving to depths greater than 100 fsw compounds the risks to the diver. The use of mixed gas, helium-oxygen (heliox), eliminated the risk of nitrogen narcosis. Dives to 300 fsw can be made without experiencing this incapacitating phenomenon when using this gas mixture. Large decompression obligations after dives to these depths make immediate ascent in the event of emergency clearly unsafe. The need for a reliable emergency breathing system is well recognized. One method to address this problem utilizing a full face mask with a switch-over block assembly that used air in the event of an emergency was explored. Decompression considerations were important in the selection of air over any other breathing gas mixture.

Introduction

SCUBA diving beyond 100 feet of seawater (fsw) increases its inherent dangers to the diver. The degree of risk compounds with increasing depth. Decompression stress, nitrogen narcosis, carbon dioxide retention and limited gas supply are major factors that make open circuit SCUBA diving with air unsafe at depths of 300 fsw. A helium-oxygen (heliox) closed circuit underwater breathing apparatus (UBA) overcomes the problems of nitrogen narcosis, excessive breathing resistance and inadequate gas supply. It utilizes a constant partial pressure of oxygen throughout the entire dive. The balance of the mix is helium.

Despite these advantages, heliox closed circuit diving has its drawbacks. Decompression concerns remain ever present and more involved. Also, increased UBA complexity and carbon dioxide scrubber limitations are factors related to the UBA itself. Furthermore, the free swimming SCUBA diver, untethered to an umbilical, takes positive control from topside supervision. This places crucial decision making in the hands of the diver affected by pressure, his environment and, possibly, trauma.

This discussion primarily addressed the problem of the emergency gas supply in the event of UBA failure. Currently used bailout procedure for rig failure has the diver breathe from his high pressure flask by activating the bypass valve thus circumventing the scrubber should it be flooded. The solution had to deliver an emergency breathing system (EBS) that was effective yet not prove to be an intolerable physical encumbrance for a free swimming diver attempting to accomplish his work effectively. Diving to depths up to 300 fsw utilizing this system is not for routine diving. Inaccessible locations for large diving platforms or operations such as ordnance disposal in which danger to the support ship is a concern are two such applications. An AGA Full Face Mask (FFM) (Interspiro, Brandford, CT) with switchover block and quick disconnect connection (QDC) provided the necessary interface between the diver and this system.

Background

Equipment

1. AGA Full Face Mask (FFM): The AGA FFM can be substituted for the mouthpiece when diving closed circuit UBA. When worn securely, it can provide nearly the same closed circuit integrity

that the mouthpiece affords. However, moderate masseter muscle fatigue has been consistently reported when the gas tight seal is rigorously maintained. The FFM has the added advantage of increased thermal protection since less facial area is exposed to the water. Hence, the lips are protected from direct contact with cold water and there is no mouthpiece to hold between the teeth. The FFM affords the diver an uninterrupted gas source despite the diver's condition such as jaw fatigue, convulsion or unconsciousness, whereas a standard mouthpiece could fall from the mouth. Loss of the mouthpiece, especially for the unconscious or convulsing diver, can ultimately result in drowning if the gas supply is not restored.

The FFM has been further modified to allow switching between closed circuit and open circuit by simply turning a lever on the face block assembly 90 degrees. Testing for human factors found no difficulty when 9 dry suit-clad divers wearing 3 fingered gloves made the change from closed to open circuit and back to closed circuit during test dives. The gas switches were made smoothly upon orders from topside to do so. These tests were performed in near freezing water while wearing closed circuit UBA (Chimiak, 1991).

2. Quick Disconnect Connection (QDC): A QDC was incorporated into the system at the Naval Coastal Systems Center. The QDC is resistant to the corrosive environment of the sea and exhibits minimal interface volume. The latter requirement is essential to prevent a pressurized slug of water from being driven into the diver's breathing circuit.

3. Umbilicals: A lightweight, small diameter hose of sufficient length to support decompression at the depth of the first stop was required. The internal diameter of the hose inversely influences the dynamic pressure loss but directly increases the size and the weight of the hose reel system. These two factors are in direct opposition in the development of a portable, lightweight, low resistance system capable of supporting the diver's breathing requirements.

4. Emergency Bottle Banks: A bank of high pressure bottles is required. Sufficient volume must be available for the decompression time anticipated. Other factors include the capability to support more than one UBA casualty and the breathing rates under which the system is to be utilized.

Operations

Diving to 300 fsw using heliox closed circuit UBA employs detailed procedures developed by the Royal Navy and the US Navy (Wright, 1983). Important points found in these procedures dictate the requirements for the emergency breathing system. The system accounts for a free swimming diver who experiences a UBA failure between 300 fsw and his first decompression stop at 150 fsw. The total decompression obligation can be over 5 hours (U.S. Navy Diving Manual). The diver swims to the EBS by activating the UBA bypass valve, which was lowered to his first decompression stop. He attaches to the EBS and actuates the switchover lever for open circuit operations. The diver is assumed to be at rest while attached to the EBS during his remaining decompression.

EBS Gas Mixture

The use of air as the breathing mixture for the EBS was incorporated. Air is readily available for use in an EBS. Heliox or Nitrogen-oxygen (NITROX) mixtures require both additional storage and mixing capabilities to supply these gases reliably. Large gas volumes are needed since the EBS is an open circuit system. The diving operations are conducted from rubber boats with limited capabilities and space. Support craft have various levels of service available. Consequently, the assumption is that any advance support the diving operation needs has to be brought to the scene. Air clearly has the advantage logistically. But more importantly, air affords decompression benefits by switching from helium to nitrogen as the inert gas. Heliox decompression is detailed in the US Navy Diving Manual. The decompression table was developed with the assumption that it uses the same partial pressure of oxygen throughout decompression and stepped decreases in the partial pressure of helium are performed in staged ascent to the surface. The substitution of air during decompression has been theorized to pose little difference in the incidence of decompression sickness (DCS). Tests at the Navy

Experimental Diving Unit using air to decompress Heliox UBA divers under constant partial pressure oxygen supported this hypothesis by demonstrating no increase in the occurrence of DCS in those dives (Thalman, 1985).

Two offsetting factors may explain this phenomenon. First, changing a diver's inert gas from helium to nitrogen in his breathing mixture quickly reduces the tissue helium partial pressure differential and greatly enhances the pressure gradient between ambient and tissue partial pressure. This increases helium offgassing. This would probably result in a safer decompression except that a second offsetting factor must be considered. The partial pressure of oxygen during decompression is less at depths shallower than 77 fsw using air than the constant 0.7 ATA of oxygen maintained by the UBA. No premix is available that could safely keep this benefit for an emergency breathing gas. Fortunately, the net effect has resulted in little actual difference noted during manned testing of such decompression practices. Air can be used as a safe EBS gas supply based on these observations.

A precaution is worthy of note. Once the inert gas in the breathing mixture has been changed from helium to nitrogen, switching back to helium later during the same dive can result in isobaric decompression sickness (D'Aoust and Lambertson, 1982). Under such conditions, DCS has been postulated to occur when a diver breathes an inert gas with a relatively slow diffusion coefficient, e.g., nitrogen, and later switches to an inert gas with a higher diffusion coefficient, e.g., helium. This can result in actual tissue partial pressures that are transiently higher than ambient pressures. Under such conditions, DCS can be a possible consequence. Isobaric DCS can result without a depth change to precipitate it, hence its name.

Discussion

The following summarizes the use of an EBS to 300 fsw:

1. Air can be used safely in emergency breathing systems for Heliox diving.

2. The AGA Full Face Mask with its quick disconnect connection is an excellent interface between diver and EBS.

3. Future possibilities could include:
   a. The AGA FFM could be modified for open circuit for both its primary and EBS mode in order to utilize its quick disconnect capability. A diver could use the SCUBA bottle merely to reach the work site where bottles are staged with QDC's. The diver could then use these staged bottles while working and switch back to his SCUBA bottles upon completion of work. If decompression is required, it could be accomplished using an EBS from topside.
   b. The use of oxygen for in-water decompression has been proposed and successfully employed for SCUBA diving. The major criticism is the potential for in-water CNS oxygen toxicity that manifests as convulsion. Use of a full face mask affords two important safety features. First, if it remains on the diver's face, drowning may be avoided since it eliminates the need for the convulsing or unconscious diver to hold a mouthpiece in place. Second, it allows a buddy or tender to quickly decrease the partial pressure of oxygen with a turn of the lever back to air. The diver himself could potentially make such a switch should he be fortunate enough to recognize the premonitory symptoms of oxygen toxicity and prevent the impending convulsion.
   c. A system for dry suit diving could utilize a large auxiliary bottle that serves both for suit inflation and as a transitional gas supply until connection to the EBS was made. The switchover lever would allow transfer between the primary breathing source and this auxiliary bottle. The QDC could attach directly to this bottle. Once attached to the EBS umbilical, the auxiliary bottle would equilibrate and serve as a volume tank. A much smaller diameter EBS umbilical hose could therefore be designed to provide adequate flow rates. In addition, this transitional gas supply would allow the diver a few breaths during the intense

moments when he makes the connection and turns the lever. This is a theoretical recommendation since this use of a volume tank has never been developed to date. It would require a new valve system that would allow the directional flow required for such an application. This concept of a volume tank is not essential but if developed would allow: 1. Hands-free operation once the diver arrives at the 150 fsw EBS; and 2. A possible decrease in the physical size of the EBS by reducing the pressure requirements to provide adequate flow to the diver.

Conclusion

In conclusion, the EBS affords some extremely noteworthy safety features that could potentially decrease the risk of SCUBA diving to deeper depths. It provides some of the safeguards found with tethered umbilical diving yet allows the advantages of free swimming SCUBA with a system that is cost effective to employ.

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Views expressed in this article are solely those of the author.

QUANTIFYING PELAGIC SPAWNING RUSHES IN LABROID FISHES: PRELIMINARY COMPARISON OF DIRECT DIVER AND VIDEO COUNT TECHNIQUES

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Group spawning in the bluehead wrasse (Thalassoma bifasciatum) was quantified by means of direct diver observation and videographic techniques. Both methods independently showed a similar trend in total spawning rushes relative to the number of spawning fish. Video counts tended to underestimate actual spawning rushes while overestimating possible rushes because gamete clouds could not be readily detected. The preliminary analysis completed in the present study indicates that videographic techniques are adequate in obtaining quantifiable, permanent, and continuous documentation of spawning activity in pelagic spawning fishes such as labroids (wrasses and parrotfishes).

Introduction

Motion photography has been used more or less successfully in quantitative studies of reef fishes by Scuba divers (Alevizon and Brooks, 1975; Bortone *et al.*, 1986). The main advantage of the cine-transect technique is that it provides a permanent record of species and habitat associations. A serious drawback of this technique is the difficulty in identifying certain species. Studies of reef fish behavior, particularly reproduction in labroids, have also used motion photography as a means to study these fast-occurring and complex behavioral interactions (Randall and Randall, 1963; Colin, 1978, 1982).

The development of compact video camera equipment and accompanying underwater housings in recent years has facilitated the use of motion photography by Scuba divers. Our objective in this paper was to compare the videographic technique with direct diver observations as means to quantify reproductive activity of pelagic spawning reef fishes. We selected a reef fish that spawns in both pairs and groups of individuals (mass spawning), the bluehead wrasse (Thalassoma bifasciatum), as our target species. This small labroid is abundant throughout the Florida Keys, Bahamas and Caribbean. Drab-colored individuals known as initial phase fish usually migrate daily to traditional spawning sites at the edge of a reef around midafternoon (Warner *et al.*, 1975; Warner and Robertson, 1978; Warner, 1988). This reproductive system provided an ideal model that allowed repeated sampling observations in excellent environmental conditions (shallow water, good light and visibility).

Study Site

Our study was conducted on a coral reef known as Key Largo Dry Rocks located within the John Pennekamp Coral Reef State Park at approximately 25°09'N and 80°17'W (Fig. 1). Observations were made on the southeastern edge of the reef at a traditional bluehead wrasse spawning site (Clavijo and

Lindquist, pers. obs.). Observations and video photography were concentrated around a small boulder coral at a depth of approximately 8 m.

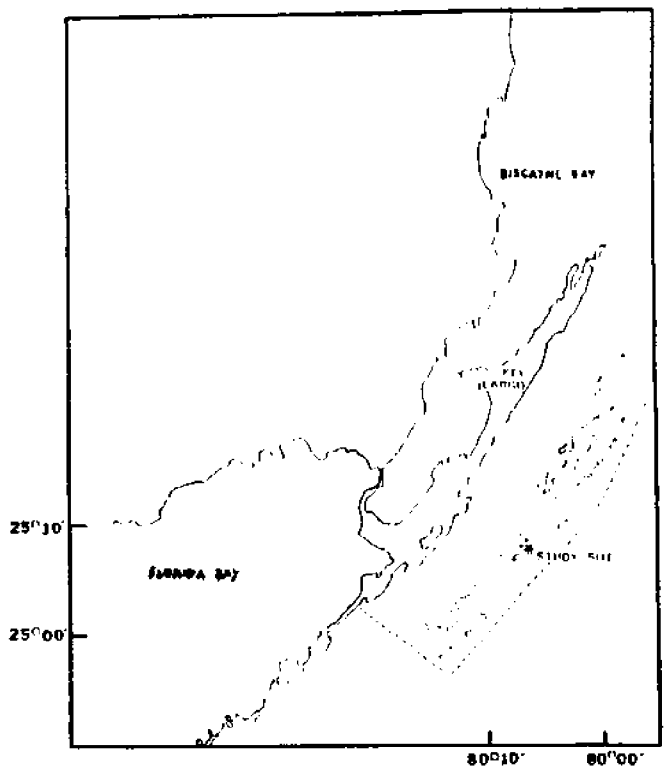

Fig. 1. Map of study site at Key Largo Dry Rocks.

Materials and Methods

A Sony V99 HI-8 mm camera equipped with a wide-angle lens in an Amphibico underwater housing was used to film spawning sequences. The camera was hand held by a diver (DGL) and maintained relatively stationary at the spawning site. The tapes were later duplicated in VHS format. A total of 5.4 video tape hrs was compared with 3.8 hrs of in situ diver observations during August 1991. The same observer (IEC) recorded spawning activity underwater on Polypaper and from video tapes to avoid introducing individual observer variability. Three quantifiable spawning behaviors were recorded: (1) spawning rushes (SR) that included a rapid upward swim by many initial phase individuals and culminated in a visible gamete cloud produced by the released milt, (2) possible rushes (PR) were similar to SRs, but a gamete cloud could not be detected and (3) false rushes (FR) in which the upward swim was interrupted before gametes could be released. Data were expressed as the number of each type of rush per hour in five observation days. Comparisons were made at the same time for each observation day whenever possible during peak spawning hours (1455 to 1720 hrs). The number of fish active at the spawning site each day was estimated visually by a diver (IEC) and rounded off to the nearest 25 fish.

Results

The data obtained from video and direct diver counts are summarized in Table 1. Diver counts consistently had higher numbers of SRs compared to the video counts. The reverse is observed in PRs,

i.e., higher numbers were obtained in video counts compared to diver counts. The number of FRs was low in both video and diver counts and no trend is seen in either counting technique.

Table 1. Estimated total numbers of blueheads and number of spawning rushes (SR), possible rushes (PR), false rushes (FR) and total rushes in video and diver observation counts.

Date	Technique	Total fish	SR	PR	FR	Total rushes	Observation time (min.)
8-1	video	150	58	38	6	102	54
8-1	diver	150	99	12	12	123	52
8-5	video	100	56	18	1	75	51
8-5	diver	100	84	0	3	87	40
8-6	video	100+	26	18	7	51	58
8-6	diver	100+	76	15	0	91	32
8-8	video	75	10	13	6	29	63
8-8	diver	75	26	11	0	37	47
8-9	video	50	9	12	0	21	95
8-9	diver	50	14	5	1	20	59

The total number of all rushes in diver counts was 14 to 21% higher than in video counts on three sampling days. On August 6, this difference was much greater, with 44% greater number of total rushes in diver counts compared to video counts. On August 9, however, the total number of rushes in video counts was 5% greater than in diver counts.

Both techniques showed a decrease in the total number of rushes over the study period with corresponding lower numbers of estimated fish present.

Discussion

The videographic technique underestimated SRs and overestimated PRs. This can be explained in part because of the difference in the distance between rushes and the camera. Direct diver counts were more efficient in detecting gamete clouds possibly because the human eye sees more effectively at longer distances underwater than the camera "eye". Occasionally, background objects interfered with camera observations. For example, a diver releasing air bubbles behind a spawning rush made video detection of the gamete cloud impossible. Human eyes with binocular vision could overcome this problem. The most common problem with video counts, however, included missing the gamete cloud because spawning fish had moved too close to the camera and rushed just beyond camera view. Again, direct diver observation was a more effective means of overcoming this problem.

Both the direct diver and videographic techniques showed similar trends in the total number of rushes relative to the estimated number of fish present. As the spawning population decreased, the difference in counts between the two techniques also appeared to decrease. On August 6, the large difference in counts between both techniques may have been due to the splitting of the spawning population into two groups. The camera recorded the spawning activity of fish close to the reef edge, while the diver attempted to count rushes in both spawning groups. The total number of fish present was underestimated since it was difficult to keep track of both groups.

The videographic technique represents an adequate method of quantifying group spawning. This method not only provides a means to count rushes, but it produces a permanent, continuous documentation of spawning activity that can later be reviewed by other researchers. In deep water (>30m), a camera deployed at a spawning site from a surface vessel or ROV would provide the only effective means of quantifying reproduction in other pelagic spawners such as other labroids (parrotfishes), serranids (groupers) and lutjanids (snappers). For example, the Nassau grouper (Epinephelus striatus), spawns in the Bahamas at depths exceeding 30m (IEC, pers.obs.). Extended documentation can be obtained

compared to the observations of time-limited divers (Colin, pers. comm.). Direct diver observation over short periods of time may still be necessary to confirm fish identifications, obtain additional information on spawning, and to estimate population size.

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SUBTIDAL OBSERVATIONS OF THE PRICKLY SHARK, ECHINORHINUS COOKEI, IN THE MONTEREY SUBMARINE CANYON, CALIFORNIA

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Prickly sharks, Echinorhinus cookei, were observed in situ near Moss Landing California, at one head of the Monterey Submarine Canyon system. Scuba dives were conducted from June 1990 through October 1991. The depths ranged from 15 m to 35 m, and the visibility was generally poor, at one to three meters. A total of 16 dives have been made by the authors, and from one to over 30 sharks were encountered on 13 dives. Approximate total length of the sharks varied from from 1.5 to 4.0 m. We are currently conducting a tagging project designed to gain more information on site fidelity and movements of E. cookei.

Introduction

The prickly shark, Echinorhinus cookei, has been taken incidentally with gill nets and hook and line off the west coast of North America in the lower Gulf of California (Galvan-Magana *et al.*, 1989), and off Los Angeles, San Diego, Santa Barbara, and Moss Landing (Hubbs and Clark, 1943; Roedel and Ripley, 1950; Varoujean, 1972). The known distribution of E. cookei includes the temperate and tropical waters of the Pacific ocean, from central California to Peru, including Hawaii and New Zealand. Specimens have been taken at depths from 10 to 280 m (Roedel and Ripley, 1950; Garrick, 1960; Miller and Lea, 1972; Castro, 1983; Eschmeyer and Herald, 1983; Compagno, 1984; Galvan-Magana *et al.*, 1989). E. cookei are considered rare, and to our knowledge have never been seen alive in their natural habitat previous to these observations.

The classification of E. cookei is controversial; they have been classified in the family Squalidae (Squaliformes), subfamily Echinorhininae (Miller and Lea, 1972), and in the family Echinorhinidae (Squaliformes) (Compagno, 1984; Nelson, 1984). The taxonomic position of E. cookei is still under debate, and it has been suggested that it is more closely related to sharks of the order Hexanchiformes (Varoujean, 1972). Sequences of the 18S rRNA and cytochrome b genes of the prickly shark, the dogfish Squalus acanthias (Squaliformes), and the sevengill shark Notorynchus cepedianus (Hexanchiformes) were found to be equally divergent, suggesting that the prickly shark is no closer to the order Squaliformes than to the order Hexanchiformes (Bernardi and Powers, 1992).

The genus Echinorhinus is comprised of two species, E. cookei and E. brucus (bramble shark) (Garrick, 1960; Miller and Lea, 1972; Eschmeyer and Herald, 1983). The type specimen of E. cookei, caught off Hawaii, was described by Pietschmann (1928), although the specimen itself is missing (Garrick, 1960). Due to the lack of information in the original description, E. cookei has been confused with E. brucus. The two species are similar, but differ considerably in morphology and placement of the

denticles, which are smaller (3 to 5mm at the base) and more uniform in E. cookei (Garrick, 1960; Silas and Selvaraj, 1972; Varoujean, 1972; Bass *et al.*, 1976; Tinker, 1978).

Observations

E. cookei has been observed by Scuba divers at one head of the Monterey Submarine Canyon system near Moss Landing, California (Fig. 1). Scuba dives were conducted from June 1990 to October 1991. The depths of sightings ranged from 15 to 35 m, and the visibility underwater was generally poor, at 1 to 3 m horizontally. Prickly sharks were encountered on 13 of 16 dives, in numbers ranging from 1 to over 30 sharks on one dive (Table 1).

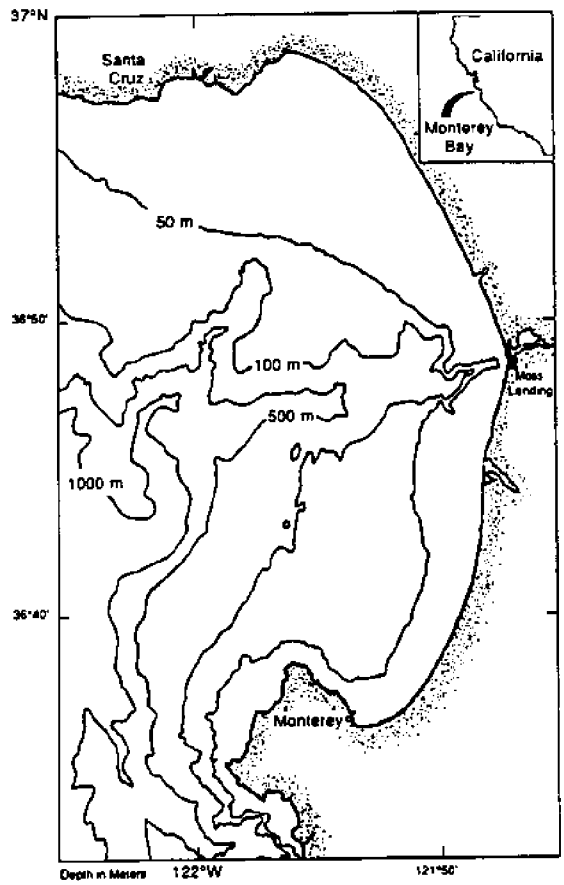

Fig. 1. Monterey Bay, showing depth contours to 1000 m. The head of the canyon where the prickly sharks are sighted is located at Moss Landing.

Once the canyon axis was found, divers descended to 30 meters, and swam along the canyon slope on a heading of 270 degrees. Estimated distance travelled along this bearing was 150 meters (using kick cycles). Counts of E. cookei were taken to one meter above and one meter below the divers, who swam one meter apart. This gave an approximate area of 450 square meters for each transect. At the termination of the first transect, divers ascended to 20 meters and swam a second transect on a heading of 90 degrees.

The bottom and edges of the canyon axis are primarily soft sediment, becoming compact and clay-like as one proceeds westward along the north wall of the canyon head. This section supports a relatively diverse, primarily benthic community of organisms. The walls begin to drop steeply at approximately 20 m, and it is in this area, to 35 m, that most of our observations of E. cookei were made.

Table 1. Number of prickly sharks, Echinorhinus cookei, observed in the Monterey Canyon.

Date	Number of sharks sighted	Visibility (m)	Depth (m)	Density (per m^2)
June 15, 1990	10	1.0	32	.022
June 20, 1990	30+	2.0	19-24	.066
June 21, 1990	12	2.0	32	.027
July 31, 1990	3	2.0	26	.006
Aug.1, 1990	0	3.0	20-33	0
Aug 16, 1990	0	1.5	20-33	0
Sept. 13, 1990	10+	2.0	26	.022
Sept. 14, 1990	8	3.0	26	.017
April 21, 1991	1	2.0	26	.002
May 26, 1991	1	3.0	34	.002
June 10, 1991	2	1.0	33	.004
June 22, 1991	0	1.5	20-33	0
June 26, 1991	3	1.5	35	.006
July 7, 1991	5	1.0	23	.011
Sept. 7, 1991	3	1.5	15-24	.006
Sept. 8, 1991	5	1.5	28	.011

Prickly sharks seem to be sluggish swimmers, although they move quickly when alarmed. They were often observed with sediment on their dorsal surface which is indicative of slow movement (see Fig. 2). They generally oriented close to the bottom or canyon slope. During one dive when approximately 40 prickly sharks were observed, the sharks appeared to swim haphazardly in many directions. Even in the poor visibility of two meters on that particular dive, up to 4 sharks could be seen together, indicating their close proximity to one another. The sharks didn't seem disturbed by our presence, and even tolerated physical contact.

Of the more than 90 E. cookei encountered, several had distinctive markings. On three, the upper lobe of the caudal fin was stunted (almost missing on one), and two had bumps approximately 1-2 cm in diameter along the posterior trunk and caudal fin area (the only area seen). In addition, several individuals were seen with circular and elongate wounds and scars (approximately 10 cm in diameter) along the length of the body. A video record of these observations was taken by the senior author, but we were unable to determine if the same individuals were seen more than once.

Consistent with others' observations (Garrick, 1960; Tinker, 1978), the larger sharks were thick bodied, and the girth on one shark (estimated length 4 m) appeared close to 1/4 the total length. Eschmeyer and Herald (1983) suggest that although E. cookei is a deep water species, immature sharks frequent shallow waters, while the adults probably remain in deep water. We observed several large females and males over three meters in length, and large males had well developed claspers. Of the 34 specimens collected by Varoujean (1972), total lengths of males ranged from 0.47 meter to 2.2 meters, while total lengths of females ranged from 0.44 meter to 3.0 meters. Our observations indicate that adult E. cookei, as well as juveniles, are found periodically nearshore in water less than 35 meters depth.

We are currently tagging E. cookei in an attempt to gather more information, through resightings, on site fidelity. Because prickly sharks are easy to approach and are not aggressive, They are tagged by divers using modified pole spears. To date, 18 sharks have been tagged in the canyon, and none have been resighted. We plan to explore other canyon heads in the area, both with divers and possibly using remotely operated vehicles (ROVs) to locate E. cookei. Acoustic tags, also attached to the sharks by divers, will give us more information on movement patterns. These data, and continued observations by divers, will add to the small database that currently exists on the little known prickly shark.

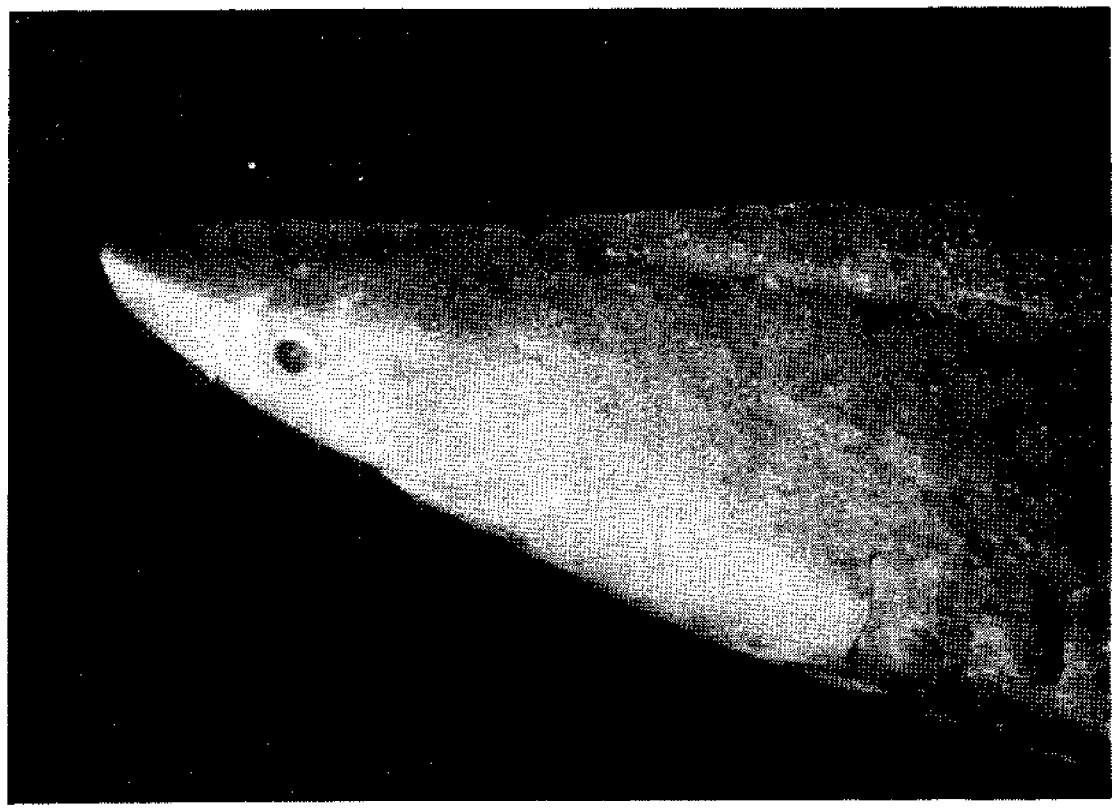

Fig. 2. Photograph of a prickly shark in the canyon (photograph by John Heine).

Acknowledgements

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SEASONAL AND ANNUAL VARIATIONS IN THE UNDERWATER LIGHT ENVIRONMENT OF AN ARCTIC KELP COMMUNITY

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The arctic kelp, Laminaria solidungula, is a predominant member of the Boulder Patch kelp bed community in the Alaskan Beaufort Sea and serves as both food and shelter for a diverse assemblage of marine invertebrate fauna. The growth and productivity of L. solidungula is related to its underwater light environment, which varies considerably on both spatial and temporal scales. Continuous measurement of the amount of photosynthetically active radiation (PAR) reaching the plants was examined in August 1984 and continuously from August 1986 to August 1991. Specially designed water-tight chambers containing LI-COR dataloggers were deployed by divers at seven locations on the seabed. Maximum daytime levels of PAR showed large seasonal differences, ranging from 0 to 15 µmol photons $m^{-2}s^{-1}$ during the ice-covered period to between 0 and 250 µmol photons $m^{-2}s^{-1}$ during the open-water season. Periods of decreased water transparency during the summer and large patches of turbid ice in winter were the major causes of low or undetectable levels of PAR. The lowest annual quantum budgets for L. solidungula ranged from 45 to 50 mol $m^{-2}yr^{-1}$, which represented only about 0.2% of total surface PAR. Although, previous studies indicate that L. solidungula possesses a very low light requirement for net photosynthetic carbon production, our data indicate that this species is living at its physiological limits in the Beaufort Sea Boulder Patch.

Introduction

This paper presents the results of a multi-year study on the underwater light environment of an arctic kelp community. Known as the Boulder Patch, this community is a unique feature of the Alaskan Beaufort Sea shelf, which is otherwise characterized by unconsolidated sediments and the absence of hard rock substrates (Dunton, 1992). Measurements of photosynthetically active radiation (PAR) were collected to assess quantitatively changes in suspended sediment concentrations that may have resulted

from the development of an offshore oil production facility (the Endicott Development Project), to examine temporal and spatial variations in underwater PAR, and to calculate annual quantum budgets for Laminaria solidungula. Only the latter two objectives are addressed here; the assessment of long term light availability in relation to the Endicott Project are addressed elsewhere (Gallaway and Martin, 1992).

Variations in annual quantum irradiance, when used in conjunction with growth and photosynthetic parameters, are critical in estimating the minimum light requirements for growth and survival of the plant in the field. For example, an earlier one year study on the light requirements for growth in L. solidungula in the Canadian High Arctic showed that the annual irradiance for the lower depth limit of this species (20 m) was about 89 mol m^{-2} yr^{-1} (Chapman and Lindley, 1980). This was similar to the result of 70 mol m^{-2} s^{-1} obtained by LHning and Dring (1979) for the lower limit (8 m) of L. hyperborea in the North Atlantic. In the Stefansson Sound Boulder Patch, the results of this long-term study suggest that the light requirements for growth may be much lower, since L. solidungula is normally not exposed to significant winter and spring contributions of PAR as is submerged vegetation in other regions.

This paper presents the results of underwater PAR measurements collected in August 1984 and continuously from August 1986 through mid-August 1991 at seven experimental sites in the nearshore Beaufort Sea. Measurements of surface PAR recorded at a nearby shore station are also presented.

Materials and Methods

Site Descriptions:

Field studies were conducted in August 1984 and 1986-1991. Seven sites, aligned on two transects extending seaward from the shore, were studied in the Stefansson Sound Boulder Patch kelp community, off Alaska's north arctic coast (Fig. 1). The transects lie on either side of an offshore oil production facility, the Endicott Development Project (EDP), which was constructed in 1985. The west transect, which is closest to the constructed islands and causeways of the EDP, contains three stations, which lie in somewhat deeper water than the stations on the east transect, located to the east of the EDP. Site DS-11 (Dive Site 11), located on the east transect, is an historical research site that has been used by investigators since 1978 (Dunton *et al.*, 1982). The EDP consists of a gravel causeway that extends offshore to join two small gravel production islands. The water depth surrounding the EDP ranges from 2 to 4 m. The seven sites range in depth from 4.6 to 6.4 m. Research has been conducted at DS-11, farthest offshore, at least annually since 1978. A geological and biological description of this area is summarized by Dunton *et al.* (1982). Bottom water temperatures usually varied from -1.0°C to 4°C during the five summers of study, with salinities typically ranging between 18 and 25 ppm.

Quantum Irradiance Measurements:

Measurements of photosynthetically active radiation (PAR = ca. 400 to 700 nm wavelength) on the seabed were collected continuously at each of the seven sites using an LI-193SA spherical (4_) quantum sensor which provided input to a LI-1000 Datalogger (Li-Cor Inc., Lincoln, Nebraska, USA) in a waterproof case (Fig. 2). The sensor was mounted on a small tripod one-half meter above the bottom to minimize fouling by drift algae. No major biofouling was ever observed and there was no shading of the sensor by the kelp, since the fronds of Laminaria do not extend upward into the water column, but lay on the bottom. Instantaneous PAR was measured at one minute intervals and integrated over an hourly or three-hourly period. To maximize our chances of obtaining complete and continuous 12-month data sets, we changed the integration period on six LI-1000 dataloggers from a 1-hr to a 3-hr interval in August 1987 and added a large power source. Coincident measurements of surface PAR were made at a nearby shore-based field station (ca. 10 km distant) using a LI-190SA quantum sensor and datalogger. The sensor was mounted atop a 33 m tower to eliminate shading from nearby buildings and drilling structures. The sensors are accurate to ± 5% (traceable to NBS), stability is ± 2% over any one year period, and data are recorded with a precision of ± 0.01 µmol m^{-2} s^{-1}.

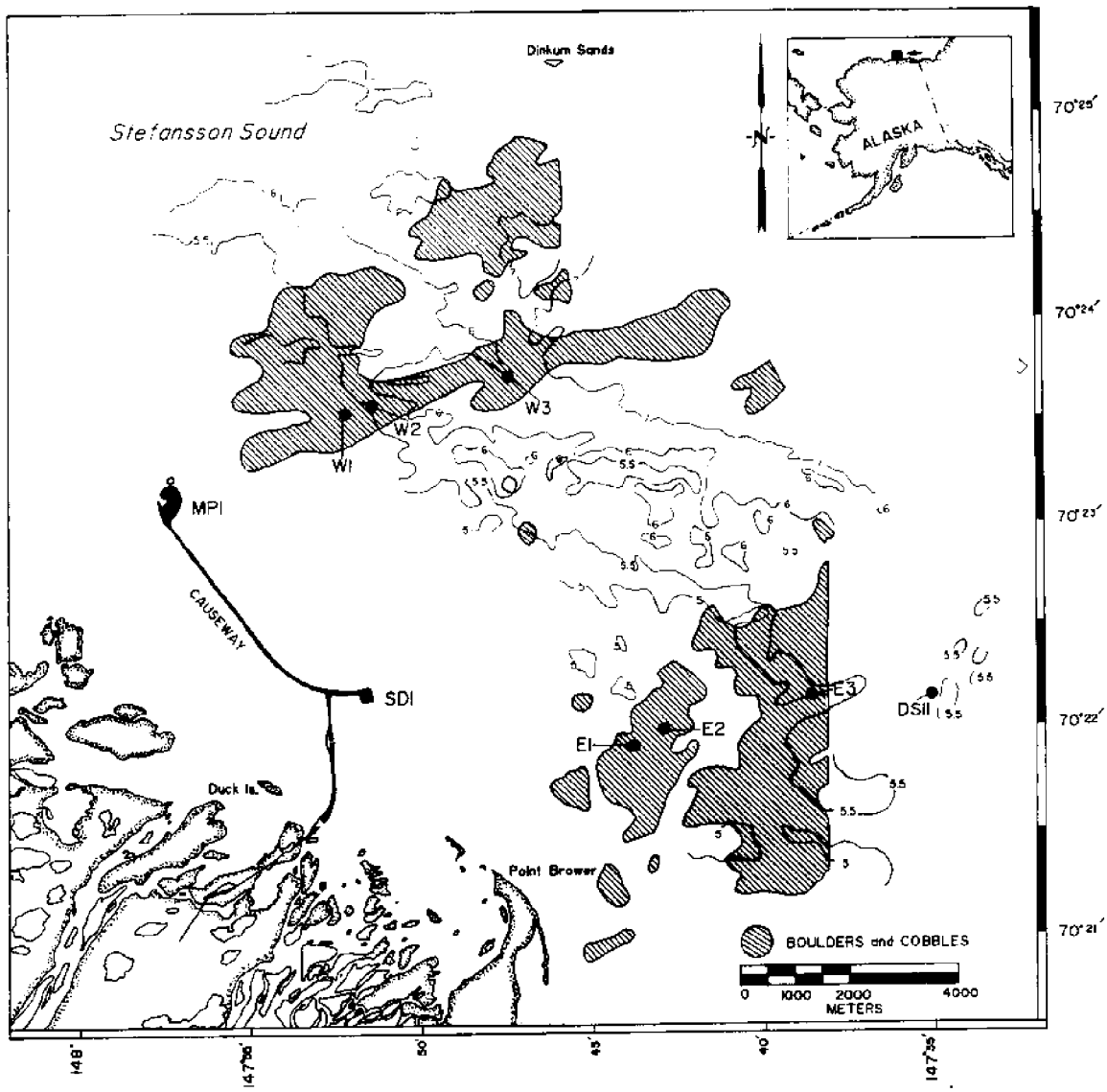

Fig. 1. Location of study sites in Stefansson Sound Boulder Patch kelp community. Depth contours are in meters. MPI: Main Production Island; SDI: Satellite Drilling Island.

A continuous record of underwater photon flux fluence rate (PFFR) was collected for a two-week period in 1984, and then for uninterrupted 12-month periods starting in August 1986. In all years, fouling of the spherical quantum sensors was minimal after one year in situ. All were coated with a thin film of silt that reduced PFFR about 10%, based on comparative measurements prior to and following cleaning of the sensors, but none were encrusted with organisms. This small reduction in PFFR was common to all sensors, which were thoroughly cleaned in early August of each summer, about three weeks following break-up of the ice canopy.

Statistics:

Statistical analyses were performed on a microcomputer using a general linear models procedure (SAS Institute 1985). Significant differences in PFFR among sites and years were tested using a two-way analysis of variance (ANOVA) using time as a block. In this case, "time" is the date and hour

recorded with each integrated light measurement. A one-way ANOVA was used to test significant differences in surface PFFR between years using time as a block. When a significant difference for a main effect ($P \leq 0.05$) was observed, the means were analyzed by a Tukey multiple comparison test to identify significant differences among years. Analyses were performed with log transformed data for light measurements since they yielded residuals with fewer departures from normality and homogeneous cell variances.

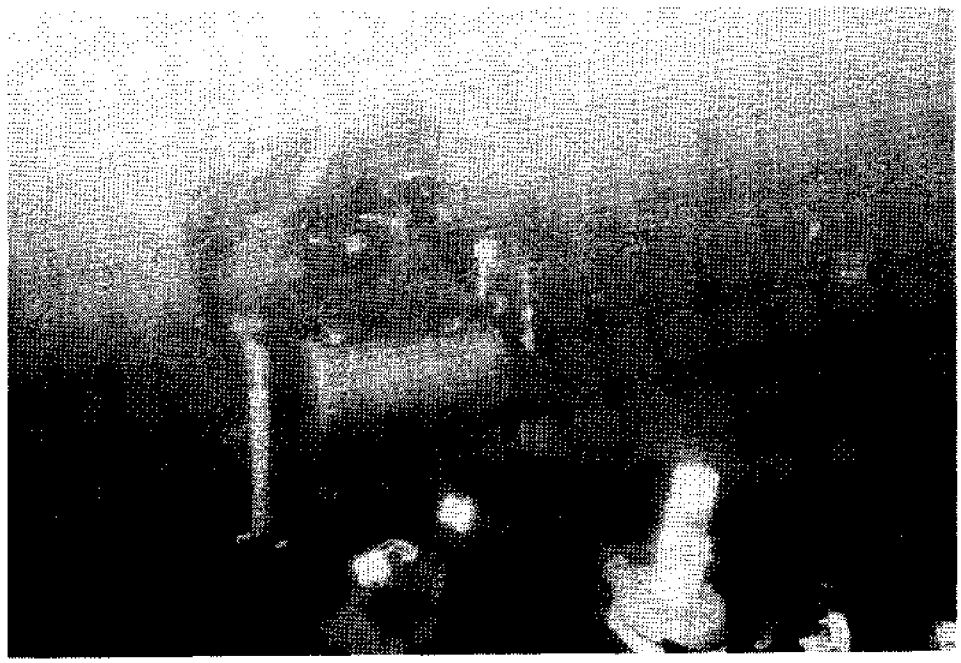

Fig. 2. The aluminum underwater housing containing the LI-COR datalogger used during the earlier years of this study. The aluminum housings were later replaced by heavy PVC chambers. Tagged kelp plants and a blue pinger (used for site location) are shown in the foreground.

Results

A large contrast between surface incident PAR and underwater PAR was common throughout the study and is exemplified in Fig. 3. Elevated levels of surface PAR are present through the autumn, spring, and summer months, but underwater light is nearly undetectable except during the 3 to 4 month summer open-water period. The large difference in surface and underwater light is largely a consequence of turbid ice, which effectively blocks light transmission through the ice canopy, even during periods of 24-hr daylight (Dunton *et al.*, 1982). In contrast, Chapman and Lindley (1980) reported measurable levels of underwater PFFR through most of the iced-over period in the Canadian High Arctic.

Summer Open-Water Period:

A complete summary of mean PFFR values of all available summer open-water light data collected at the surface and at the seven experimental sites since 1986 is shown in Table 1. Average PFFR in 1986 and 1991 does not include light levels for the earlier and later portions of the summer respectively, which prevents a direct comparison of these data with other years. However, an interannual comparison of the data collected at three sites (W-2, E2, and DS-11) which are characterized by the most complete summer open-water records since 1987 for the period 15 July - 18 August showed that PFFR in 1991 was significantly lower than in all other years except 1988 (Table 2).

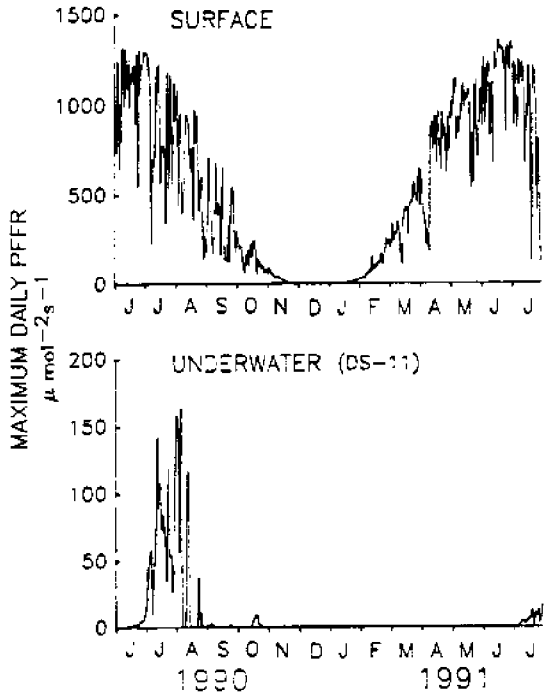

Fig. 3. Maximum daily surface and underwater PFFR at DS-11 from 1 June 1990 through 1 August 1991.

Table 1. Laminaria solidungula. Mean PFFR (μmol $m^{-2}s^{-1}$) of all available summer irradiance data collected at sites from 1986-1991 (3 hr integrations). nd: no data

SITE	YEAR					
	1986 (11 Aug-30 Sep)	1987 (7 Jul-30 Sep)	1988 (1 Jul-30 Sep)	1989 (15 Jul-30 Sep)	1990 (25 Jun-30 Sep)	1991 (11 Jul-18 Aug)
W-1	nd	nd	nd	8.6	6.8	1.8
W-2	11.0	6.3	4.0	6.1	7.5	3.1
W-3	--	--	5.6	8.3	9.4	2.5
E-1	--	--	--	12.9	11.4	5.0
E-2	20.6	8.9	4.8	13.0	11.9	4.8
E-3	--	--	6.9	15.3	15.6	5.8
DS-11	17.1	7.3	4.9	9.0	13.3	3.6
Surface	200.4	211.8	221.2	227.0	278.4	349.3

Table 2. Laminaria solidungula. Mean PFFR (μmol $m^{-2}s^{-1}$) of underwater PFFR for the period 15 July - 18 August from 1986 - 1991. Mean values are based on PFFR data from sites W-2, E-2 and DS-11; values with the same letter are not significantly different.

Year	N	Mean PFFR	Tukey grouping
1990	834	20.8	A
1989	840	17.5	B
1987	838	13.6	C
1991	828	4.0	D
1988	840	2.4	D

A summary of underwater PFFR (as reflected in daily 3-hr integrated maximum values) at these three sites is shown in Fig 4 for 1990 and 1991. In general, the ablation of most land-fast ice in Stefansson Sound occurs by mid-July, although it can occur much earlier (Table 3). Break-up is usually followed by a rapid increase in light levels which remain elevated throughout most of July and August. In 1990, break-up of land-fast ice occurred by 25 June (Fig. 4), and was followed by rapid increases in PAR to 150 to 250 μmol m^{-2} s^{-1}. In 1991, break-up did not occur until 11 July, and was not followed by a substantial increase in underwater PAR. Decreases in water transparency usually occur by early September, such that PFFR seldom rises above 10 μmol m^{-2} s^{-1}, although favorable weather conditions occasionally result in PFFR values of up to 25 μmol m^{-2} s^{-1} for short periods before the onset of ice formation in early October. The abrupt drop in light levels in early August, 1990, at all sites in Steffanson Sound (Fig. 4) is entirely related to large decreases in water transparency, a product of large westerly and northeasterly storms characterized by winds exceeding 40 km h^{-1} that predominated during this period. The short period of higher light levels in mid-October, 1990, appears to be coincident with sea ice formation and a period of decreased water turbulence and thus lower turbidity. However, light is rapidly attenuated as sea ice accretion continues and attains a thickness of 30 to 50 cm by early November.

Table 3. Dates of break-up of the ice canopy in Stefansson Sound based on light data collected at the seven experimental sites over the duration of this study.

Year	Date of Break-up
1987	8 July
1988	1 July
1989	15 July
1990	25 June
1991	11 July

An interannual comparison of in situ underwater irradiance at site DS-11 from 11 to 23 August is shown in Fig. 5 alongside recorded levels of surface solar radiation. All PFFR data presented are measurements collected once per minute and integrated every three hours. Underwater PFFR was highest in 1986 and lowest in 1988 and 1991, while surface levels varied little among years (no incident PFFR data are available for 1984). Most years were characterized by 2-4 day periods when PFFR levels remained below 2 μmol m^{-2} s^{-1} due to high water turbidity that resulted from intense storms. Measurements from three sites (W-2, E-2, DS-11) for which a complete data set is available for the entire open-water period (from break-up to 30 September) between 1987 and 1990 revealed that mean PFFR was significantly greater in 1989 and 1990 than in all other years. The mean PFFR of the three sites in 1987 was 7.6 μmol m^{-2} s^{-1}, compared to 4.6 (1988), 10.1 (1989), and 11.0 (1990). In contrast, surface PFFR was lowest in 1987. Values ranged from a high of 278 in 1990 to a low of 212 in 1987 for the same period. A complete data set is not available for these sites in 1986 or 1991.

Winter Ice-Covered Period:

The substantial decrease in PFFR by mid-October marks the onset of ice formation in Stefansson Sound (Fig. 4). A concurrent decrease in surface light in the late autumn to low winter levels also results in nearly undetectable levels of underwater light through November, December, and January (Fig. 3). In previous years, we have also clearly documented that light levels remained nearly undetectable through February, but increased steadily through March and April at locations where turbid ice concentrations were minimal. Short term temporal variations do occur in under-ice PFFR, but these are attributed to differences in surface snow cover and ice accretion during the winter and early spring. The character of the ice canopy is determined at freeze-up and consequently defines the light environment for the next eight to nine months. Previous studies (Dunton 1984, Dunton and Schell, 1986) have demonstrated that low winter levels of PAR are related to high sediment concentrations in the ice canopy (*i.e.* turbid ice). These sediments are almost entirely incorporated into the ice canopy during

freeze-up in October (Dunton *et al.*, 1982). In this study we found measurable under-ice levels of PAR at all sites, but values varied considerably. For the period 1 March to 15 May, average under-ice PFFR ranged from 0 to 2.25 μmol m^{-2} s^{-1} between 1987 and 1991 (Fig. 6). These mean values reflect daily 3-hour maximum PFFR values that range from 0.01 to 15.7 μmol m^{-2} s^{-1}. These light levels reflect the temporal and spatial heterogeneity in turbid ice cover based on a process that occurs entirely at random.

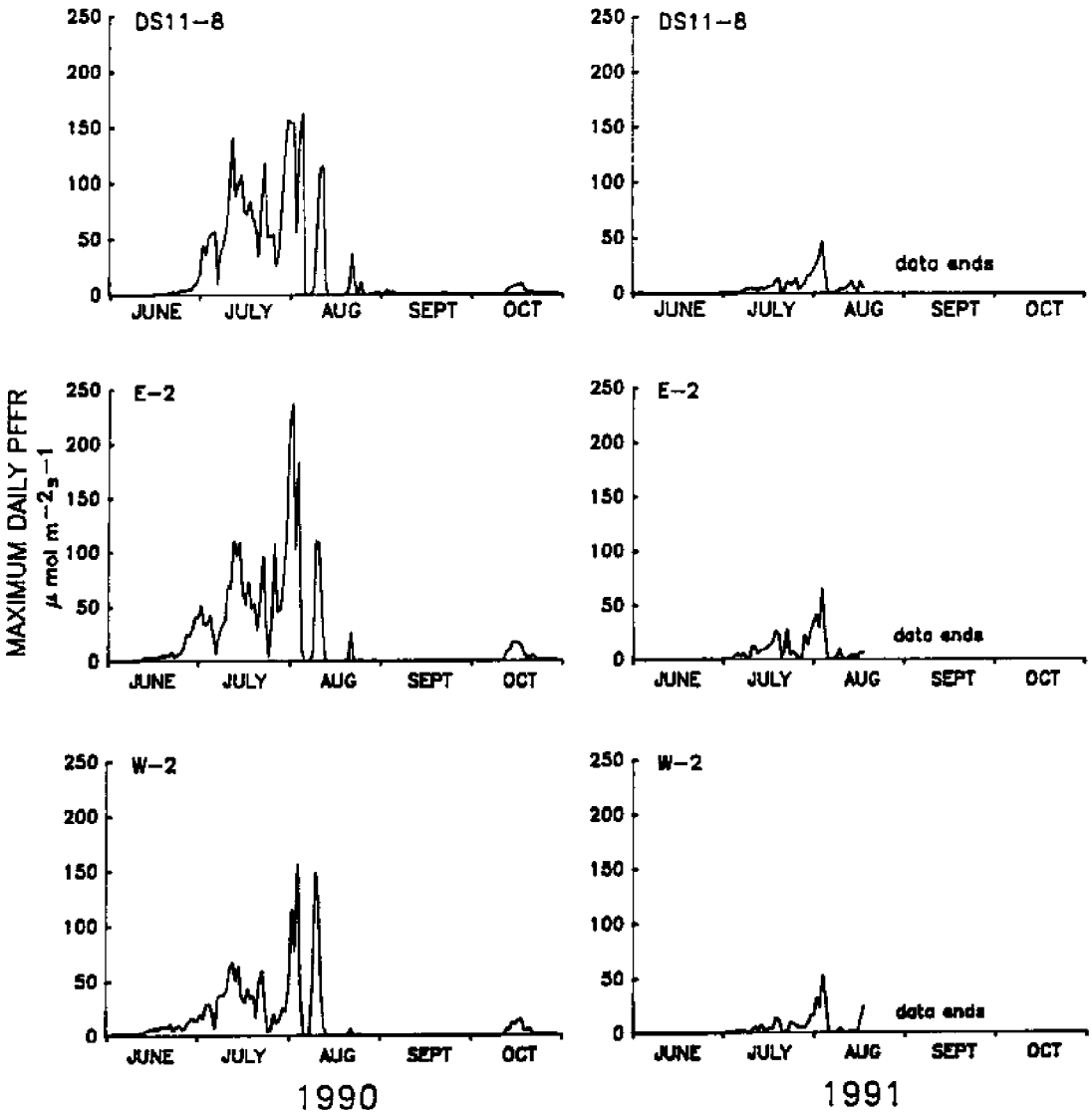

Fig. 4. Average daily maximum underwater PFFR at sites W-2, E-2 and DS-11 from June through late Septermber, based on continuous 3-hr integrations in 1990 and 1991. Break-up of ice canopy occurred by 25 June in 1990 and 11 July in 1991.

Quantum Budgets:

The annual quantum budgets for Laminaria solidungula at sites W-2, E-2, and DS-11 in Stefansson Sound are shown in Table 4. Values range from a maximum of 140 mol m^{-2} yr^{-1} in 1990 to a low of 45 mol m^{-2} yr^{-1} in 1988. It is likely that plants at site E-2 received well over 100 mol m^{-2} yr^{-1} in 1986, since this total only includes the last three weeks of August, September, and October. Plants in Stefansson Sound were thus exposed to large differences in PAR between 1986 and 1991 among the different sites.

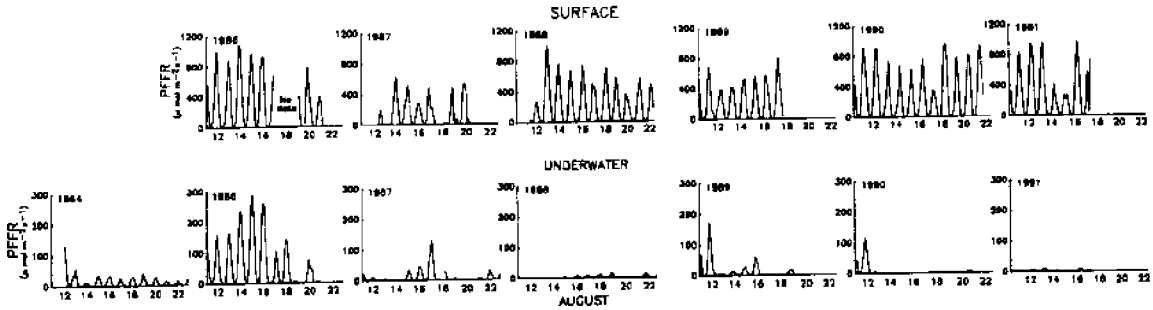

Fig. 5. Comparison of PFFR measurements collected underwater (site DS-11) and at surface from 11 to 23 August 1986-1991.

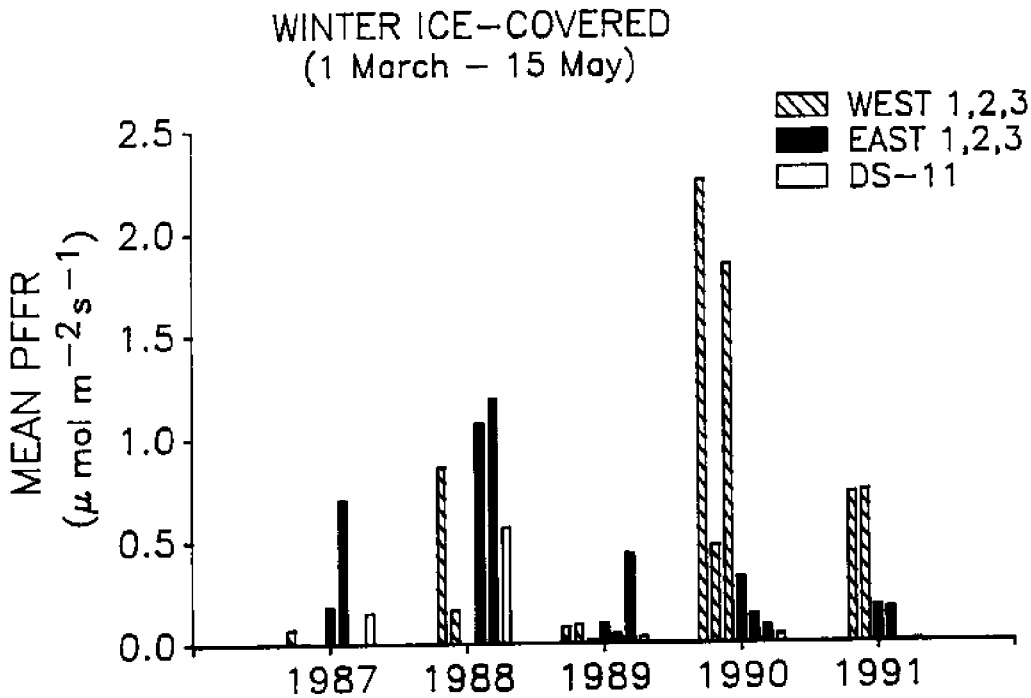

Fig. 6. Variation in mean under-ice PFFR at seven sites from 1 March to 15 May 1987-1991. Data for E-2 in 1991 reflects period from 1 March to 10 April.

Discussion

Levels of underwater PAR varied greatly among years and sites over the five-year continuous period of this study. Nearly all this variation can be attributed to distinct changes in water transparency during the summer open-water period, since the annual differences in underwater PAR cannot be correlated to variations in surface insolation. The causes of the changes in water transparency, particularly the very poor conditions that prevailed through July and August 1988 and 1991, are predominantly products of storms and associated shifts in wind induced currents. Although the light received during the nine-month ice covered period (October through June) usually represented less than 10% of the total amount of light reaching the plants in most years, it can comprise as much as 15 to 30% of the annual light budget (*e.g.*, 1988). These substantial variations reflect the highly dynamic nature of high arctic aquatic systems.

Table 4. Laminaria solidungula. Annual quantum budgets for kelp at three sites in Stefansson Sound in between 1986 and 1991. Note that data sets for 1986 and 1991 do not cover an entire 12-month period; nd: no data

Year	Total irradiance (mol m^{-2} yr^{-1})						
	W-1	W-2	W-3	E-1	E-2	E-3	DS-11
1986 (11 Aug-31 Oct 1986)	nd	51	nd	nd	89	nd	74
1987 (1 Nov 1986-31 Oct 1987)	nd	68	nd	nd	81	nd	61
1988 (1 Nov 1987-31 Oct 1988)	nd	45	52	nd	50	69	47
1989 (1 Nov 1988-31 Oct 1989)	62	61	58	91	90	110	64
1990 (1 Nov 1989-31 Oct 1990)	82	74	98	124	106	140	115
1991 (1 Nov 1990-18 Aug 1991)	nd	18	19	19	18	nd	12

The 1990 summer was unique in that benthic plants were exposed to a prolonged period of elevated light conditions early in the summer. This resulted in part from an early break-up of the fast ice and relatively low frequency of storms and high winds. As a consequence, the amount of light reaching the plants was much greater than in previous years, with values ranging from 74 to 140 mol m^{-2} yr^{-1}. In 1991, ice break-up occurred much later, water transparency remained low, and the plants were thus exposed once again to record low levels of PAR.

Comparison of surface and underwater PAR measurements reveals an annual transmittance of incident PAR that ranges from close to 0.001 to 0.6%. This transmittance corresponds to about 45 to 50 mol m^{-2} yr^{-1} and is substantially lower than the annual irradiance of 89 mol m^{-2} yr^{-1} reported for Laminaria solidungula at its lower depth limit in the Canadian High Arctic (Chapman and Lindley, 1980). It is also lower than the 70 mol m^{-2} yr^{-1} obtained by LHning and Dring (1979) for the lower limit (8 m) for L. hyperborea in the North Atlantic. The large temporal and spatial variations in underwater PAR in Stefansson Sound contribute substantially to differences in the growth and productivity of L. solidungula (Dunton, 1990). In addition, the ability of L. solidungula to survive at more reduced levels of total annual PAR may be related, at least in part, to substantial carbon production during the ice-covered period. Under-ice light levels as low as 10 µmol m^{-2} s^{-1} appear to be associated with a doubling of annual growth in L. solidungula (Dunton *et al.*, 1991).

Acknowledgements

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AQUARIUS HABITAT SITE AT CONCH REEF: POSSIBILITIES FOR RESEARCH ON FORAMINIFERA WITH ALGAL SYMBIONTS

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Rubble bottom and proximity to reefal habitats makes the Florida Keys site selected by the NOAA-NURC/UNCW program for initial deployment of the AQUARIUS habitat an excellent location to collect and conduct experiments on reef-associated benthic foraminifera, particularly larger species that host algal symbionts. Symbiont-bearing species most abundant at the site are Amphistegina gibbosa and Asterigerina carinata, and Laevipeneroplis protea. Other common larger species are Cyclorbiculina compressa, Heterostegina antillarum, Laevipeneroplis bradyi, Parasorites orbitolitoides, and Sorites orbiculus. Common attached species include Homotrema rubrum and Planorbulina mediterranensis. A diverse assemblage of smaller hyalline and porcellaneous species is also present. The abundance of rubble substratum facilitates non-destructive foraminiferal sampling by SCUBA or NITROX divers equipped with small brushes and plastic bags. Sediment, foraminifera, and other loosely attached benthos can be concentrated in collecting bags and rubble returned to the reef. Interest in use of foraminiferal-algal symbioses as surrogates for coral-algal symbioses may increase as coral populations continue to decline in the Florida Keys.

Introduction

Foraminifera are shelled marine protozoans (Class Sarcodina, Order Foraminiferida) whose study can have geological, environmental, and biological significance. As a result of their relatively small size and preservation potential, they are useful in comparing present and past environments. Studies of modern species, populations and assemblages can be used to interpret changes observed in the fossil record. In addition, studies of changes in foraminiferal faunas in sediment cores can be used to interpret the recent past, including environmental changes associated with anthropogenic influences in an area.

Benthic foraminiferans are abundant and important members of coral-reef communities. Like corals, many foraminiferal species host algal endosymbionts upon which they are dependent for growth and calcification (*e.g.*, Lee and Anderson, 1991). As a result, these foraminiferans are significant primary (Sournia, 1976) and calcium carbonate producers (McKee *et al.*, 1959; Hallock, 1981) in reef systems. Because foraminiferans with algal symbionts can be sensitive to many of the same environmental stresses that influence corals, *e.g.*, heavy metal (Alve, 1991) and nutrient pollution (Hirshfield *et al.*, 1968), thermal stress (Hallock and Larsen, 1979), and increases in ultraviolet radiation (Hallock *et al.*, 1992), these protists may be useful surrogates for corals in studies of human impacts on reef systems. Foraminiferans may be particularly valuable in studies of western Atlantic and Caribbean reefs, because coral populations are severely declining (*e.g.*, Liddell and Ohlhorst, 1992;

Porter, 1992) and managers of reef resources are becoming increasingly reluctant to sacrifice corals for research.

Foraminiferans, particularly larger species with algal endosymbionts, are relatively easy to collect alive for physiological, cytological and culture experiments. The purpose of this paper is to discuss the foraminiferal species that are readily collectible in the vicinity of the site that has been selected by the National Oceanic and Atmospheric Administration's National Undersea Research Center at the University of North Carolina, Wilmington (NOAA-NURC/UNCW) for deployment of the AQUARIUS Underwater Habitat (Miller and Hulbert, 1992). With this information, other researchers interested in foraminiferans may be able to plan experiments using the in situ capabilities of the Habitat.

Site Description and Methods

The site selected for the AQUARIUS Habitat is in the vicinity of Conch Reef, which is offshore of the southern end of Key Largo in the Florida Keys. Primary substratum types include coral-algal reef, reefal sands, and reef rubble. While foraminiferans can be found on all of these bottom types, reef rubble is the subject of this report because it provides a permanent, relatively immobile substratum for both attached and motile foraminiferal species, which can be sampled with minimal environmental impact. Mobile sediments have a more restricted biota, while continuous hard substratum is very difficult to sample nondestructively.

Because the Conch Reef site ranges in depth from 15 to 30 m, sampling is carried out by divers using SCUBA or enriched air (NITROX) containing 36% oxygen (NURC/UNCW, 1991). Two techniques are routinely employed to collect living foraminiferans (Hallock *et al.* 1992), depending upon depth, available bottom time, and purpose for sampling. Either pieces of reef rubble are directly collected or debris concentrates are obtained from the rubble. Rubble samples consist of roughly hand-sized pieces (*i.e.*, covering 50-100 cm^2 of bottom) of coral debris or algal nodules, whose upper surfaces are coated with a stubble of filamentous algae. Each piece is carefully placed into a plastic bag to avoid detachment and loss of living foraminifera. Debris concentrates are collected by placing pieces of reef rubble, one at a time, inside a 4 liter plastic bag and brushing the rubble free of sediment, algae, and meio- and microfauna; the rubble is returned to the substratum.

Samples are taken to the surface where they are further processed. For faunal surveys, samples consisting of one or two pieces of rubble are either frozen or treated with preservative to kill the foraminiferans quickly, and later washed in freshwater, dried on filter paper at 40-50°C, examined microscopically and picked for foraminiferans (*e.g.*, Hallock *et al.*, 1986a). Useful quantities of living foraminiferans for experimental studies require debris concentrates, either collected directly or obtained from bulk rubble samples by brushing the rubble in a bucket filled with seawater. Rubble is disposed of and debris is washed several times to remove as much loose organic matter as possible. The remaining debris concentrate is spread thinly over the bottom of large, flat dishes (*e.g.*, 150 x 20 mm Petri dishes), covered with 1-2 cm of seawater and allowed to sit overnight. Many of the foraminiferans are negatively geotaxic, so they climb the walls of the Petri dish or to the top of the layer of sediment and algal debris, where they can be readily picked. If species that attach directly to the rubble are desired, rubble pieces must be saved.

Results and Discussion

Table 1 presents relative abundances of symbiont-bearing foraminiferal species commonly collected live at Conch Reef. Individuals of symbiont-bearing species that are living when collected are readily distinguished by their color in fresh or dried samples (Hallock *et al.*, 1986a). Chlorophyte-bearing species retain at least some of their grass-green color, rhodophyte-bearing species are red and white

"candy stripe", diatom-bearing species are golden brown, and dinoflagellate-bearing species are usually a purplish brown.

Table 1. Attached and symbiont-bearing foraminiferal species collected from rubble at Conch Reef, Florida Keys. A = >10 individuals/50cm² rubble piece; C = 1-10/50 cm²; P = typically find several in rubble concentrates from 5-10 pieces of rubble. Attached species are noted by *.

Suborder	Species	Symbiont	Abundance
Rotaliina	*Amphistegina gibbosa* d'Orbigny	Diatom	A
	Asterigerina carinata d'Orbigny	Diatom	A
	Heterostegina antillarum d'Orbigny	Diatom	P
	*Homotrema rubrum**(Lamarck)	None	C
	*Planorbulina mediterraneansis** d'Orbigny	None	A
	*P.variabilis** d'Orbigny	None	C
Miliolina	*Archaias angularus* (Fichtel and Moll)	Chlorophyte	C
	Borelis pulchra (d'Orbigny)	Diatom	P
	Cyclorbiculina compressa (d'Orbigny)	Chlorophyte	C
	Laevipeneroplis protea (d'Orbigny)	Chlorophyte	A
	Laevipeneroplis bradyi (Cushman)	Chlorophyte	C
	Parasorites orbitolitoides (Hofker)	Chlorophyte	C
	Peneroplis pertusus (Forskal)	Rhodophyte	C
	Sorites orbiculus (Forskal)	Dinoflagellate	P

Amphistegina gibbosa, Asterigerina carinata, and Laevipeneroplis protea are the most abundant species with algal endosymbionts. Parasorites orbitolitoides and L. bradyi are also relatively common. Dead tests of Archaias angulatus and Cyclorbiculina compressa are abundant, but living individuals are much less common. Of these species, Amphistegina gibbosa is the most studied (*e.g.*, Hallock *et al.*, 1986b) followed by A. angulatus, C. compressa, and Sorites orbiculus (Hallock and Peebles, in press). Virtually nothing is known of the biology, symbionts, and life history of the other species.

Attached species are also relatively large and abundant at Conch Reef, particularly Homotrema rubrum and Planorbulina spp. (Table 1).

Smaller species are taxonomically diverse; typically tests of 30-40 species can be found in a fully picked sample. As is typical for reef samples (*e.g.*, Murray, 1973), rotaliine and milioline smaller taxa are about equally represented, while agglutinated individuals are few. Unfortunately, because most of these species lack symbionts and because the reliability of vital staining techniques is questioned (*e.g.*, Martin and Steinker, 1973), careful observation of live samples is required to determine living assemblages with full confidence. Common and easily identified taxa are listed in Table 2. Detailed species lists and species illustrations of Florida Keys foraminiferans can be found in Bock *et al.* (1971).

Table 2. Common smaller foraminiferal species observed in samples from Conch Reef, Florida Keys.

Suborder Textulariina
- *Textularia agglutinans* d'Orbigny
- *Valvulina oviedoiana* d'Orbigny

Suborder Miliolina
- *Articulina mexicana* Cushman
- *A. mucronata* (d'Orbigny)
- *Hauerina bradyi* Cushman
- *Hauerina speciosa* (Karrer)
- *Miliolinella circularis* (Bornemann)
- *M. fichteliana* (d'Orbigny)
- *Nodobaculariella cassis* d'Orbigny
- *Pyrgo fornasinii* Chapman and Parr
- *Quinqueloculina agglutinans* d'Orbigny
- *Q. bicarinata* d'Orbigny
- *Q. bicostata* d'Orbigny
- *Q. bosciana* d'Orbigny
- *Q. horrida* Cushman
- *Q. subcuneata* Cushman
- *Q. polygona* d'Orbigny
- *Quinqueloculina* spp.
- *Schlumbergerina alveoliniformis* Cushman
- *Triloculina oblonga* (Montague)
- *Triloculina trigonula* (Lamarck)
- *Triloculina* spp.

Suborder Rotaliina
- *Bolivina* spp.
- *Buliminella elegantissima* (d'Orbigny)
- *Discorbis rosea* (d'Orbigny)
- *Discorbis* spp.
- *Elphidium* spp.
- *Haynesina depressula* (Walker and Jacobs)
- *Neocorbina terquemi* (Rzehak)
- *Nonionoides grateloupi* (d'Orbigny)
- *Rosalina floridana* (Cushman)
- *Rosalina* spp.
- *Tretomphalus atlanticus* (Cushman)

The abundance and diversity of readily collectible foraminiferans at the Conch Reef site provide the potential for a variety of kinds of studies. We are currently monitoring symbiont loss (bleaching) in Amphistegina gibbosa (Hallock *et al.* 1992) and are collecting this species for physiological and cytological studies to document the bleaching process and its causes. We are also using specimens of several species in biochemical taxonomic research aimed at determining the affinities among taxa with chlorophyte endosymbionts and their relationships to other porcelaneous taxa (Toler *et al.*, 1991).

Once the AQUARIUS Habitat is operational, the kinds of studies that can be conducted in situ are limited only by the imagination of the potential researchers and their ability to promote their ideas to NURC/UNCW and other funding agencies. in situ growth rate, sediment production rate, habitat specificity, competition, and disturbance-recovery rate studies are a few examples that would provide ecologists and paleoecoloists with insight into niche separation in these fascinating protists. Determining the effects of transplantation or small-scale release of fertilizers or other chemicals on foraminiferal assemblages are also possibilities. The outcome of such studies will make foraminiferans even more useful in paleoenvironmental analysis of ancient reefs, and also more useful for predicting environmental impacts on benthic communities.

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NOCTURNAL AND CREPUSCULAR ACTIVITY OF REEF FISHES IN ONSLOW BAY, N.C.: SCUBA, VIDEO, AND REMOTELY OPERATED VEHICLE OBSERVATIONS

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We observed the nocturnal and crepuscular behavior of reef fishes in Onslow Bay, North Carolina, and evaluated four basic direct observation methods: SCUBA; SCUBA with Sony video; SCUBA with Osprey video; and a Remotely Operated Vehicle (ROV). The primary species actively foraging at night were tomtate, cubbyu, and Carolina hake. Secondary nocturnal species were oyster toadfish, spotted soapfish and twospot cardinalfish. Many of the reef fishes exhibited distinct color patterns at night. In general, diurnally active fishes usually changed to a distinctly barred pattern after dusk and nocturnally fishes usually changed to a blanched or lighter pattern after dusk. SCUBA offered the most realistic and accurate observations of reef fishes. Combined intermittently with video, SCUBA observations most accurately defined and documented nocturnal behaviors.

Introduction

While most observations of the nocturnal and crepuscular behavior of fishes have largely emphasized feeding behavior (or lack of it) in tropical zooplanktivores (Collette and Talbot, 1972; Gladfelter, 1979; Hobson, 1965, 1968, 1973, 1974; Hobson and Chess, 1978), fewer studies have reported on nocturnal benthivory over soft bottom adjacent to reefs (Davis, 1967; McFarland *et al.*, 1979; Ogden and Quinn, 1984; Starck and Davis, 1966). The latter studies have been confined primarily to the grunts. Still fewer studies on the nocturnal and crepuscular behavior of temperate reef fishes have been done and have taken place primarily in California waters, *e.g.*, Hobson *et al.* (1981) in the western hemisphere. Virtually no studies of the nocturnal and crepuscular behavior of reef fishes have been reported for the warm temperate and subtropical reef fishes of Onslow Bay, North Carolina.

Our interest in the crepuscular and nocturnal behavior of fishes associated with reefs in Onslow Bay, North Carolina originates with the hypothesis that many reef fishes derive their foods from the sand bottom around reefs and that the primary function of reefs is to provide daytime cover for these fishes. We further hypothesize that some of the reef fishes feeding away from the reef over sand do so at night. Our objectives are to evaluate four different methods of observing the nocturnal and crepuscular behavior of reef fishes (SCUBA; SCUBA with Sony video; SCUBA with Osprey video; and a Remotely Operated Vehicle), and to report our observations on the reef fishes.

Methods and Materials

We used four basic methods (SCUBA, SCUBA with Sony video, SCUBA with Osprey video, and ROV) at three different sites in Onslow Bay, North Carolina during 1986 and 1989 to study the

crepuscular and nocturnal activities of reef fishes (Table 1). Methods for each site are discussed separately below.

Tug wreck surveys:

A 24-hour visual survey sampling of reef fishes associated with the tug wreck, R. R. STONE [see Lindquist and Pietrafesa (1989) for description of wreck], was carried out on June 16-17, 1986. In order to compare relative abundances of nocturnally active fishes, we conducted five daylight and three night surveys using a modification of the stationary survey method developed by Bohnsack and Bannerot (1986). We used a 4 m radius for our counts instead of the 7 m radius used by Bohnsack and Bannerot (1986). Night surveys were accomplished with standard underwater diving lights. We also made two daylight and one night video documentation of the fishes associated with the tug using a hand-held Osprey video camera connected to a shipboard Cyclops control console via an umbilical. The console allowed remote shipboard operator control of camera focus, intensity control for two flood lights attached to the camera, and on-screen overlay of real time, elapsed time, station number, and other annotations that were then recorded on one-half inch video tape along with the video image from the camera. In addition, the diver operating the camera wore an AGA full face mask with microphone and bone-conduction speaker that allowed real-time annotation of the video image as well as communication with the console operator.

Five nautical mile site:

We conducted surveys with the Superphantom II Remotely Operated Vehicle (ROV) and SCUBA. This site has been described by Lindquist *et al.* (1989). We used the ROV from an anchored vessel in conjunction with SCUBA (except final dawn dive at train cars) for dusk and dawn surveys at the natural ledge and train car reefs. In each case, one of us (DGL) narrated the ROV video in real time on board the research vessel while the other (IEC) made briefer SCUBA observations usually midway into the ROV dive. The ROV was outfitted with color video camera, two flood lights, and a Silicon Intensified (SIT) black and white video camera capable of imagery under low ambient light conditions. Both camera systems were used as follows. The SIT camera was used without lights during the 20 min before or after sunrise/sunset. After or preceding this twilight period both SIT and color camera were used intermittently with lights. The ROV was connected to a surface console where ROV direction, camera switching, and flood light intensity were controlled. Video images and real-time narration were recorded on one-half inch video tape. ROV and SCUBA surveys were made along the main axis of the reef and perpendicular to the main axis, over surrounding sand bottom, to a distance of 40 m to 130 m from the reefs. Distances were estimated from length of umbilical cable out.

Twenty-three nautical mile ledge:

We made six night and crepuscular SCUBA dives in June and August, 1989 (Table 1). On two of the four June SCUBA dives, we used a self-contained 8 mm Sony Handycam video camera in an underwater housing with single flood light to document reef fish behavior. During the August dives, we used the Osprey/Cyclops system with red gelatin filter hoods attached to each of the two flood lights. On each of these dives, we attempted to document fish behavior along the main axis of the rock ledge as well as up to 15 m away from the ledge over the adjacent and deeper sand bottom.

Use of common names for fishes follows Robins *et al.* (1991).

Observations

Tug wreck:

Results of our quantitative night surveys compared to the daylight surveys are shown in Table 2. Round scad formed massive schools that oriented upcurrent and above the tug in the water column during the daytime and were essentially absent from the tug during the night. Tomtate formed moderately large, inactive, milling aggregations all around and often inside the tug compartments. These aggregations were absent from the tug at night except for a small aggregation observed at the

stern keel of the tug (Table 2). Spottail pinfish, black sea bass and cubbyu were present in small numbers on all parts of the tug during the day and during the night as well. Carolina hake were restricted primarily to the stern keel area.

Table 1. Summary of crepuscular and nocturnal video and visual observations in Onslow Bay, North Carolina. Osprey = umbilical video; nm = nautical mile; Sony = self-contained video; Osprey (r) = video with red filters on lights.

Date	Time Observations	Sunrise/set	Method	Location
6-16-86	2119-2153	2027	SCUBA/Osprey	10 nm tug wreck
6-17-86	0146-0205	0600	SCUBA	10 nm tug wreck
6-17-86	0304-0323	0600	SCUBA	10 nm tug wreck
6-17-86	0511-0542	0600	SCUBA	10 nm tug wreck
5-24-89	2000-2130	2010	Superphantom ROV	5 nm ledge
5-24-89	1958-2028	2010	SCUBA	5 nm ledge
5-26-89	0424-0702	0603	Superphantom ROV	5 nm ledge
5-26-89	0552-0643	0603	SCUBA	5 nm ledge
6-8-89	1929-2208	2022	Superphantom ROV	5 nm train cars
6-8-89	1957-2044	2022	SCUBA	5 nm train cars
6-9-89	0404-0640	0559	Superphantom ROV	5 nm train cars
6-14-89	2004-2040	2024	SCUBA	23 nm ledge
6-14-89	2052-2126	2024	SCUBA/Sony	23 nm ledge
6-14-89	2204-2239	2024	SCUBA/Sony	23 nm ledge
6-15-89	0514-0552	0559	SCUBA	23 nm ledge
8-14-89	1941-2015	2000	SCUBA/Osprey (r)	23 nm ledge
8-15-89	0612-0745	0631	SCUBA/Osprey (r)	23 nm ledge

The Osprey video documentary occurred just after the end of the evening crepuscular period (Table 1). The diver operating the video camera (DGL) descended onto the stern deck of the tug in the area of the "Dutch bar" (towing device). Small numbers (20-25) of spottail pinfish, and one each of black sea bass, gag, and Carolina hake were seen here. Descending further to the stern keel, small numbers of Carolina hake and spottail pinfish were recorded as well as three cubbyu. Further investigation of the drive shaft tube revealed that a large conger eel and a twospot cardinalfish that were present during the day were now absent. Proceeding forward along the starboard keel where the tug had worn down into the soft limestone, the video documented four cubbyu, a spotted soapfish, and a small number of Carolina hake that appeared particularly active and possibly feeding. At the bow, six spottail pinfish, one round scad, and a Carolina hake occurred. Ascending the tug, one scaled herring was in the wheel house and a Carolina hake was inside the stack entry door. Inspection of the engine room compartment that had been full of tomtate during the day revealed a single spottail pinfish.

Five nautical mile site:

ROV and SCUBA surveys commenced simultaneously on May 24, 1989 at the rock ledge ten minutes before sunset (Table 1). An aggregation of cubbyu was observed to move from the main ledge to a secondary ledge closer to the sand bottom at 2005 hrs. All other reef fish activities were typical of diurnal behaviors until 2053 hrs when Carolina hake and oyster toadfish were observed out on top of

the ledge moving about. At 2100 hrs the ROV was driven out over the sand on the deep side of the ledge on a perpendicular bearing. We recorded four round scad as individuals nestled down into the sand bottom.

Table 2. Comparison of day (D) and night (N) stationary counts of fishes associated with the 10 nm tug wreck on June 16-17, 1986. Numbers are rounded means of five day and three night counts.

Species		Main deck				Upper deck		Stern keel
		Stern	bow	Port	Starbd	Forward	Aft	
Round scad	D	750	2333	10000	0	4375	2125	500
	N	2	1	1	0	0	0	0
Tomtate	D	210	1060	210	267	410	610	108
	N	0	3	0	0	2	0	67
Spottail pinfish	D	22	10	9	12	17	17	4
	N	1	1	0	0	12	3	12
Cubbyu	D	2	7	3	3	0	0	19
	N	2	1	2	1	0	3	17
Black sea bass	D	5	6	2	3	3	2	8
	N	1	1	2	0	1	2	0
Carolina hake	D	0	1	0	0	0	0	10
	N	1	0	0	1	1	0	3
Greater amberjack	D	2	3	0	0	2	1	0
	N	2	0	0	0	0	0	0
Belted sandfish	D	1	1	1	0	0	1	1
	N	1	1	0	0	0	2	0
Great barracuda	D	0	1	1	0	0	1	1
	N	1	0	0	0	0	0	0
Pigfish	D	3	0	0	0	0	0	2
	N	0	0	0	0	0	0	0
Spanish mackerel	D	0	2	0	0	2	0	0
	N	0	0	0	0	0	0	0
Spotted goatfish	D	0	0	0	0	0	0	4
	N	0	0	0	0	0	0	0
Slippery dick	D	1	1	0	0	0	0	1
	N	0	0	0	0	0	0	0
Gag	D	0	0	0	0	1	1	1
	N	0	0	0	0	0	0	0
Spotted soapfish	D	0	0	0	0	0	0	0
	N	1	1	1	0	0	0	0
Plainhead filefish	D	0	0	0	0	0	0	1
	N	0	0	0	1	0	0	0
White grunt	D	0	0	0	1	1	0	0
	N	0	0	0	0	0	0	0
Tautog	D	0	0	0	0	0	0	0
	N	0	1	0	0	0	0	0
Vermilion snapper	D	0	0	0	0	0	1	0
	N	0	0	0	0	0	0	0
Bar jack	D	1	0	0	0	0	0	0
	N	0	0	0	0	0	0	0

We made a 2.5 hr ROV pre-dawn survey with a 51 min SCUBA dive midway through at the rock ledge on May 26, 1989 (Table 1). Upon descending to the ledge, six round scad were seen individually resting on the bottom. Carolina hake were active on top of the ledge at 0429 hrs. Spottail pinfish, scup, and a honeycomb boxfish were all appeared inactive in the area of the ledge. AT 0503 hrs, we commenced ROV operations over the sand bottom. We noted little activity, with spottail pinfish, inshore lizardfish, round scad, and southern flounder all appearing inactive. At 0517 hrs, at 130 m from the reef, two tomtate and a round scad were seen over sand. At 0530 hrs, having reversed our course, we saw three tomtate at 20 m from the reef. Beginning at about 0540 hrs, we noted that the diurnal fishes were becoming active. Scup, belted sandfish, greater amberjack, and spottail pinfish all appeared to be exhibiting their normal daytime behaviors. We also noted both tomtate and white grunt on the ledge at this time. Concurrent SCUBA observations of round scad by one of us (IEC) suggested that massive schools began forming at 0556 hrs.

On June 8, 1989, we also made concurrent ROV and SCUBA dives at the train car artificial reef (Table 1). At 1930 hrs, the ROV descended upon a large piece of train car wreckage and began a SIT

camera survey in a northwesterly direction along a ground line connecting train car wreckage. Typical diurnal fish behavior was noted. Upon approaching a smaller piece of wreckage, we noted an aggregation of 15 cubbyu that streamed out to meet and gather under the ROV. The color camera and lights were switched on and immediately the cubbyu streamed back to the wreckage. At 1943 hrs, we noted that the cubbyu aggregation had increased to about 30 individuals. At 2030 hrs, three of these cubbyu wandered out over the sand about 2 m from the wreck and then returned. At 2042 hrs, the cubbyu aggregation remained under the wreckage. At 2049 hrs, Carolina hake were moving about under the wreckage. Traversing away from the wreckage over sand bottom, we noted the following (and time): two cubbyu (2052, 2105); two scup (2105, 2137); two round scad (2111, 2135); two tomtate (2130, 2135); black sea bass (2136); pinfish (2136); and five inshore lizardfish (2140). One SCUBA diver (IEC) noted a spotted soapfish exiting from under wreckage at 2009 hrs and a tomtate over sand with nose down, mouthing the substrate while apparently feeding at 2024 hrs. The diver also noted a white grunt over sand at 2029 hrs. Returning the ROV to the wreckage at the descent line, we noted a cubbyu on the side of vertical section of train car at 2145 hrs. At 2200 hrs we returned to the site of the aggregation of 30 cubbyu and found none present and, upon returning to the descent line, one cubbyu was encountered at 2201 hrs over sand bottom.

We began a predawn ROV dive at 0404 hrs on the following morning at the train car reef (Table 1). At the main wreckage beneath the descent line, we saw one tomtate, one scup, two spotted goatfish, and six individual round scad between 0410 to 0412 hrs. At 0416 hrs, we saw one tomtate over the sand about 6 m from wreckage. Five spottail pinfish and two round scad were under wreckage at 0415 hrs. A summer flounder swam from adjacent sand to wreckage at 0420 hrs. An inshore lizardfish and cubbyu were on sand bottom about 5 m from the reef at 0426 hrs. Returning via ground line to the site of the aggregation of 30 cubbyu the night before, one tomtate was on sand bottom about 15 m from the reef at 0456 hrs. A series of small excursions away from the reef revealed no other fishes. At 0530 hrs, tomtate were seen back at train cars previously occupied by the 30 cubbyu. Round scad schools began to gather at the wreckage at 0530 hrs. Twelve cubbyu were apparently feeding over sand about 5 m from the reef at 0540 hrs. Between 0545 and 0630 hrs, fishes returned to their typical diurnal behaviors.

Twenty-three nautical mile ledge:

We began our dives of June 14, 1989 about one-half hour before and after sunset (Table 1). During the first dive (Table 1), thousands of tomtate were noted on top of the ledge with about 40 moving downslope toward sand bottom. On the second dive, between 2056-2101 hrs, a cubbyu and two twospot cardinalfish were active along the vertical ledge break while a purple reeffish and a blue angelfish were inactive within holes in the ledge. Four tomtate, a white grunt, a cubbyu and a vermilion snapper were over sand about 3-5 m from the ledge at 2058-2108 hrs. The cubbyu appeared to be feeding on organisms in or on top of the sand bottom. Gag and bank sea bass were noted at the reef/sand interface at 2110 hrs. Between 2110-2115 hrs, we saw three cubbyu, four two spot cardinalfish, a white grunt, a tomtate, four spottail pinfish, a plainhead filefish, a bank sea bass, and a purple reeffish on top of the ledge adjacent to the thick algal beds. The last dive on this day was restricted to the ledge. We noted two tomtate, a bank sea bass, three spottail pinfish, and three twospot cardinalfish on top of the ledge. Along the vertical break of the ledge, we noted again a purple reeffish and a blue angelfish within holes while two spotted soapfish, two cubbyu, and a twospot cardinalfish were active at the reef/sand interface. Our predawn dive of the following morning documented a mixed school of tomtate and white grunt streaming in from deeper water over sand bottom to the ledge between 0520-0525 hrs. Purple reeffish became active between 0525 and 0530 hrs and twospot cardinalfish and spotted soapfish still appeared active between 0520-0540 hrs. Normal daytime activities of reef fishes appeared to begin about 0540 hrs.

During our next dives in August, we tried to avoid disturbing the reef fishes with the harsh white light from the flood lamps attached to the Osprey camera by hooding the lamps with red filters. This proved to be only partially successful. The lights were effective in illuminating fishes only at full intensity. At this light level we still detected some avoidance behaviors by the fishes. A more serious problem was that the red gelatin hoods caused the lamps to overheat, resulting in a tripped fuse on the

Cyclops control panel. Consequently we were not able to note many fishes on this and the following dive. Between 1948 and 1957 hrs we saw cubbyu and spotted soapfish on top of the reef and a large number of fish that appeared to be tomtate hovering over the top of the reef. During the dawn dive of the next day we saw groups of about 100 tomtate coming in from deeper sand onto the reef between 0619-0630 hrs. About five yellowtail reeffish and one purple reeffish were feeding near the bottom at 0624 hrs. We saw purple reeffish males in nuptial color patterns beginning at 0632 hrs. At 0636 hrs, large numbers of tomtate were milling about in aggregations over the top of the ledge and normal daytime activities for reef fishes appeared to commence at this time.

Nocturnal color patterns of reef fishes:

During the course of these observations, it became apparent that many of the reef fishes exhibited distinct color patterns at night. In general, diurnally active fishes usually changed to a distinctly barred pattern after dusk and nocturnally active fishes usually changed to a blanched or lighter pattern after dusk. Some patterns were particularly striking and are described in more detail below.

One of the main features of the scup's diurnal color pattern is a dark crescent shaped bar on the side. Occasionally, we saw larger scup with dark abdominal areas during the day and we have interpreted this as a possible nuptial color pattern. At night, scup often exhibit an intensely barred pattern consisting of five thick bars that are angled slightly rearward and connected from the upper third of a preceding bar to the top of the next bar with a thin upward slanting stripe. The bars begin just behind the head and stop at the caudal peduncle. Black sea bass often show a distinctly pattern also with five bars at night. Spottail pinfish are also barred at night with nine thin yellowish bars on the body. Round scad have been observed with six to seven bars on the body at night. In contrast, tomtate and cubbyu exhibit a striped pattern during the day. At night, both species appear to lose the color pattern altogether and have a pattern that is washed out or blanched, hence whitish in color.

Discussion

Observations of natural behaviors of fishes at night are obviously a difficult task given the necessity of using an artificial light source except under some conditions when sufficient ambient light from the moon may allow observations. Unfortunately, this was not the case for our situation where depths and visibility precluded use of ambient light except during the crepuscular period. Typically only about twenty minutes of twilight were available to us before we had to resort to artificial lights. We attempted to keep lights to a minimum. For instance, we tried to use the low light setting on the ROV with the SIT camera. However, this was not entirely satisfactory since even the low light setting tended to overpower the sensitive SIT camera and render a "burnout" of the video image. We think that our lights may have affected some behaviors. For instance, lights probably inhibited some feeding behavior in the nocturnal fishes we observed. Overall, though, we believe that our observations of general activity, or lack of it, at night and during crepuscular periods are biologically meaningful.

We chose a variety of methods to observe nocturnal and crepuscular behavior in reef associated fishes. SCUBA offered the most realistic and accurate observations of reef fishes. Combined intermittently with video, SCUBA observations most accurately defined and documented nocturnal behaviors. Unfortunately, the Sony video we used did not offer on-screen annotation in real-time nor were we able to record voice-over narration in real time with this early system. This made it more difficult to decipher the video tapes at a later date. However, the portability of the Sony system was excellent and we could easily move along the reef and out over sand.

The Osprey video system suffered from lack of portability and required umbilical tenders topside and at depth with the diver operating the video camera. The Osprey/Cyclops system allowed maximum on-screen annotation of date, real time, location, and running time and the diver could also narrate the video in real-time. The latter made analysis of the videos much easier and more accurate.

SCUBA observations were always limited by the amount of bottom time available to the diver. However, the Osprey camera was clumsy and caused diver fatigue, especially in the hand and arm holding the camera. The ROV did not have this limitation but had other problems.

The Superphantom II ROV is best described as a clumsy-looking apparatus underwater. Despite expert operation, the ROV umbilical was subject to entanglement on the reef and the ROV itself sometimes caused disturbance of the sand bottom via the propeller wash or contact with the frame. The ROV video system also did not allow on-screen annotation although real-time narration at the surface monitor was possible. We found that the narration was often inadequate, especially in terms of updating clock time during the ROV dive. We found that the SIT camera could be used effectively for the first twenty minutes of twilight without the use of lights. After this, we alternated between the color and SIT cameras. The SIT camera was not effective at close range because even the lowest flood light intensity caused image burn out. The ROV also acted as an undesirable positive attractor for some reef fishes (*e.g.*, cubbyu) since it probably appeared as wreckage when still and when no lights were in use.

Of course, verification of nocturnal feeding activity is required through the analysis of stomach fullness at early morning hours and by other techniques such as nocturnal gill netting. This information is reported elsewhere for the tomtate (Bolden, 1990). Stomach fullness data for the cubbyu is presented in Figure 1 (Lindquist *et al.*, manuscript). Nocturnal foraging patterns for these species and some of their congeners have been verified by other workers (*e.g.*, Collette and Talbot, 1972; Darcy, 1983; Davis, 1967; Hobson, 1965; 1968; 1973; McFarland *et al.*, 1979; Ogden and Quinn, 1984; Starck and Davis, 1966 for grunts and Hobson, 1965; 1968 and Longley and Hildebrand, 1941 for close relatives of the cubbyu, the rock croaker and the high hat.

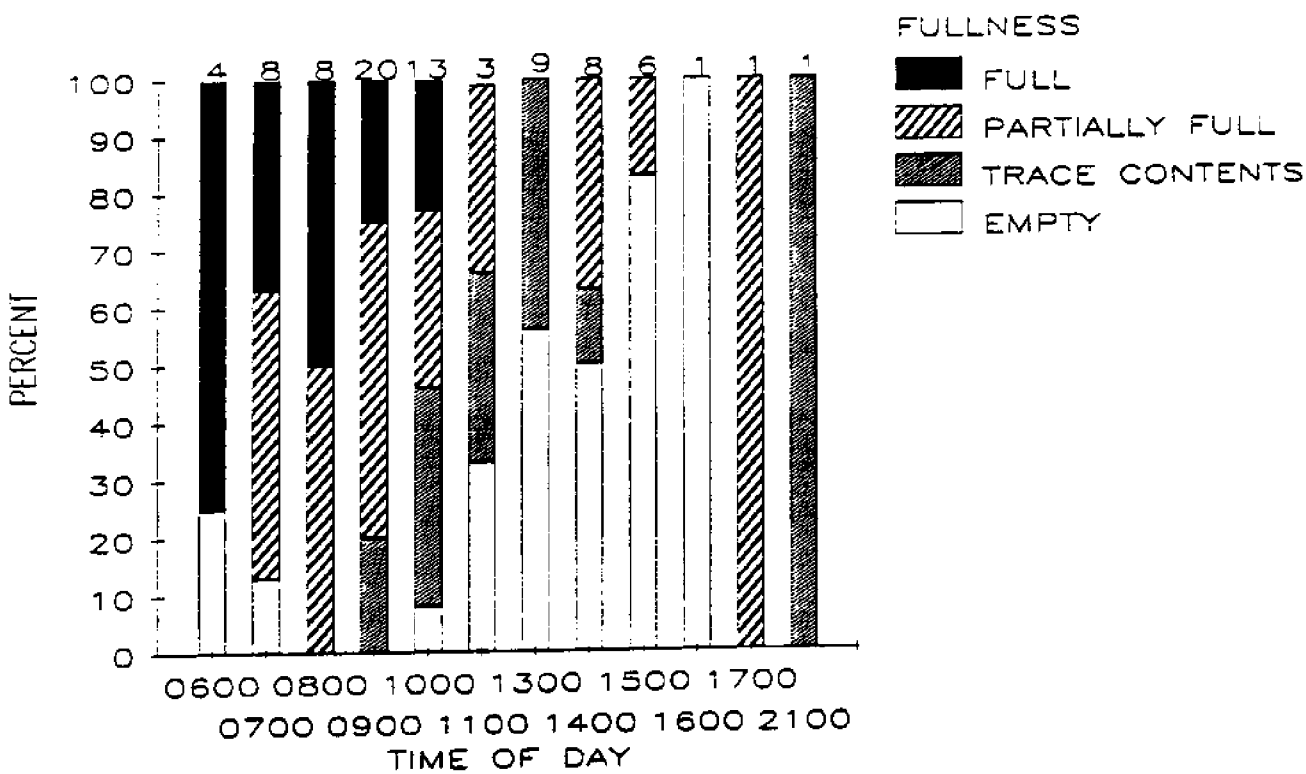

Figure 1. Estimation of stomach fullness for cubbyu taken from offshore reefs in Onslow Bay, North Carolina. Numbers above bars represent thte sample size for that time period.

It is clear from our observations that most of the fishes associated with the tug wreck during the day departed during the night (Table 2). Our observations at the other sites and our stomach fullness studies indicate that at least the tomtate and cubbyu forage away from the reef over sand bottom at night. Many of the diurnally active fishes such as scup, round scad, and black sea bass also are found over the adjacent sand bottom even though they do not appear to be feeding at this time. These observations suggest that the reef offers cover and some forging area for reef fishes during the day. At night many fishes abandon the reef for cover and the reef is no longer important for protection (most piscivores are diurnal). Some of our observations and those of Hobson (1965; 1968) indicate that reef croakers and cubbyu may also feed on prey in direct association with the reef at night.

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DEVELOPMENT AND APPLICATION OF A LOW-COST PAIRED-LASER MEASURING DEVICE

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An underwater paired-laser measuring device was developed as a training aid to help reduce bias associated with in situ estimation of fish sizes. The device was constructed out of low-cost materials and incorporated two relatively inexpensive laser pointers. Observers were asked to estimate the size of targets with and without the use of the paired-laser apparatus. There was a significantly higher accuracy of estimation associated with the laser measuring device and no significant difference in accuracy between mobile and stationary targets while using the device. More testing needs to be conducted comparing differences in accuracy between the paired-laser measuring device and "accepted" methods that are currently being employed, and to find other practical applications for paired-laser systems.

Introduction

Several publications have been released in recent years that provide a critical review of visual assessment techniques used for the quantification of reef fish assemblages (*e.g.*, Jones and Thompson, 1978; Brock, 1982; Bortone *et al.*, 1986). The original intent of these visual assessment techniques was to develop and standardize non-destructive comparison methods (Jones and Thompson, 1978). The information that can be obtained from visual assessment techniques ranges from simple species abundances to biomass and stock size comparisons.

Although the visual assessment methods currently employed attempt to obtain quantitative information regarding species composition, abundance and frequency of occurrence, few have dealt with ways to reduce bias associated with in situ fish length estimation. Bohnsack and Bannerot (1986) used a ruler attached perpendicularly to the end of a one meter rod to avoid magnification problems in estimating fish size. Short of spearing a fish after its size is estimated, there is currently no way to "ground truth" the actual size of the fish. The error associated with this method of length estimation is probably acceptable, provided that the same observer conducts the estimations every time.

Recent advances (and associated cost reduction) in laser technology have made it possible to develop a "low-cost" paired-laser measuring device to aid observers in the estimation of fish lengths. Lasers are now commonly used during undersea research for camera guidance and size estimation (Caimi and Tusting, 1987). We designed and constructed an adjustable, paired-laser device to be used as a learning tool to estimate fish size more accurately.

The objectives of this study were to:

1) Determine if observers using a paired-laser measuring device can produce more accurate estimations of size than the same observers using vision alone.

2) Determine the difference in observer estimation error between a mobile and a stationary target while using the laser measuring device.

Methods and Materials

Paired-laser construction

The 5.0 mW helium-neon (red) laser pointers were obtained from Edmund Scientific at a cost of $199.95. Housings were constructed from schedule 80 PVC. Lens caps were constructed out of acrylic and contained an o-ring seal. The paired laser housings were mounted on a 1/4" x 2" x 48" aluminum bar in a manner that allowed them to be moved parallel to each other (Fig. 1). One laser was stationary and the other moved relative to it.

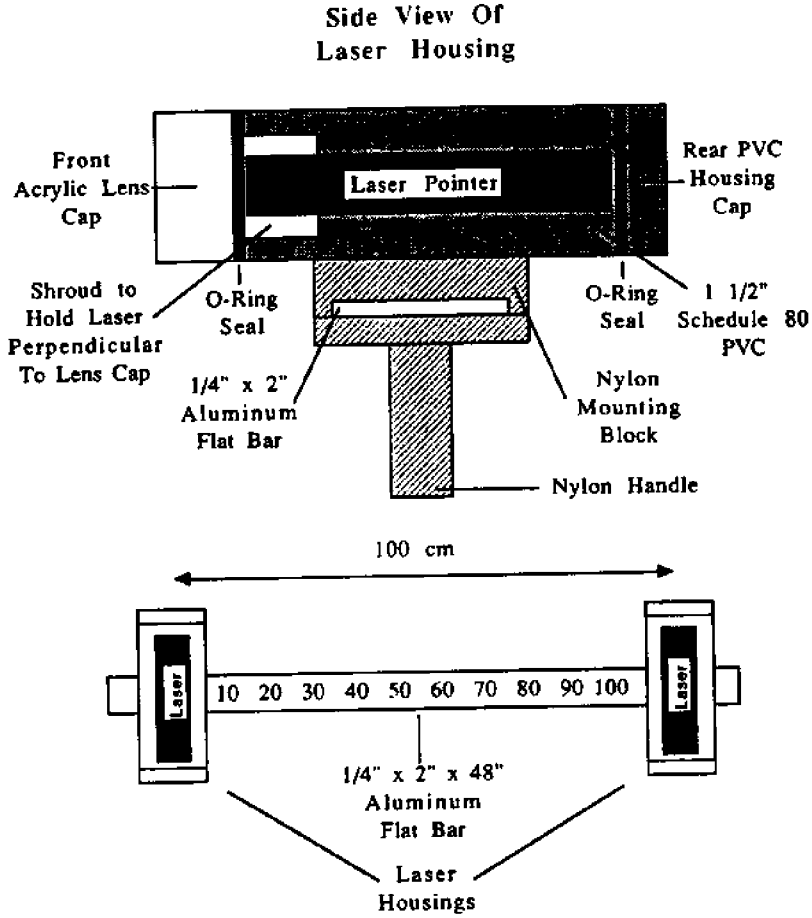

Figure 1. Design and construction of a paired-laser measuring device used to reduce error associated with in situ fish size estimation.

Experimental design

"Fish" targets were constructed from 1" x 6" pine boards that were cut to desired lengths. An 11/16" hole was bored into the center of the boards and a 3/4" PVC elbow was hammered into the hole with a soft mallet. The elbow was used to mount the targets on a pole for presentation to the observers.

Ten individuals inexperienced in visual assessment procedures were selected. Using SCUBA, these individuals were located in a pool and presented ten random targets that ranged in size from 10-100 cm. The subjects were placed at a distance of 5.0 m from the presentation area. The targets were moved

laterally for ten seconds at a rate of 1.0 m/s. The observers recorded the estimated size of the targets on underwater slates. Three trials were conducted in this manner. The same targets were presented in each trial, but the order of appearance was randomized.

We were interested in comparing how accurate observers were at estimating fish size, and considered accuracy to be a function of size, *i.e.*, a 5.0 cm error for a small fish is more inaccurate than a 5.0 cm error for a large fish. Also, we were not interested in whether individual estimates were larger or smaller than the actual size. Thus, estimation error was calculated as:

$$Z = | (E-A)/A |$$

where:
Z = estimation error (accuracy)
E = estimated size of the target
A = actual size of the target

An ARCSIN square-root transformation, appropriate for ratios, was used before parametric analysis using ANOVA.

From the ten original observers, five were selected that were most similar in their size estimations, based on the results of a Tukey's multiple range test. This step was necessary in order to reduce bias associated with differences among observers. These five observers were presented targets in the same manner as described above to re-establish a baseline for comparison to the paired-laser measuring device. Once this baseline was established, an ANOVA was conducted to ensure that there were no significant differences in estimation errors among observers.

For the laser validation portion of the experiment, observers were placed 5.0 m from the presentation area and asked to estimate the size of mobile targets with the laser measuring device to the nearest 5.0 cm. The targets were presented in the same fashion as described above. One set of trials was conducted on stationary targets to determine if there was an increased error due to the physical manipulation of the apparatus required to place the laser spots on a moving target. The observers had ten seconds to estimate the target's size. The transformed estimation errors from the mobile and stationary targets were also compared by one-way ANOVA.

Results

The observers were able to estimate the size of the targets more accurately ($P < 0.0001$, ANOVA) with the laser measuring device than by observation alone. The observers had a mean estimation error of 8.96% (±0.74%) without the lasers, and a mean estimation error of 6.26% (±1.4%) with the laser device (Fig. 2A).

Observers using the paired-laser measuring device estimated the size of mobile targets just as accurately as stationary targets ($p > 0.05$, ANOVA), although the error of the estimations was slightly higher for the mobile targets. The mean estimation error for the stationary targets was 2.86% (±0.85%) and 4.91% (±1.58%) for the mobile targets (Fig. 2B).

Discussion

Limitations of the device

The helium-neon laser pointers that were used for the device produce a red beam. The visible projection of the lasers into the water column is only 5.0 m due to the attenuation of red wavelengths at

that distance. Lasers that produce a green beam would probably project further into the water column, but the cost is significantly higher.

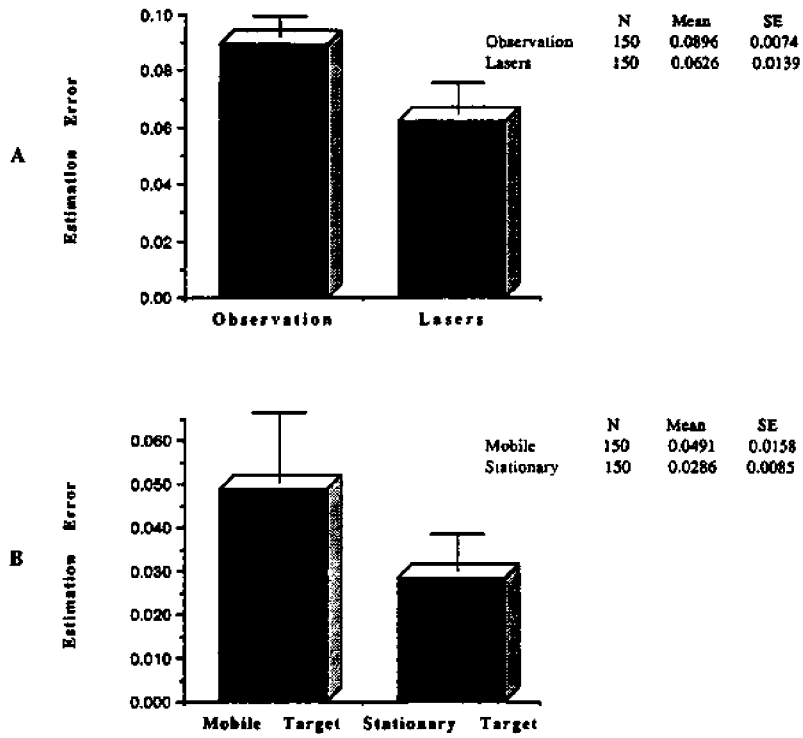

Figure 2. A) Target size estimation errors for observation with and without use of the paired-laser measuring device and B) estimation errors between observers using the device on mobile and stationary targets.

The design of the laser housings and the choice of materials proved to be more than adequate. The housings were pressure tested to 300 fsw and maintained watertight integrity for three hours at depth. Caution should be exercised to prevent the acrylic lens caps from becoming scratched because scratches on the inside of the lens cap severely impede projection of the laser beam.

The precision of the laser measuring device is dependent on the lasers being perpendicular to the mounting bar. The precision of the paired-laser system is $\pm$ 2.0 cm at 5.0 m, which takes into account the beam width and play inherent in the apparatus design.

Future directions

Further testing needs to be conducted by observers using the measuring device in a field-oriented situation to assess adequately the applicability of the apparatus to a marine environment. The next test that needs to be conducted should be a comparison of the ability of a laser-trained observer to estimate actual fish sizes as compared to an observer using methods such as those described by Bohnsack and Bannerot (1986). If the laser-trained observers are no more accurate in their estimations than those obtained by observers using other methods, then investment in a paired-laser system may not be advisable.

Other possible applications for a low-cost paired-laser system are for use on submersibles and ROV's (Tusting, 1990; Auster *et al.*, 1989). The lasers could be mounted on a bar that allows for the traditional 10.0 cm beam separation. Lasers have also been adapted for use in this manner on the hand-held underwater video systems used by the National Undersea Research Program at the University of

North Carolina at Wilmington. When used in this manner the lasers provide a reference scale on the video tape that would be difficult to discern otherwise.

This simple tool should have multiple applications to in situ research in addition to measuring fish sizes. For example, measurements of stationary objects or fauna, *e.g.*, coral diameters and heights, could be made with greater accuracy and less damage to the subject. Hard to reach areas and cryptic species could also be measured more quickly and easily.

Conclusion

Observers equipped with a paired-laser measuring device appear to be able to assess more accurately the size of targets underwater. Evaluation should continue to determine if the same observers can learn to estimate the size of targets as accurately without the use of the lasers, once they have become familiar with this form of in situ visual assessment. Future applications and feasibility will be determined through further field testing and evaluation.

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NO-STOP ENRICHED AIR (NITROX) DIVING WITH SURFACE INTERVAL OXYGEN BREATHING - FIELD VALIDATION

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The objective of the Surface-Interval Oxygen (SIO2) dive trials was to evaluate the decompression stress associated with diving procedures developed for oxygen-enriched air (nitrox) diving and surface oxygen breathing. The Phase I trials consisted of chamber tests with dry, resting divers. The Phase II field trials were conducted in water of nearly uniform temperature with divers maintaining a light, constant workload throughout each dive. A total of 362 subject dives were completed. Extending chamber based studies to an open water setting creates a number of challenges. This paper reviews the basic procedures of the study and the field staff involved. Planning considerations that proved to be critical in the project are discussed and recommendations made for future studies of this type. A strategy for summarizing experimental data into a computer readable "clock" is introduced and encouraged. Improvements in dive computer software and development of clock databases are expected to facilitate the comparison of findings from independent studies.

Introduction

The Surface-Interval Oxygen (SIO2) dive trials were developed to evaluate the decompression stress associated with diving procedures for oxygen-enriched air (NITROX or EAN) diving and surface oxygen breathing (Gerth *et al.*, 1992). Nitrogen partial pressures are reduced in EAN mixtures. This will decrease the rate of nitrogen uptake for a given pressure exposure. Nitrogen elimination may also be increased by breathing 100% oxygen following the dive.

Phase I consisted of chamber trials to evaluate four dive profiles. A total of 286 subject dives were conducted with dry, resting divers. Total bottom times were 34-120% longer than equivalent NOAA EAN dives and 65-267% longer than USN air dives. A single case of knee pain occurred during the trials (Vann *et al.*, 1992a).

Phase II trials were planned to validate the schedules under more realistic diving conditions. Dives were to be conducted in water of nearly uniform temperature (68-72°F). Divers would maintain a light, constant workload throughout each dive.

A total of 362 subject dives were completed in the Phase II trials. The preliminary results are described elsewhere (Vann *et al.*, 1992b). The purpose of this paper is to review this project as a pilot for future open water trials. Considerations that proved to be critical to the success of the project and lessons learned that may aid future projects are discussed.

Procedures

The Phase II SIO2 trials involved four separate subject groups. Each group completed three dives a day for six consecutive days. Subjects were pre-screened, informed of all protocols, medically cleared and evaluated anthropometrically prior to the trial period. Table 1 lists the baseline measurements that were made. Each group of eight subjects reported the evening prior to their first dive. They remained with project staff for the duration of the experimental period. Meals and accommodations were provided.

Table 1. Baseline Measures.

Anthropometrics

Age
Height
Weight
Percent body fat
Vital capacity
Residual lung volume
Pulmonary diffusion rate

Table 2. Phase II Profiles.

Dive #	Depth (feet)	Bottom Time (min)	Oxygen Breathe (min)
1	80	51	35
2	80	37	35
3	80	37	35
4	120	25	30
5	120	22	30
6	120	22	30
7	80	51	35
8	80	37	30
9	120	22	30
10	120	25	30
11	120	18	30
12	120	17	30
13	80	51	30
14	80	40	35
15	120	20	30
16	80	51	30
17	80	40	30
18	80	40	30

Each of the six days of diving involved three dives in combination to either 80 feet (36% oxygen EAN) or 120 feet (32% oxygen EAN) for a specified period. Table 2 lists the planned dive profiles and post-dive oxygen breathing periods for each of the 18 dives. Diving was conducted in two teams of four subject divers, each accompanied by an in-water divemaster and monitored by surface personnel. Pre- and post-dive exertion was minimized by providing assistance to subjects donning their gear and instructing them to remove their gear prior to leaving the water. The underwater workload was normalized by having the divers maintain a 0.5 knot swimming speed along a flagged course for the entire time at depth. Subjects breathed 100% oxygen for 30 or 35 minutes following each dive.

Decompression stress was monitored using Doppler ultrasonic bubble detectors. Daily baseline recordings were made as were post-dive assessments at 20 minute intervals as allowed by the repetitive dive schedule. Hydration was assessed on a daily basis through urine hydrometry and weighing. Subjective impressions of health status and performance were recorded throughout each diving day and reviewed with each diver at the end of the day. Exposure profiles were monitored by a combination of diver carried time/pressure recorders and observation by surface personnel. The presence of pulmonary oxygen toxicity was assessed through carbon monoxide diffusing capacity tests conducted at the start and end of the six days of diving.

Staffing

A review of the administrative staff requirements involved in establishing this program will be discussed in a separate paper. The responsibilities discussed here are restricted to the field component of the study.

Breathing Gas Delivery and Equipment Support:

A total of 576 subject and 105 staff dives were initially planned for the SIO2 Phase II trials. A total of 362 subject dives were completed. The estimated gas requirement to meet the initial plan approached 120,000 cubic feet. Provision of this amount of gas is much more difficult in field, as opposed to chamber, operations. In addition to a supply of the two EAN mixtures, a filling (and/or mixing) system capable of reliably providing over 5,000 cubic feet per day was required.

The longest dives of this study required a 51 minute bottom time at 80 feet. Twin 80 and twin 120 cubic foot tank systems were used to ensure adequate gas supply for the divers. Because of the weight of these systems, the availability of staff to provide donning assistance and to move the cylinders between the water and fill station became significant considerations.

Basic equipment repair and replacement demands were the final responsibilities of this group. Adequate backup equipment, parts, and servicing skill were required on-site to maintain the dive schedule.

Chamber/Medical Support:

A transportable multi-lock recompression chamber, gas charging facility and operational staff were made available for the duration of this study by the NOAA/National Undersea Research Center at UNC Wilmington. Staff members trained as Diving Emergency Medical Technicians (D-EMTs) conducted baseline neurological examinations on all subjects, responded to any medical complaints from the divers, and operated the fill station with the assistance of the equipment staff. A Diving Physician was on call throughout the experimental trials.

Dive Monitoring:

Dive monitoring required a Diving Supervisor overseeing all in-water operations and a divemaster to accompany each dive. Divemasters alternated between diving and serving as surface Stand-By Divers. The subject divers breathed EAN mixtures of 36% and 32% oxygen at 80 feet and 120 feet, respectively. The Divemasters breathed 40% oxygen mix at 80 feet and a 35% oxygen mix at 120 feet to

minimize their decompression stress. These oxygen partial pressures of the Divemaster's gas supply represented a maximal value of 1.4 ATA.

Data Collection:

Data collection staff were responsible for coordinating the operating schedule, monitoring and recording of all activity throughout the study period. Two Doppler Technicians were each assigned a group of four divers to monitor throughout the trial period. Data collectors also monitored the dive schedule, recorded and collated a range of data throughout the day, and downloaded each diver-carried computer at the end of the day. Table 3 lists data collected during the trial period.

Table 3. Field Measures.

State of hydration (daily)
- Weight
- Urine specific gravity

EAN mixes
Time of start of dive
Dive profile
- Descent rates
- Maximum depth
- Bottom time
- Ascent rate

Surfacing time
Gas consumption
Post-dive time to start of oxygen breathing period
Oxygen breathing time
End of oxygen breathing to repetitive dive time
Doppler scores (daily baseline and post-dive)
Maximum doppler grade
Time to maximum doppler grade
Ambient temperature (hourly)
Ambient relative humidity (hourly)

Planning Successes

The goal of SIO2 Phase II was to extend the Phase I trials with the addition of a more normal workload and more realistic field conditions. The list of considerations important to developing this work is beyond the scope of this paper. The focus here is on three considerations that proved to have significant impact on both the planning effort and final outcome of this study.

Site Selection and Emergency Planning:

Access to emergency services requires careful attention in field trials. The underwater site must be proximate to a convenient and controlled entry and exit point. The site must be able to accommodate all of the staff, subjects, and equipment required for the trials. While a shipboard operation may offer the greatest flexibility, convenience, and control, the cost of conducting four weeks of trials in this manner made the option untenable for the current project. Instead, a controlled inland site was identified.

The SIO2 Phase II site (Wakulla Springs, south of Tallahassee, Florida) had the required depths, visibility well in excess of 100 feet during the season the study was conducted, and minimal horizontal distance between entry point and the depths required. It also had an elevated dock to allow observation of in-water activity, and road and helicopter access to facilitate emergency evacuation plans, and an on-site commercial lodge that could provide meal and washroom facilities.

The importance of these considerations became paramount. Since the subjects were required to stay together for the entire six day trial period, the provision of convenient and adequate support was critical. More importantly, however, was the response to an accident that occurred during the first day of the study. A subject who failed to disclose a medical history of seizures or current anti-convulsive medications went into a full seizure at depth during the first day of diving. The implementation of

emergency procedures, including helicopter evacuation directly from the site, was so effective that a situation that had the potential to threaten the entire study only disrupted the first week of scheduled dives.

Data Reporting:

The inevitable variability between nominal and actual exposures and inter-individual differences makes direct comparison of individual dives and different studies difficult. Compilation of computer readable databases offers the best means to analyze such data. In this study, downloadable diver-carried Orca Delphi dive computers were used to attain accurate records of all exposures (Heinmiller, 1989). Table 4 provides an example of the basic Delphi output.

Table 4. Delphi computer output example.

```
(a)
     (b)     (c) (d)
   19.00    0.00
    0.50    8.15 D1
    0.50   26.70 D1
    0.50   40.28 D1
    0.50   50.24 D1
    0.50   62.91 D1
    0.50   74.68 D1
    0.50   85.99 D1
    0.50   90.07 D1
    1.50   90.97 D1
    0.50  106.36 D1
    0.50  114.96 D1
    0.50  115.41 D1
    0.50  116.32 D1
    0.50  119.03 D1
    0.50  121.30 D1
    0.50  119.03 D1
    0.50  117.68 D2
    0.50  117.22 D2
    0.50  116.77 D2
    0.50  112.24 D2
    0.50  103.65 D2
    0.50   97.76 D2
    0.50   94.59 D2
    0.50   86.45 D2
    0.50   72.87 D2
    0.50   58.84 D2
    0.50   45.71 D2
    0.50   45.26 D2
    0.50   38.47 D2
    0.50   31.23 D2
    0.50   19.91 D2
    0.50    9.50 D3
    1.00    1.81 D3
    0.50    1.36 D3
    4.00    0.00 D3
88888.88   33.94
  110.00    0.00
    0.50    9.50 D1
    0.50   33.94 D1
    0.50   60.65 D1
    0.50   93.24 D1
    0.50  105.91 D1
    0.50  115.87 D1
    0.50  116.32 D1
    ....
    ....

(e)   Delphi .Sim File Key

      (a) two blank comment lines available during download

      (b) time at this depth (measured every 30 s)

      (c) depth (feet) at sample point

      (d) Orca code

      (e) 88888.88 - tank change (or line depressurized)
          (9s indicate cpu power turned off/on)
```

The depth/time profiles generated by the dive computers were downloaded on a daily basis. These daily files were joined to form a single continuous file for each subject for the duration of the dive series. Breathing gas switches, durations, and real time references were then integrated with the basic profile information into computer readable summary files. These files then serve as time lines, or "clocks", to which all events throughout the experimental period can be referred. Simple programs can then be

compiled to compare any of the variables. Table 5 provides an example of the clock files generated through these trials.

Table 5. Clock summary format

```
SIO2  Wahulla Springs  (a)
Subject XXX  (b)
(c)  (e)     (f)          (g)     (i)   (i)
(d) 0.50    9.05         0.363   1300  Day 1-Dive 1  911028
    0.50   37.11                       cpu 15596  Buddy 304
    0.50   66.08
    0.50   79.12
    ....
    ....
    0.50   53.00
    0.50   39.00
    0.50   26.50
    1.50    0.00
    1.00    0.45
    5.00    0.00         0.210   1206
   27.00    0.00         1.000   1211
   99.00    0.00         0.210   1238
    0.50    7.69         0.317   1417  Day 3-Dive 3
    0.50   24.44                       cpu 15401(*304)  Buddy 304
    0.50   63.36
    0.50   68.15
    ....
    ....
    0.50    5.12
    0.50    3.34
    0.50    2.72
    0.50    2.26
    5.00    0.00         0.210   1533
   30.00    0.00         1.000   1538
            0.00         0.210   1608  (j)
```

Clock File Key

(a) First File Line - project identification

(b) Second File Line - subject identification

(c) Third File Line - blank

(d) Fourth File Line - start of subject "clock" data

(e) First Column - time reference (minutes)

(f) Second Column - depth reference in feet

(g) Fourth Column - oxygen partial pressure; blank indicates continuation of last pressure listed

(h) Fifth Column - real time reference: 24 hour clock

(i) Comment Space - includes:
Day/Dive Reference (based on planned dives)

Start Date (e.g. 911028) of experimental period

Computer (cpu) Used (NB. 15596 indicates data from diver's own cpu; 15401(*304) indicates case where cpu 15401 data were taken from diver 304 to substitute for missing data for this diver); all substituted dives are marked with *; if data from diver's own cpu, only noted at start of day unless cpu changed during the day

Buddy or Buddies (each group should have consistent exposures but buddy pairs were assigned)

Miscellaneous comments/explanations

(j) End of File - indicated by blank time column coinciding with oxygen partial pressure change

Recommendations for Future Project Development

Protocols and Experimental Schedule:

Subjects completing chamber dives typically require minimal effort to exit the chamber at the end of a dive, minimal time for gear removal or changing, and have immediate access to washrooms or other amenities. Open water diving demands additional time to allow for surface swimming, pre- and post-dive and equipment setup, donning and removal. It was clear in the early planning that the chamber-based schedules had to be relaxed to accommodate open water requirements.

Open water trials also require significantly more manpower than chamber trials. While the coordination of multiple subject groups is an attractive means of minimizing in-water group size while maximizing data collection, two concerns arise. The first involves the increase in scheduling complexity. In this case, repeated post-dive ultrasonic assessment and in-water times were the most difficult to arrange. Additional staff must be available to make sure that complex schedules can be maintained. The second concern of multiple subject groups is the potential of a single disruption to affect a greater number of individual trials. While two subject groups of four divers each were run concurrently in the present study, it is strongly recommended that the relative simplicity of single trial groups be considered.

Pilot trials must also be conducted. All staff and equipment should be employed if possible. In multi-week, multi-group studies such as this one, the first set of dives should be scheduled with a single group to maximize the quality of data gathered during the initial trials. Subsequent sets can then include the full schedule with greater confidence.

Staffing:

One of the strategies successfully employed in the current study was to compartmentalize areas of responsibility. Tasks can be accomplished more efficiently when efforts can be focused. The field staff used in this project and the staff recommended for future projects of a similar size and complexity are summarized in Table 6. A 60% increase in staff would have been ideal.

Table 6. Field Staff

Responsibility	SIO2 Phase II	Recommended
Breathing gas delivery and equipment support	1	2 or 3
Dive monitoring		
Diving supervisor	1	1
Assistant supervisor	0	1
Divemasters	2-3	3
Chamber/Medical support	2	2
Data collection		
Doppler technicians	1/4 subject	1/2 subjects
Data coordinators	2	3
Total	10-11	16-17

Breathing gas delivery and equipment support was provided by one person on a full-time basis, with the support of staff from other areas as demanded. Optimally, two or three on-site staff members should be exclusively assigned to this area. The number will vary based on the distance between

entry/exit point and the fill station, the degree of assistance required to dress the divers, and the complexity of the required mixing operation.

Dive monitoring was conducted by a Diving Supervisor and two or three Divemasters as available. Staff in this area should consist of Diving Supervisor, three Divemasters, and an assistant to the Diving Supervisor if multiple subject groups are to be scheduled. The Supervisor will typically maintain the dive logs, coordinate and monitor the Divemasters and assist subjects in analyzing their breathing mixtures prior to every dive. With multiple teams, when one group is in the water, the supervisor is unable to contend with the divers preparing for their next dive. At this point, the assistant is needed to ensure the completion of critical checks of gear setup, gas analysis, etc.

The chamber/medical support staff in the current study consisted of two individuals. This number is sufficient, but in this study, these individuals were committed to carry out filling and mixing responsibilities in addition to those requiring D-EMT skills. Optimally, the filling operations would be handled by the equipment support staff. All tasks assigned to the D-EMTs should keep them in direct contact with the divers. These may include the responsibilities of Assistant Diving Supervisors as described previously.

Data collection staff can be divided into Doppler Technicians and General Coordinators. In this study, a single Doppler Technician was assigned to four subjects, one pair from each of two subject groups. This created some difficulties when both groups required repeated monitoring. Optimally, one Doppler Technician should be available for each pair of divers.

The responsibilities of general data coordination were assigned to two individuals in this study. Optimally, three would be assigned. Between daily recording and collation and end-of-the-day computer downloading, long days are required even before the day's data can be reviewed. The extra person could serve an important role in the daily review and data entry. This would help to ensure that shortcomings in the procedures are corrected in a timely manner and would reduce the post-study handling time required to reduce the data.

Data Reporting:

The clock file data summary may be the most exciting development from this study. If subsequent projects employ the same format and increase the available database, it is expected that the ability to carry out useful cross-trial analysis will be improved. It is for this reason that this pattern of data storage is recommended.

One recommendation to improve future clock files would be to include constant ultrasonic monitoring results to present the timing of bubble grade progression. Inclusion of Doppler monitoring results was not appropriate in the current study since sampling was discontinuous and somewhat irregularly timed due to the constraints of having a single technician for four divers.

While most of the information must currently be manually entered into the basic computer file generated by a dive computer, developments in dive computer software can be expected to assist in automating the system. This would dramatically reduce the handling time of the individual data and move data collection in the direction of a paperless system.

Conclusions

This paper has reviewed the overall research plan, staffing requirements, and data reporting strategies involved in advancing the SIO2 Phase I chamber trials to the Phase II open water trials. It is a practical review to provide recommendations for future projects of a similar nature. The "clock summary" of exposure data is presented as a strategy that may be used to facilitate future comparison of

dive exposure information. It is hoped that interest on the part of diving researchers and equipment manufacturers will improve the software available to support this approach.

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SHORT-TERM RESPONSES OF BENTHIC INFAUNA TO THE ESTABLISHMENT OF AN ARTIFICIAL REEF

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Increased attention is being focused on the use of artificial reefs to enhance populations of certain fishery species. However, relatively little is known about the functioning of these systems, including the major trophic pathways supporting reef communities or their links with other habitats. As part of a larger project designed to examine multiple aspects of artificial reef systems, we examined the distribution of benthic infauna adjacent to two artificial reefs to determine if a halo of reduced faunal abundance was present. Several major taxa exhibited changes in abundance immediately adjacent to the reefs (within 10 m of the reef structure), indicating the potential for predation by reef-associated predators or physical effects from the reef. Distributional patterns observed were on the same spatial scale as reported for other temperate artificial reef communities and help support a the concept of a general pattern for infaunal distributions around artificial reef structures.

Introduction

Hardbottom communities, including both natural and artificial reef systems in warm temperate areas, provide important habitat for recreational and commercial fishery species along the Atlantic and Gulf coasts of North America (Lindquist and Harris, 1979; Sale, 1980; Grimes *et al.*, 1982; Chester *et al.*, 1984; Sedberry and Van Dolah, 1984; Bohnsack and Sutherland, 1985). Recent research on natural and artificial reef systems has indicated that there may be important trophic linkages between these reef systems and adjacent sand-bottom habitats (Nelson *et al.*, 1988; Cahoon *et al.*, 1990). Rather than being members of self-sustained systems, many reef-associated fish and crustaceans may use the reef primarily as a structural refuge, obtaining much of their food by foraging on infauna in the sand bottoms or on planktonic food sources (Bray *et al.*, 1981; Parish, 1989; Heuckel *et al.*, 1989; Frazer *et al.*, 1991).

Much of the evidence for trophic linkages between reef and sand bottom habitats comes from studies of fish foraging and diets. Studies of the foraging behavior of many reef-associated fishes (including tomtate, black sea bass, porgy, grouper, and white grunt) indicate that several species are foraging over sand-bottom areas adjacent to the reefs (Helfman *et al.*, 1982; Bolden, 1990; Burk, 1990; Frazer *et al.*, 1990; Sedberry, 1990). Analyses of gut contents indicate that sand-bottom macrofauna comprise an important portion of the diets of many reef-associated fishes (Harris, 1979; Manooch and Raven, 1984; Bolden, 1990; Burk, 1990; Lindquist *et al.*, 1990; Vose, 1990).

Distributions of soft-bottom fauna around reefs also provide important information concerning links between these two habitats. Halos of decreased macro-infaunal abundances adjacent to artificial reefs have been reported by Ambrose and Anderson (1990), Nelson *et al.* (1988), and Navratil (1988) and analogous halos have been reported adjacent to coral reefs (Alongi, 1989; although patterns around coral reefs have often been complicated by depth variations or the presence of thalassinid crustaceans). Halos of decreased infaunal abundance near a reef have been suggested to indicate foraging by reef-associated predators on the adjacent sand plain (Turner *et al.*, 1969; Davis *et al.*, 1982). However, relatively little is known concerning the prevalence or dynamics of halos. First, certain studies have yielded conflicting evidence about the existence of macro-infaunal halos around artificial reefs (Davis *et al.*, 1982). Second, little is known about the geographical variations in infaunal distributions relative to distance from a reef, such as may occur with differences in vertebrate or invertebrate assemblages. Third, the degree of infaunal density decrease near a reef may change seasonally with seasonal variations in feeding activity and recruitment patterns. Finally, little work has critically examined the time frame of halo formation after an artificial reef has been deployed or the long-term persistence of halos around either natural or artificial reefs.

As part of a long-term study designed to examine the effects of reef structure (size and spacing) on the composition and dynamics of artificial reef communities, we examined short-term macro-infaunal responses to the establishment of an artificial reef array. Presented here are preliminary results of studies on the distribution of higher taxa with respect to distance from an artificial reef within one year after the reef was established.

Materials and Methods

A. Study Location

The study was conducted off the Suwannee Regional Reef System. This artificial reef system is located offshore of the Cedar Keys, Florida, U.S.A., in approximately 13 meters of water depth, and extends along 24 nm of coastline in the Gulf of Mexico (Fig. 1). It is a series of artificial reefs that are composed of standardized concrete modules of approximately 2500 lbs. weight in air. Each reef array is composed of a group of 6 patches set up in an hexagonal pattern. Replicate reef arrays represent combinations of two treatments of patch size (4 or 16 modules per patch) and two treatments of interpatch distance (25 or 225 m between patches). The artificial reef modules were deployed from a barge and subsequently arranged with lift bags into square monolayer patches. The results presented here are from preliminary sampling at two of these reef arrays: one array with 16 blocks per patch and 25 m spacing between patches and the other array with 4 blocks at 225 m spacing (these two array types spanned the extremes of patch size and spacing). Both reef arrays were in place for approximately 9 months before sampling of benthic macro-infauna.

B. Sampling

Macrobenthic cores were collected in duplicate at 1, 10, 25, 50, and 75 m distances away from 3 patches at each reef array, and at a control bottom area with no artificial reef (6 cores). The 3 patches used for sampling at each reef array were selected to have minimal emergent rock within the intended transect distances. Cores were 10 cm in diameter and 12 cm deep and coring devices had 500 micrometer mesh at one end to facilitate draining of the cores before fixation and preservation in a separate container. The other end of the corer was fitted with a rubber stopper to retain the sample. Cores were collected by hand with SCUBA during May 1991 and were fixed in 10% formalin solution with rose bengal dye after being sieved on a 0.5 mm screen.

C. Statistical Analyses

Macro-infauna were divided into major taxonomic groups for analysis: polychaetes, bivalves, crustaceans, gastropods, and echinoderms. Abundances of fauna were compared between distances, blocking for transect differences, separately for each reef array type and then for the two array types combined using Analysis of Variance (ANOVA). ANOVA's were run on log-transformed abundances

and were conducted separately for each infaunal group. Variances were heterogeneous before transformation but were non-heterogeneous after log transformation (F-max test, Sokal and Rohlf, 1981). Where differences between distances were observed, means were contrasted using Ryan's Q-test.

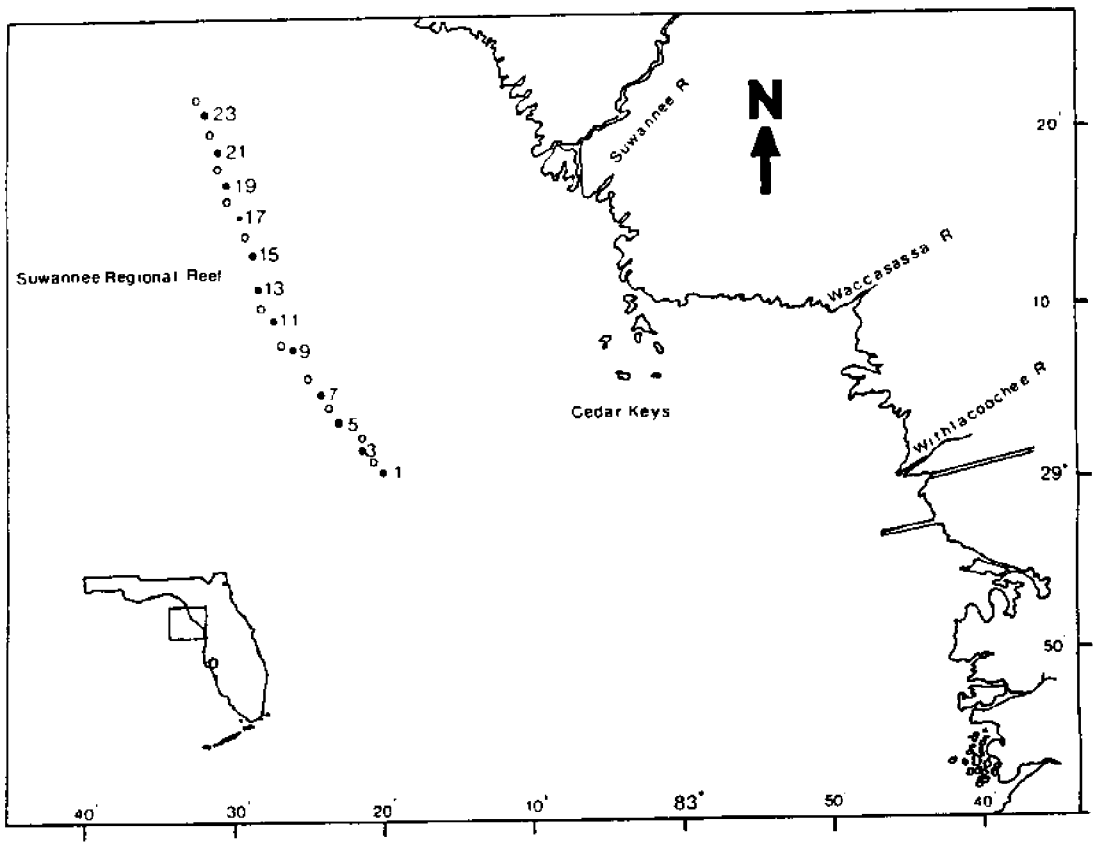

Figure 1. Location for Suwannee Regional Reef. Reef #3 (25m spacing) and Reef #7 (225m spacing) were sampled for this study.

Results

The major taxa in the sand-bottom community surrounding the reefs were polychaetes and crustaceans. Polychaetes included 77 species with 10 species comprising at least 1% of the total individuals collected in this preliminary sampling. Crustaceans were dominated by amphipods (26 species), isopods (9 species) and decapods (11 species). Among the remaining taxa, bivalves included 8 species, echinoderms were dominated by 1 brittle star species and two sand dollars, and gastropods included 9 species.

When examined separately, there was no clear evidence of a halo pattern at either reef array type for the major taxa. At the dense, close-spaced reef there were significantly higher abundances of gastropods 1 m from the reef compared to 50 m away ($F=2.89$, $p<0.05$; Figure 2), but there was no difference between 1 m and 75 m distances. There was also a non-significant trend towards higher abundances of bivalves away from the dense reef up to a 50 m distance ($F=1.62$). Polychaetes and echinoderms showed a relatively lower abundance adjacent to the reef at both reef array types (Figure 2), but the pattern was not statistically significant (dense, close reef: $F=0.52$; low-density, dispersed reef: $F=0.90$).

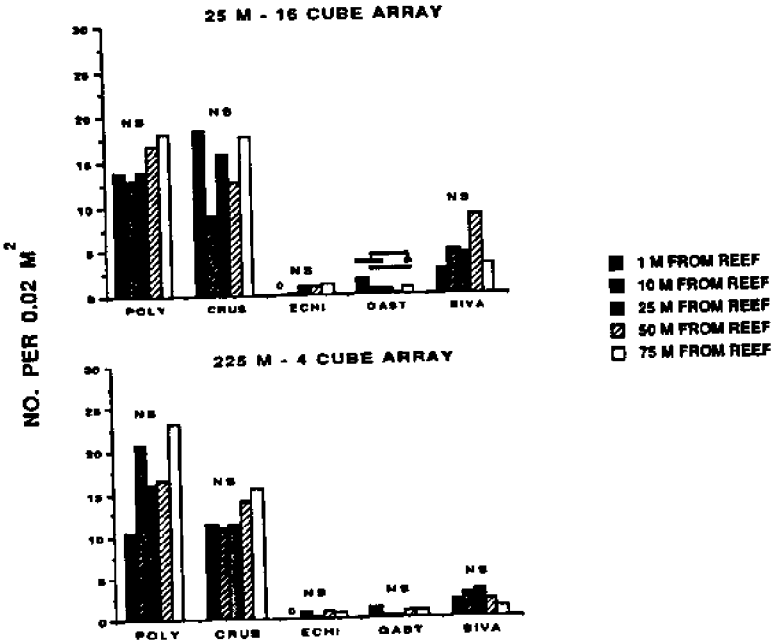

Figure 2. Abundances of polychaetes, crustaceans, echinoderms, gastropods, and bivalves at varying distances from each reef type. NS: no significant difference between distances; where differences occur, bars not connected by a line differ significantly.

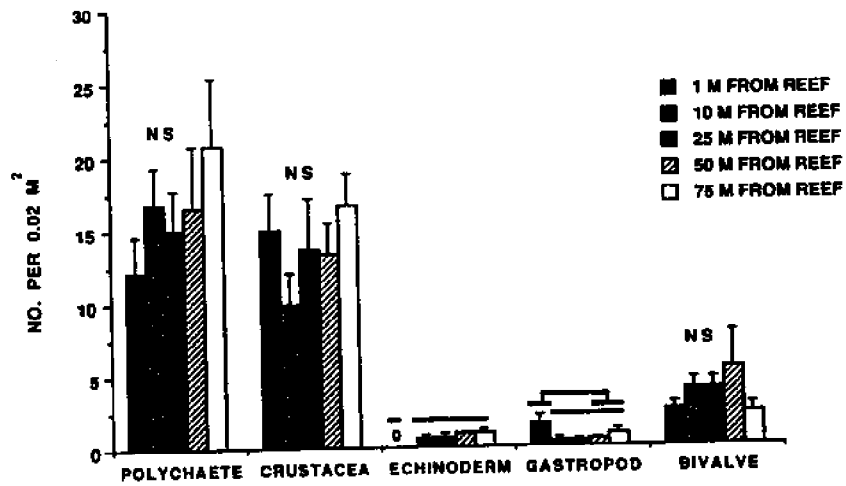

Figure 3. Faunal abundances at varying distances for both reef types combined (mean per core ± 1 SE). NS: no significant difference between distances; where differences occur, bars not connected by a line differ significantly.

When the data for the two reef array types were combined, a significant decrease of echinoderm abundances was observable immediately adjacent to the reef ($F=3.16$, $p<0.025$; Figure 3). A non-significant trend of decreased polychaete abundances near the reef is also apparent. Gastropods exhibit higher abundances near the reef than 10-25 m distant ($F=2.68$, $p<0.05$), but there was no statistical difference in abundances compared to the furthest sampling distances.

Discussion

Distributions of higher taxa at varying distances from the artificial reefs in this study provide limited support for the existence of a halo. Several taxa exhibit a tendency for decreased abundances near the reef (polychaetes and echinoderms) and one taxon (gastropods) exhibited a tendency for higher abundances near the reef. For both echinoderms and gastropods, the most dramatic differences in abundance occurred between the 1 m location and other distances from the reef.

The results of this study suggest that variations in faunal abundance around a reef may occur within a relatively short time (9 months) of reef establishment. However, the patterns for higher taxa were variable among taxa, and abundances of most fauna did not exhibit a consistent increase or decrease with increasing distance from the reef. This may reflect species-specific variations in responses, the short time since reef establishment, or the potential presence of natural hardbottoms. In a study of infaunal distributions around a California artificial reef, Ambrose and Anderson (1990) also found that several taxa did not exhibit consistent variations in abundance with increasing distance from the reef, with some taxa being more abundant at intermediate distances than either close to the reef or at the farthest distance. Of 5 species exhibiting distributional halos around the California reef, two were more common immediately adjacent to the reef (one polychaete and one crustacean species) while the others were less abundant adjacent to the reef (one polychaete, nemerteans, and cumaceans) (Ambrose and Anderson, 1990).

The spatial scale of infaunal responses observed in this study is also consistent with patterns reported for other artificial reef systems. Halo effects on the order of 1-5 m have been reported by Nelson *et al.* (1988), Ambrose and Anderson (1990) and Davis *et al.* (1982) for other artificial reefs, although the scale of effects may be greater off natural reefs (Kinsey, 1985; Alongi, 1989; Meesters *et al.*, 1991; Posey and Ambrose, unpublished data). With respect to the temporal scale of effects observed in this study, immediate (within 1 year) responses of benthic fauna to the establishment of an artificial reef system also have been reported along the east coast of Florida (Navratil, 1988; Nelson *et al.*, 1988).

The similarity of short-term infaunal responses observed in this study with those reported in other systems suggests a general pattern for distributional halos of sand-bottom fauna around artificial reefs. Such general patterns may indicate foraging by reef-associated predators on sand-bottom fauna or physical effects of the reef, with most previous studies assuming the patterns are the result of predation interacting with physical changes (Davis, 1982; Ambrose and Anderson, 1990). The results presented here, however, are only from preliminary sampling at two reef arrays. This work is the first step in a long-term project designed to examine structural and functional aspects of the interrelationship between artificial reefs and adjacent sand-bottom communities. Future efforts will be aimed at sampling replicate reef arrays, examining the interactive effects of reef density and spacing on infaunal community distributions, examining long-term and seasonal variations in infaunal distributions, and experimentally assessing relative predation pressures at varying distances from an artificial reef.

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THE USE OF FISH AGGREGATING DEVICES (FADs) AS AN ALTERNATIVE TO SMALL-SCALE ARTIFICIAL REEFS.

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Data from a study conducted in 1989 and 1990 in Onslow Bay, North Carolina, demonstrated that use of three-dimensional mid-water artificial habitats are effective at aggregating fish populations. The mobility, ease of deployment and retrieval, and availability of inexpensive construction materials make these habitats a viable alternative to small-scale benthic artificial reefs. The durability of these habitats allows for year-round deployment with minimal maintenance.

Introduction

It is widely known that many fish species tend to congregate in areas of pronounced topographical changes of the sea bottom (Mottet, 1985). Many studies have examined this phenomenon by artificially replicating natural structures responsible for aggregating fishes. Particular structures of interest include large sunken vessels (Chandler *et al.*, 1985; Stephan and Lindquist, 1989), fabricated steel modules (Sonu and Grove, 1985), surplus concrete pipe (Brock and Norris, 1989), concrete bells and cubes (Feigenbaum *et al.*, 1985; Brock and Norris, 1989; Hixon and Beets, 1989), tire modules (Spanier *et al.*, 1985; Brock and Norris, 1989), plastic cones and hemispheres (Bell *et al.*, 1989), abandoned offshore platforms (Quigel and Thornton, 1989), transplanted kelp (Carter *et al.*, 1985), floating fish aggregating devices (Bombace, 1989), and midwater fish aggregating devices (Wickham *et al.*, 1973; Wickham and Russell, 1974; Murray *et al.*, 1987; and Rountree, 1989). A census conducted by Grove and Sonu (1991) concluded that use of artificial reefs worldwide, in 40 countries on six continents, resulted in increases in fish catches from 20% to 4000%. Yet knowledge of fish behavior related to artificial habitats, an issue central to technology, still remains largely quantitative.

International conferences on artificial habitat technology have been an appropriate forum for scientists engaged in this area of research since 1974. However, certain topics of interest have not been as vigorously examined as others. Grove and Sonu (1991) tallied the papers and abstracts submitted to four international conferences and separated them by region, country and topic. They found that only 18 papers and abstracts, 10 of which were submitted by U.S. researchers, addressed the topics of fish aggregating devices (FADs). This total represents only 5.5% of the papers and abstracts submitted, indicating that FADs are not considered topics of key interest.

We explored the feasibility of using FADs as an alternative to small-scale benthic artificial reefs. This portion of our research examines the construction, cost and deployment of FADs near the coast of Wilmington, North Carolina, in 1989 and 1990. The driving scientific premise of our study examined the use of FADs as experimental habitats to test the hypothesis that fish species composition and abundances are directly influenced by the size of the habitat and the degree of habitat complexity. We artificially simulated size and complexity characteristics of a natural hardbottom rock outcrop through the use of experimental habitats and assessed fish species composition and abundances at the habitats and a natural rock outcrop.

Materials and Methods

Experimental Habitat (FAD) Construction:

Two types of experimental habitats were designed to simulate separate structural aspects of the reef. The first habitat, termed "V-Habitat," and two additional replicates were designed to simulate the vertical relief component of a natural rock outcrop. This was accomplished by using two 1.5 m lengths of 1.3 cm diameter schedule 40 PVC pipe connected to one another by clamps and stainless steel screws in a cross shape. Holes drilled through the ends of the pipe provided a means by which 136 kg monofilament fishing leader was strung, thus reinforcing the structure while providing a frame to which a fiberglass-reinforced polypropylene tarp was attached (Fig. 1a). Tie-wraps were used to secure the tarp to the x-shaped frame. Estimated volume of this habitat was 0.1 m^3.

Polypropylene mooring line was threaded through two 15.2 cm sections of tygon tubing attached to the back of the structure. Foam pot-buoys sectioned into halves were spliced into the mooring line above and below the structure to keep it vertically stationary on the mooring line while allowing the structure to freely rotate 360_ to offset strains imposed by currents. One whole foam pot-buoy was spliced into the unsecured end of the mooring line approximately 0.9 m above the structure to provide buoyancy that kept the structure virtually upright under most current conditions. A 0.6 m loop was spliced into the bottom end of the mooring line as a place of attachment to the mooring (Fig. 1b).

The second experimental habitat, referred to as "H-Habitat," and two additional replicates were designed to simulate the three-dimensional aspects of a natural rock outcrop in addition to vertical relief. The general design of this experimental habitat was modified from Workman *et al.* (1985). This was accomplished by cementing two 0.9 m lengths of 3.8 cm diameter schedule 40 PVC pipe to opposite ends of a PVC T-joint. The procedure was repeated resulting in two 1.9 m sections connected to one another by a 1.2 m length of pipe resulting in an H-shaped structure. The four open ends of pipe were capped with PVC endcaps.

A 1.6 cm diameter hole was bored through the 1.2 m cross-piece, in which a hollow PVC dowel was cemented. A 15.2 cm stainless steel eyebolt was threaded through the dowel and permanently secured by means of a machined locknut. The resulting H-frame was airtight and, thus, needed no additional flotation once deployed in the water. A stainless steel thimble was fitted around the circle end of the eyebolt to offer a place of attachment for the polypropylene line which was wrapped around the thimble and spliced back into itself, providing the maximum holding strength while protecting the line from chafing against the metal eyebolt. A 0.6 m loop was braided into the free end of the mooring line which was used to attach the structure to the mooring (Fig. 2a).

To give the structure its three-dimensional characteristics, two 1.8 x 2.4 m polypropylene tarps were attached to the 1.9 m sections of the H-frame by tie-wraps. The tarps were sliced into five streamers by cutting the tarps from the free ends to within 0.3 m of the frame. On alternate streamers (3), 56.7 g weights were attached by tie-wraps to keep the streamers in a vertical position (perpendicular to the H-frame) in the water column (Fig. 2b). This increased the surface area and compartmentalized the experimental habitat design. Estimated volume of this habitat was 5.5 m^3.

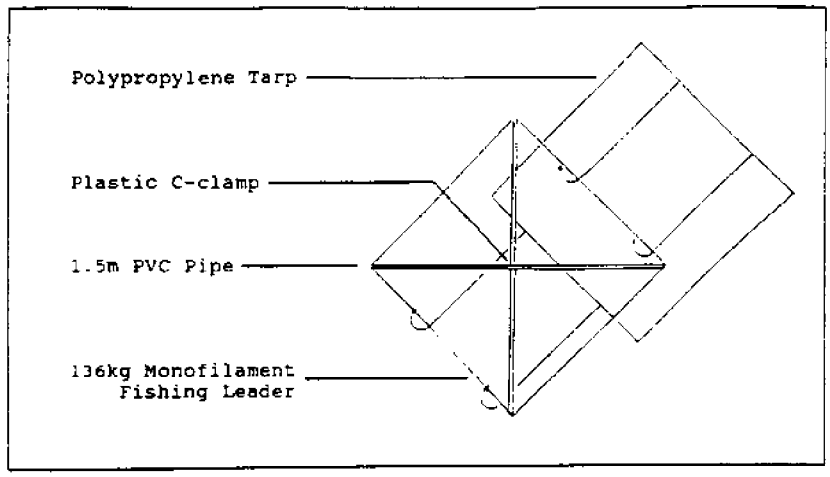

Figure 1a. Schematic diagram showing V-Habitat construction.

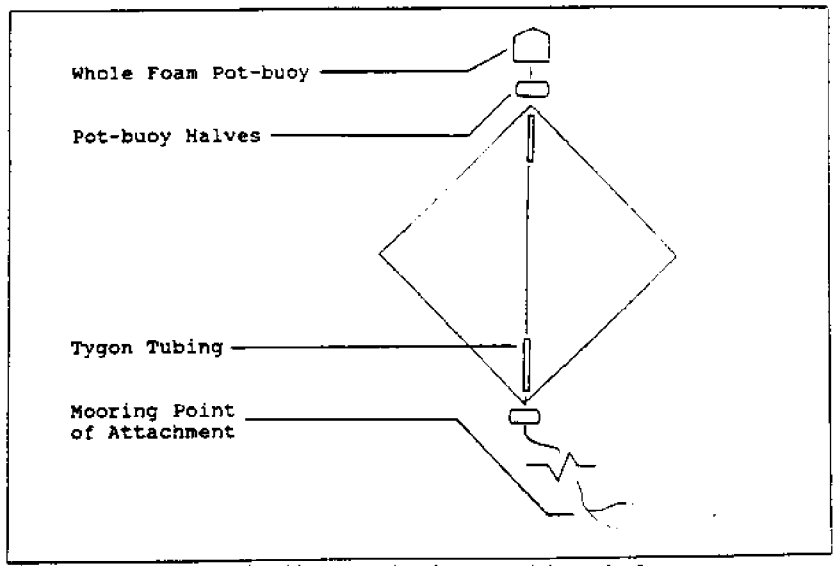

Figure 1b. Schematic diagram showing V-Habitat before deployment.

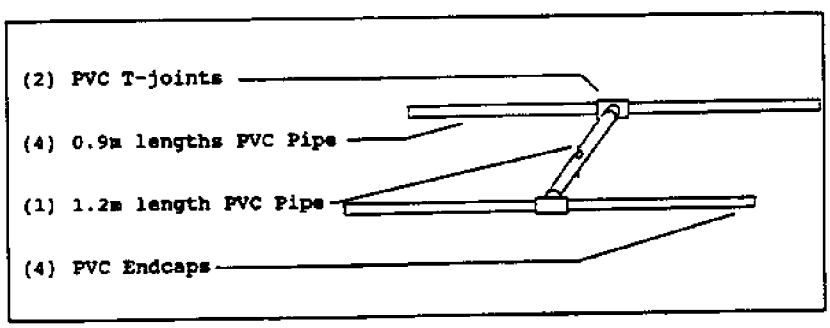

Fig. 2a. Schematic diagram showing H-habitat frame construction.

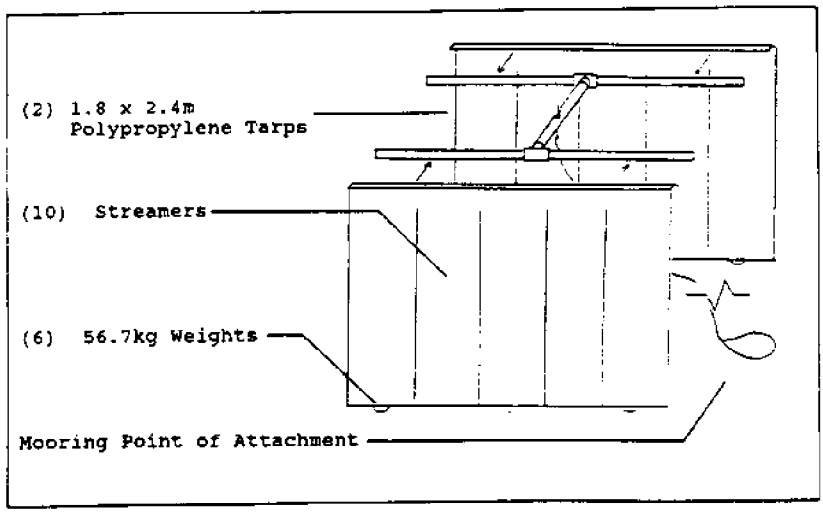

Fig. 2b. Schematic diagram showing H-habitat tarp attachment.

Two controls were constructed and consisted of a concrete anchor, a 0.7 m length of 1.3 cm diameter chain, a 2.7 m length of mooring line, and a pot-buoy. Without a structure attached to the mooring line, the control provided a way of measuring the impact that the moorings had on aggregating fish.

Each structure was anchored to the substrate by a concrete anchor (approximately 15.9 kg), a 1.2 m length of 1.3 cm diam chain (approximately 9.1 kg), and four 30.5 cm galvanized spikes. The concrete anchor was constructed with a section of rebar threaded through a totally embedded chain link to make the chain steadfast in the concrete anchor.

Table 1. Experimental habitat materials and costs.

V-HABITAT CONSTRUCTION: ONE UNIT

Amount	Item	Unit Price	Extended Price
2	1.5m lengths 1.3cm diameter PVC pipe	$ 1.14	$ 2.27
2	Plastic clamps	0.20	0.40
3.3m	136kg monofilament fishing leader	0.83/m	2.74
2	0.15m lengths tygon tubing	0.59	1.18
2	Foam pot-buoys	2.00	4.00
5.5m	0.65cm diameter polypropylene line	0.26/m	1.45
1	11kg bag dry concrete	4.48	4.48
1	polypropylene tarp	9.29	9.29
1	19L bucket	5.00	5.00
1.2m	1.3cm diameter chain	13.20/m	15.84
	Miscellaneous hardware (spikes, screws, etc.)		5.50
		TOTAL	$ 52.15

H-HABITAT CONSTRUCTION: ONE UNIT

Amount	Item	Unit Price	Extended Price
4	0.9m lengths 3.8cm diameter PVC pipe	$ 2.04/m	$ 7.34
1	1.2m lengths 3.8cm diameter PVC pipe	2.04/m	2.45
2	PVC T-joints	1.25	2.50
4	PVC endcaps	0.72	2.88
1	15.2cm stainless steel eyebolt	3.00	3.00
1	Stainless steel thimble	0.88	0.88
2	1.8 x 2.4m polypropylene tarp	15.99	15.99
6	15.2kg weights	1.00	6.00
1	19L bucket	5.00	5.00
1	11kg bag dry concrete	4.48	4.48
1.2m	1.3cm diameter chain	13.20/m	15.84
	Miscellaneous hardware (cement, spikes, etc.)		5.50
		TOTAL	$ 71.86

All materials used to construct the experimental habitats and controls were purchased locally. The cost of materials for constructing one "V-Habitat" was $52.15 while the cost for constructing one "H-Habitat" was $71.86 (Table 1).

Experimental Habitat Deployment:

Ninety meters of groundline were laid in three 30 m sections by SCUBA divers using a NITROX/EAN 36% oxygen mixture (Mastro, 1989) to increase bottom time and decrease surface intervals. The groundline originated from the reef (water depth of 26.6 m) and ran perpendicular from the reef to the experimental habitat study site (water depth of 25.5 m). The groundline was comprised of 0.3 cm polypropylene line that was attached to the sand-covered limestone substrate by 30.5 cm galvanized spikes hammered into the substrate and bent over the line at approximately 6 m intervals. A surface float was attached to the end of the groundline to mark visually the experimental habitat study site and to aid in deploying the experimental habitats from the surface. The groundline served two purposes; first, it allowed divers to locate the experimental habitat study site in the event that the surface buoys were removed, and, second, it allowed the divers to locate the rock outcrop study site from the experimental habitat study site without having to surface.

Three additional 18 m groundlines were laid at the experimental habitat site. Two groundlines were laid perpendicular to the original reef groundline while the third groundline was laid as a continuation of the reef groundline. This configuration allowed the experimental habitats and the control to be deployed in a diamond-shaped pattern 21.4 m apart from one another. The groundline configuration expedited structure location underwater and in periods of low visibility (Fig. 3).

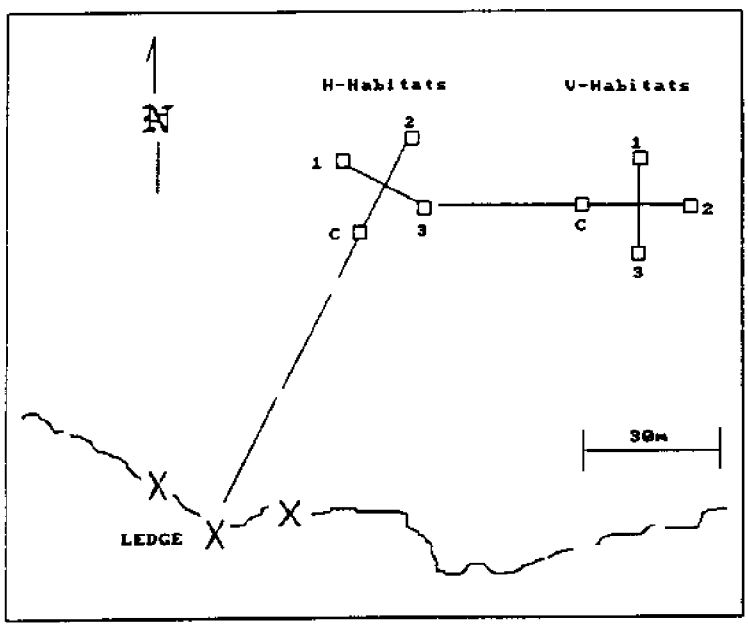

Figure 3. Position of the ledge study site (X, 3 replicates); H-habitat study site (3 replicates); V-habitat study site (3 replicates); and the controls (C, 3 replicates).

Artificial habitats were deployed by connecting the habitat to the anchor at the surface and lowering the assembled structure to the substrate from above using a 1.9 cm polypropylene down-line. Once an individual experimental habitat was deployed in the water, SCUBA divers moved it to the appropriate location and secure it to the substrate by four galvanized spikes and a length of 0.3 cm polypropylene line. The anchor remained virtually stationary on the substrate while the length of chain catenary took up any strain from currents and/or surge acting on the experimental habitats.

Visual Assessment of Fish Assemblages:

Fish assemblages at the natural rock outcrop, the six experimental habitats, and the two control structures were censused using a stationary visual census modified from Bohnsack and Bannerot (1986). Visual censuses were conducted from 10 July to 16 November 1989, and again from 15 May to 22 November 1990. Forty one stationary visual censuses were performed on the H-Habitats, 15 on the V-Habitats, 14 on the control structures, and 30 on the natural rock outcrop.

Results

Fish Censuses

Three-dimensional structures (natural rock outcrop and H-Habitats) aggregated significantly higher numbers of fishes than did two-dimensional structures (V-Habitats). The natural rock outcrop aggregated higher numbers of fishes than did either experimental habitat and the control. The H-Habitats aggregated more fishes than both the V-Habitats and the control, while the V-Habitat did not aggregate significantly more fishes than the control ($P > 0.1$, Table 2). A total of 52 fish species were observed in association with the natural rock outcrop, the experimental habitats, and the control over the two year survey period (Table 3).

Table 2. Comparisons of fish abundance categories at the natural rock outcrop, experimental habitats, and the controls. Means that are not joined by a line indicate a significant difference (multiple range test, $P<0.05$).

	H-Habitats	Outcrop	V-Habitats	Control
No. Censuses	41	30	15	14
Mean number total ind.·census^{-1}	129.5	498.9	15.1	18.3
Mean number baitfish ind.·census^{-1}	100.7	235.7	2.8	0.0
Mean number predator ind.·census^{-1}	14.3	2.0	1.9	12.2

Experimental Habitat Effectiveness

Habitat integrity and longevity were excellent, with most of the structures lasting throughout the study period. Hurricane Hugo, with tropical storm strength off North Carolina, had a moderate effect on the structures. One H-Habitat was lost and two H-Habitats broke free from the substrate at the galvanized stake/mooring interface and moved across the sand plain. These structures were located, untangled from the groundlines, and returned to their original location.

PVC material proved to be extremely durable, but for studies lasting longer than two years it is probable that the polypropylene tarps would need to be replaced. Accumulation of biofouling organisms was quick and probably had some aggregating effect on grazing fishes. Additional weight caused by biofouling organisms made two structures somewhat negatively buoyant and forced us to add flotation.

The mooring design was adequate; however, alternate materials used to secure the anchor to the substrate should be investigated. If the substrate consistency is appropriate, screw anchors would probably yield the best results. If the structures were to be deployed for an extended period of time (over three years), eyebolts drilled and cemented into the substrate would be a logical choice.

Table 3. Mean number of individuals ($\bar{x}$) and frequency of occurrence (%) for each species by structure.

Species	Structure											
	Control			V- Habitat			H- Habitat			Outcrop		
	($\bar{x}$)	(SE)	(%)	($\bar{x}$)	(SE)	(%)	($\bar{x}$)	(SE)	(%)	($\bar{x}$)	(SE)	(%)
Acanthuridae												
Acanthurus sp.										<0.1	0.5	6.6
Apogonidae												
Apogon pseudomaculatus										<0.1	(-)	3.3
Balistidae												
Monacanthus sp.				0.1	(-)	13.3	0.4	0.3	28.0			
M. hispidus	<0.1	(-)	7.1	0.2	(-)	20.0	0.2	(-)	23.3	0.7	0.2	53.3
Blenniidae												
Hypleurochilus geminatus				<0.1	(-)	6.7	<0.1	(-)	2.3	0.2	(-)	16.7
Parablennius marmoreus										<0.1	(-)	3.3
Carangidae												
Caranx sp. *							0.1	(-)	4.7	1.0	2.7	10.0
C. crysos **	6.9	28.5	14.3	1.3	(-)	6.7	11.3	52.9	11.6			
C. latus **							0.2	(-)	2.3			
C. ruber **										0.7	(-)	3.3
Decapterus punctatus *							98.1	106.0	32.6			
Decapterus sp. *				2.6	4.2	40.0	2.6	2.0	25.9			
Seriola dumerili **	5.3	17.1	21.4	0.5	2.0	13.3	2.4	4.2	23.3	5.2	3.0	46.7
Chaetodontidae												
Chaetodon ocellatus										0.2	(-)	3.3
Clupeidae												
Sardinella aurita *										235.7	1434.3	10.0
Congridae												
Conger oceanicus										<0.1	(-)	3.3

* denotes pelagic baitfish species, ** denotes pelagic predator species

Discussion

The experimental habitats used in this study may offer an inexpensive alternative to studies of structure-related fish ecology. Results of fish censuses (Table 2; Table 3) support our hypothesis that fish species composition and abundance are directly influenced by the size of the habitat and degree of habitat complexity.

Field observations of the behavior exhibited by associated pelagic baitfishes suggested that these fishes utilized the natural rock outcrop and the experimental habitats for predator avoidance by forming tight schools and positioning the structure between themselves and potential predators. Demersal fishes (*e.g.*, Centropristis striata, C. ocyurus, and Diplectrum formosum) associated with the natural rock outcrop, the experimental habitat moorings, and the controls showed similar predator avoidance behavior.

The relative ease of structure deployment and the availability of inexpensive construction materials make the experimental habitats a viable alternative to small-scale benthic artificial reefs. The mobility and versatility of these habitats allow for both precise deployment and easy retrieval. Habitats can be deployed for a minimum of one year without structural failure. However, it is advisable that structures be assessed and maintained on a regular basis to facilitate repairs (*e.g.*, replacement of chafed lines).

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SUBMERSIBLE STUDIES OF DEEP-WATER OCULINA AND LOPHELIA CORAL BANKS OFF SOUTHEASTERN U.S.A.

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Two types of deep-water coral banks occur off the coast of southeastern United States: Oculina and Lophelia/Enallopsammia. The Oculina banks form an extensive reef system at depths of 70-100 m along the shelf edge off central eastern Florida. These reefs are comprised of >100 individual pinnacles and ridges which are up to 24 m in height. Each pinnacle is actually a bank of unconsolidated sediment and coral debris that is capped on the slopes and crest with living colonies of Oculina varicosa, the ivory tree coral. In comparison, deep-water banks of Lophelia and Enallopsammia corals occur at depths of 490-870 m along the base of the Florida-Hatteras slope on the west side of the Florida Straits and also on the Blake Plateau off South Carolina and Georgia. The morphology and functional structure of both the Oculina and Lophelia banks are similar. This paper summarizes 10 years of submersible studies on the deep-water Oculina reefs and describes recent submersible reconnaissance of the Lophelia banks off southeastern U.S.A.

Introduction

Deep-water coral banks typically consist of mounds of unconsolidated sediment and coral rubble. They are found in regions of fairly strong currents where the coral structures capture suspended sediment and build up mounds to heights of a few meters to >150 m. Average depths are from 70 m to >1000 m. At these depths the corals lack zooxanthellae, the algal symbionts found in shallow, hermatypic reef corals; however, the deep-water banks still form a thriving reef community.

Two types of deep-water coral banks are common off the southeastern United States, primarily between Florida and South Carolina. Oculina coral banks form an extensive reef system at depths of 70-100 m along the shelf-edge off central eastern Florida (Avent *et al.*, 1977; Reed, 1980). In contrast, banks of Lophelia and Enallopsammia corals occur at greater depths, 490-870 m, in the Florida Straits and on the Blake Plateau off the coasts of Florida, Georgia and South Carolina (Stetson *et al.*, 1962; Milliman *et al.*, 1967; Uchupi, 1968; Neumann and Ball, 1970; Emery and Uchupi, 1972).

This paper compares these two systems of deep-water banks off southeastern U.S.A. and contrasts them with the deep-water lithoherms (Neumann *et al.*, 1977) in the Florida Straits off the Bahamas.

Methods

Data on the Oculina banks are based on research over a ten-year period with Johnson-Sea-Link (JSL) submersibles. The four-person JSL submersible is capable of dives to 915 m and is outfitted with an array of photographic and collection equipment including a manipulator arm with clam-shell grab and suction hose; 12-bin rotating collection buckets; environmental data recorder to log temperature,

conductivity, salinity, depth, and light; a modified Edgerton 35-mm camera with 35 or 80 mm lens and 750 exposure film; and a color video camera system (Tietze and Clark, 1986). Lockout dives to depths of 100 m were utilized on the Oculina banks. Data on the Lophelia banks and lithoherms were gathered with Harbor Branch Oceanographic Institution's (HBOI) JSL submersible and CORD, a remotely-operated-vehicle (ROV). Additional information was summarized from published literature on submersible dives with ALVIN (Milliman *et al.*, 1967; Neumann *et al.*, 1977) and ALUMINAUT (Neumann and Ball, 1970) and from surveys using echo-soundings, dredges, and camera sleds (Stetson *et al.*, 1962; Mullins *et al.*, 1981).

Results and Discussion

Coral Description and Distribution:

The dominant corals forming deep-water banks in this region are Oculina varicosa, Lophelia prolifera, and Enallopsammia profunda, although other branching Scleractinia may also occur, including Solenosmilia variabilis and Madrepora oculata. Numerous solitary coral species are also common.

Oculina varicosa (Lesueur, 1820): In deep water (>60 m), O. varicosa forms spherical, dendroid, bushy colonies that are 10 cm to 1.5 m in diameter and height (Fig. 1). The branches average 6 mm in diameter near the tips and frequently anastomose. Individual corals may coalesce forming linear colonies 3-4 m in length or massive thickets of contiguous colonies on the slopes and tops of the banks (Reed, 1980). The deep-water form lacks zooxanthellae, whereas in shallow water O. varicosa is usually golden brown with the algal symbiont and colonies average <30 cm in diameter with thicker branches. O. varicosa ranges from the Caribbean to Bermuda and the Gulf of Mexico, at depths of 5-152 m. Deep-water banks of the coral, however, are only known from 27°32'N and 79°59'W to 28°59'N and 80°07'W (Fig. 2, Site A and A1).

Lophelia prolifera (Pallas, 1766): Similar in gross morphology to Oculina, this coral also forms massive, dendroid, bushy colonies, 10-50 cm in diameter, with anastomosing branches (Fig. 1). Its distribution ranges in the western Atlantic from Nova Scotia to Brazil and the Gulf of Mexico, and also in the eastern Atlantic, Mediterranean, Indian, and eastern Pacific Oceans at depths of 60-2170 m (Cairns, 1979).

Along with Enallopsammia profunda, it is the primary constituent of banks at the base of the Florida-Hatteras slope and at depths of 500-800 m from Miami to South Carolina (Fig. 2, Sites B and C). In addition, over 200 banks have been mapped at depths of 640-869 m (Site D) on the outer eastern edge of the Blake Plateau (Stetson *et al.*, 1962). Elsewhere Lophelia banks are known from the Gulf of Mexico (Ludwick and Walton, 1957; Moore and Bullis, 1960) and the eastern Atlantic off Norway and Scotland (Teichert, 1958; Wilson, 1979). On the Lophelia banks in the eastern Atlantic, Madrepora oculata commonly occurs with Lophelia rather than E. profunda.

Enallopsammia profunda (Portales, 1867) (=Dendrophyllia profunda): This species also forms dendroid, massive colonies up to 1 m in diameter (Fig. 1). Its distribution ranges from the Antilles in the Caribbean to Massachussetts at depths of 403-1748 m (Cairns, 1979). E. profunda occurs with L. prolifera at Sites B, C, and D (Fig. 2). It appears to be the primary constituent of the banks at Site D except at the tops of the mounds where L. prolifera is more prevalent (Stetson *et al.*, 1962).

Site Descriptions:

Site A: Dozens of isolated banks have been mapped within Site A along a 90 nmi stretch near the shelf-edge break at 70-100 m depths (Reed, 1980; Thompson and Gulliland, 1980). A typical bank is a pinnacle-shaped structure with a maximum relief of 24 m and several hundred meters in diameter (Fig. 3 top). The tops of the banks are usually one or more linear ridges with east-west orientation. Greatest concentration of live coral occurs on the 30-45° southern slopes whereas the northern slopes are often

more gradual (<25°) with more dead coral rubble and scattered live colonies, 0.5-2 m in diameter. Some of the banks are completely covered with dead coral rubble with no live coral colonies.

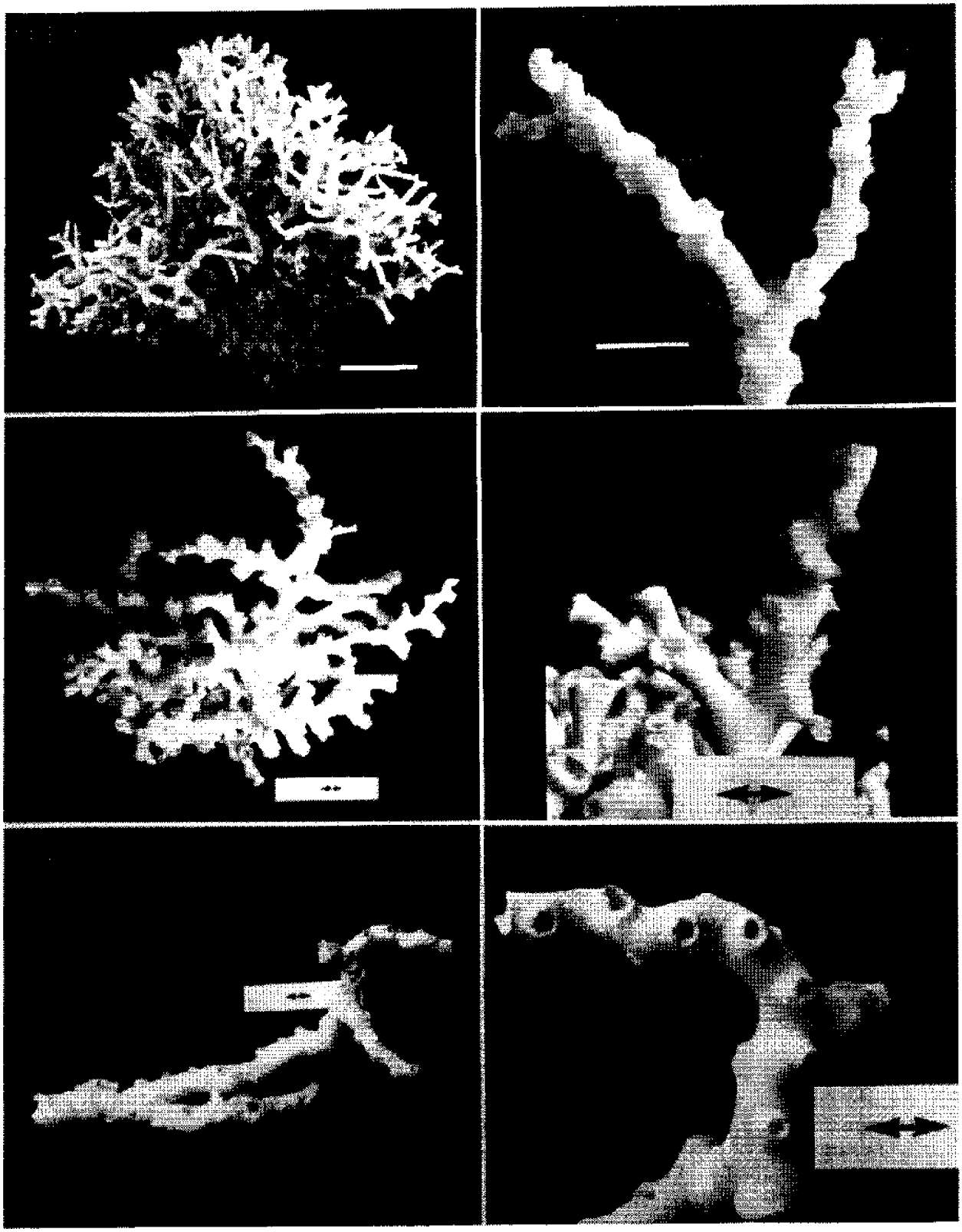

Figure 1. Deep water Oculina varicosa and Lophelia prolifera.

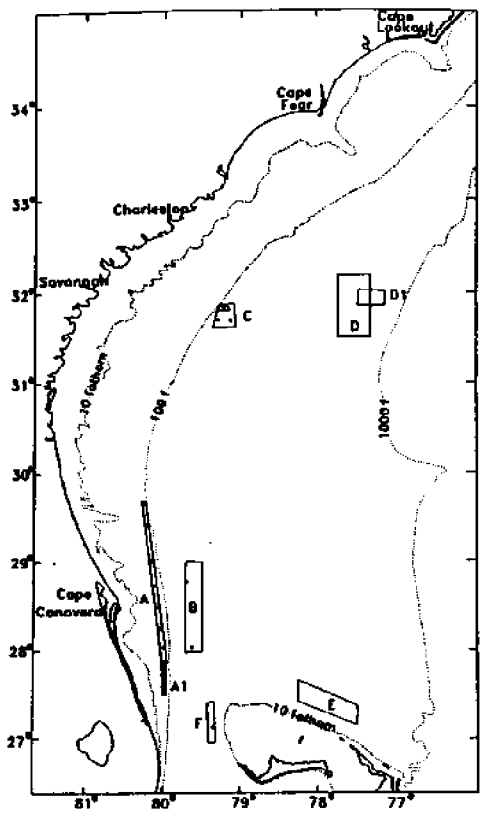

Figure 2. Deep-water coral banks off southeastern U.S.A. •=*Johnson-Sea-Link* I and II Sites, ▲=*Alvin* Sites, A=*Oculina* Bank, A1=*Oculina* HAPC Site, B-E=*Lophelia* Banks, F=Lithoherms

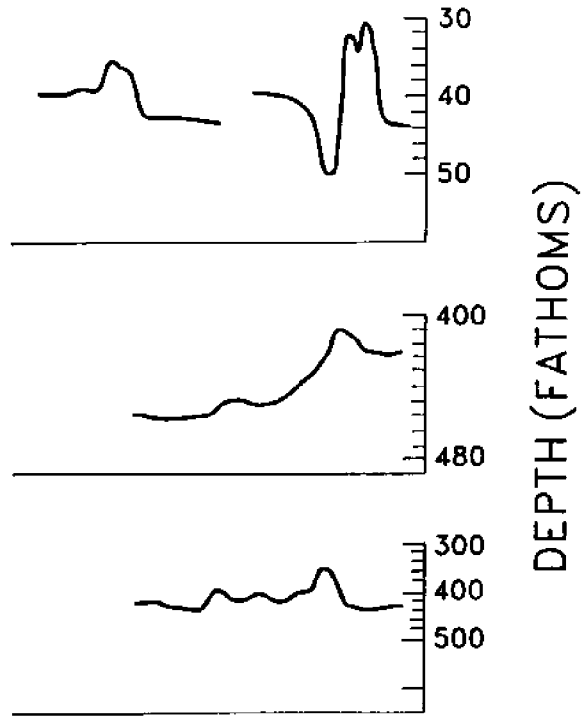

Figure 3. Bottom profiles of deep-water coral banks. Top-*Oculina* coral banks (Site A1); Middle-*Lophelia* coral bank (Site B); Bottom-*Lophelia* coral bank (Site D).

Greater growth on the southern facies may indicate exposure to the northerly flowing Gulf Stream (Florida Current); however, the clear, warm waters of this current rarely penetrate below the upper 50 m in this region. Current meters recorded average currents of 8.6 cm/s (0-58.5), which consisted of east-west tidal currents, a northerly flow (16% of total flow) and a southerly countercurrent (11% of total flow) (Hoskin *et al.*, 1987). Temperatures averaged 16.2°C and ranged from 7.4 to 26.7°C (Table 1). Intrusions of cold-water upwelling drop the temperature below 10°C episodically throughout the year (Reed, 1983). Nutrient levels of nitrates also increase nearly 10-fold during upwelling events.

A 92 sq.mi. portion (Fig. 2, Site A1) of the Oculina bank system is protected as a Habitat Area of Particular Concern within the Fishery Management Plan for Coral and Coral Reefs (NOAA, 1982) and was selected to the final site evaluation list of potential National Marine Sanctuaries (Federal Register, vol. 48, 1983; Reed, 1992).

Site B: Isolated Lophelia banks at the base of the Florida-Hatteras slope occur at depths of 700-850 m along the western edge of the Florida Straits and 15-25 nmi east of the Oculina banks. At a site east of Cape Canaveral (JSL-I dive 2474) a few small (<30 cm) colonies of Lophelia? were observed on slopes of nearly 100% dead coral rubble. At the southern end of Site B eight pinnacles were traced near a dive site documented by an ROV (CORD dive 85), and one pinnacle had 97 m relief (Fig. 3 middle). Near the peak a steep 45° slope consisted of coral rubble with a 5% cover of live coral colonies, 30-50 cm in diameter. Some upright dead colonies were also present. The northwest slope was muddy with less coral rubble. Temperatures ranged from 6.5 to 8.4°C and currents were northerly at 15 cm/s. Further

south in the Florida Straits off Miami, Neumann and Ball (1970) using the ALUMINAUT submersible found thickets of Lophelia, Enallopsammia (=Dendrophyllia), and Madrepora growing on elongate depressions, sand ridges and mounds. It is uncertain whether these are true coral banks. Large quantities of L. prolifera and E. profunda have also been dredged from 738-761 m at 26°22-24'N and 79°35-37'W (Cairns, 1979).

Table 1. Site summary for *Oculina, Lophelia,* and Lithoherm Banks off Southeastern U.S.A.

Site Reference	Depth (m)	Max. Relief (m)	Temp. (°C)	Current (cm/s) (Dir. to:)	Salinity (ppt)	Visibility (m)	Coordinates
* A) Reed, 1980	70-100	24	7.4-26.7 (x̄=16.2)	0-58.5 (N,S) (x̄=8.6)	35.7-36.4	0-30	27°32.8'N, 79°58.8'W to 28°59.2'W, 80°06.6'W
B) *JSL* I-2474	762-793	30	6.5	15 (315°)		15	28°46.72'N, 79°41.17'W
CORD-85	741-838	97	7.6-8.4				28°02.04'N, 79°36.51'
C) JSL II-1690	490-503	13					31°41.23'N, 79°17.46'W
JSL II-1697	541		8.75	25-40 (50°)		15	31°41.82'N, 79°08.60'W
JSL II-1698	499-532	33	7.97-8.4	25-45 (50°)			31°41.5'N, 79°18.06'W
ALVIN-203	500-550	54	7.5	35-60 (NE)		30	31°48'N, 79°15'W
D) Stetson, *et al.* 62	640-869	146	7-10		35		31°30'N, 77°45'W to 32°10'N, 77°20'
E) Mullins, *et al.* 81	1000-1300	40	4-6	50			27°40'N, 78°15'W to 27°10'N, 77°30'W
F) Neumann, *et al.* 77	639-675	50		2-7 (N)			~27°N-27°25'N, 79°20'W
JSL II-1522, 1523, 1533	610-631		8.25-9.58	0-15 (N)		15-30	26°56.72'N, 79°16.02'W to 27°02.66'N,79°18.29'W

* Sites A-F (see Fig. 1). *JSL* and *CORD* = Harbor Branch Oceanographic Institution's *Johnson-Sea-Link* submersibles and *CORD* ROV. *ALVIN* = Woods Hole Oceanographic Institution's submersible.

Site C: This is a continuation of the Lophelia banks along the base of the Florida-Hatteras slope from Site B. Not much information is available between these sites. Site C is at the western edge of the Blake Plateau and occurs in a region of phosphoritic sand, gravel and rock pavement. Coral banks occur at depths of 490-550 m and have maximum relief of 54 m. JSL-II dives 1690, 1697 and 1698 found a coral rubble slope with <5% cover of 30 cm, live coral colonies. On top of the bank were 30-50 cm diameter colonies covering ~10% of the bottom. Some areas consisted of a rock bottom with a thin veneer of sand, coral rubble, and 5-25 cm phosphoritic rocks. At ALVIN dive sites 200 and 203, Milliman *et al.* (1967) reported elongate coral mounds, approximately 10 m wide and 1 km long, that were oriented NNE-SSW. The mounds had 25-37° slopes and 54 m relief. Live colonies (10-20 cm diameter) of E. profunda (=D. profunda) dominated and L. prolifera were common. No rock outcrops were observed. Currents at all dive sites within Site C were to the northeast at 25-60 cm/s and temperatures averaged 7-9°C (Table 1).

Site D: This site is on the outer eastern edge of the Blake Plateau at depths of 640-869 m. Over 200 coral mounds up to 146 m in height occur over this 1800 sq.mi. area (Stetson *et al.*, 1962; Uchupi, 1968). These are steep-sloped structures with active growth on top of the banks (Fig. 3 bottom). Live coral colonies up to 0.5 m in diameter were observed with a camera sled. E. profunda (=D. profunda) was the dominant species in all areas although L. prolifera was concentrated on top of the mounds. Densest coral growth occurred along an escarpment at Site D1 (Fig. 2).

Site E: This is a deeper site (1000-1300 m) north of Little Bahama Bank and consists of 5-40 m high mounds of unconsolidated sediment with coral debris (Mullins *et al.*, 1981). These contrast with the other sites in that Lophelia sp. and E. profunda (=Dendrophyllia sp.?) were absent. The dominant live branching coral was Solenosmilia sp.

Site F: On the east side of the Florida Straits and along the western slope of Little Bahama Bank a region of lithoherms occurs at depths of 600-700 m (Neumann *et al.*, 1977). In contrast with Lophelia and Oculina banks which are unconsolidated, these are mounds of lithified carbonate sediment. Dives with ALVIN found these 30-50 m high lithoherms to be elongated north-south in a northerly flowing current which averaged <15 cm/s. The 20-30^0 slopes have a thin veneer of sediment. Although individual colonies of Lophelia and Enallopsammia are a common component on top of the mounds, these are not true coral banks.

Bank Geomorphology:

The internal structure of deep-water coral banks is not well documented. Attempts were made on an Oculina bank (Site A1, Figs. 2 and 3 top) to determine whether live coral capped a mound of unconsolidated sediment or lithified rock. Using a JSL submersible, a lockout dive was made at a depth of 71 m in a small flat sand area on the flanks and midway between the top and base of a 16 m high Oculina bank. A 1.3-cm diameter steel rod was used to probe to a depth of 4 m on the mound without hitting bedrock. Rock outcrops were not observed on the bank although rock pavement occurs within 50 m of the base on a flat sand bottom. A 6-cm diameter aluminum tube was used to core the flank of the bank. The cores consisted of coral branch fragments and mud sediment but only penetrated 22 cm. An Oculina branch taken at a depth of 8-12 cm within the core had a radiocarbon age of 480+/-70 yr B.P. (Hoskin *et al.*, 1987).

These results support the hypothesis that deep-water coral banks are accumulations of coral debris and sediment that are initially built upon a hard substrate. The formation of a deep-water bank may progress through the following hypothetical sequence as proposed in part by Mullins *et al.* (1981): 1) coral larvae initially settle and develop into isolated colonies on rock pavement or outcrops; 2) a coral thicket forms as other colonies grow nearby either by sexual reproduction or by branch fragmentation and regrowth; 3) a coppice stage or mound develops from trapped sediment and coral debris; 4) and finally the coppice develops into a coral bank which is a large structure of unconsolidated coral debris and sediment and is capped with live coral.

Seismic profiles of Lophelia/Enallopsammia banks do not adequately show their internal structure (Stetson *et al.*, 1962; Mullins, 1981). The banks, however, are probably associated with hardbottom. The banks within Site B are concentrated along the rims of linear depressions that may be erosional features of the Gulf Stream (Emery and Uchupi, 1972). The banks on the Blake Plateau (Site D) are best developed on the crest of an escarpment and also tend to follow bathymetric trends and depressions that may indicate rock outcrops (Stetson *et al.*, 1962; Uchupi, 1968).

Sediments:

Sediments from deep-water coral banks and nearby interbank areas have been analyzed for both the Oculina and Lophelia banks (Stetson *et al.*, 1962; Mullins *et al.*, 1981; Hoskin *et al.*, 1987). Each of these studies reported a greater percentage of mud (silt + clay) in the reef sediments than the non-reef sediments, indicating that the reef structure was trapping the finer sediments. The percentage of gravel, mainly from coral debris, was also generally greater at the reef sites. As the coral dies and erodes (Hoskin *et al.*, 1983), a portion of the sand and mud components from the coral may be transported from the reef by currents while the gravel-size branch fragments remain behind to form the bank structure.

Hoskin *et al.*(1987) found the sediment components of the Oculina banks to be more similar to shallow, hermatypic reefs than to other deep-water banks. Sediments of both Oculina banks and shallow reefs have a greater percentage of mollusc components whereas the Lophelia banks have

higher percentages of planktonic sand components such as foraminiferans and pteropods. The Oculina bank sediments, however, lack sand components from calcareous green algae that are abundant on shallow reefs.

Coral Growth:

Coral from both the Oculina and Lophelia banks lack zooxanthellae, the algal symbiont that enhances the growth rates of hermatypic corals. Average growth rate of Oculina varicosa at a depth of 80 m was 16 mm/yr (Reed, 1981). Light levels at this site averaged 0.33% of transmitted surface light but did not support the growth of algae (including zooxanthellae). Comparable growth rates of 6-15 mm/yr have been estimated for colonies of Lophelia prolifera collected from deep-water cables (Teichert, 1958; Wilson, 1979). Greatest coral growth for both the Oculina and Lophelia banks is on the top or on the current-facing side of the mound. The banks are in areas of fairly strong currents (up to 60 cm/s), undoubtedly contributing to the growth of the corals.

Coral Communities:

The deep-water banks support very rich communities of associated invertebrates. Faunal diversity on the Oculina banks is equivalent to that of many shallow tropical reefs. Over 20,000 individual invertebrates were found living among the branches of 42 small Oculina colonies, yielding 230 species of molluscs, 50 species of decapods, 47 species of amphipods, 21 species of echinoderms and numerous other phyla and species (Reed *et al.*, 1982; Reed and Hoskin, 1987; Reed and Mikkelsen, 1987). A striking difference between the Oculina and Lophelia banks is that larger sessile invertebrates such as massive sponges and gorgonians are not common on the Oculina banks. The Oculina coral itself is the dominant component on these reefs. The maximum percentage of live coral coverage is less on the Lophelia banks (5-10% at Sites B and C) compared to the Oculina banks (100% on some banks); however, both types of banks have extensive areas where the bottom is covered with 100% dead coral rubble and no live coral.

The Lophelia banks at Site C support large populations of massive sponges and gorgonians in addition to the smaller macroinvertebrates that have not been studied in detail. Dominant macrofauna include large plate-shaped sponges (Pachastrellidae, Choristida) and stalked, fan-shaped sponges (Phakellia ventilabrum?, Axinellida), up to 90 cm in diameter and height. At certain sites (JSL-II dive 1697), these species were estimated at 0.1 colony/m^2. Densities of small stalked spherical sponges (Stylocordyla sp., Hadromerida) were estimated in some areas at 17 colonies/m^2. Hexactinellid (glass) sponges such as Farrea? sp. are also common. Dominant gorgonacea include Eunicella sp. (Plexauridae) and Plumarella portalessi? (Primnoidae). At this same site, colonies of these two species averaged 10-25 cm in height with maximum densities of 3-10 colonies/m^2 and 1 colony/m^2, respectively. The axes of all these fan-shaped sponges and gorgonians were perpendicular to the current, which was constantly to the northeast during all ALVIN and JSL dives. Piles of sediment were on the lee side of these colonies.

At the Lophelia banks of Site D, Stetson *et al.* (1962) reported an abundance of hydroids, alcyonaceans, echinoderms, actiniaria, and ophiuroids, but a rarity of large molluscs. The flabelliform gorgonians were also current-oriented.

The lithoherm banks at site F (JSL-II dives 1522, 1523, and 1533) also share some species of large sessile macroinvertebrates with the Lophelia banks. Large current-oriented fan sponges up to 90 cm in diameter (Phakellia ventilabrum?) are common, as well as several species of plate sponges (Pachastrella sp., Choristida) and hexactinellid sponges (Euplectella? sp. and Farrea? sp.). Fan-shaped gorgonians are common (*e.g.*, Paragorgia johnsoni?, Corallium sp., Paramuricea sp., and Narella sp.) but are of different genera than those found on the Lophelia banks. Unstalked crinoids are also common on the rock substrate (Neocomatella pulchella and Crinometra brevipinna). In addition, numerous stalked crinoids (Neocrinus decorus, Endoxocrinus parrae, Isocrinus blakei, and Diplocrinus maclearanus) are common on the lithoherms but absent on the Oculina banks or Lophelia banks at Sites B, C and D.

Summary

The geomorphological structure of the deep-water Oculina banks is similar to that of Lophelia banks. Their occurrence in high current regimes where fine sand, mud and coral debris are trapped results in similarly functioning ecosystems that support a rich community of invertebrates. Lacking zooxanthellae, Oculina varicosa and Lophelia prolifera have comparable growth rates. The primary difference appears in the species associated with these banks. The Oculina banks are on the shelf edge and have moderate faunal affinities with the shallow shelf reefs. The Oculina banks also lack large sessile invertebrates common to the Lophelia banks and lithoherms. The different faunal assemblages are reflected in the components of the sediment that also differ between the two types of bank systems.

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RED BAND DISEASE: A NEW CYANOBACTERIAL INFESTATION OF CORALS

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"Red band disease" is a previously undescribed interaction of cyanobacteria ("blue-green algae") and scleractinian corals in which a microbial population dominated by cyanobacteria forms an obvious line, or "band", between live coral tissue and (dead) coral skeleton. The band moves horizontally across the surface of the coral, degrading the coral tissue. The disease looks similar to black band disease except that it is brick red in color (as opposed to brownish black) and contains a different genus of cyanobacteria as the dominant microorganism. Whereas black band is composed predominantly of the cyanobacterium Phormidium corallyticum, red band consists of two species of the genus Oscillatoria. Red band also exhibits a different daily migration pattern than black band. This paper describes this new disease.

Introduction

There has been a significant decline in coral species diversity and vitality in coral reef ecosystems in the recent past (Dustan, 1977; Dustan and Halas, 1987; Williams and Bunkley-Williams, 1990). Much of this decline is believed due to coral diseases. Several diseases of corals have been described, and include black band disease (Rutzler et.al., 1983), white band disease (Gladfelter et.al. 1977), Shut Down Reaction (Antonius, 1981), infection by a eukaryotic green alga (Goldberg and Makemsom, 1981), bleaching (see Williams and Bunkley-Williams, 1990) and cancerous tumors (Peters et. al., 1986). In addition to these documented afflictions, there are numerous undescribed diseases that are seen to occur on corals.

The most commonly known diseases are coral bleaching, and "line", or "band", diseases. The latter are characterized by a sharp line of demarcation between healthy coral tissue and bare coral skeleton, with the line actively moving across the coral colony, destroying coral tissue. Two have been described in the literature: black band and white band.

Black band disease was originally described by Antonius (1973) and later in more detail by Rutzler *et al.* (1983). Black band consists of a dense population of the cyanobacterium Phormidium corallyticum plus assorted microbes (Rutzler and Santavy, 1983). The band appears dark brownish/black due to the presence of the light-harvesting photosynthetic pigment phycoerythrin in P. corallyticum. Black band width varies from 1 mm to 4 cm, and horizontal rates of movement range from less than 1 mm/day to 1 cm/day. The disease is highly destructive, as it occurs most commonly on scleractinian ("stony") corals that exhibit growth rates on the order of 1 cm (in height) per year. In a recent study by Edmunds (1991) it was shown that some scleractinian coral colonies infected with black band can lose greater than 75% of their tissue in six months. Although it has been reported that P. corallyticum is the causal agent of black band disease (Rutzler and Santavy, 1983) experiments on infectivity using pure cultures of the cyanobacterium have never been carried out. It has also been postulated that bacteria (Garrett and Ducklow, 1975) and fungi (Ramos-Flores, 1983) cause the disease. The actual band is comprised of a suite of microorganisms, including sulfate reducing bacteria, sulfide oxidizing bacteria and non-

photosynthetic heterotrophic bacteria. The microbial community in black band is in many ways analagous to microbial mat communities (Richardson, manuscript in preparation). Black band occurs throughout the Caribbean, the Florida Keys, the Bahamas and the Indo-Pacific.

Much less is known about white band disease, which was originally reported by Gladfelter *et al.* (1977). In white band disease there is no obvious band. There is simply a line between healthy coral tissue and bare coral skeleton, which moves across the coral head. There is no reported dominant microbe associated with white band, and attempts at isolating an infective agent have simply yielded a suite of bacteria that do not infect other corals (Peters *et al.*, 1983). White band disease has been found in the Caribbean (Gladfelter *et al.*, 1977) and the Florida Keys (Dustan, 1977).

The work presented in this paper is a description of a new band disease of corals, "red band" disease. This disease is similar to black and white band in the overall characteristic of a line that traverses and destroys the coral tissue, but is different in that it is brick red in color, dominated by two species of the cyanobacterial genus Oscillatoria, and exhibits a distinctive migrational pattern. So far this disease has been seen only in the Bahamas.

Materials and Methods

Research was performed during a cruise on the R.V. BELLOWS, between May 7 and May 10, 1991. The research site was an area SW of Bimini in the Bahamas, 1.04 nautical miles SSW of the Victory Cays, and 2.75 nautical miles off-shore from South Cat Cay at latitude 25° 28.51" N, longitude 79° 15.40" W. Loran coordinates were 14256.44 and 61934.39. All field work was performed underwater using SCUBA.

The site was comprised of a sandy bottom, 50 to 52 feet in depth, with large, scattered coral heads up to 15 feet tall. Numerous smaller corals were present on areas of high relief (predominantly recolonized coral skeleton). The study area was approximately 50 m by 25 m.

Infected corals were tagged by tying small numbered floats to nails inserted in dead areas of coral. Nails were also inserted adjacent to the active red band (in the freshly exposed coral skeleton). Progress of the band was documented by measuring the distance traversed from the stationary nail heads, using a ruler, and by macrophotography using a Sea and Sea Motomarine II with a 1:2 macro lens. The black band photograph in this paper was taken using a Nikonos V with a 1:2 macro lens. Measurement of band progress was carried out during different times of the day and once at night.

Samples were collected using a sterile 3 ml syringe, and examined on board using an Olympus compound microscope. Samples were preserved in 3% formalin. Photomicrographs were taken using an Olympus Photomicrographic System (model PM10AD).

Results

Twelve coral colonies in the study site exhibited red band, which occurred either as small patches or as a line across a coral. Of these twelve corals, five coral species were represented: Diploria strigosa, Montastrea annularis, Montastrea cavernosa, Porites astreoides and Siderastrea radians. Depth of these corals ranged from 41 to 51 feet. Measurement of movement of the band across corals showed that motility was variable, and ranged from 1 mm per day (during daylight hours) to no movement (data not shown). There was no movement of any band during the night.

There was a distinct diel pattern to the band motility. Fig. 1 shows four photomacrographs of two patches (marked by arrows) of black band on a colony of Porites astreoides. The colony was 1 meter high and 1/2 meter wide, and was present at a depth of 52 ft. These photographs were taken in the

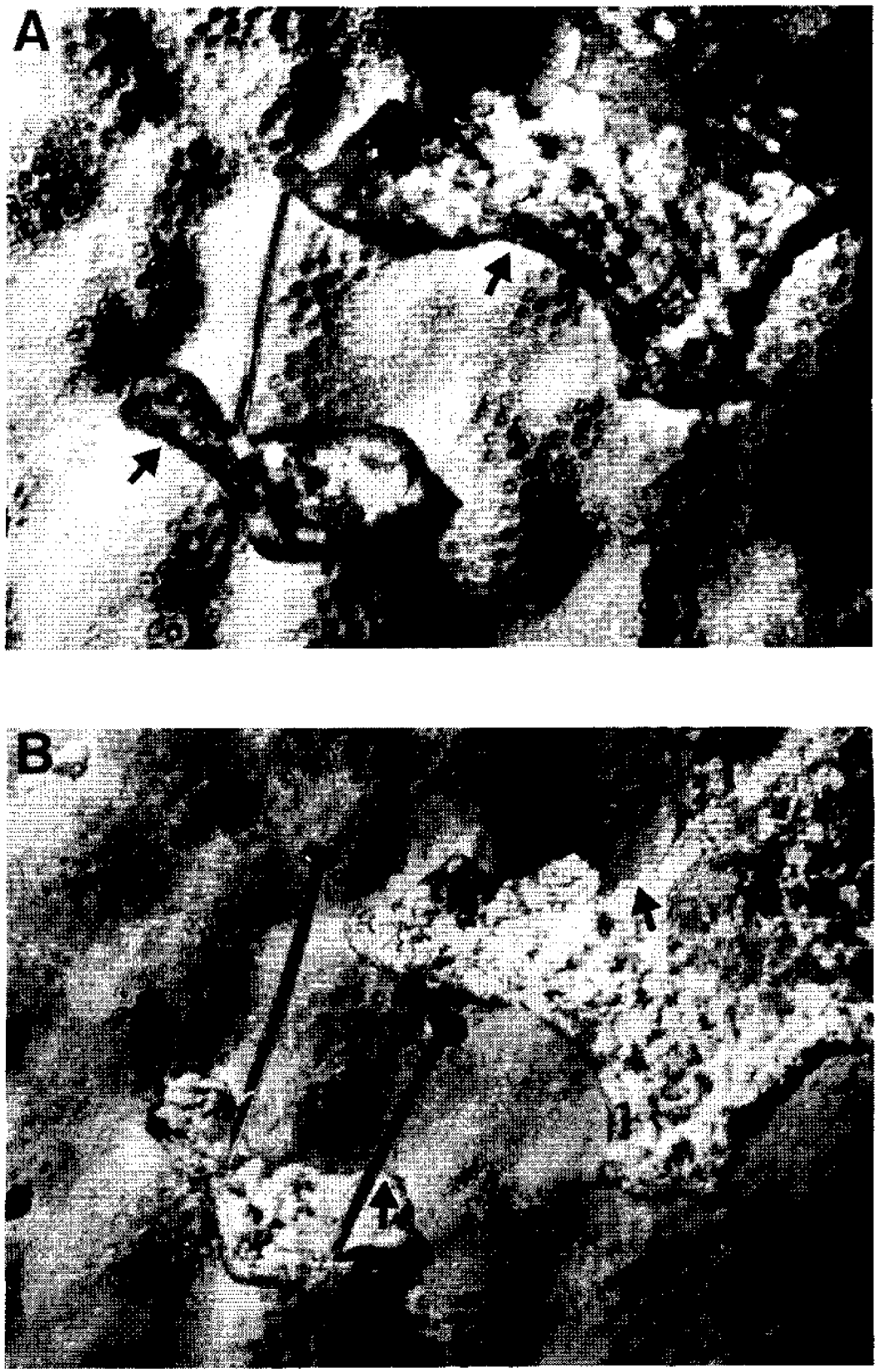

Figure 1. Diel behavior of red band disease on Porites astreoides. Nail heads are 3 mm in diameter.

Figure 1. Continued.

morning and at dusk over a two day period (May 8 and 9, 1991). Fig. 1a, taken at 8:15 am, shows the characteristic daytime appearance of red band, which is diffuse and spread out both on live coral and on the exposed coral skeleton ("inside" the patches). Throughout the day, filaments spread out and randomly move, but maintain the diffuse appearance. Fig. 1b shows the same two patches at dusk (7:15 pm). At this time, the band had contracted to form a very thin (less than 1 mm), compact band positioned exactly at the live coral/coral skeleton interface. In the compact state, the band appeared dark brown. The band remained compact throughout the night, as seen in one night dive (not shown). Fig. 1c shows the same area the next morning (8:00 am), and again the band is diffuse. In addition, it can be seen that the band has spread to cover more of the live coral tissue. By 7:30 pm (Fig. 1d), the nighttime compaction of the band revealed that there had been loss of coral tissue in areas where the band had spread across live coral during the day (see arrows for comparison in both 1b and 1d).

Red band disease appears quite different from black band disease, the only other described band disease of corals that is dominated by a cyanobacterium. An example of black band (from the Florida Keys) is shown in Fig. 2 for comparison with Fig. 1. Black band is typically much wider (up to 4 cm) and denser, and moves at a much faster rate with maximum rates of 1 cm/day (Antonius, 1981). As mentioned above, red band moved a maximum of 1 mm/day during the four day study period.

Figure 2. Black band disease on Diploria strigosa, Key Largo, FL. Nail (N) diameter = 9 mm.

While red and black band are both dominated by cyanobacteria, the genera are different. Figs. 3a and 3b show photomicrographs of a preserved sample of red band from the colony depicted in Fig. 1 . There are two species of Oscillatoria present, of two distinct sizes. The large species (open arrows) has cells 11 µm wide, while the smaller species (closed arrow) is 9 µm wide. Fig. 3b shows an enlarged view of the larger species. Figs. 3c and 3d are photomicrographs of a laboratory culture of Phormidium corallyticum isolated from black band in the Florida Keys. The cell width of P. corallyticum is 4.0 µm. Besides size, red band Oscillatoria are distinguishable from black band P. corallyticum in that individual cell shapes are different and filaments of P. corallyticum are typically rounded at one end and pointed at the other. The two Oscillatoria that dominate red band have not yet been classified to the species level, and are not in culture at this time.

Discussion

Red band disease has only been seen once, during the four day study period detailed above in the Bahamas. While some corals in the upper Florida Keys exhibit patches of red cyanobacteria which look superficially like red band, these patches do not migrate like a band disease, and are composed of

varying species of cyanobacteria including Oscillatoria of many sizes and Spirulina (personal observation). After one year of searching throughout reefs of the upper Keys for red band, none has been found.

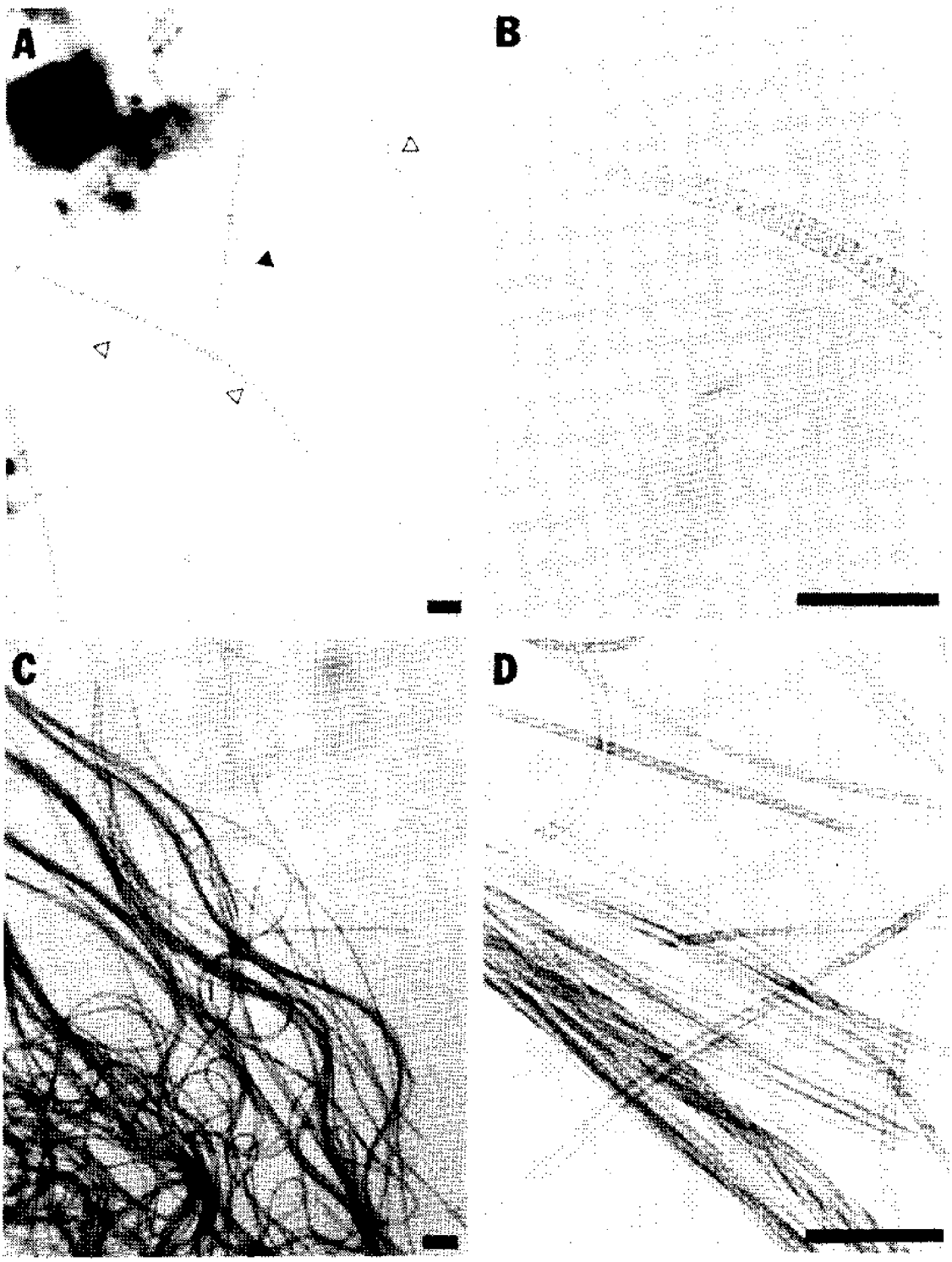

Figure 3. Photomicrographs of cyanobacteria from red band (3a, 3b) and black band (3c, 3d). [3a, 3c x100; 3b, 3d x400. Scale = 50 μm].

It is currently unknown if red band is seasonal (as in black and white band) and the extent to which it can destroy coral colonies. The work described above was done in May, which is typically the beginning of the active season of black and white band disease at latitudes of the Bahamas. Black and white band are known to occur only during the warmer months of the year when the water temperature is above 25°C (Antonius, 1981). At the time of the study described in this paper, the water temperature at 45 ft was 27.5°C. The small patches documented above may have been the beginning stage of a progressive band. Two of the study corals were approximately half dead, but the reason for the tissue death cannot be inferred by observation alone.

There are both similarities and differences between red and black band, to date the only described cyanobacteria-dominated band diseases of scleractinian corals. Both contain as the dominant microorganism gliding, filamentous, phycoerythrin-rich cyanobacteria that do not have distinct sheaths. Both red and black band move across living coral colonies, destroying coral tissue and leaving behind bare coral skeleton. Red band disease, however, "behaves" differently than black band disease. In red band disease, the migration across the coral is much slower and occurs exclusively during the day, when filaments can be seen to spread out in a diffuse fashion over the coral tissue. During darkness, the band condenses precisely at the edge between live and dead coral tissue, forming a compact band that is stationary throughout the night. In contrast, black band migration occurs both during the day and the night, although night migration is approximately one half the distance of day migration (Richardson, manuscript in preparation). In contrast to red band, black band does not contract, but remains the same width during the day and night.

The underlying reason for the observed difference in motility patterns is not known. Most of the work on cyanobacterial motility response has shown that movements are controlled by light, specifically phototaxis, photokinesis, and step-up and step-down photophobic responses (Castenholz, 1982). It has recently been shown that black band movement across coral colonies is not controlled by light (Richardson, manuscript in preparation), suggesting that the movement is a response to a chemical. To date there has been only one report of migration of a naturally occurring population of cyanobacteria controlled by a chemical cue, specifically a chemokinetic response to sulfide (Richardson and Castenholz, 1987a). In this case, the vertical migration of Oscillatoria terebriformis in response to sulfide in microbial mats is physiologically significant in terms of the metabolic properties of this particular cyanobacterium (Richardson and Castenholz, 1987b). It is conceivable that movements of red band and black band are also based on response to a chemical.

Relatively little is known about coral diseases in general. In addition to many unanswered questions regarding the most well-studied diseases, many undescribed diseases of coral are commonly observed on reefs. It is important to document such coral diseases, especially in the context of validating (or contradicting) the general opinion that coral diseases are contributing significantly to the overall observed decline of coral reef vitality.

Acknowledgements

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BENDS IN A FEMALE UNDERWATER SCIENTIST

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A variety of precipitating factors can lead to bends even in an unlikely individual such as a female scientist. Untreated pneumonia permits bronchiolar air trapping and air embolization of small volumes mimicking the usual de novo generation of intravascular bubbles from repeated ascents. Prevention by controlled ascent and NITROX breathing is recommended. A CNS hit usually does not give focal findings; instead it is manifested by a change in affect or personality. Sometimes changes such as UBOs can be seen on the MRI scan. Pharmacologic additions to the body chemistry may contribute to symptoms and group concern for the diver. A new development of a transportable 8 pounds per square inch fabric chamber with an oxygen bib that weighs under 10 kg could provide field management of a subject in this predicament. The advantage of knowing the square root formula to improvise a table is advised. The management of blowup, the unconscious diver, and avoiding bends are outlined. An illustration of UBOs in a CNS bends case is provided.

Introduction

Bends or decompression sickness is a physical event that can be related to bubble formation after exposure to supersaturation of a gas. Oxygen is used up by metabolism and it binds with hemoglobin. Therefore, I am concentrating on the inert gas, usually nitrogen, in this definition. The case of C.C. illustrates that bends occurrence after a low-level supersaturation of 50 fsw or a high-level supersaturation of 133 fsw (Beyer *et al.*, 1976) can be mimicked by underlying lung pathology such as pneumonia. Rapid ascent (blowup) contributed to the clinical picture.

C.C., a 42 year-old nonsmoking female, dove Palancar reef at Cozumel for 6 days. She made 2 dives per day to 80 fsw and on the last day she made three dives: 80ft./28 min., S.I. 60 min.; 50 ft./34 min., S.I. 305 min.; 20 ft./60 min. Her 3rd dive included a rapid ascent in a strong current. She had neurologic symptoms within 30 minutes of surfacing. She developed symptoms in this order: numbness/tingling in both knees, dizziness and vertigo, extreme fatigue, nausea and vomiting, headache, difficulty breathing, and low back pain. The first symptom appeared in 30 minutes, the second in 6 hours, the third in 24 hours, the fourth in 40 hours, and the last two in 48 hours. She flew home on a commercial airliner 41 hours after surfacing. She called DAN 60 hours after diving and her recompression began about 3 hours after that. The first aid consisted of a Nuprin (ibuprofen) tablet for headache in her hotel room. On examination the day of her treatment she was 68 inches tall and weighed 150 pounds, she had no bubbles in her fundi and her lungs were clear to auscultation. C.C. mentioned that she was aware of a clicking sensation and sound at various times, on the plane, while sleeping at night, and on arising in the morning. Ten days later I made a followup phone call to C.C. and she told me that she had had pneumonia a few weeks before diving but she had not been compliant with her doctor's instructions to take antibiotics. A couple of days after her recompression on U.S.N. Treatment Table 5 she coughed up a large mucus plug. She also noticed the clicking sound in her chest and about that time she had a febrile episode. A chest x-ray after her recompression demonstrated middle lobe pneumonia that was clinically related to her complaints of fever and a clicking noise in her chest. After the

hyperbaric treatment she noted that she had difficulty finding the correct words when lecturing to her students and she felt "spacey". An MRI of her head was normal. She intended to complete her present course of antibiotics. There are eight issues that I am going to discuss further in relation to the example of C.C. The first is bends; second, a new transportable device for field use; third, mental dive table arithmetic; fourth, surface interval activities; fifth, blowup; sixth, HBO; seventh, disappearing wave; and last of all, UBOs, unidentified bright objects, seen on MRI scanning of the head in SCUBA divers who have bends.

Bends

Bends may be ameliorated by O_2 breathing after air or NITROX diving. At the surface oxygen has very low saturation concentrations in plasma, and hemoglobin is already saturated while breathing air. There has been very good experience with oxygen breathing from a demand regulator at 1 ATA on the surface. Use of surface O_2 can relieve bends symptoms. 1 ATA O_2 should be available and utilized in every case of suspected or evolving bends. Evolution of bends depends on bubble formation and growth, which is influenced by the depth of saturation and temperature (Berghage, 1976; Beyer *et al.*, 1976). Doppler scores (Dunford and Hayward, 1981) may be influenced by the diver's temperature, which is higher in dry-suit divers than for divers with other protective garments. The risk of bends may be greater in a diver wearing a dry-suit because vasodilation in the warmer environment permits fatty tissue to load nitrogen faster than if there were vasoconstriction induced by a cold environment. But Doppler scores are not a good predictor of clinical bends unless continuous high volume bubbling is observed. NITROX may have made a difference in this illustrative case, because of the blowup.

Field hyperbaric bag

A transportable 1.5 ATA hyperbaric bag has been developed (Bower, pers. comm., 1992). This fabric zippered bag weighs about 20 pounds and when inflated has a volume of 17 cu.ft. There is a manifold that can accept a bib for breathing oxygen inside the bag. There is a danger of combustion if the bag is inflated with oxygen-enriched air. Exhaling through the bib and venting of the bag can prevent dangerous concentrations of oxygen (>23.5% O_2). The bag should be maintained in an oxygen clean state, without dirt or hydrocarbon film buildup. The subject being treated should have on clean, static-free, 100% cotton clothing and no hair grooming solutions. A fire-safety check before sealing the bag should also include a request for any matches, lighters, batteries and combustibles. Brief instructions should include a warning not to let the uninflated bag rest against the face. The hands and arms should extend the material allowing a sufficient pocket of air from which to breath until the inflation has proceeded to shape the material into a chamber. Encouragement to clear the ears should be followed by verification that equalization has been accomplished before continuing with the compression, increasing the pressure in the chamber. The subject should rest for a few minutes after return to the surface before standing. The fabric should be inspected and dried before storing it in its sack. The bib may be cleaned with soap and water before drying and storing. A dilute solution of Cold-Spor (Cold Spor Product MX5310, Metrex Research Corp., Parker, CO 80134) may be used as an overnight soak if more complete sterilization is desired.

The 1.5 ATA compression is not a substitute for a complete medical evaluation and chamber treatment. It is a means of transporting a patient to a chamber while providing emergency oxygen breathing. In a remote location where transport to a chamber is impossible it may provide more benefit than other presently available procedures. Plans for more definitive treatment should follow the recommendations of the Diving Medical Officer. This device provides thermal stability and protection both by air compression, which is insulating, and by wind deflection by nonporous fabric material. The heat generated by the patient is thus stored. The comparison with a fiber-filled sleeping bag is being studied by the manufacturer.

Mental dive table arithmetic

There is an old formula that can be used mentally to calculate a rough dive table (Behnke, 1979). For example one considers the maximum amount of nitrogen that one can absorb without getting bends as

a no-decompression limit of time that one can stay at a depth. For 100 fsw this would take 25 minutes for air. The constant, k, equals depth, d, times the square-root of time, t, ($k=dt^{1/2}$), 500 with these units. We can extend this formula in a mental exercise to predict approximately the minutes of stop time required for a decompression dive, *(k-500)/6* . For a more conservative table use 5 as a divisor.

Surface interval activities

Consuming food and fluids, including oranges and jello, between dives is important to rehydrate the body and promote vasodilation to facilitate degassing of nitrogen. Metabolism of the food, by specific dynamic action, will also aid rewarming.

Blowup

Case C.C. may be considered a blowup from a depth of 20 fsw or less in a diver within the USN tables. She developed symptoms half-way through the recommended one hour of observation at the surface. This requires, according to the USN Dive Manual, (U.S. Navy, 1989) an immediate USN Table Five recompression, or at least O_2 breathing with a demand valve for 30 minutes, and if there is improvement continue until out of O_2 or 6 hours has elapsed. If severe type 2 symptoms (unconsciousness, paralysis, vertigo, respiratory distress or shock) evolve then in-water recompression as an alternative to transport to a chamber may be contemplated on USN Table 1A, the 100 foot table for 6:20. If the depth is inadequate for full treatment take the diver to the maximum available depth for 00:30 and bring the diver up on USN Table 1A taking the full time for all stops. After surfacing O_2 breathing for several hours is advisable. If symptoms persist transport the patient to a recompression chamber as soon as possible. To facilitate degassing of nitrogen elevated pressure is necessary to increase the partial pressure of dissolved O_2 outside the bubble. Increasing the concentration of oxygen increases the gradient and tendency of the nitrogen to decrease as equilibrium is established. In bends of long duration much of the pain is from infarcted tissue resulting from blood-vessel occlusion by bubbles. The gradient of high to low tissue oxygen pressure is a potent stimulus encouraging new blood-vessels to restore tissue O_2 to the damaged area and oxidize lactic acid, relieving pain.

Hyperbaric oxygen

HBO, hyperbaric oxygen, treatment schedules in the recompression chamber room fall into 5 categories.

1. USN Treatment Table 6 for divers (U.S. Navy, 1989).
2. USN Table 5 for divers and with modifications for smoke or carbon monoxide inhalation (U.S. Navy, 1989).
3. 45 fsw for 90 minutes on O_2 for medical treatments such as gangrene and osteoradionecrosis.
4. 33 fsw for 60 minutes in a monoplace chamber for medical treatment and burn care.
5. 16 fsw transport bag for field use.

Disappearing wave

Shallow hangs can be hazardous especially in rough seas due to the disappearing wave. An 8 foot wave crest passing above equals a rapid ascent of 8 fsw. AGE, acute gas embolization, is an expected result as the diver can't see the approaching wave to coordinate his breathing. This is akin to the blowup situation and may result in an unconscious diver at the surface.

Unidentified bright objects

Central nervous system (CNS) bends with symptoms including vertigo, fainting, passing out, attention deficit and glassy-eyed staring may be observed on magnetic resonance imaging (MRI) to produce unidentified bright objects (UBOs) in brain scans. I have seen UBOs (Messina, pers. comm., 1992) in CNS bends patients on MRIs taken with the 1.5 Tesla magnet (Fig. 1).

Figure 1. MRI scan of a CNS bends patient. Unidentified bright objects (UBOs) can be seen as small bright spots in middle right portion of brain.

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A PORTABLE, DIVER-OPERATED PLANKTON SAMPLER FOR NEAR SUBSTRATUM USE

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A portable, inexpensive, diver-operated plankton sampler was developed to enable plankton collection near the substratum. This device has been used in studies on coral feeding and planktonic larval dispersion near subtidal rock walls. Its portability allows a diver to position the sampler wherever a sample is needed, over a broad depth range. Plankton are sampled near the substratum with intake heads that draw water laterally from a thin layer. The unit is driven by a 12 volt DC bilge pump powered by a housed battery pack or by a cable to the surface. It can be switched on or off by a diver so that sampling bags can be replaced and multiple samples collected during a dive. A flowmeter inside the pump chamber records water volume sampled.

Introduction

Zooplankton are known to distribute themselves spatially in relation to the substratum. Demersal plankton of coral reefs (Ruetzler et. al., 1980), and plankton near subtidal rock surfaces (Sebens and Koehl, 1984) show such patterns. Towed nets are unable to sample near the substratum, whereas plankton pumps can potentially sample from a defined layer of water if the intake structure is properly designed.

Ruetzler *et al.* (1980) used a near bottom reef plankton sampler powered by a trolling motor, but this apparatus sampled from one large opening and zooplankton passed through the propeller before entering the net. Sebens and Koehl (1984) used a submerged bilge pump and hose to sample plankton near rock walls at Nahant, Massachusetts in 1979. Because of limitations in that design, an intake head that takes plankton from a specified horizontal layer was needed. The present design allows better spatial sampling, places the mesh sampling bag upstream of the pump, and allows rapid changing of sample bags underwater.

The apparatus described in this paper was developed by K. Sebens in 1987 - 1988 to sample coral reef zooplankton within a few centimeters of coral surfaces. It was tested in a flume at the Marine Science Center by A. Johnson and K. Sebens in 1988 to determine flow characteristics around the intake heads, and was first used in St. Croix during an Aquarius underwater habitat mission in 1988 (Sebens and Johnson, 1991). The apparatus was adapted for underwater battery power by J. Witting in 1991, and was used by K. Graham for a two-year study of invertebrate larvae near subtidal rock walls at Nahant, Massachusetts (Graham, 1992).

Sampler Design

The plankton sampler consists of a 80 cm length of 10 cm Schedule 40 PVC pipe. At one end a Rule 2000 GPH (enclosed battery system) or 3500 GPH (cabled system) 12 volt DC bilge pump is attached. The 3500 GPH pump fits over the PVC pipe. The 2000 GPH pump is hot melt glued into a 10 cm PVC coupling so it will attach to the end of the pipe.

Inside the pipe at the pump end, a General Oceanics digital, mechanical plankton net flowmeter is mounted with stainless steel threaded rod and positioned in the middle of the pipe. A hole is cut into the pipe wall and a piece of plexiglass is cemented over the hole to create a see-through port for flowmeter readings.

The other end of the pipe is covered with a 10 x 4 cm PVC reducer coupling. This should fit snugly but must be removable. The plankton sample bags are inserted and draped over the end of the pipe. Attaching the reducer coupling holds the sample bag in place. The plankton sample bags are made from 40 μm (or larger) Nitex sewed and/or glued together to make a tapered bag (40 cm long) that fits over the PVC pipe. The collection end of the bag contains a 50 ml polyethylene centrifuge tube. Removable plankton sample bags allow for multiple samples to be collected during a dive.

A 1 - 2 m piece of 4 cm flexible plastic hose is connected to the reducer coupling. This connects at the other end to the plankton sampler head. The sampler head is constructed with a 50 cm piece of 4.4 cm ID x 5 cm OD clear acrylic tubing with one end capped off. On the other end a 5 x 3.8 cm reducer coupling is glued on to connect to the flexible plastic hose. Four 2.5 cm equidistant holes are drilled into the tubing and 5 cm lengths of 1.9 cm ID x 2.5 cm OD clear acrylic tubes are glued to each hole.

The intake heads are made from two 5.5 cm diameter circles of 3 mm Plexiglas with a 2.5 cm hole drilled into the center of one of the circles. These are glued together with four 6 mm Plexiglas posts separating the two circles. A 5 cm length of 1.9 cm ID x 2.5 cm OD clear acrylic tube is glued to the hole. Each intake head is attached to the sampler head with 2.5 cm ID Tygon tubing. We chose to make removable intake heads because they are fragile and a spare can be easily attached underwater. Removable intake heads also allows the extension of the head by adding variable lengths of acrylic tubing.

An earlier version of the plankton sampler was powered by a Rule 3500 GPH 12 volt DC bilge pump that was wired with a 30 m heavy duty outdoor extension cord to a 12 volt marine battery in a boat. A mercury light switch was wired in line at the pump end to turn the sampler on and off. The switch was embedded in an empty film canister filled with hot melt glue to prevent breakage. This system was very effective, but had some drawbacks. First, the diver was limited to the length of the power cord so the boat had to be moored directly over the study site. Second, the long power cord had high resistance that affected pumping power. Third, the cord was susceptible to leakage caused by abrasion.

The most recent version of the plankton sampler uses a Rule 2000 GPH 12 volt DC bilge pump powered by 2 Power Sonic 12 volt, 12 Amp (PS-12120) rechargeable sealed lead acid batteries in an Ikelite housing (# 5810). A hydrogen catalyst is put into the housing to absorb hydrogen gas that may develop, reducing the risk of explosion. This unit is completely portable and has stronger pumping power (approximately 1 liter s^{-1}) than the cabled system using the Rule 3500 pump. A more reliable switch was built using an Ikelite 10 cm shaft, round knob control assembly (# 5008) which operates a push button on/off switch enclosed in 2.5 cm PVC pipe sealed at both ends.

Sampler Operation

The sampler must be calibrated prior to use to determine the volume of water sampled per revolution of the flow meter. The time and number of revolutions of the flowmeter needed to pump a known volume of water must be recorded. By recording the "start" and "stop" flowmeter revolution numbers, the volume of water pumped through the plankton sampler can be calculated. Calibration is done in shallow water with a sampling bag in place and the outlet hose directed into a large bucket of known volume held just at the water surface to minimize head. The plankton pump is deployed at the study site by divers. The reducer coupling is removed and a clean plankton bag is inserted. The end of the bag is draped over the pipe and the coupling is replaced to secure the bag. The sampler head is placed at the desired sampling area and the pump is started. If more samples are desired a new plankton bag can be reinserted and the above procedure repeated. Fig. 1 illustrates the plankton pump set up for use.

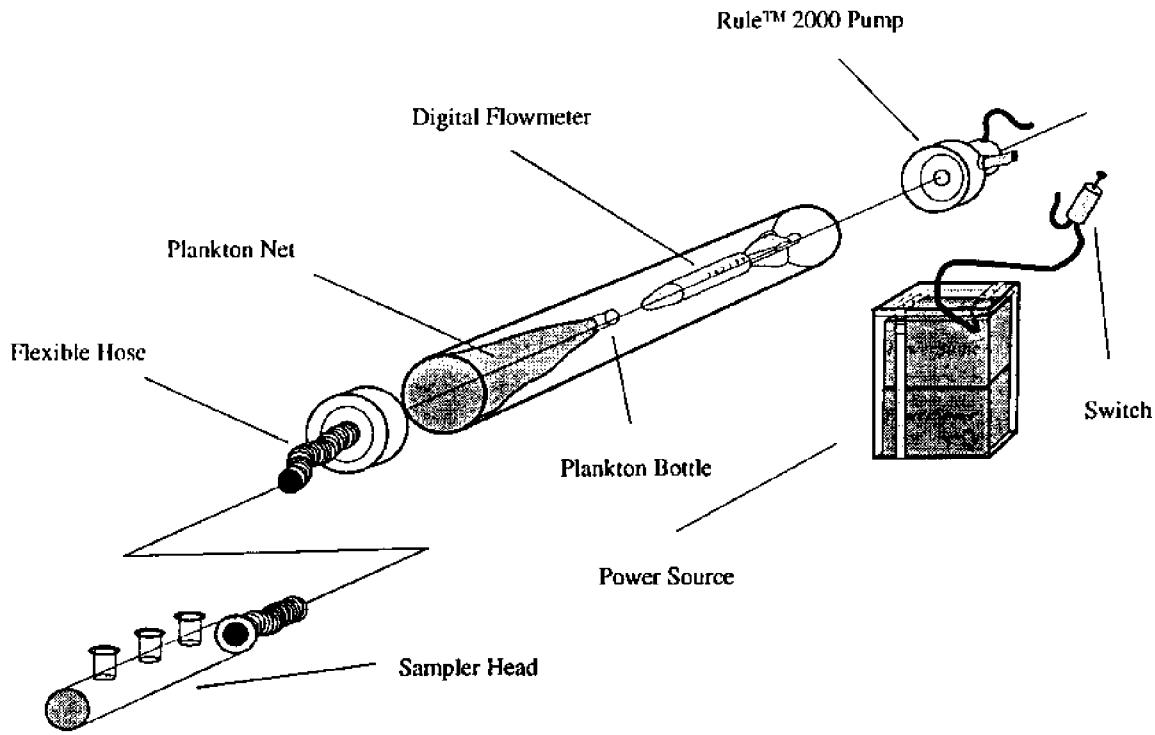

Figure 1. Diagram of the portable plankton sampler.

Sampler Performance

Each intake head of this apparatus samples a horizontal layer of surrounding water where the layer width depends on local flow speed. Flume tests using suspended hydrated brine shrimp cysts, illuminated for video by a 5 mm light slit, indicate this layer is about the same width as the gap between the plates (6 mm) at flows of $\geq$ 10 cm s^{-1} (Fig. 2). In still water, the head draws particles from at least 3 - 5 cm above and below the plates, and at 5 cm s^{-1}, this is reduced to 1 - 2 cm. Even in slow moving water, most particles taken in come from directly around the plate, entering the head at 30 - 60 cm s^{-1}. Particles two centimeters away are moving at about a tenth of that speed even if they are going toward the plate opening. Given ambient flows of 5 - 20 cm s^{-1} (means of oscillating wave-induced flow), it is probable that the heads are sampling a layer of 0.6 to 2 cm thickness at most times. Fig. 3 shows fluorescein dye entering an intake head.

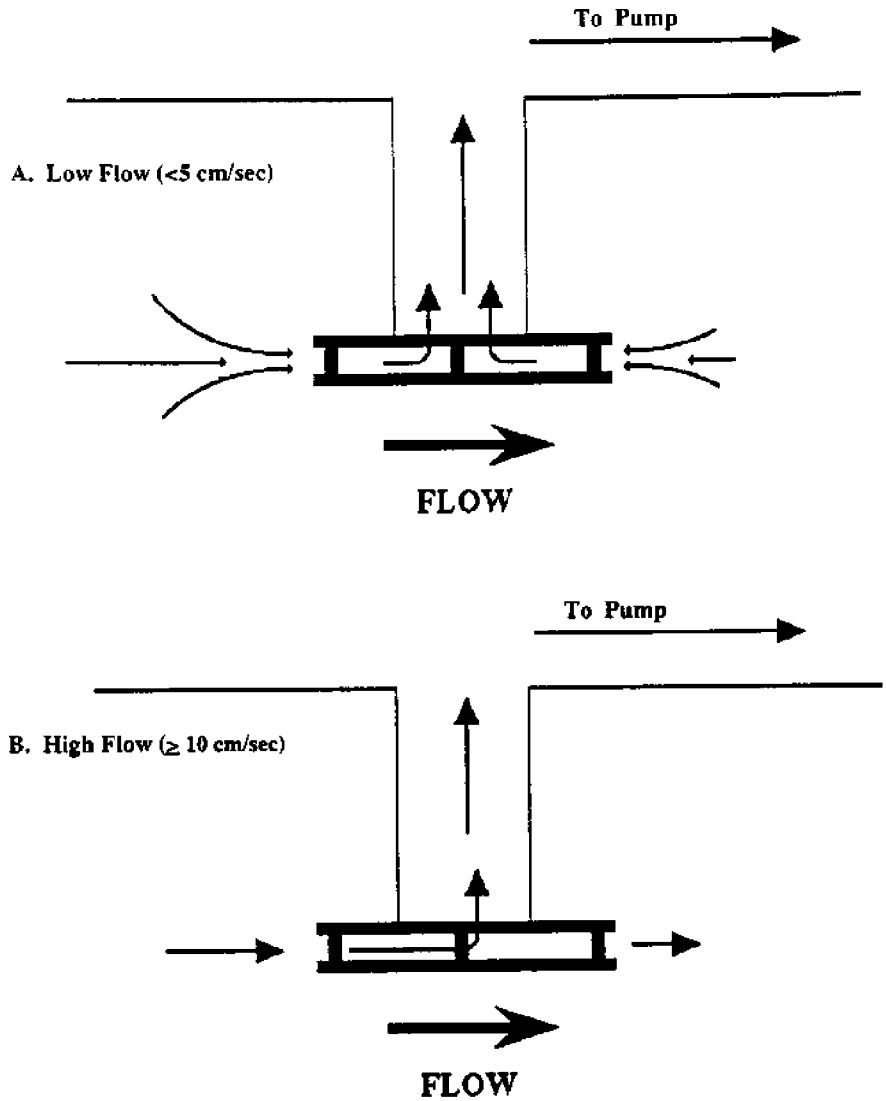

Figure 2. Flow diagram of plankton pump sampler head in A. low flow (<5 cm/sec) and B. high flow (≥ 10 cm/sec)

All plankton pumps are selective depending on the characteristics of the zooplankton sampled. Large, strong swimming plankters can avoid rapidly towed nets, pump intakes, and tube traps (Taggart and Leggett, 1984; Butman, 1986; Yund et. al., 1991). It is likely that large zooplankton, i. e. > 10 mm, can avoid the intakes of this system as well. Videotapes of plankton swimming near the intake heads in Jamaica (1989) showed chaetognaths (>3 cm) reversing direction rapidly as they approached. It is difficult to test pump selectivity. If they are tested against nets, the nets are also selective. We tested this sampling apparatus at Discovery Bay, Jamaica (10 m depth), by attracting plankton into a (1.5 x 0.5 x 0.3 m) Plexiglas enclosure mounted on cement blocks, 15 cm off the reef surface. The pump was allowed to run for for 3 minutes, and the composition of zooplankton in the sample was compared to a sample of all the remaining zooplankton in the enclosure. The latter sample was taken by pushing a tight plunger through the enclosure and collecting all the plankton in a 40 _m mesh bag covering one end of the enclosure, then tying off the bag. Except for a slight difference in the percentage of chaetognaths (which were in low numbers in both samples), copepods and other zooplankton were captured in proportions very close to those in the enclosure (Sebens, unpublished data). The enclosure method

provides a complete (non-selective) sample of available plankton for comparison to pump samples. A test similar to this one is suggested for each use of the sampling system because of differences in local zooplankton sizes and potential avoidance behavior.

Figure 3. Plankton pump intake head with fluorescein dye entering from left.

Overall, this sampling apparatus is easy for a diver to deploy, and allows collection of multiple samples per dive. There are a few cautions for its use, however. First, sample bags must be inspected each time for small holes, especially around the seams, and for gaps at the net/vial interface. Second, the sample bag must be closed off and removed rapidly once the pump is shut off to prevent plankton escaping back out of the bag opening. Bags can be tied off and new ones inserted. Third, when the apparatus is first put in place, a few minutes of running the pump without a bag in place should be done to remove detritus or sediment from the tubing before the first sample bag is used. Fourth, sample bags should be placed directly into a container of formalin/seawater either underwater or at the surface to ensure proper preservation. Fifth, if a bag slips down the PVC tube, be sure a second diver is available in case the pump turns on while the operator reaches for the bag. The pump suction is strong enough to make arm removal difficult (K. Graham pers. observation).

Acknowledgements

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THE TWILIGHT ZONE: THE POTENTIAL, PROBLEMS, AND THEORY BEHIND USING MIXED GAS, SURFACE-BASED SCUBA FOR RESEARCH DIVING BETWEEN 200 AND 500 FEET

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Recently mixed gas diving methods have started to be applied to scientific research needs in place of more expensive submersible techniques. This paper brings to the fore what is being considered and what is actually being done. The authors endeavor to present a short, review of the physics and physiology topics that relate to mixed gas diving and decompression theory as well as examine, with special emphasis on diving tables, hardware, diver training and safety considerations, the use, advantages and disadvantages of mixed gas, surface-based scuba for projects in the 200 to 500 foot depth range.

Introduction

During the past few years the authors have, through separate paths, come to consider and use scuba techniques for research diving at depths greater than the normal scientific diving boundary of 190 feet of sea water (FSW). One author (Pyle) did so as the only way to accomplish his personal research, which was (and is) conducted outside of the oversight of a Diving Control Board, and the other author (Sharkey) did so as part of an ongoing inquiry on behalf of research divers at his institution into a broad spectrum of low cost alternatives to conventional submersibles for conducting manned deep research. Other alternatives being studied include the use of One Man Atmospheric Diving Systems (OMADS) and mixed gas, surface-supplied diving.

A report sponsored by the National Science Foundation, the National Oceanic and Atmospheric Administration and the Undersea and Hyperbaric Medical Society observed that:

> **Some of the most experienced leaders in the scuba world are dead set against releasing information, let alone encouragement, on the diving methods discussed here - Bill Hamilton on "High-tech diving".**
>
> *There was a time in the history of scientific scuba diving when it was unregulated and a pioneer activity. As it became more regularly utilized, recognition of the need for standards and guidelines brought about their development* (Hanson, 1990).

What was true of scuba in its early days is true of mixed gas scuba today. It is a pioneer activity and not an accepted, routine diving activity. As these pioneer techniques are refined they may, like air

scuba, diving computers and more recently NITROX scuba, become part of the suite of tools available to the general scientific diving community at large.

There is a time and depth envelope available to research divers which at shallow depths extends to many hours of bottom time and at its deepest limit of 190 FSW is only five minutes wide. The 190 FSW depth was initially established as a limit within the scientific diving community by the Scripps Institution of Oceanography standards (Stewart, 1971). In establishing this limit the primary consideration was that 190 FSW was the deepest depth on the U.S. Navy Standard Air Tables (Egstrom, 1988). Some researchers, even back in the 1950s, anticipated the need to work deeper (Given, 1982) but the technology and knowledge of diving physiology were not sufficiently developed to make this kind of diving a reasonable undertaking under university auspices. This may still be the case, but scuba pioneers especially in the cave and wreck diving portions of the newly emergent technical diving community have pushed significantly deeper with apparent safety.

Since the advent of conventional scuba gear marine biologists have been busy exploring coral reef habitats and collecting and documenting the wide diversity of organisms which inhabit them. Since virtually all of these scuba explorations have been conducted using air as a breathing gas thorough biological investigations have been generally limited to 190 FSW. To study the marine life at greater depths scientists have had to rely on such devices as traps, trawls, remote operated vehicles (ROVs) and submersibles. Traps and trawls are not very selective and are ineffective at collecting the many small, cryptic organisms typical of coral reef habitat.

Submersibles, both manned and remote, are cumbersome and expensive; most are designed for thousands of feet of water and there has been a tendency to see them as wasted on such *shallow* dives. Those researchers fortunate enough to obtain funding to use such systems tend to concentrate their efforts at the extreme limits of the vehicles' capabilities. An additional problem is the amount of energy that such systems put out into an environment in which many of the organisms have highly evolved detection systems. Attempting to study fish from submersibles and ROVs has been likened to trying to study jack rabbits from the back of a moving locomotive.

There remains a zone of coral reef habitat, at depths between 200 and 500 FSW, throughout the tropical seas, which has escaped extensive exploration and documentation. This zone, the biological *Twilight Zone*, undoubtedly harbors vast numbers of undiscovered species of marine life, and represents a new frontier for underwater science. Using mixed gas scuba techniques Pyle and veteran fish collector Chip Boyle recently collected seven new species and one new genus of marine fish, all of which were restricted to depths of 200 to 500 FSW in the Cook Islands. The extent of their success is appreciated fully when one considers that: 1) these fish were all collected with less than one hour of working bottom time; 2) all were collected without ichthyocides; and 3) the Cook Islands lie geographically well outside of the center of reef fish species diversity. One can only imagine the wealth of deep-reef fish which would be discovered given more bottom time and the use of scientific ichthyocides at localities in the western Pacific and Indonesia, where reef biodiversity is at its greatest.

> **Would you venture into the unknown out of greed? Greed only works in the world of ordinary affairs. To venture into that terrible loneliness one must have something greater than greed. Love. One needs love for life, for intrigue, for mystery. One needs unquenching curiosity and guts galore - Carlos Castenada - *The Fire Within***

Efforts to use OMADS such as WASP and JIM that are appropriate to this range were pioneered by Bruce Robison and Sylvia Earle. When the OMADS DEEP ROVER (Sharkey, 1986) became available its deeper range of 3300 FSW tended to shifted the emphasis to work in greater depths.

An example of what may be found in the 200 to 500 foot range is two rocks that were recovered from about 500 FSW by Mike Jordon in 1986 using DEEP ROVER as part of a project at the Perry Oceanographic's Caribbean Marine Research Center. The organisms on this rock created a great deal of

excitement amongst the project's participants, several animals were difficult to even place in a phylum and the diversity of life on the rocks was astounding. There were 11 phyla of animals (foraminifera, sponges, corals, brachyopods, bryozoans, arthropods, molluscs, polychaetes, a sipunculid and several small, green, flat crinoids) and 3 divisions of plants (cyanophyta, cholorophyta and rhodophyta) represented (Earle, 1991). Keep in mind that these were two small, randomly selected rocks that were picked up as an afterthought by Jordon during his ascent from a dive. It is exciting to consider what might be found if fairly routine access to these depths were possible coupled with the freedom that a scuba diver enjoys.

> **This small rock hosted a fair cross-section through the history of life on the planet, reflected in the genetic code of creatures that were more than a little different from each other. Such a large scale diversity is not likely to be found in non-marine areas of comparable size - Sylvia A. Earle**

Scuba is not the only *wet* way to approach this problem. Deep Diving Systems (DDSs) can provide access to great depths for lock-out divers (Orzech, 1985), but with a complexity and expense that eclipses even large manned submersibles. Another alternative that has been shown to work well in areas where there is little or no current and where there is a fixed study site is mixed gas surface supplied diving (Wood, 1990).

Conventional submersibles are very complex and expensive systems. Costs range from $10,000 to $25,000 per day including a support vessel. OMADS are much simpler and less expensive, but still cost between $3,000 and $5,000 per day once all costs are factored in. Mixed gas scuba dives can be conducted for less than a few hundred dollars.

Limitations of Diving with Air

Physics Background

The depth and time limitations of using conventional scuba (*e.g.*, breathing compressed air) are imposed by the physiological responses to breathing elevated partial pressures of the two major constituents of air, nitrogen and oxygen. These limitations may be overcome only with an understanding of certain basic physical laws and the physiological dynamics of gasses and gas mixtures. An understanding of *Dalton's Law of Partial Pressures* and *Henry's Law of Dissolved Gasses* are particularly important.

Acute Central Nervous System Oxygen Toxicity

Oxygen is the only constituent of air that is *required* by the diver since it is part of the metabolic process, but the inspired PO_2 must fall within certain limits. Below a PO_2 of about 0.15 ATM there in insufficient oxygen to sustain life. Since the PO_2 of air starts at about 0.21 ATM and can only increase with descent, too little oxygen (hypoxia) in never a problem when diving with air. Too much oxygen, however, can lead to acute central nervous system (CNS) oxygen toxicity. The recommended maximum allowable PO_2 for a diver ar rest is 1.6 ATM and 1.4 ATM for a diver subjected to a moderate workload (U.S. Navy, 1989). At greater partial pressures oxygen may lead to convulsions. Convulsions are always dangerous for a diver and are likely to be fatal for a diver using a conventional regulator. A diver breathing pure oxygen is limited to a safe depth of only 20 FSW at rest and 13 FSW while working. In order to descend to greater depths the oxygen in a diver's breathing medium must be reduced by diluting it with another constituent gas. In the case of air the dilutent is nitrogen. Using simple algebra it is possible to calculate that the maximum depth to which a diver breathing air may descend without undue fear of acute CNS oxygen toxicity is 220 FSW for a resting diver and 190 FSW for a working diver.

Decompression Sickness

Acute CNS oxygen toxicity is not the only limitation of deep diving on air. Nitrogen, the other major constituent of air, also creates problems at elevated pressures. Since nitrogen is not metabolized by the body, it accumulates in the tissues until the diver ascends, or until the tissues become saturated with nitrogen at an amount proportional to the depth of the dive. Whenever a diver descends, even if *saturated*, the surrounding pressure increases and the diver begins to absorb additional nitrogen. By ascending to a shallower depth, no matter how slight, the diver decreases the surrounding pressure and starts to decompress. When a diver has absorbed enough nitrogen to have any of the theoretical tissues used in decompression models exceed their surface supersaturation ratio, the diver is no longer able to return directly to the surface and a *Staged Decompression Stop* is called for.

Nitrogen Narcosis

The other limitation imposed by breathing elevated partial pressures of nitrogen is the onset of Nitrogen Narcosis. Different individuals have different tolerances for nitrogen, and the biochemical physiology is not well understood. In most cases subtle effects are encountered at a depth of 100 FSW, which results in a PN_2 of just over 3 ATM (Fowler, 1987). The narcotic effect increases substantially with increased depth and most divers are significantly impaired by the time they reach 190 FSW.

> **At depths greater than 180 feet (6.5 ATA), no trust should be placed in human performance or efficiency while breathing compressed air - Peter Bennett - "Inert Gas Narcosis and HPNS.**

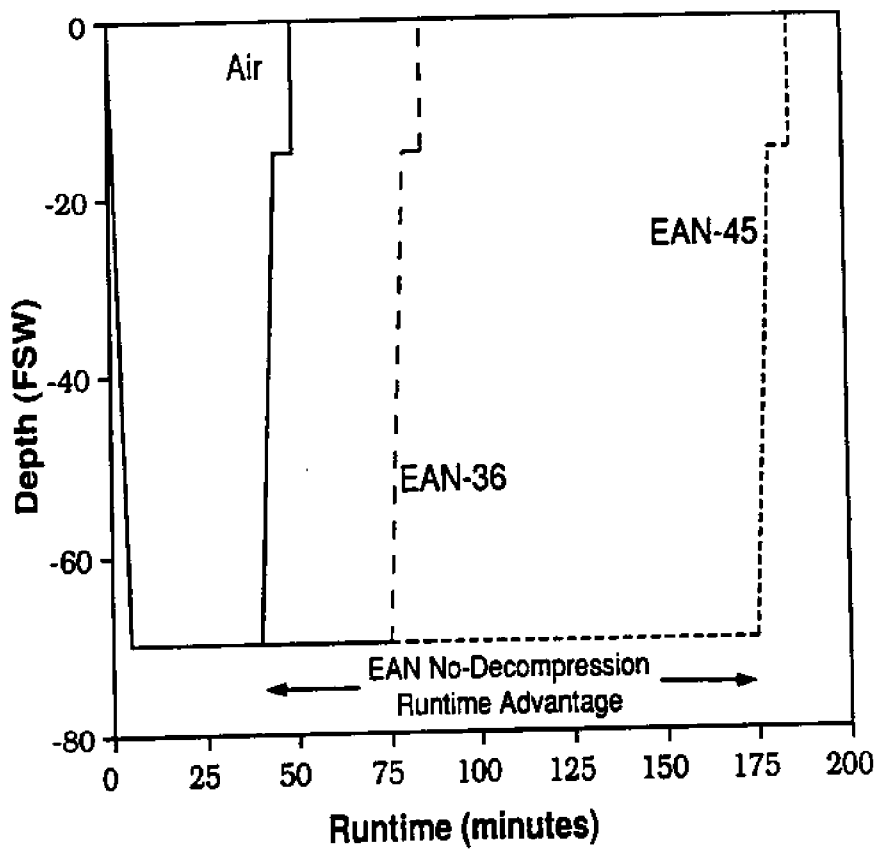

Figure 1. No Decompression Limits for Air, EAN-32 & 36.

In order to overcome the limitations imposed by diving with air the relative concentrations of the constituents of the breathing media need to be altered. If the selection of gasses is limited to oxygen and nitrogen, then there is a trade-off between oxygen and nitrogen related problems. Carefully working within the limitations of these gases has yielded excellent results. Man-made breathing mixtures of nitrogen and oxygen are known as NITROX and are widely accepted within the scientific, military, commercial and recreational diving communities.

Nitrox

Definitions

The term NITROX refers to any breathing mixture containing just nitrogen and oxygen, and is usually followed by a number which represents the percentage of oxygen in the mix. For example, NITROX-36 is a mixture of 36% oxygen and 64% nitrogen. The term Enriched Air NITROX (EAN) is in common usage today to describe NITROX that has an oxygen fraction greater than 21% to differentiate from NITROX that has a lower oxygen percentage which is sometimes jokingly called NARTOX because of its increased narcosis. EAN is not used for deep diving, it is used at moderate depths (between 40 and 150 FSW) where it has two advantages, less narcosis and decreased nitrogen uptake that results in either longer no-decompression dives (as shown in) or shorter decompression schedules.

Equivalent Nitrogen Depth

The concept of Equivalent Nitrogen Depth (END) is an important one for quantifying both narcosis and decompression obligation. END is simply the depth at which air would have the same PN_2 as the breathing mix. It is calculated by obtaining a ratio between the PN_2 of air and that of the mix and applying that ratio to the depth of the mix dive. Two examples follow:

1) A mix with 85% nitrogen has a nitrogen to air ratio of about 1.06 [0.85_0.79]. So it is about 6% more narcotic than air. A diver making a 275 foot dive with such a mix would experience the narcotic effect of an air dive to about 290 feet [275/1.06].

2) Using EAN-36 reduces the maximum depth at which the PO_2 reaches 1.4 ATM from 190 FSW to 110 FSW, but the END is about 0.9 [0.21/0.36] of the actual dive depth or about 100 FSW for a 110 FSW dive.

Is 10 FSW less narcosis on a 110 FSW dive worth the trouble of obtaining EAN-36 and the safety concerns of its manufacture? Probably not. However the increased no-decompression dive time might be. A no-decompression 110 FSW air dive is limited to 20 minutes, but with EAN-36 the limit is increased to 30 minutes. That is half again as much bottom time and significantly greater proportional gains are available at shallower depths!

Decompression Diving

EAN also offers advantages for decompression dives conducted within the depth limits appropriate for the mix. An air dive to 110 FSW for 40 minutes requires a two minute stop at 20 FSW and a 21 minute stop at ten FSW. An identical dive using EAN-36 requires only a single seven minute stop at ten FSW.

The Use of Oxygen for In-water Decompression

Breathing pure oxygen at the twenty and ten foot stops can significantly shorten decompression time for any dive. It serves to speed the *wash-out* inert gas by creating a high diffusion gradient. This technique was used successfully during Phase 1 of the Warm Mineral Springs Project (Wood, 1990) and also during 1988 when it was used for over 3,000 safe two-a-day decompression dives to depths between

160 to 190 FSW by divers affiliated with the Department of Nautical Archaeology at Texas A & M University (Fife, 1990). While this technique was once considered radical and potentially dangerous, it has recently been gaining acceptance.

Multiple Gas Mixes on a Single Dive

It is possible and advantageous in some cases to switch breathing gasses during a dive. Doing so can reduce decompression obligation, and the reduce dangers of acute oxygen toxicity and narcosis. The terminology (fig. 2) used within the cave diving community to describe this sort of dive is somewhat different from that conventionally used within the scientific diving community, but is very useful when discussing such *multiple mix dives*. The length of the entire dive, surface-to-surface is the *runtime*. Runtime is divided into two phases, a *working phase* (descent, time at depth and ascent to the first stop) and a *decompression phase*. The breathing mix used for the working phase is known as *bottom-mix*. A *travel-mix* may be used during descent to avoid nitrogen loading or hypoxia resulting from a bottom-mix with a low oxygen fraction. There may be one or more *intermediate decompression mixes* used on the ascent. The overall objective of this technique is as best as possible to hold the PO_2 close to 1.4 ATM during the working phase and 1.6 ATM during the decompression phase. This type of diving often requires custom tables, although some divers combine conservative use the U.S. Navy Exceptional Exposure Air Tables with mix changes for increased safety.

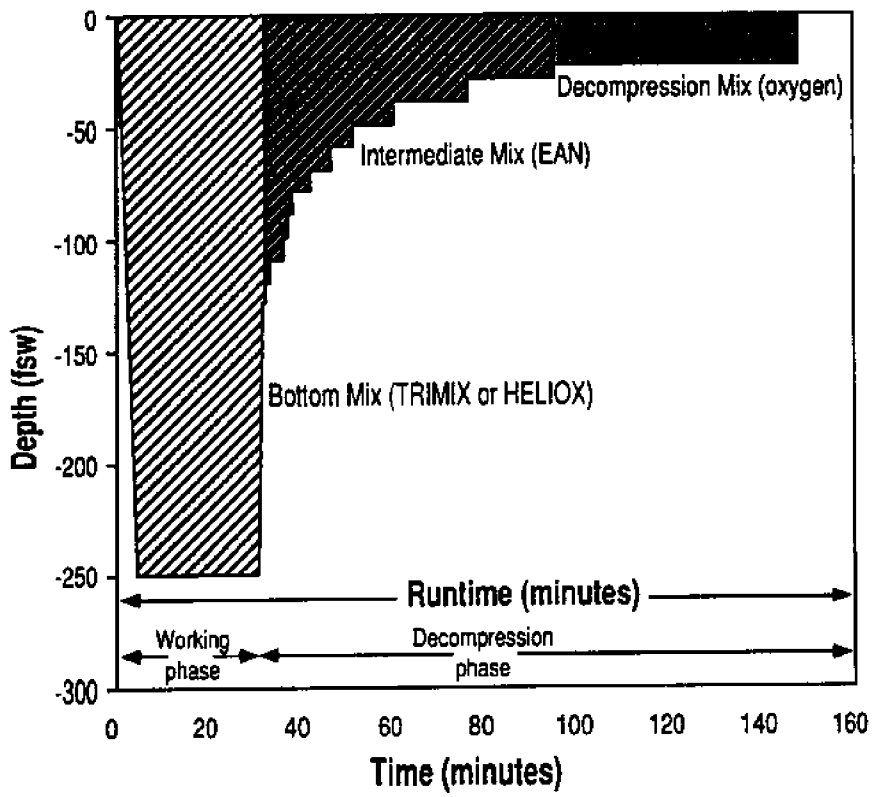

Figure 2. Mixed gas dive nomenclature.

An example of this technique is an air dive to 220 FSW (PO_2 of 1.6 ATM) for 30 minutes. The U.S. Navy decompression schedule for this dive would be one minute at 50 FSW; seven minutes at 40 FSW; ten minutes at 30 FSW; 23 minutes at 20 FSW and 47 minutes at 10 FSW. This is not considered a dependable or well tested schedule and diving on air to this depth will result in significant narcosis. Additional ascent safety may be obtained by: (1) switching to EAN-40 at 100 FSW thereby obtaining a PO_2 of 1.6 ATM; (2) using variable ascent rates (Hamilton, 1989); (3) lessening the change in pressure

with respect to time in the shallower regions by adding some short stops (one to two minutes every ten FSW from 100 to 60 FSW); and (4) switching over to pure oxygen at the 20 FSW stop. While the use of either custom tables or the approach used in the example above can make for a safer ascent, the problem of narcosis will still greatly limit the work most divers are capable of. The only way to reduce narcosis is to reduce the partial pressure of nitrogen that the diver is breathing.

Using Mixed Gas to Reduce Narcosis

EAN offers significant advantages in available no-decompression bottom time and minor advantages in terms of narcosis, but has the drawback of being useful only at shallow to moderate depths. In order to effectively push beyond the limits of NITROX mixtures we must explore the possibility of using gases other than nitrogen for diluting the oxygen in the breathing medium.

Various binary gas combinations have been used for deep diving. At depths to 600 FSW HELIOX (helium and oxygen), HYDROX (hydrogen and oxygen) and NEOX (neon and oxygen) do not exhibit the narcotic effects of NITROX (Stone, 1991). Of these mixes HELIOX is preferable since HYDROX is dangerous to prepare and NEOX is much denser and as a result harder to breathe at depth, which leads to CO_2 buildup. HELIOX completely eliminates narcosis, but there are two problems that have been, somewhat incorrectly, laid at its door.

When a diver breathes HELIOX it feels cold because it has six times the heat conductivity of nitrogen. Helium is very light and its thermal capacity (which actual controls the number of calories carried away from the body) is quite low. Despite the feeling of cold, the actual heat loss from the body as a result of breathing helium is not excessive. Helium does not work very well as an insulating gas in a dry suit, argon or carbon dioxide are much better choices for insulation. Argon is the best choice since CO_2 when combined the water in a moist dry suit creates a weak acid that results in skin irritation (Taylor 1992).

Helium is a very small molecule with a rapid diffusion rate. This means that as compared to nitrogen, it is quickly taken up into the body. But it is also quick to diffuse out of the tissues. The off-gassing speed is significantly increased when a high diffusion gradient is established with a decompression-mix (like EAN) that has no helium in it.

The major problem with HELIOX is that helium is an expensive gas. This expense may be reduced by moving away from a binary combination to TRIMIX, a combination of oxygen, helium and nitrogen.

Using Trimix to Optimize Decompression, Narcosis and Cost

The characteristics of the available mixes for deep diving can be illustrated with dive profiles prepared for the Wakulla Springs Project using the Hamilton Research DCAP computational program (Stone, 1991). These profiles are 300 foot dives with either a 20 minute or 80 minute working phase for each of three different breathing mixes containing 14% oxygen. The mixes are NITROX-14, TRIMIX-14/34 (34% helium and the remaining 52% nitrogen) and HELIOX-14. Each profile planned for decompression inside a dry bell with air as a decompression mix from 60 to 40 feet and pure oxygen from 30 feet to the surface.

Table 1 shows that for the 20 minute dives the runtime reduction in favor of HELIOX was only about 5%, but that for the 80 minute dives the reduction was a significant 18%. As illustrated in , Helium mixes of up to 50% are increasingly advantageous for longer dives in the 300 foot range. This is because helium more rapidly approaches saturation level and out-gasses quickly. When helium is used for shorter dives it yields little decompression advantage. However, the more helium in the mix the less

narcosis. HELIOX-14 results in no narcosis, while for a 300 foot dive NITROX-14 has an unacceptable END of 325 feet and TRIMIX-14/34 has an END of about 200 feet which may be barely acceptable for some divers.

Table 1. After Stone (1991)

Runtime Comparisons for 300 foot dives		
Bottom Mix	**20 minute**	**80 minute**
NITROX-14	174	818
TRIMIX-14/34	165	698
HELIOX-14	163	668

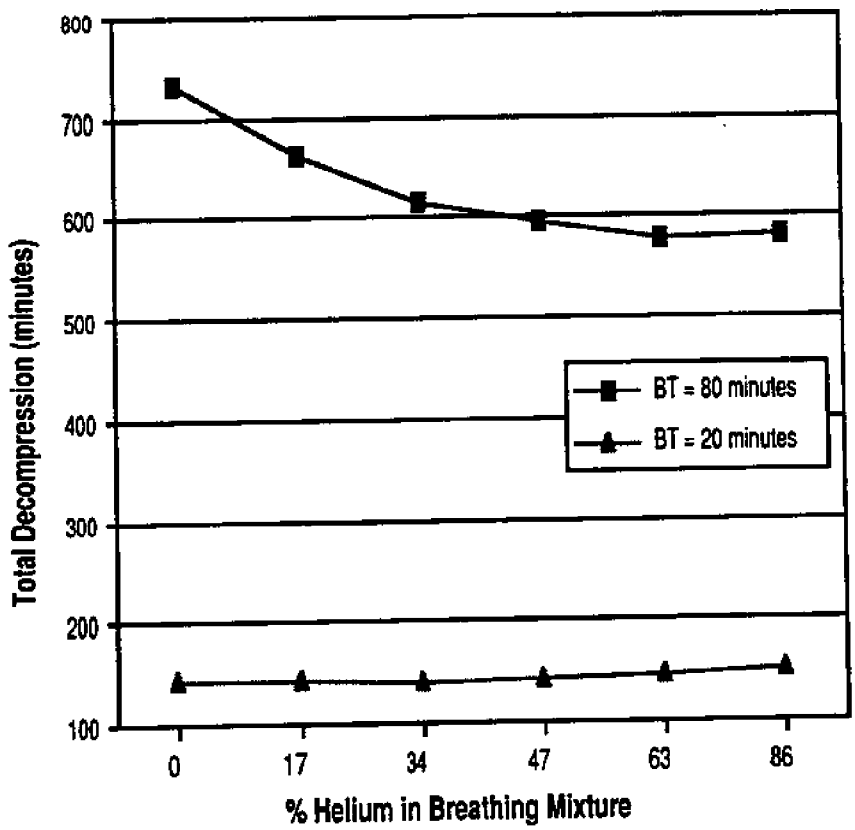

Figure 3. From Stone (1992)

All things being equal HELIOX-14 is the mix of choice from a decompression and narcosis perspective. But helium is expensive. A K-bottle costs $75 and that translates to $55 of helium for a 20 minute HELIOX-14 dive and $215 for an 80 minute HELIOX-14 dive contrasted with $25 and $85 for the

TRIMIX-14/34 dives. The more helium the less narcosis and the higher the cost. It becomes a question of how much sobriety can you afford?

An additional advantage to TRIMIX is that it is easier to prepare in the field. To fill a scuba cylinder with HELIOX, pure oxygen and helium must be combined together. This requires a special booster pump to top off scuba cylinders to the desired pressure, and introduces the risk of exposing various components of the system to high pressure pure oxygen. TRIMIX, can be easily generated in the field. In order to *brew* TRIMIX-10/50 (10% oxygen, 50% helium and 40% nitrogen) all one need do is decant about 1500 PSI of helium into a an empty aluminum cylinder and top it off with air from a standard air compressor. It should be noted that the ideal gas laws do not rigorously apply at the high pressures in a scuba cylinder, so slightly more than 1500 PSI of helium is actually required. Proper numbers, very accurate gauges, gas analysis equipment and slow, cool fills are a must.

Equipment for Mixed Gas Diving

Using open circuit gear for mixed gas dives requires switching regulators and tanks to optimize the mix during different phases of the dive. Closed circuit systems have a major advantage. The PO_2 can be held at 1.4 ATM for the working phase of the dive and at 1.6 ATM for decompression phase of the dive, since exact mix can be continuously adjusted as the diver descends and ascends. This approach, when combined with dive computer technology, holds the hope of significant reduction in runtime. But at the current time closed circuit equipment in not readily available in the civilian market. Carmellan Research Inc. produces closed circuit equipment only for the military, and though Cis-Lunar Labs is likely to produce a closed circuit system in the near future which will be available to the scientific community, it is not here yet.

While closed circuit equipment is more expensive than open circuit gear, its operational costs may be low enough to makeup for the initial outlay. The open circuit rig discussed below represents an initial outlay of $3,500 to $5,000. Cis-Lunar Labs is hoping to market its closed circuit rig for $10,000 to $15,000. With a $10,000 difference between an open and closed circuit rig, the break even point for dives in the 300 foot range is reached after about 60 hours of diving, even sooner if the cost of support equipment such as scuba compressors, gas mixing systems, gas analyzers, etc. is factored in.

> **Your most important piece of diving equipment is a free and clear head - Carlos Eyles "The Inner Experience of Diving".**

Pyle has designed and used an open circuit, multiple mixed gas diving rig (fig. 4) which attempts to optimize package size and the life support needs of the diver by incorporating an adequate amount of equipment redundancy into a system that is small enough to be entirely self contained by the diver through all phases of the dive (Pyle, 1992). This system was designed for use on deep (200 to 500 FSW) coral reef exploration projects which do not involve any penetration into an overhead environment such as a wreck or cave. Furthermore, the system was designed to enable the diver to carry all required breathing mixtures throughout all phases of the dive, eliminating the requirement to return to stage bottles attached to a boat (a serious consideration in the reef environment where currents can quickly and unpredictably change direction). The difficulties surrounding the design of such a rig involve incorporating enough redundancy of critical items to minimize the effect of an otherwise life threatening system failure, while keeping the rig small and streamlined enough to provide for diver mobility and maneuverability in the presence of moderate to strong currents, even when hampered by additional gear such as fish collecting equipment and bulky underwater camera housings.

In general, the rig design incorporates two 100 ft^3 cylinders that are overcharged and hold 130 $foot_3$ of breathing gas each. One is filled with bottom-mix (TRIMIX or HELIOX) and the other is filled with intermediate-decompression-mix (usually NITROX-32). Two independent regulators are attached to

the decompression-mix cylinder with a slingshot valve. One side of the valve is maintained in the open position and the other is kept closed after pressuring its regulator to prevent the entry of water during the dive. The closed side is the backup and is opened and used only in the event of a primary regulator failure. The primary bottom-mix cylinder has a single regulator maintained in the pressurized, open state. A 30 foot^3 cylinder containing identical bottom-mix is carried by the diver and is maintained in the pressurized, open state for use in the event of a malfunction in the primary bottom-mix system. In addition to these three cylinders, a small (22 to 30 ft^3) cylinder of pure oxygen for the final decompression stops is carried by the diver. This cylinder, equipped with a single oxygen clean regulator, is maintained in a pressurized but closed state. It is a backup supply, the primary oxygen system is a stage bottle or surface supplied oxygen system at the boat. The backup oxygen is only opened only in the event the diver is unable to return to the boat during the final stages of decompression or if there is a failure in the primary oxygen system. The buoyancy compensator is a set of Dive-Rite Wings providing a primary bladder connected by a power inflator to the EAN-32 bottle and a backup bladder connected to the primary bottom-mix bottle.

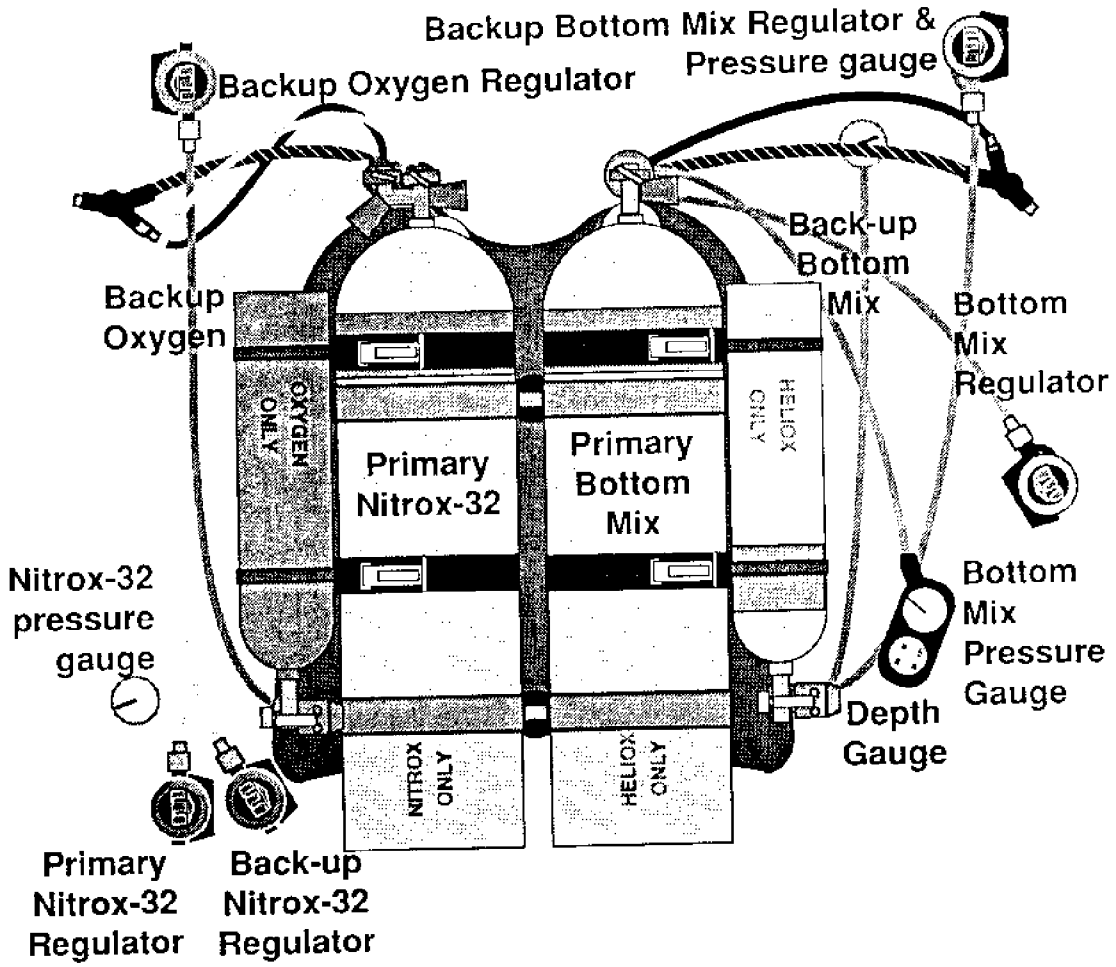

Figure 4. Deep Reef Set.

Redundancy is increased by the divers' option to use a buddy's backup gas supplies with standard *octopus-breathing* procedures. The authors are considering the use of modified blue water tethered diving techniques (Heine, 1986), with a third diver serving as pivot diver and staged EAN-32 backup bottles.

A single pony bottle will give you enough time at 250 to say the Lord's prayer - Mike Hanna

The EAN-32 and oxygen backup regulators need to be specially prepared, oxygen cleaned and maintained for oxygen service. At this time the authors only know of two manufacturers that provide data on how to prepare their equipment for use with breathing gases that have an oxygen fraction greater than 21%, MARES and Poseidon. Pyle uses Poseidon Odin regulators for EAN-32 and Sharkey uses left handed MARES MR-12 BETA regulators. For bottom-mix Pyle uses ScubaPro MX-X/G250 regulators and Sharkey uses MARES MR-12 NAVY regulators. A *second-generation* system is being designed that will use 160 foot^3 primary bottles (that will obviate the questionable practice of overcharging the aluminum 100s) and composite material flasks for backup bottom-mix and oxygen.

Deep Rig Failure Mode Analysis

Deep mix diving shares some commonality with cave diving. In both cases there is an overhead obstruction that prevents a diver from returning to the surface. For cave diving this obstruction is physical rock over the diver. For a deep reef dive the obstruction is *virtual*, the diver's decompression ceiling. This ceiling is the shallowest depth to which the amount of absorbed inert gas will permit a safe ascent. Under normal circumstances, both overheads are the same to the diver, but in the event of an emergency the differences are significant.

A diver whose concern is a decompression ceiling needs a different redundancy design than a diver who must travel a distance through a cave before obtaining access to the surface. A diver lost in a silted up cave at 10 feet of depth will die when the air runs out. A diver with a twenty foot decompression ceiling will likely survive a short trip to the surface and quick reimmersion with a fresh tank with no ill effects. Each TRIMIX cave diver must carry a sufficient quantity of bottom-mix reserve so that if one of the dive team suffers a complete primary gas equipment failure at the furthest point of penetration both divers will be able to make a long horizontal swim to gain access to a depth where it is safe to switch to EAN. Solving this problem requires the use of the rule-of-thirds (one third going in, one third coming out, and one third for my buddy) and a set of crossed-over-doubles with two regulators and an isolation valve. TRIMIX dives in the ocean have a virtual ceiling that, by limiting bottom time, is always kept above 130 FSW where EAN-32 may be safely used. Complete failure of the primary bottom-mix system thus only requires a direct ascent to 130 FSW where EAN-32 may safely be used.

All deep rigs demand attention to redundancy. Design must ensure that equipment malfunctions may be dealt with under actual and psychological pressure. The rig design takes into consideration the malfunction of pieces of equipment during a dive and permits the diver access to sufficient breathing gas to always permit a safe ascent to the surface. The design process is complicated by the need to have access to different breathing mixes at different points in the dive. On a deep dive using separate bottom, intermediate and decompression gases, each gas system must have a backup. Three times as much gear is needed to arrive at the same level of redundancy as would be needed for an air dive.

There are three failure modes that must be considered for each system: No-Gas-Delivery, Too-Much-Gas-Delivery and Gas-Management-Error. A No-Gas-Delivery failure mode is characterized by a regulator that suddenly stops working, even though there is still gas in the cylinder. This is perhaps the most serious potential crisis, but fortunately is rare with modern, well-maintained, two stage regulators. A Too-Much-Gas-Delivery failure would result from a free flowing regulator and a Gas-Management error could result from an inaccurate pressure gauge or human error.

The only gas, carried by the diver, that can be safely breathed below 130 FSW is bottom-mix. EAN-32 and oxygen may not be relied upon as deep backups. Therefore, the bottom-mix backup regulator must be maintained in the open state in order to allow the diver immediate access to safe backup gas in the event of any failure mode. A slingshot valve would provide adequate regulator

redundancy for the bottom-mix system. This would result in the exactly the same redundancy deemed acceptable by cave divers. But this approach effectively doubles the probability of a Too-Much-Gas-Delivery failure. Such a failure would result in the rapid loss of the primary, and now only, bottom-mix supply. By using a separate backup bottle, with the valve open, an adequate redundant supply of bottom-mix is always accessible to the diver to provide a means of ascending to 130 FSW.

In contrast, a slingshot valve on the EAN-32 cylinder is a good choice since only regulator redundancy is required. Any bottom-mix with 12% or more oxygen can be breathed at 20 FSW, or deeper, without fear of hypoxia. Since EAN-32 is only used between 130 FSW and 20 FSW, the bottom-mix system provides a short term emergency backup for a No-Gas-Delivery or Too-Much-Gas-Delivery failure. The diver can use bottom-mix while closing the primary EAN-32 slingshot valve (if necessary) and opening the secondary slingshot valve. Then the diver may switch back to EAN-32 from the secondary regulator. Mismanaging EAN-32 is unlikely since there is more gas then needed. In fact, the cylinder has 130 ft^3 of gas, enough to perform the required decompression more than twice over.

Primary gas for oxygen decompression is a staged bottle or surface supplied system located at the boat. The diver carried backup oxygen bottle is a redundant system and is used only in the event that the diver is unable to return to the boat at the end of the dive or if the primary oxygen system malfunctions. In an *immediate danger* situation EAN-32 and even bottom-mix may be used as a temporary backup while the diver sorts things out.

Besides safeguarding against equipment failure, the rig is designed to reduce human error as well. To minimize the possibility of accidently breathing the wrong mixture at the wrong depth all regulators are color coded to indicate the gas they deliver. The use of different regulator designs, side-breather vs. standard and left vs. right-handed also helps to differentiate mixes. The rigs are not without weaknesses, all of the systems are at the mercy of a double failure, such as a blowout plug rupture in the primary bottom-mix combined with a regulator malfunction in the backup bottom-mix cylinder. This would leave a diver totally dependent upon the other diver for bottom-mix until they reach 130 FSW.

Buoyancy

Buoyancy is an important consideration since about 20 pounds of gas are used up during a typical dive. These dives are made in relatively warm water and a Darlexx diveskin covered by a 3 to 5 mm wetsuit jacket and hood provide sufficient warmth, so wet suit crush only creates a few pounds of buoyancy change. One of the reasons for using aluminum 100s is so that the entire rig is only slightly negative at the end of the dive. Larger capacity cylinders are simply too negative at depth after the wet suit crushes. The second generation system should balance out properly because of the positive buoyancy of the composite flasks. Small weights and syntactic foam will be used, if necessary, to precisely trim the rig. The possibility of tearing a BC requires the use of a double bladder BC or a backup BC.

Training

Although these rigs are not perfect the authors feel that they pose no more risk than the accepted rigs used for diving in overhead environments. Training and overlearning the correct responses to emergencies is critical. Putting in the training time required to assure that all divers function properly both as individuals and as a team is also an important undertaking. Before considering this kind of diving a diver needs to be a highly skilled deep diver with sufficient experience to be able to predict his or her response to significant narcosis. A series of shallow dives with full rig, during which independent and dependent responses to simulated system failures are practiced must be conducted.

Mixed Gas SCUBA Diving Operations

A typical mixed gas scuba dive begins with preparing the breathing mixtures and assembling the rigs. The oxygen content and total pressure of each cylinder is checked with two calibrated, reliable analyzers, once after filling and once again before entering the water. Criteria for dive abort, that depend on the dive conditions, objectives and the general plan, are reviewed during a pre-dive briefing.

All tank valves are opened and then those valves that should be closed are shut. Since the rig is too heavy to be comfortably handled by a diver out of the water, it is lowered over the side of the boat by support divers and donned at the surface. After checking and buddy checking that all the proper valves are open and the others are closed, divers initialize their timing devices and begin their descent using their primary EAN-32 regulators.

At 130 FSW the switch to the primary bottom-mix regulator is made and all team members ensure that their buddy has made the proper switch before continuing the descent. Experienced deep divers instinctively use the level of narcosis as a rough guide to their depth, so great care must be taken to monitor actual depth during the dive. With reduced levels of nitrogen, there is a tendency to forget how deep you really are.

The dive is conducted according to a plan and all team members carry out their respective duties. Because of the high level of equipment redundancy carried by each diver independent emergency procedures are favored. If an individual experiences a problem that leads to a decision to abort the dive he or she reacts immediately, independent of other team members. Although it is preferable for all team members to be informed of an individual's decision to abort, it is usually more important that the aborting diver take steps to ensure his or her own safety, rather than take time to notify other team members of the problem. Once the other members of the team become aware of the problem they also abort the dive and join, if at all possible, the aborting diver.

The ascent and decompression are best conducted in a group, but all divers are responsible for initiating their own ascent and managing their decompression profile. Decompression along a vertical drop-off or other reef feature is less dreary than hanging in mid water on a line, but the later mode is better. When distracted by creatures of the reef it is too easy to make errors in actual depth and time for each decompression stop.

Throughout decompression all team members should continuously account for each other. If for some reason this is not possible and a team member is unaccounted for during decompression no attempt is made to locate the missing diver. In this circumstance it is best to assume that he or she is decompressing somewhere else using the equipment on his or her back. A search or rescue attempt is not likely to accomplish anything, and may lead to the unnecessary endangering of other team members.

It is always advisable to have additional trained divers on hand, either waiting at the boat or in shallow water, ready to assist any team member who requires help. A slate and pencil is a must as a means of accurate and reliable communication. It may be possible by using manifolds and band masks with both demand and free-flow features to utilize wireless diver-to-diver communications for mixed gas scuba. This kind of communication equipment might significantly increase diver safety.

Summary

Mixed gas scuba can provide a low cost alternative to ROVs, submersibles and OMADS for work in the 200 to 500 FSW range. Presently the techniques and equipment are being pioneered by highly knowledgeable and experienced individuals at their own risk. The END of these dives is always below 150 FSW so, all other considerations aside, these types of dives should only be considered by research

divers who hold a 190 foot card. These dives are experimental and the authors do not, at this time, advocate Diving Control Board consideration for more general use.

If you live outside the law you must be honest - Bob Dylan

These diving rigs and procedures are intended only to permit short duration dives in the absence of physical overhead obstructions. They are not intended for cave or wreck diving. The application of these techniques to other environments, especially those far removed from recompression assistance are not reasonable. Interest in using these techniques has been expressed by geologists and blue water biologists but these applications must await future developments.

The items needed before any consideration of general use are undertaken include more operational experience and validated decompression schedules. Experience will come with time. Statistically based decompression evaluation procedures (Albin, 1991), hold great promise for the validation of schedules based on this experience rather than more time consuming experimental human trials. The availability of redundant mixed gas rebreathers will be a great aid. At least one manufacturer says that closed circuit equipment will be here soon. Advanced dive computers and diver-to-diver communications systems can also help to move mixed gas scuba out of its pioneering phase into an acceptable and routine environment.

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NO-STOP ENRICHED AIR (NITROX) DIVING WITH SURFACE INTERVAL OXYGEN BREATHING: PRACTICAL CONSIDERATIONS FOR FIELD VALIDATION

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Surface-breathed 100% oxygen was demonstrated to increase the no-stop dive times relative to current equivalent air depth (EAD) dive procedures while participants breathed enriched-air (nitrox). This validation study represented the second phase of NOAA-National Undersea Research Center, University of North Carolina at Wilmington (NURC-UNCW) funded research at Duke Medical Center, Durham, North Carolina. Phase I consisted of controlled chamber trials where subjects breathed 100% oxygen during surface intervals. Phase II consisted of time/depth computer profiled fresh water trials using similar procedures including doppler technology to monitor subject's intravascular bubbles as a measure of decompression stress. This paper will focus on the practical aspects of conducting field dive table validation studies. Challenges included securing and maintaining Institutional Review Board, Dive Control Board and Department approval, legal, risk management and fiscal cooperation, site and technical consensus, permits, a medical plan, staff and funding, all from multiple agencies. This project demonstrated that field validation trials of dive tables could be conducted both safely and economically.

Introduction

Field validation of dive tables is an important step in the implementation of new dive profile procedures previously tested in chambers. Hamilton and Schreiner (1989) divide the process of table validation into an experimental and operational component. The experimental component includes chamber and field tests requiring informed consent where the driving purpose is human testing. The operational component, where the procedure is applied in support of underwater botanical investigations, for example, may not require informed consent as the driving purpose is not human testing. The focus of our study, breathing 100% oxygen between dives to permit increased bottom time, fell under the experimental component of their model at this time.

Surface breathed 100% oxygen was demonstrated to increase the no-stop bottom time of dives without excessive DCS risk while participants breathed NITROX at depth (Vann, 1992b). This

validation study, conducted at Wakulla Springs, represented the second phase of research at Duke University Medical Center, Durham, North Carolina. Phase I consisted of resting, dry chamber trials (Vann *et al.*, 1992a). Phase II consisted of fresh water trials held at Wakulla Springs using similar Doppler technology to monitor subject's decompression stress (Vann *et al.*, 1992b). This paper will focus on logistical considerations of Phase II. Pollock *et al.* (1992) review data collections.

The underlying purpose of this Surface Interval Oxygen (SIO2) study was to evaluate diving procedures (dive profiles) for NITROX (EAN) followed by breathing 100% oxygen at the surface (Gerth *et al.*, 1992). The National Oceanic and Atmospheric Administration's National Undersea Research Center at UNC Wilmington (NURC-UNCW) funded the project in search of ways to increase working bottom time within the research diving community.

Project Statistics

A total of 481 "subject" and "staff" dives (of the 681 proposed) were conducted during this project. Two hundred and four subject dives were made to 80 feet while breathing 36% NITROX for a total of 149:05 hours:minutes. One hundred and fifty eight subject dives were made to 120 feet breathing 32% NITROX for a total of 55:21 hours:minutes. Sixteen subjects completed the entire series of dives. Twelve subjects completed less than the full series of dives. Collectively 362 subject dives were completed and 119 staff dives (51 hours) were completed for an approximate grand total of 255:30 hours:minutes of bottom time. A total of 26 FSU staff were involved on the SIO2 project in a diving or non-diving, volunteer or paid capacity (an estimated additional 7 from NURC). An estimated 60 hours of Doppler recordings were stored on 53 metal tapes.

By combining the resources of NURC-UNCW and FSU, the one month intensive validation study became feasible. A total of $40,000 was secured for FSU (with $30,122 in-kind NURC-UNCW support) to complete the project. An estimated inventory value of over $65,000 worth of FSU equipment was involved on this project. An estimated additional $70,000 of equipment came from NURC-UNCW. This did not reflect the required in-kind contributions of the Duke Medical Center, Orca (dive computers) and FSU in terms of staff, facilities, and expense beyond the grant.

Pre-Project Preparations

A summary of the tasks undertaken during the year prior to implementation of the SIO2 project are listed below:

1. Write an experimental protocol to a detail that would pass University Institutional Review Boards (IRB)'s scrutiny including the Medical Plan. Arrange for medical backup (alternate chamber) and medical supervision of DEMTs and subjects.
2. Secure and schedule a reliable site (Wakulla State Park), including appropriate permits and letters of understanding & a community consensus (cave diving) on a variance for the site designating the area under the ledge as open water.
3. Secure, train to level of project's technology, evaluate (from a training, medical and physiological perspective), process, and schedule at least 40 volunteer subjects.
4. Secure lodging and a reliable place to eat for the staff and subjects.
5. Secure a chamber, gas charging facility, and the technology to keep subjects reliably and accurately monitored at depth for extended times on bottom gas and on the surface breathing 100% oxygen or air (with appropriate back-ups).
6. Secure facilities for anthropometric, pulmonary diffusion and pregnancy testing.
7. Secure and train reliable and qualified staff to collect the data, administer the project, select and train the subjects, operate the chamber and fill station and supervise the dive site.

8. Pass the proposal through two IRB's, legal offices, FSU Diving Control Board, and FSU Contracts & Grants.
9. Secure funding and a University project account.
10. Setup the surface support facility & move all the subjects, staff and equipment into place by the proposed start date.

1. The Protocol

The full text of the SIO2 protocol covered the physiology of surface breathed 100% oxygen between dives, what was accomplished through Phase I of the research to date, proposed project logistics in Florida, anticipated risks from the research and at the site, and a detailed budget. An alligator risk paper was generated by the Wakulla Springs Park Biologist and included in the required (by the Human Subject's Committee) "Informed Consent" document to be signed by all subjects. The vast majority of problems were anticipated in advance and adequately managed in this protocol.

NURC-UNCW's willingness to function through the terms of reciprocity recognizing FSU/ADP's Standard Operating Procedure (SOP) and the AAUS Standards for Scientific Diving was equally critical to the success of this project. Staff, subjects and diving protocol met FSU/ADP standards regardless of the institutional affiliation of the individual, as the project was conducted under the auspices of FSU. Considerable benefit occurred as cooperating institutions learned from each other.

Compartmentalization, worked reasonably well as a management scheme proposed for this project. Four major areas of activity were identified and a coordinator was assigned to each. They included Medical, Dive, Data, and Subjects. Each coordinator then organized and trained his respective staff based upon the project's objectives and available funds. A lack of funds resulted in sharing of the Subject's duties between other compartments and the administration. Due to the ownership of the gas charging facility, the NURC-UNCW Dive Emergency Medical Technicians (DEMT) provided cylinder charging services.

Funding was requested for food and lodging for the subjects, salaries for the staff, travel for the subjects (to and from the FSU Marine Lab), expenses such as tapes, batteries etc., site prep and cleanup, rental of equipment, and gasses.

2. The Medical Plan and Supervision

Dr. Kepper, FSU/ADP Program Physician, served as the medical supervisor of the site and the subjects. He was backed up by Dr. Lowenhertz of Mercy Hospital in Miami, Florida. Locally, he was also backed up by Dr. Walker, of Family Practice of Tallahassee and the Tallahassee Community Hospital Hyperbaric Facility. NURC-UNCW DEMTs served as the Medical Supervisor's assistants on site since Dr. Kepper also had a private practice to maintain.

Medical pre-screening consisted of a participant filling out the standard AAUS medical history review form, and submitting to a full medical evaluation (to AAUS standards) conducted by a physician and reviewed by Dr. Kepper. Subjects were asked to read and sign "informed consent" forms. These forms were revised as the project progressed and signed again prior to the first day of diving on the project.

Medical screening of 60 subjects was not as complicated as it might have been since more than half were already currently registered within the FSU/ADP system. Those that required further attention were processed through Dr. Kepper's office during the last month pre-project and during the project, creating a rush on his staff and facilities.

Subjects were asked to meet on Sunday evening at the campus FSU/ADP office for a briefing with the Project Manager, Data Coordinator and Medical Supervisor. A subject, an epileptic under medication who had lied on his health forms (see incidents later in this paper), convulsed on the first dive of the project. Subsequent medical screening of subjects included an isolated interview with Dr.

Kepper who discussed the incident and asked if anyone had anything else they would like to add to their medical files before starting the dives. After the briefing, subjects drove to the FSUML (50 miles) and checked into the dorms. The next morning, the DEMTs took them to Tallahassee Community Hospital for their pulmonary diffusion tests.

Mr. Curt Varner of the Tallahassee Community Hospital provided pre- and post-project pulmonary diffusion tests for all subjects at the hospital. His charge to the project was for cost only. Pregnancy testing was donated by Smith Kline and Associates of Tallahassee, Florida, to help keep the medical screening costs to subjects to a minimum. Finally, subjects were taken to the Lab the morning of the first day of diving and then bused back to Wakulla Springs (20 miles) for a late start of the dives. Here again the NURC-UNCW DEMTs played a very helpful hand by driving the subjects up from the coast to the hospital for these tests.

The NURC-UNCW DEMTs provided excellent medical support during this project. They conducted on-site pre-dive medical evaluations, including neurological exams, on every diver (staff included), Dr. Kepper worked closely to support the staff while on site. Each day, subjects were asked how they felt and were medically monitored, again, by the on-site ever watchful DEMTs. The same DEMTs stayed with the subjects for the entire week, including meals and in-residence at the FSU Marine Lab (FSU-ML). Subjects were accompanied by Dive Masters (usually certified diving instructors) while underwater. Any problems (such as ear & respiratory infections, anxiety, and panic) were capably evaluated by the Dive Masters or DEMTs and relayed to Dr. Kepper immediately. No closer supervision could have been expected without violating the subject's right to privacy.

A medical evacuation procedure using Life Flight was organized pre-project with the Tallahassee Memorial Hospital (including site visits with the staff) to be certain that medical evacuation to a hospital-based chamber was efficient.

Dr. Kepper was also the medical director of the back up chamber to this project at the Tallahassee Community Hospital. Medical-based chambers are clearly preferred by physicians as the full range of services are available nearby. Considering this, when would the project use the NURC-UNCW chamber? This dilemma was resolved by Dr. Kepper requiring the DEMTs to permit him to determine the severity of the emergency at the time of the injury and recommend which chamber to use at that time. Procedurally, if the NURC chamber were used on site, all diving would be stopped until the victim was released (as much as 36 hours later), but no Workman's Comprehensive claim would be filed (as per FSU Environmental Health & Safety <EH&S> policy) unless follow up treatments were required. If the Tallahassee Community Hospital chamber were used, however, FSU's EH&S would be billed under Workman's Compensation Act and a full investigation would shut the project down for an undetermined time.

This procedure proved to work best as the two cases treated clearly required different protocols. The convulsion required evacuation to a hospital and ultimately was diagnosed as near drowning (not requiring recompression). The second case, a shoulder bends case, required a very standard recompression treatment and was handled at Wakulla Springs using the NURC chamber. Both cases are briefly described later in the paper. Ear and respiratory infection cases were handled through Dr. Kepper's office.

Post-project medical reviews were conducted by the DEMTs prior to the subjects' departure from the site. Several respiratory infections were reported following the project. Dr. Lowenhertz visited the site in mid-project, reviewed the facility and the backup chamber in Tallahassee, and the medical procedures post in week one of the project.

3. The Site

One of the first steps taken by the FSU/ADP staff was to secure a variance to dive open-water trained SCUBA certified people under the ledge in Wakulla Springs. Depths below 80 feet in Wakulla

Springs fall under a gentle overhead slope, which classifies the site as an overhead environment. Expensive cave training would have been required by rigidly applied dive community standards. Representatives from the major overhead diving community associations (NACD, NSS-CDS, CMAS) were asked to attend a meeting, dive, and evaluate this specific site. A consensus report was then generated in which the conditions under which this site could be used with open water SCUBA divers were defined. The SIO2 project was later obligated to follow these standards by the Florida Department of Natural Resources when the site permit was issued.

A permit to use the Florida Department of Natural Resources Parks and Recreation Wakulla Springs State Park's basin was not easily obtained. The entire Park at Wakulla Springs is dedicated to research, especially the spring basin and associated caves. Use of the facility involved addressing at least three major areas of concern: safety (& liability), damage to the environment (noise, slope, and surface support areas) and disturbance of the basin's artifacts (early man and paleontology). We did not permit the operation of the on-site LP compressor unless in an emergency, and subjects (closely supervised for the entire week of their participation) were reminded not to touch anything while underwater. When the site was unloaded, steps were taken to stabilize the slope damaged while loading the chamber.

A window of opportunity was selected for mid-October to the end of January as water quality could change during this time and render the Wakulla Springs unusable. As it turned out, the October 14 through November 9 period was optimal. Had we postponed the project, we would have lost many subjects due to an unusually cold and early winter. As predicted, heavy rains shut down diving in this region early in February.

Park personnel were very helpful throughout the project. They helped with the selection of the surface support site, trash collections, power supply from the conference center, unloading and loading of the chamber and support facilities, and the security of the area. SIO2 staff were asked to carry ID cards that would permit their easy access to the Park. Park staff and visitors were always welcome to the site and accommodated with explanations and tours when possible. A poster board provided by the NOAA-NURC-UNCW personnel provided a national perspective on their program and related it to what was going on at the springs.

4. The Subjects/Board & Room

Academic Diving Program's science divers were proposed as volunteer subjects for this project. Originally, NURC-UNCW was to provide the project's subjects from their staff in Wilmington (at great logistical expense). FSU/ADP was already recruiting heavily (in-house mostly) and found it possible to shift from air to NITROX with some additional training. This reduced the costs to the project by shifting the responsibility of the medical exam (for those not yet evaluated) to the FSU/ADP participant (saving the project over $9000 in medical expenses alone), the cost of training to the FSU/ADP (saving the project another $10,000) and the salaries and travel of the NURC-UNCW staff (saving NURC-UNCW an estimated $6000). FSU/ADP still had to train staff not already certified in NITROX, Accident Management & Oxygen administration, and, in several staff cases, full cave diving as required by the FDNR site permit. The savings to NURC-UNCW permitted them to make much needed additional funds available to the project.

Selection for subjects began in April, 1991, a full six months before the project began, and continued right up through the project. Over 60 subjects were recruited and trained (four NITROX courses were offered and booked to capacity) at the FSU/ADP during that period. As expected, some dropped out after training due to scheduling and medical problems, others failed their medical or administrative FSU/ADP screening, 5 subjects were unable to re-schedule the first week when the project was temporarily suspended, and at least one withdrew after carefully reviewing the risks. Subjects were not restricted from participating on the SIO2 project based upon race, gender, years of experience or discipline of study. As long as they were registered as Science Divers in the FSU/ADP (also meeting AAUS standards), were trained to our standard in the use of NITROX and were willing to volunteer for

the project, we accepted their application and continued the screening tests specific to the project. Twenty-eight subjects participated in experimental dives.

FSU/ADP science divers are typical of those found in any university environment. Most are students who are either working on their own research or working with faculty or staff on someone else's underwater research projects at FSU. A respectable number are past students or associates of FSU faculty who now work off campus and voluntarily participate on recognized FSU underwater projects. Ten SIO2 applicants came to the FSU/ADP as off- campus divers.

The relative merits of using highly trained divers that should, in theory, reduce the risk of injury should a problem occur must be matched against the bias introduced when the results from less trained subjects (though more reflective of the intended user community) are excluded from the database. The bias we reference is the physical conditioning that an active diving technologist maintains over that of a diving scientist. While we encourage diving scientists to maintain good physical fitness, the demands of the science profession seldom allow cooperation. When factoring in the limited availability of highly trained and physically conditioned diver subjects, the extra safety and supervision precautions taken during SIO2 and the physiological nature of this project, the selection of subjects from an existing science diving program seemed very defensible.

Subjects were medically screened by Dr. William Kepper, the FSU/ADP Program Physician (see Medical Plan). Subject anthropometric assessment was conducted in the exercise physiology lab on the FSU campus. They were tested for pulmonary diffusion pre- and post-project at the Tallahassee Community Hospital Respiratory Physiology Lab.

The enormous task of Subject Coordinator was absorbed by the FSU/ADP secretary and assisted by the DEMTs. The Secretary coordinated the subjects and their medical evaluations and served as communications central in Tallahassee. The DEMTs assisted in subject coordination by supervising them while they were at the Marine Lab. DEMTs were to be close at hand by design anyway.

Incentives for subjects had to be carefully selected so as not to violate the ethics for human testing. The SIO2 staff focused on providing an informative, supportive, and relaxed environment, a social meal and evening period with an occasional slide show, free NITROX training with certification options through International Association of Nitrox Divers (IAND), a sweat shirt with the project's logo printed on the back (purchased with non-project funds), a certificate of participation, and the opportunity to participate in a NITROX-based physiology study at Wakulla Springs. Subjects completed 18 dives each during a six day period.

Subjects were compensated for food at the FSU per diem rate of $21.00. They ate as a group with the DEMT and usually at the Wakulla Springs Conference Center.

Lodging was provided at the FSU Marine Lab 35 miles away, because it was inexpensive to the project. Subjects tended to be very tired at night, so little socializing occurred once back at the Lab.

5. The Technology

A. Gas and Gas Delivery

Two blends of NITROX and 100% oxygen were required for the project: 32% for the 80 ft profiles, 36% for the 120 ft profiles and 100% for the surface profiles. A final figure, based on an estimated consumption rate of 1 surface cubic foot of gas per minute per subject for the proposed dive profiles was established at 99,000 cf of blended gas (33,382 cf of 32% NITROX and 65,746 cf of 36% NITROX). Twelve thousand cf of 100% oxygen were forecast for surface breathing. The staff required an additional 20,000 cf of NITROX for a projected total of 120,000 cf of NITROX. The volume of gas require on several dives on the 80 ft profiles exceeded what could be provided by an 80 cf twin set (160 cf) rig and still have 500 psi buffer to return to the surface. Twin set 120 cf (Sherwood Genesis) rigs were provided to accommodate the shallow long dives.

Twin 5 cfm Rix Sweet Air compressors provided by NURC-UNCW as part of their blending system were not able to keep up with the demand without running for an estimated 9 hours each day. This assumed that the compressors and fill technicians did not break down.

The FSU/ADP dive locker converted 96 cylinders into doubles, including 32 - 120 cf Genesis cylinders for the shallow (80 ft) dives, and dedicated half to an exclusive 36% NITROX blend and the other half to a 32% NITROX blend for the deep (120 ft) dives. In this way the blenders would not need to bleed down the cylinders between dives since they were now dedicated to a single gas mix. Dive Masters were asked to provide their own cylinders when possible (since they had to be configured for "full" cave). This strategy reduced the compressing time considerably, but not enough. The fill station was to be operated by the DEMTs while on site during the day (along with their many other responsibilities).

A proposal was made to Air Products and Chemicals, Inc. of Allentown, Pennsylvania, to provide two premix gasses to the site in quantities that would accommodate our entire gas requirement. This would eliminate mixing time on-site, and only require topping off using a special pneumatic Haskel pump. Premix NITROX, by Air Products, was achieved by blending nitrogen and oxygen into "K" cylinders, which are each tested for water vapor and gas content before shipping.

Air Products agreed to donate 99,000 cubic feet of premix gas in proportions requested. They undertook to assess the market potential of pre-mix NITROX and to see if they could reach the mix accuracy needed in this emerging NITROX field. They delivered half the supply needed at the start of the project along with documentation of quality control. Their cost of production was ultimately found to be just under $0.20 per cubic foot (=just under $16 for a 80 cf SCUBA cylinder). Their contribution to the project amounted to $10,000.

Connecting these pre-mix cylinders into the NURC-UNCW fill system proved to be considerably more expensive than expected. FSU/ADP also provided a LP compressor and 10 new 4500 psi "K" cylinders which were also connected into the NURC-UNCW fill station. Over $1000 was spent unexpectedly for additional hoses, adapters and fittings.

The NURC-UNCW brought a Haskel pump and planned to drive it using the diesel driven low pressure (LP) compressor. The gas required to drive the Haskel pump proved to require more than FSU/ADP's LP compressor could muster. Since running the chamber's LP diesel compressor was considered too loud for the site, the fill station could only use the pre-mix in a cascade mode. By combining the limited Rix (2 x 5 cfm) compressors consti-blending NITROX and topping off cylinders cascaded with Air Products pre-mix, the fill station was able to keep up with charging 60 cylinders a day on site. Half of the requested Air Products pre-mix was used. Air Products wisely held off mixing the second half of the requested amount and were alerted to the change in gas requirements in time to prevent wastage.

In addition to the 170 cylinders (47,600 cf) of pre-mix used on this project, 109 cylinders (30,520 cf) of oxygen were used either for surface-interval breathing (about 36) or for blending (73). This quantity exceed the expected volume considerably, doubling our projected gas cost! While these figures are crude (cylinders are seldom drained completely), an estimated 78,000 cf of compressed gas was brought to the site. If an estimate may be made that the Rix compressors pumped an average of 65% air while blending, say 73 cylinders of oxygen (20,440 cf) an estimate of 43,000 cf of air was pumped (at 10 cfm this means the compressors ran for between 72 and 100 hours, up to 5 hours a day). A cumulative estimate of 120,000 cf of gas was expended by this project.

A modification was made to the surface oxygen delivery system during the last week of the project, based upon recommendations made by Dr. Lowenhertz. During the first three weeks of the project, the surface interval 100% oxygen was delivered to subjects from an oxygen manifold at a rate of approximately .66 cf/min through a standard SCUBA diving second stage, which required subjects to

wear nose clips. It was observed that these clips were uncomfortable and may have been omitted by the subjects at times. Once omitted, there was no way to be sure that the subject received 100% oxygen while at the surface oxygen station. Just before the last week of the project, the FSU/ADP was able to purchase eight oxygen-clean AGA full face masks, and replaced them for the regulator second stages on the oxygen manifold in the tent. The full face mask AGA units reduced the chance of subjects inhaling anything but 100% oxygen. Further investigations are needed to evaluate the effectiveness of oxygen delivery equipment.

The FSU/ADP dive locker staff (not paid from the project) worked long hours oxygen cleaning all cylinders, valves and first stages of all regulators, and configuring all 48 twin sets during the week leading up to the start of the project. During the last few days, the FSU/ADP locker mixed and pumped all 100+ cylinders with the correct NITROX blend for the first two days of diving, giving the NURC-UNCW staff extra time to get on line.

A week and a half into the project, after a new 120 cf Genesis twin set leaked and required retro-fitting, 5 ml of water was found in both cylinders. This information was relayed to the NURC-UNCW at Wilmington. The water separators on the Rix compressors were identified as either failing or inadequate to remove all the moisture. Air Products pre-mix dew point was checked for moisture content and found to be very dry. A large water separator was sent from Wilmington and installed by the start of week three of the project. When opened at the end of the project cylinders brought to the fill station after the installation of the new water separator did not have water or water damage inside. Those that were used throughout the project all had between 3 and 5 ml of water at the end of the project with corrosion damage.

NURC-UNCW staff did a fine job keeping up with the charging of cylinders. This task required long and hard hours. FSU/ADP hired a person to be at the site to assist in hauling cylinders from the water to the fill station, but he became a subject for one week, placing an additional burden on the DEMTs. Subjects were not permitted to carry cylinders, which is consistent with FSU/ADP standards regarding arduous diving.

Subjects were outfitted with twin cylinders, configured with an Orca dive computer, single hosed regulator (complete with pressure and depth gauge, octopus second stage and inflator hose), back mounted "wings" with mechanical inflator, aluminum back plate harness and data slate. The Genesis 120 cf rig weighed approximately 90 pounds while the twin 80 cf aluminum rig weighed approximately 80 pounds out of the water. Subjects wore wet suits with hoods, and provided their own masks, fins, and snorkels.

B. Site Facilities

NURC-UNCW provided both a Chamber van and Nitrox blending van. Across the weekend before the project, the DEMTs from NURC-UNCW configured the facility, working closely with the ADP and FSU staff for power and additional parts needed to bring all systems on line. The FSU physical plant staff spent the day bringing three phase power from a pump spring-side to power boxes installed on a fence near the NURC-UNCW vans. Dr. Kepper visited the site during this first weekend and discussed emergency procedures including cellular communications.

In front of the chamber and blending van, the FSU/ADP staff erected a 20 by 20 foot tent provided by Air Products. An out-door rug was set under 3/4 of the tent over a sand bed. Eight cots and two low tables were erected under an oxygen manifold which supplied each subject with a personal 100% oxygen breathing device. Two extra oxygen deliver hoses were provided at the end of the table for the Dive Masters to use when not out serving as standby divers. Oxygen "K" cylinders were set up at the perimeter of the tent and attached to the manifold by way of oxygen cleaned regulators. Two full "K" cylinders of oxygen (approximately 500 cf) were required per day to support the surface breathed oxygen needs of the project.

Techno Scientific Ultrasonic Doppler Monitors with Marantz recorders were used to record intravascular bubbles. The two doppler technologists set up their station on a table set between the cots so that subjects could stay on 100% oxygen during the required 30-35 minutes while being tested. The Data Coordinator had up to two assistants throughout the project. To assure complete hydration, (which was tested periodically) cold drinks and hot cinnamon apple juice was available from the blending van and tent throughout the day. Subjects consumed about 50 gallons of apple juice during the four weeks of the project.

Wind was controlled by placing Visqueen panels horizontally hung from the tent's rafters. During the fourth week of the project, the temperature dipped from the mid 70's to the low 20's. A jet blower/heater, which brought the temperature in the tent up to an acceptable 75-80 degrees F. was rented during this last week.

The area adjacent to the water and in front of the tent became the staging area where the Dive Supervisor and his staff configured equipment, tested the gas, and briefed subjects. A tower located to one side provided a near vertical view of the dive site and swimming lane out from the staging area. The entire area was fenced providing a measure of security. The NURC-UNCW Wells Fargo trailer was parked next to the tent and used to secure all the cylinders, vests and regulators after hours.

NURC-UNCW provided a cellular telephone link at the site, which permitted the Project Manager to carry the other cellular phone and stay in constant contact with the site. Two pay phones 200 feet away, and the Park phone system (also 200 feet away) served as emergency backup communications links. On-site VHF radios connected the dive station located on the tower over the springs and the tent/chamber complex 150-200 feet away. Data Recorders were able to track exposure times, coordinate the subjects and respond to emergencies quickly due to this on-site link.

Vehicles were needed to move equipment to and from the site, subjects to and from their lodging at the FSU Marine Lab, and staff to and from their offices throughout the project. FSU/ADP's 3/4 ton pickup truck was used often to ship oxygen cylinders to the site and recover (or repair) broken equipment and empty cylinders.

6. Project Staff

A. Administration

The Project Manager was envisioned as being on site part time (daily, but not full-time) and otherwise coordinating the various compartments (listed below) and the various agencies and institutions. The Project Manager's responsibilities on campus continued, teaching a class with 75 students, serving as the University's Diving Officer and Director of the FSU/ADP.

The Project Manager found working with the staff of the SIO2 project pleasant and challenging. Any time so many people are concentrated under such a work load and time constraint there are bound to be problems. In all cases, the Project Manager found everyone focused on the need for safety first and quality data a very close second. At the end of each day he communicated with at least the Dive Supervisor to be sure the dive site was safely secured, and to get the latest data before calling Dr. Vann each night with a progress report. Weekly reports were generated with a focus on lessons learned.

B. Dive Supervision

The Dive Supervisor was instrumental in securing the site, site variance, and the excellent caliber of Dive Masters used during this project. He met the restrictions of the site variance by hiring diving instructors who where cave trained and had plenty of diving experience. He also set up an alternate Dive Supervisor who could step in and replace him in the event of an illness. On site, the Dive Supervisor supervised the Dive Masters and the subjects. He made sure that gas mixtures were checked by the subjects, dive profiles were accurately followed, and the life-support equipment was functional. In an emergency, he coordinated the rescue and medical evacuation of a victim at least back to the

DEMTs chamber or other Emergency Medical Service as required. The bulk of his work was done long before the first dive of the first week was made.

Dive Masters were the in-water subject supervisors assuring that the profiles and conditions laid down by the proposal would be met. They were all experienced in rescue and subject management. Most "problems" never came to the surface, as they were resolved long before they became a problem in the first place. None of these staff members participated solely for the money (at $50/day) but rather to gain a better understanding of the new techniques tested.

The concept of in-water supervision proved to be most valuable on this project. While both surface and in-water supervision was applied, the surface support added very little additional assistance over what was possible with the Dive Masters closest to the subjects. These in-water supervisors also provided encouragement to the subjects to stay on task during the trials.

Dive Masters (who were not supposed to be subjected to the same stressors as the subjects) were often subjected to the same profiles as the subjects. Precautions to avoid complications (such as DCS) included staying shallower (by 10 feet) than the subjects, breathing a richer blend of NITROX (up to 40%), wearing dry suits, and breathing 100% oxygen longer at the surface. For one week the Dive Supervisor brought a third Dive Master into the schedule, which unexpectedly added to the cost of the project. For another week, he alternated with his Dive Masters and spent some time underwater himself. During the last week, the Project Manager spent several days as a Dive Master filling in for a Dive Master who came down with a respiratory infection. No cases of decompression sickness occurred among the staff.

Chamber operators were provided by NURC-UNCW. To back them up, three FSU/ADP staff members were sent to Hyperbarics International (at no cost to the project) and passed a course in chamber operations. Two of these staff also had considerable experience with chambers, one working full time with the Tallahassee Community Hospital chamber just prior to the project.

C. Data Supervision

The Data Coordinator came into the project later than most of the staff, arriving at FSU in late August 1991. He identified and began working with two Doppler technologists (advanced graduate students) and one alternate, who served as his assistant throughout the project. The extra load of data collection was anticipated, with some of the discretionary funds intended to pay for a full time assistant if the volume became too much. Additional volunteers were also brought in as the workload increased.

Anthropometric assessment of subjects was completed at FSU's Exercise Physiology Lab prior to the project. These tests, discussed in greater detail in Pollock *et al.* (1992), included body fat, weight, height, skin fold thickness, age, vital capacity, and a medical history. Subjects were given instructional sheets regarding what to expect during the study, what to do and avoid, and encouraged to discuss any problems throughout the study. The Data Coordinator personally debriefed each subject on a daily basis. This provided the subjects with a more realistic grasp of the project, giving them a chance to articulate their concerns, and gave the Data Coordinator a chance to monitor their well being. The Data Coordinator spent near full time on site, which was not considered necessary in the proposal, but which proved to be pivotal to the quality of the data collected.

The prone or sitting position for Doppler monitoring was used in Phase I. This technique did not produce enough bubbles to monitor intravascular bubbles during Phase II. Evaluating the prone or sitting technique before the project, however, was very difficult without dives that generated a good spectrum of bubbles. Generating bubbles pre-project was abandoned without the availability of an on-site chamber. Doppler monitors were given a number of practice tapes from which to master bubble grade evaluation.

During the second day of the second week of the project, Doppler scores from use of the sitting technique were not matching the scores generated during spot checks (of the standing technique). At that time, one subject with a low bubble score reported shoulder pain and was treated in the chamber. Since all diving was stopped for the rest of the day, all subjects were re-tested using the standing Doppler monitoring technique (Eatock, 1986) and when compared to the sitting technique performed at the same time, were found to have very different Doppler scores. The standard Kisman & Masurel (DCIEM) standing Doppler monitoring technique was subsequently adopted for all subsequent evaluations. Since all Doppler monitoring was recorded, a double blind analysis of the signals was possible after the field evaluation was completed.

7. Securing the Project on Campus

The budget status for this project at FSU was very important. Overhead costs exist for all research conducted at FSU whether on campus or off campus. On campus "indirect" charges are as high as 46% of the modified total direct cost <mtdc> while off campus indirect charges are 26% mtdc. Since this project was extremely limited in funding and promising in future prospects, a request to waive the indirect charges was approved by the Vice President (Office of Research). This saved the budget a much needed $10,000.

The Office of Research also assisted us to secure, manage, coordinate purchase supplies and meet fiscal reporting requirements of the contract from NURC-UNCW. A theft of FSU/ADP cylinders, regulators, BCs, tools, a computer, first aid kits, etc., which occurred from the dive locker just weeks before the project, was accommodated by a much needed advance to purchase this equipment back by the Office of Research.

Over the six to eight months prior to the project, several FSU Boards and administrative offices needed to approve the SIO2 proposal. The FSU Human Subjects Committee had met on a previous mix-gas project, but had determined that was not a "human subjects" related activity. The FSU Diving Control Board was informed of the research. The office of Legal Council was asked to work with DUMC regarding liabilities. The FSU Marine Laboratory regarding facilities and of course the office of Contract & Grants to manage the financial monitoring of the project.

The FSU Human Subject's Committee requested that the SIO2 project be reviewed only after the Duke University Medical Center IRB had approved the project. Duke's approval occurred in August 1991, permitting the FSU Board to approve the project shortly thereafter (in September) citing Duke as the primary IRB.

After the convulsion incident in week 1, the project leadership and the FSU Human Subjects Committee shut the SIO2 project down until a full review was conducted. This took Dr. Kepper and the Project Manager the remainder of the week preparing documents to get the project back on track by week 2. The success of that documentation resulted in a clearance to continue the project and a letter congratulating the project on saving the subject's life.

A special FSU Dive Control Board meeting was called during the last week of the project to discuss the risks of this type of project and to review the two cases that had occurred. Dr. Vann attended. The entire project was reviewed and, while the discussions demonstrated concern that diving projects at FSU should not expect to result in injury, the final consensus was that human subject testing was a component of the University's research environment. The University's Diving Officer was asked to propose amendments to the FSU/SOP to include human subject testing diving standards, and to isolate his office's participation on such projects so that there would be no possibility of a conflict of interest.

8. Site Setup & Day 1

There are times when everything comes together right on the schedule. A year's worth of hard work came to focus on the weekend of October 12-13 when the chamber, cylinders, staff and subjects arrived ready to start on Monday. The FSU/ADP locker and NURC-UNCW spent long hours

configuring, labeling and charging the system. The Data Coordinator and his staff were busy assembling the tent and oxygen delivery system. When Monday arrived along with the press (channel 6 WCTV), we thought our troubles were over and looked forward to an exciting month of data collection. After the first dive came off with few problems, the Project Manager was called away to resolve a problem on campus. Before he could get there, dive two (the first dive of team B) had ended in a convulsion, documented for all to see on the evening news, which was to ultimately test the mettle of us all.

Incidents

Case 1

A 25 year old male was injured on October 14, 1991 while serving as a subject for the FSU/SIO2 project. The first dive of the second team began at 1311 hours. The profile called for 51 minutes at 80 FSW breathing 36% nitrox. At 35 minutes into the dive, the victim experienced a seizure. His buddy and the in-water Dive Master immediately began the rescue. The victim was brought to the surface and towed to the beach a short distance away. He was conscious and breathing on the surface. While the EMS was activated the victim was carried to the on-site chamber and waiting DEMTs.

Dr. Kepper was advised by phone and while consulted, the victim's partner brought forward two vials found in his diving bag. One contained Dilantin and the other Tegretol (anti convulsive drugs). Dr. Kepper directed the EMS (Life Flight had arrived) to transport the victim to the Tallahassee Community Hospital for more tests. The gas content of the victim's cylinder was tested at the site and found to contain 36.8% oxygen, which at a depth of 80 FSW would expose the diver to a PO_2 of 1.26 for 35 minutes. While the victim was eventually recompressed on USN-T 6 as a precaution, the injury was listed as a near-drowning. The victim was released from the hospital on October 16, 1991 with a strong recommendation not to continue diving.

Upon closer inspection of the medical screening, the victim had three preliminary opportunities to disclose his medical condition, and did not. His evaluating doctor did not disclose the condition either. Just prior to making the dive, he signed yet another form (#4), which stated that he was not taking any medication for "seizure disorders that could produce a loss of consciousness". After the incident, he acknowledged that he has had and kept hidden this condition since childhood. His diving knowledge and skills were excellent. He carried a cave diving and NITROX certification and had participated on other diving physiology studies as a subject.

Case 2

A 22 year old female was injured on October 22, 1991 while serving as a subject on the FSU/SIO2 project. Her first dive on the project was to 120 FSW for 25 minutes. Surface Doppler monitoring detected no intravascular bubbles using the sitting technique. After 30 minutes on 100% oxygen and a total of 120 minutes of surface interval time, she entered the water and completed dive #2 with a profile of 120 FSW for 25 minutes. This was three (3) minutes longer than was planned, a mistake in reading the schedule that the Dive Master made and no one caught. She consumed much more gas than her partners. Both dives were on a blend of 32% nitrox.

Her sitting Doppler score during the second surface interval (while on 100% oxygen) was between group I and II bubble grade. Ten minutes after surfacing she felt a deep but minor (1-10 scale=4) pain in her right shoulder that she reported ten minutes later. The standing (K-M) technique was performed at the same time as the sitting technique and recorded a group III+ bubble grade on this same subject. Dr. Kepper advised a USN-T5 recompression therapy, which was carried out at the site. During the remainder of the day (all dives were canceled while the chamber was active), a comparison study between the sitting and standing techniques was completed on all subjects present. While the subject's files were all in order and she wanted to complete the study, she was not permitted to continue the dives.

Lessons Learned

1. Funding for projects like this must be secured months in advance of the proposed research. A last minute NURC-UNCW budget allocation made the task of FSU's early investment of funds difficult. Purchases were put off until the very last minute wondering if the project was going to be funded at all. Each obligation to staff and technology had to be matched by internal resources to back up the real possibility that the funds would not come through.

2. A pilot study must be conducted in the future. Several problems would have been identified pre-project and steps taken to improve the quality of the data. A full week extra (or more) should be scheduled a month in advance of the real data collections to allow a pilot study. We realize this was not possible as costly facilities arrived just days before the start of this project. Since all diving stopped the first week due to the convulsion incident and all diving stopped while the case was reviewed, and problems with Doppler monitoring, etc., were identified in week two, we were left with two weeks' worth of good data collections. Technical considerations could also have been trial tested.

3. Additional subjects committed to additional dates beyond the project would permit us to accommodate shifts in the schedule such as bad weather, injury or technical failures. The hastily proposed fifth week of this project (to make up for the lost first week) was finally canceled specifically because we had only two subjects available. The reason for so few subjects at this point in time was mostly due to the volunteer nature of the subject pool, the approach of the Thanksgiving holidays, final exams and the early, unseasonably cold (mid 20 degrees F.) arrival of winter. Had the cold snap occurred just a few days earlier, the fourth week of the project may also have been lost. The number of backup subjects who are ready to step into a project like this should double the number of subjects anticipated. To achieve this number four times the number of needed subjects should be interviewed.

4. The staff requirements should be increased to facilitate longer projects and reduce exhaustion. A Subjects Coordinator and assistant should be a requirement for a project such as this, starting as much as six months to a year in advance of diving. The assistant should serve as the project "go-fer" when the subjects are at the site. The blending or charging facility should function under the diving supervisor and include two people: one to help haul equipment and one to monitor the compressors. Three Dive Masters per week (one for rotation) will be required to minimize burnout and maximize safety supervision. Increasing from one Doppler monitor for every four subjects to one for every two subjects will double the data available.

5. In future projects the Project Manager should appoint a site supervisor who will assist in coordinating those on the site. At times, the various compartments needed some assistance in coordination, and the Project Manager was not there to assist. Indeed, people often sought for one person to be "in charge", not recognizing that the Project Manager was a call away on the cellular phone and never more than 30 minutes away by car.

6. Medical screening and supervision of subjects, especially this many subjects, will require additional funds in the future. The grant did not provide any funds to be used for medical supervision. Half way through the project, funds were identified in the grant and used to help defray over 100 hours of Dr. Kepper's time beyond the initial medical screening. At $70 per hour, this should have cost the grant over $7,000, but it cost the grant less than $1000. Had the grant also covered medical screening, at $150 per subject, an additional $6000 would have been necessary. The cost of the week 1 incidents was borne by the Workman Compensation provision since the subjects were recognized as volunteers to the University, and represents another savings to the grant.

7. A per-diem stipend for food should be made available in advance of the project. Food for the staff should also be included from grant funds as they often worked late into the evening and seldom

could afford to eat with the subjects at Wakulla Lodge prices. Petty cash should be available during the project since University purchasing can be very slow. The Data Coordinator and the Project Manager both paid out considerable personal funds unexpectedly to keep the project properly supplied.

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PATTERN AND PROCESS: DIFFERENTIAL GROWTH IN AGGREGATIONS OF THE GREGARIOUS TUBE WORM, HYDROIDES DIANTHUS

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Spatial segregation and differential growth are used as evidence to support the contention that competition is an important community structuring process. Growth of the serpulid tube worm, Hydroides dianthus, was found to differ between solitary individuals and dense aggregations depending on food availability. Field data indicate that this species is gregarious and that H. dianthus is positively associated with some and negatively associated with other common fouling organisms. In food-limited cultures, aggregated animals were found to have a significantly higher growth rate than solitary individuals, whereas in cultures with super-abundant food, solitary individuals were found to have a significantly higher growth rate than aggregated individuals. These results were similar between assays of lab-reared siblings but not field-collected conspecifics. This study emphasizes that caution must be used in inferring processes from observed patterns in the field.

Introduction

Competition has been widely cited as a major structuring process in marine and terrestrial communities (*e.g.* Tansley, 1917; Gause, 1935; Connell, 1961; Mackie *et al.*, 1978; Hairston, 1980; Bellows, 1981; Lawton & Hassell, 1981). Competition is an interaction between individuals, derived from a shared requirement for a resource in limited supply, which results in the reduction of fecundity, survivorship, or growth of a given individual as a result of resource exploitation or interference by other individuals. Competition occurs when two or more organisms require and obtain common resources from a supply that is insufficient to support all, and thus, can only occur if that resource is in limiting supply (Begon *et al.*, 1986). Therefore, even two individuals with identical resource requirements will not be in competition with one another if the resources available to them are super-abundant (Begon *et al.*, 1986). In other words, as long as the combined demand of the organisms for a given resource does not exceed the available supply of that resource, competition does not occur.

Studies examining the importance of competition in structuring communities have traditionally been conducted in communities where there is reason to believe that competition will be important. These communities have generally been terrestrial systems in which the resource overlap of the species of interest is such that the likelihood of competition occurring is high. Despite the fact that these studies have usually been conducted in communities where a positive result is expected, Schoener (1983) found that about 90% of studies (±160 papers) showed evidence of competition but only about 76% of species were demonstrated to show evidence of competition. Connell (1983) suggested that although roughly 95% of species are subject to competition in single species analyses (±70 papers), only about 48% of species showed any evidence for competition in multi-species analyses. In fact, according to Roughgarden (1989) only about one-third of these studies (±200) have been able to demonstrate the importance of competition in structuring communities. Furthermore, many of the studies examining competition in the field have been inadequate to document the patterns upon which the conclusions are

based (Underwood, 1986). A survey of the competition literature used by Connell (1983) and Schoener (1983) determined that approximately 16% did not support the conclusions, and a further 28% of studies lacked appropriate replication, controls, or experimental design (Underwood, 1986). Finally, many studies of competition have involved demonstrating patterns of niche segregation rather than experimental manipulations of the system (*e.g.* Brown, 1975; Brown & Kodric-Brown, 1979; Pyke, 1982; Bowers & Brown, 1982).

One problem with the approach of pattern documentation is that niche segregation, by definition, indicates that competition is no longer a structuring process (even if it once was). In such systems the assumption is made that organisms were subject to competition during some period in the past, which has resulted in the partitioning of resources. However, it is impossible to know if the pattern which we see today is the result of long-term selection or simple stochastic processes, because we were unable to experimentally manipulate the system during the period when competition was hypothesized to be important. Differences in the conclusions reached from studies examining competition as a potential structuring force in communities have slowly evolved into a debate in the literature. This debate has revolved around two main schools of thought on the formation of simple communities. The main focus of these studies have been terrestrial communities found on isolated islands, which have then been used as model systems for community development as a whole. One school of thought supports the idea that communities are limited to a fraction of the organisms which could potentially exist there - the "limited membership" concept proposed by Elton (1933). The other school of thought supports the contention of Gleason (1926) who argued that communities were simply the product of "fluctuating and fortuitous immigration...and an equally fluctuating and variable environment" (from Roughgarden, 1989). There has traditionally been a third school of thought - the "super-organism" concept proposed by Clements (1916) - which considers communities to be tightly co-evolved entities in which each species functions as part of a greater whole. The Clementean view of community structure has fallen out of favor, however, due in part to the unpredictability of communities in space and time (Begon, *et al.* 1986).

There has been a profusion of literature generated on the opposing view points of Gleason and Elton (reviewed in Roughgarden, 1989), however, but no answers to the question reiterated by Roughgarden in the first sentence of his review: "Do the populations at a site consist of all those that happened to arrive there, or of only a special subset - those with properties allowing their coexistence?". Roughgarden has also proposed a model which combines the two ideas and predicts the formation of communities based on chance colonization events followed by competition - the so-called 'taxon-cycle'. In such a scenario, competition may result in either competitive exclusion or niche segregation, depending on the system and the characteristics of the species in question (Roughgarden, 1986). It is easier (both energetically and financially) for one to determine patterns of species distribution and abundance in the field than to perform large-scale manipulative experiments to elucidate the mechanisms by which these patterns are derived. In this time of limited funds, the result has been a proliferation of studies which document patterns in the field and infer the mechanisms (processes) from which these patterns have been derived. One result of such an unfortunate bias is the potential for incorrect inferences to lead to further complications in the ecological models which are based on such studies. If we are to develop ecological models of any predictive value, accurate descriptions of the mechanisms by which communities are structured are an essential basis for these models.

The main danger in inferring causal mechanisms from observed patterns is that many processes could conceivably have produced very similar patterns of organismal distribution and abundance in the field. For example, the intensity of competition experienced by an individual is not really determined by the overall density of the local population, but rather by the extent to which it is inhibited from deriving resources by its immediate neighbors. This is particularly true of sessile organisms, such as the fouling tube worm Hydroides dianthus (Polychaeta: Serpulidae). Organisms are, of course, more likely to be crowded as the local population density increases, but if the density of organisms is not uniform, some individuals in the population may experience levels of crowding far different from other individuals in the same population. This is especially important for gregarious species. In gregarious

species, many individuals are highly crowded while other individuals experience levels of crowding that are far from typical, and the level of crowding is relatively independent of overall density. In such populations, density tends to be a measure of the number of aggregations within a region rather than a measure of the mean degree of crowding among individuals of the population. Density is, therefore, a concept which applies to the population as a whole, but need not apply to each individual within that population.

Hydroides dianthus (Verrill, 1873) is a gregarious fan worm that is common in marine fouling communities along the coast of North Carolina and the rest of the East coast (Sutherland & Karlson, 1977). This species is an economically important fouling organism (the U.S. Navy spends approximately $10-30 million a year on biofouling control - T. Dowd, Naval Systems Command, 1984 estimate) for which several marine biofouling assays have been developed by the Electric Boat Division of General Dynamics (Leone, 1970: Zuraw & Leone, 1968, 1972; Gaucher *et al.*, 1967). Besides being an economically important species, H. dianthus has several other characteristics which make it an attractive study organism: (1) H. dianthus has a high fecundity and growth rate; (2) adults are easily obtained from local fouling communities, where the density of individuals can be quite high; (3) adults are easy to spawn, and the larvae are relatively easy to culture; (4) the calcareous tubes of H. dianthus do not adhere well to glass, and so can be easily moved to new locations where the animals rapidly reattach; and (5) new growth in laboratory cultures is easily determined because the coloration of new tube growth is distinctly different in laboratory culture (white) than in the field (brownish-grey).

The purpose of this study was to examine the growth rate of H. dianthus in culture to determine if there was any evidence of intraspecific competition among individuals in dense aggregations of these animals.

Methods and Materials

Local animals were collected from hard substrata (shell hash, consisting mainly of Mercenaria mercenaria shells) found in the low intertidal zone of the Atlantic Intracoastal Waterway beneath the drawbridge access to Wrightsville Beach, North Carolina. These animals were brought into the laboratory on 21 January, 1992, and divided into two groups: aggregated and solitary. Aggregated individuals were defined as those with more than five tubes/cm^2, while solitary individuals were defined as those more than 1cm away from the nearest conspecific. In this study density was not used as a measure of the degree of crowding which an individual experienced for the reasons listed above. Rather, aggregation was used as a relative indicator of the typical degree of crowding which an individual experienced on a daily basis.

Several representatives of each group were placed together into 4 l glass culture jars containing filtered seawater that were constantly aerated and maintained at 20 $\pm$ 0.5°C. The culture jars were cleaned and water was changed every other day. Super-abundant food trials were fed monospecific cultures of the diatom Phaeodactylum tricornutum at densities of roughly 5x10^5 cells/ml/day (125ml of stock culture at every cleaning). At this food density the water was always slightly discolored with diatoms at the end of the feeding period. The food limited trials were also fed monospecific cultures of the diatom, but rather than daily, worms were fed 125ml of stock culture every 14 days, resulting in densities around 5x10^4 cells/ml/day. A "moderate density" of tube worms is capable of clearing roughly 5 ml/l/day (Leone, 1970). In this study the food limited cultures received approximately 1 ml/l/day, while the super-abundant food cultures received approximately 15 ml/l/day. Under food-limited regimes, the water was changed after two days, removing any remaining diatoms, and the animals were `starved' for the remaining 12 days. For each trial, both solitary and aggregated individuals were cultured on opposite sides of the same culture vessel. These animals were cultured for six weeks in the laboratory, after which time animals were removed from their culture vessels and measured for new growth. There was no mortality observed in any of the laboratory culture vessels. Growth was

measured with calipers to the nearest millimeter for 25 individuals (five from each culture vessel) in each feeding regime (limited and super-abundant) and each settlement pattern (solitary and aggregated). Differences in the growth of individuals were analyzed on JMP with an ANOVA.

Large concrete blocks were collected from the same location as the animals for laboratory culture. These blocks were scrap dumped into the lower intertidal sometime in the recent past, which had since been colonized by fouling invertebrates. The position of the blocks was such that they would be exposed for only a short period of time on very low tides (-20cm or better). These blocks were brought into the laboratory and assayed for nearest neighbor and quadrat analyses of the associations between H. dianthus and other fouling marine invertebrates. The other species discussed in this study included: Botryllus planus (tunicate), Chthamalus fragilis (barnacle), Schizoporella unicornis (encrusting bryozoan), Spirorbis sp. (tubeworm), Aplysilla longispina (sponge), Ascidia interrupta (solitary tunicate), Microciona prolifera (sponge), Halocordyle disticha (arborescent hydroid), Tubularia crocea (stalked hydroid), and Brachidontes exuctus (mussel). Focal animals for nearest neighbor analyses were chosen haphazardly by blindly selecting a spot on the block with a probe, until an individual H. dianthus was chosen. This animal was then used as the center of a 2x2cm quadrat, the contents of which were identified and counted. The data were manually analyzed with Nearest Neighbor and Index of Association (v) tests.

Local H. dianthus for spawning were collected from settling tanks from an old desalination plant at Wrightsville Beach, North Carolina. These animals were brought into the laboratory and spawned (after Scheltema *et al.*, 1981) on 11 March, 1992. Gametes were placed on a shaker table in finger bowls at low RPM for 24h to increase the likelihood of successful fertilization. Drops of dilute sperm from a single male were slowly added to the swirling eggs for 30 minutes after which 1ml of dilute sperm was added to each finger bowl to ensure fertilization of all viable eggs. Trochophore larvae were decanted off the next morning and cultured in the laboratory on monospecific cultures of P. tricornutum. Competent larvae were settled out on biofilmed glass microscope slides. These slides were then separated according to the degree of aggregation of the individuals attached to it. Five trials were run, each consisting of the larvae from one of five females all fertilized by a single male. In each of these trials all individuals were full siblings, minimizing the potential complicating influences of genetic differences between individuals. Several aggregated and solitary individuals were placed into food-limited and super-abundant food trials. These individuals were cultured in the same way as the field collected individuals, and raised from settlement until the end of the experiment under the feeding regimes described above. Twenty five individuals of each settlement pattern and feeding regime were measured for growth: five from each culture vessel containing both solitary and aggregated individuals.

Results

Nearest neighbor analyses indicated that H. dianthus is distributed non-randomly ($P < 0.05$, $n = 25$ individuals), with 16 of 25 nearest neighbors being conspecifics. An index of association (v) indicates that H. dianthus tends to be positively associated with conspecifics (displays an aggregated dispersion in the field - $P < 0.05$, $n = 25$ quadrats), and is negatively associated with several other common fouling invertebrates: (1) Spirorbis sp. ($P < 0.01$, $n = 25$ quadrats); (2) Botryllus planus ($P < 0.01$, $n = 25$ quadrats); (3) Microciona prolifera ($P < 0.01$, $n = 25$ quadrats); and (4) Ascidia interrupta ($P < 0.01$, $n = 25$ quadrats). There were no significant associations found between H. dianthus and these other common fouling species: Chthamalus fragilis, Schizoporella unicornis, Aplysilla longispina, Halocordyle disticha, Tubularia crocea, and Brachidontes exuctus ($P > 0.05$, $n = 25$ quadrats).

Growth of field-collected H. dianthus in the laboratory indicated that there is differential growth of aggregated and solitary individuals in vitro (Fig. 1). The mean growth of individuals cultured under food limited conditions was significantly different (ANOVA, $P < 0.01$, $n = 50$

individuals) between aggregated (2.4 ± 0.4mm SD; all errors and error bars are 1 SD) and solitary individuals (3.1 ± 0.4mm). The mean growth of individuals cultured with super-abundant food was also significantly different (ANOVA, $P < 0.01$, $n = 50$ individuals) between aggregated (3.2 ± 1.4mm) and solitary individuals (4.5 ± 1.9mm).

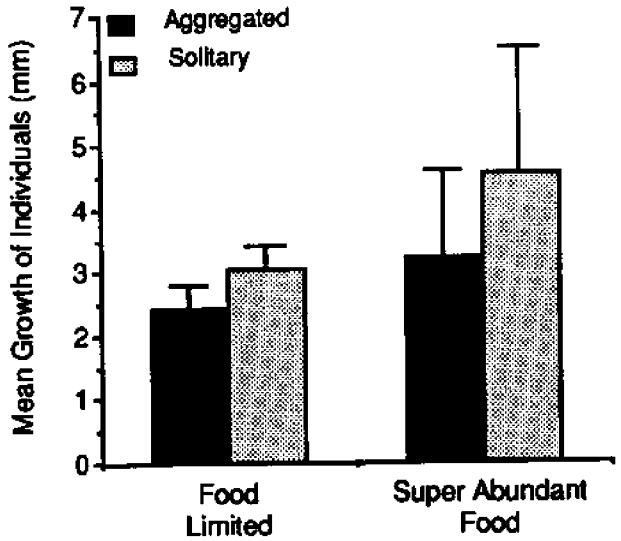

Figure 1. Mean growth of solitary and aggregated field-collected Hydroides dianthus in laboratory cultures under food-limited and super-abundant food conditions.

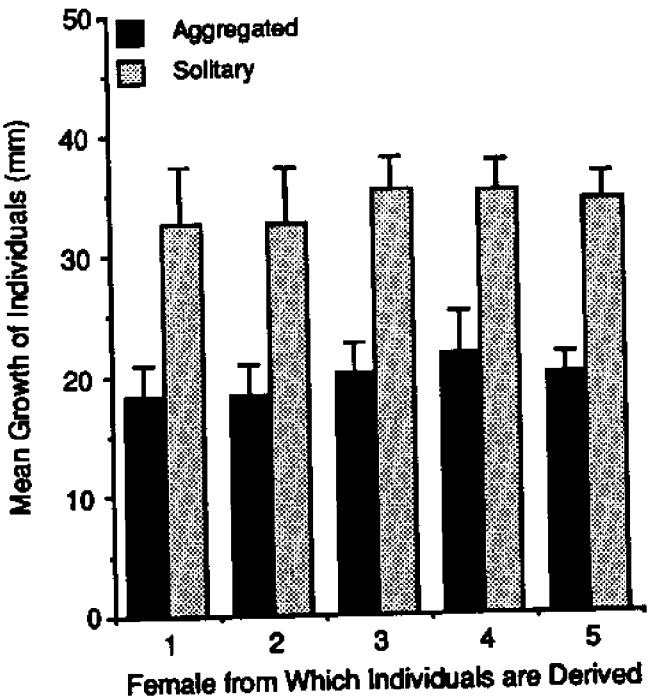

Figure 2. Mean growth of solitary and aggregated Hydroides dianthus in laboratory cultures with super-abundant food. The female corresponds to the parent from which larvae were obtained.

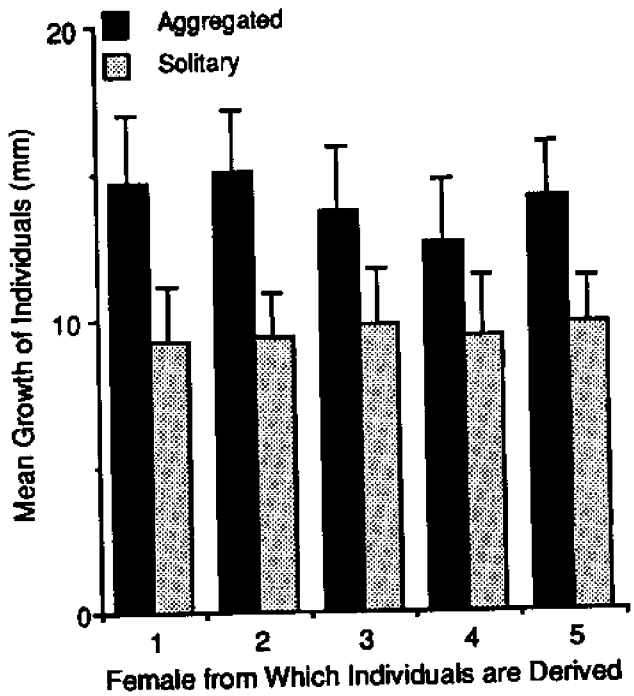

Figure 3. Mean growth of solitary and aggregated Hydroides dianthus in laboratory cultures with limited food. The female corresponds to the parent from which larvae were obtained.

In full sibling laboratory cultures there were significant differences between the growth of aggregated and solitary individuals in all cases (ANOVA, $P < 0.01$, $n = 50$ individuals per trial). In super-abundant food cultures, aggregated individuals demonstrate decreased growth relative to solitary individuals over the culture period (Fig. 2). In food limited cultures, however, aggregated individuals demonstrate increased growth relative to solitary individuals over the culture period (Fig. 3). This pattern is strikingly different than that of the field-collected cultures (Fig. 1), where aggregated individuals always have lower growth rates than do solitary individuals. Furthermore, there was differential growth between sibling cultures (ANOVA, $P < 0.01$, $n = 5$ cultures - Figs. 2 & 3).

Discussion

Data indicate that H. dianthus is a gregarious species that tends to demonstrate an aggregated dispersion in the field. The extreme proximity of individuals in these aggregations suggests the potential for 'upstream' individuals to interfere with the feeding of 'downstream' individuals by stripping available food before it reaches the 'downstream' animals. Although many other species of fouling marine invertebrates live in close proximity to H. dianthus in the field, most of these species are randomly distributed in relation to H. dianthus: Chthamalus fragilis, Schizoporella unicornis, Aplysilla longispina, Halocordyle disticha, Tubularia crocea, and Brachidontes exuctus demonstrated no significant association with H. dianthus ($P > 0.05$, $n = 25$ quadrats). However, it appears that several common species are non-randomly avoided by or avoiding colonies of H. dianthus: Spirorbis sp., Botryllus planus, Microciona prolifera, and Ascidia interrupta are all negatively associated with H. dianthus in the field ($P < 0.01$, $n = 25$ quadrats). Thus, it seems likely that there is at least the potential for both intraspecific and interspecific competition in this system.

The growth of field-collected H. dianthus that are cultured in the laboratory is always uniformly lower than that of lab-reared cultures (Figs. 1, 2, and 3). There are several potential explanations for the relatively lower growth of these individuals. First, all lab-reared individuals were raised from larval settlement through the most rapid growth phase of the life cycle of these animals, and, therefore, one would expect that young animals would show more growth than would mature animals during the same time period. Second, these animals live in calcareous tubes, and thus, they must expend relatively more energy to grow as they get larger to the construction and maintenance of a larger diameter tube. Third, the culture density of animals in each trial was relatively constant (as closely as possible), so the effects of crowding would be more pronounced with older animals than younger ones. Finally, the field collected animals were cultured in the laboratory at 20°C (± 0.5°C) after being directly removed from cold water (±5-10°C), while the larvae were raised for their entire lives in the same constant environmental conditions. Therefore, it is not surprising that there is significantly lower growth in the field-collected animals relative to the lab-reared conspecifics.

Measurement of the calcareous tubes of these animals as a relative measure of growth assumes that: (1) there is some relation between the tube size and the size of the worm inhabiting that tube; (2) there is some selective advantage to increased worm growth; and (3) tube growth is a plastic character that is capable of responding to fluctuations in food. I have not substantiated these assumptions, although there is literature to suggest that shell growth is a plastic character which responds to environmental stimuli (*e.g.*, Palmer, 1979), and it seems reasonable to assume that larger worms make larger tubes (*e.g.*, Dixon, 1980). It was impractical in this study to examine differences in tube weight (a relative measure of tube length, tube thickness, and crystal density) as a measure of growth because all individuals were attached to pieces of hard substrate. Furthermore, aggregated individuals were usually so closely intertwined that it would be impossible to separate them precisely. However, although conventional wisdom tells us that invertebrate reproductive success is positively correlated with body size (Begon *et al.*, 1986), there seems to be no relationship between the size of the animal and the total egg production or fertilization success (Toonen, Pawlik & Butman, in prep.).

The interesting results of this study, however, are: (1) in the field-collected animals, aggregated individuals always showed significantly lower growth than did solitary individuals; and (2) aggregated individuals grow better than do solitary individuals in food limited systems, while solitary individuals grow better than do aggregated individuals with super-abundant food. I believe that the relatively higher growth of the aggregated individuals in both cultures is an artifact of the experimental design. Although six weeks appears long enough to detect differences in the growth of newly settled individuals, it is most likely not long enough to detect differences in the growth rate of mature animals. Mature animals all showed less than 5mm (approximately 5-10% growth in these animals) of growth during the experimental period, which is probably insufficient to draw conclusions on differences in the growth rate. Furthermore, there is no control for the relative ages of the animals used in the field-collected cultures, nor is there any control of the parents from which these animals derived. Growth in the different sibling cultures showed significant differences between the cultures, and thus parental influences may play a significant role. Finally, these animals were all collected from the same habitat in a relatively small area, and environmental constraints on development may have also played a role. This result could be real, however. These animals may have been acclimated to super-abundant food in the field, and were not able to overcome the resultant pattern in the subsequent short-term experiment; especially given that they were switched from cold into warm water at the beginning of the experiment and may have needed time to acclimate to the temperature change. The area in which the animals were collected was located just downstream of a relatively large housing development, and with the productivity of coastal waters, along with the recent problems of eutrophication of coastal waters, there is the distinct possibility of food not being limiting. Although there is no reason to expect food to be limiting in eutrophic estuarine waters under normal conditions, this species is widely distributed and food limitation may be important in many of the habitats in which they are found. However, if one were to simply examine the observed pattern of differential growth in the laboratory, and the patterns of spatial segregation in the field, it would be easy to

conclude that competition was driving this pattern. This study has not given any conclusive evidence concerning the mechanisms controlling field-collected animal growth patterns, however, despite that being the original purpose of the study.

Unlike the field-collected cultures, the laboratory reared cultures had all of the above complicating factors controlled. All larvae were derived from a single male, and each culture was derived from a single female. All larvae were cultured under identical conditions from the fertilization of gametes through the end of the experiment, and all animals were of identical age (42 days ± 2 hours). One potential explanation for the increased growth of aggregated individuals in food limited cultures relative to solitary individuals is the harvesting of food. In dense aggregations, the collective feeding current of the colony could entrain relatively more food per individual than can the feeding current generated by a solitary individual. This would result in not only relatively more growth in aggregations relative to solitary individuals, but also to the increased growth of individuals on the outer edges of aggregations relative to more centrally located siblings. This appeared to be the case in this study, with some of the largest tubes measured being on the outer edges of aggregations. Pullen and LaBarbera (1991) worked with barnacles and demonstrated that "in general, rows upstream of and at the peak of hill-shaped profiles captured significantly more particles than downstream rows". On the other hand, in super-abundant food cultures, obtaining sufficient food is no longer a factor limiting to growth. In such conditions, the growth of solitary individuals becomes greater than the growth of aggregated individuals. This is likely due to physical parameters affecting the growth of individuals in dense aggregations of H. dianthus. Physical limitation of growth could also account for the huge difference in growth of solitary and aggregated siblings (Figs. 2 & 3). When food is super-abundant the growth of solitary individuals relative to food limited siblings is at least double in all cases; whereas growth in aggregated individuals is only slightly higher relative to food limited siblings in super-abundant food cultures. This hypothesized physical limitation to growth could result from any number of factors from space available for tube growth, to interference with feeding by overlapping of radioles, to depletion of nutrients (such as calcium, for example) in the microhabitat (although nutrient depletion is highly unlikely given the experimental procedure).

There are differences in the growth of individuals derived from different females, thus maternal influences may be important in the differential growth of individuals. It is conceivable that if female were to put more energy into her eggs, a newly settled juvenile may be able to put more energy into the development of feeding radioles, which would, in turn, allow that juvenile to harvest relatively more food than conspecifics which did not have that ability. Longer radioles would only be advantageous to an animal which is growing away from conspecifics if the limitation to growth is radiole overlap. In dense aggregation, longer radioles would lead to more overlap than would short radioles. Thus, aggregations would favor individuals with shorter radioles, while solitary individuals would do relatively better with long radioles. Thus, individuals inclined to develop long radioles would do relatively better as solitary animals in super-abundant food conditions, while individuals inclined to develop short radioles would do relatively better in aggregations under any conditions. This potential for maternal effects and radiole interference needs to be explored further.

This study demonstrates the potential for incorrect inferences being drawn based on documentation of field patterns. If one were to simply examine the pattern of growth in field collected cultures, it would be relatively easy to conclude that there is intraspecific competition which leads to decreased growth of individuals in aggregations relative to solitary individuals. However, competition for micronutrients obtained from the water column (essential minerals, for example) is unlikely among few animals with frequent water changes and strong circulation. Also, because both aggregated and solitary individuals were cultured together in the same culture vessel, both would suffer simultaneously from any such nutrient deficiency. Furthermore, competition for food can not, by definition occur in super-abundant food cultures, and therefore, some other process must be important in regulating the growth of those individuals. The culture of laboratory siblings presents a possible explanation for this pattern, but the data are not conclusive for the importance of processes structuring distribution in the field.

Although this study has not demonstrated any conclusive results concerning mechanisms structuring the distribution of this species in the field, new questions to be addressed before any conclusions can be reached are proposed. In order for mechanistically accurate conclusions to be drawn it seems necessary to do long-term (to account for sufficient environmental variability), large scale (to account for sufficient spatial heterogeneity) manipulations of systems in the field concurrent with carefully controlled laboratory simulations. Only by combining laboratory precision and field realism will any progress be made in useful ecological modelling.

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