Dive Operations Plan for the Assessment of the Status and Risk Posed by the Invasive Lionfish in North Carolina Hardbottom Communities

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Project Description

The Indo-Pacific Lionfish (*Pterois volitans/miles* complex), a venomous predatory fish with few natural enemies, is established (reproducing and dispersing) along the southeast shelf of the United States. Within the last three years lionfish have spread from Florida to Cape Hatteras NC and Bermuda (Whitfield et al. 2002, Hare and Whitfield in press). To our knowledge this is the first Pacific marine fish to become established in Atlantic waters. However, it may not be the last (Semmens et al. in review).

Since there has been little research to examine the consequences of marine fish invasions, it is difficult to accurately predict their impact on native communities (Hare and Whitfield, in press). This proposed research will increase our understanding of the current status and risk posed by the lionfish invasion. We will accomplish three goals; 1) establish a baseline of population abundance for lionfish and native fish communities to determine population status, 2) characterize the ecological role of lionfish to determine the risk associated with their presence, and 3) evaluate laboratory-derived thermal tolerances to predict geographic distribution.

Our multi-disciplinary approach combines *in-situ* lionfish and native fish community surveys with information on genetics and basic life history characteristics collected from lionfish specimens in Atlantic waters. Our quantitative field surveys will be conducted at two regions off the coast of North Carolina over a two-year period. From these surveys, we will obtain estimates of lionfish density, thereby providing a baseline from which the status of the invasion and rate of population increase can be estimated. These surveys will also provide critical baseline data on native fish community diversity. In addition we will evaluate laboratory-derived lionfish thermal tolerance limits in the field through collection of *in-situ* bottom water temperatures and from ROV/diver observations of lionfish during winter. From lionfish specimen collections we will examine the genetics and basic life history characteristics of lionfish. Genetic studies will allow us to estimate effective population size, potentially identify the number and source of the invasion event(s), and determine the extent of the bottleneck accompanying the invasion. From life history characteristics we will examine, population demographics (size/age structure) of lionfish, their trophic role, and the reproductive potential for lionfish in their introduced range. An evaluation of *in-situ* lionfish winter temperature tolerances and winter bottom water temperatures will allow a projection of the potential geographic distribution of lionfish. This research is a first step toward understanding the consequences of the lionfish invasion and possibly of marine fish invasions in general, and directly supports NOAA and NURP program goals “to characterize and monitor ecosystem health and stressors”.

Objectives

Our overall goal is to learn as much as possible about the biology and the effect of the lionfish invasion and the first aspect of this will be to develop a baseline estimate of abundance for both lionfish and the native fish communities. This baseline will allow future population trends to be determined. Further, we will provide information on lionfish environmental tolerance and several aspects of lionfish ecology and biology. All of this information is necessary to determine the status and the risk posed by an invasive species (Bax et al. 2001). Specifically we will:

1. Quantify lionfish abundance at two regions off the North Carolina shelf to provide a baseline for future assessment of trends in population size.

2. Quantify native fish communities to provide baseline population estimates essential for future assessments of lionfish impact.

3. Evaluate results of lionfish thermal tolerances with field observations and *in-situ* bottom water temperature collection.

4. Use genetic data to characterize population structure in order to better understand the biology of North Carolina lionfish.

5. Provide preliminary life history and ecological information to describe population demographics and the trophic role of North Carolina lionfish

Methods and Approach

Objective 1: Quantify lionfish abundance at two regions off the North Carolina shelf to provide a baseline for future assessment of trends in population size.

In order to maximize our time and resources we will be incorporating a multi-stage sampling design to address these objectives (Menges and Gordon 1996, Philippi et al. 2001). This approach is often used in surveys of rare plant species to intensively monitor locations where rare individuals are found and to monitor more extensively (larger geographic area) those locations where individuals may be rare or absent (Philippi et al. 2001). Because lionfish are still relatively rare in their new environment and exhibit considerable site fidelity in their native range we determined that our study would be an appropriate application of this design. There are two stages in this experimental design:

1) We will intensively survey areas where lionfish have been reported
2) We will explore and survey new locations over a larger area that is likely to support lionfish populations (Figure 1).

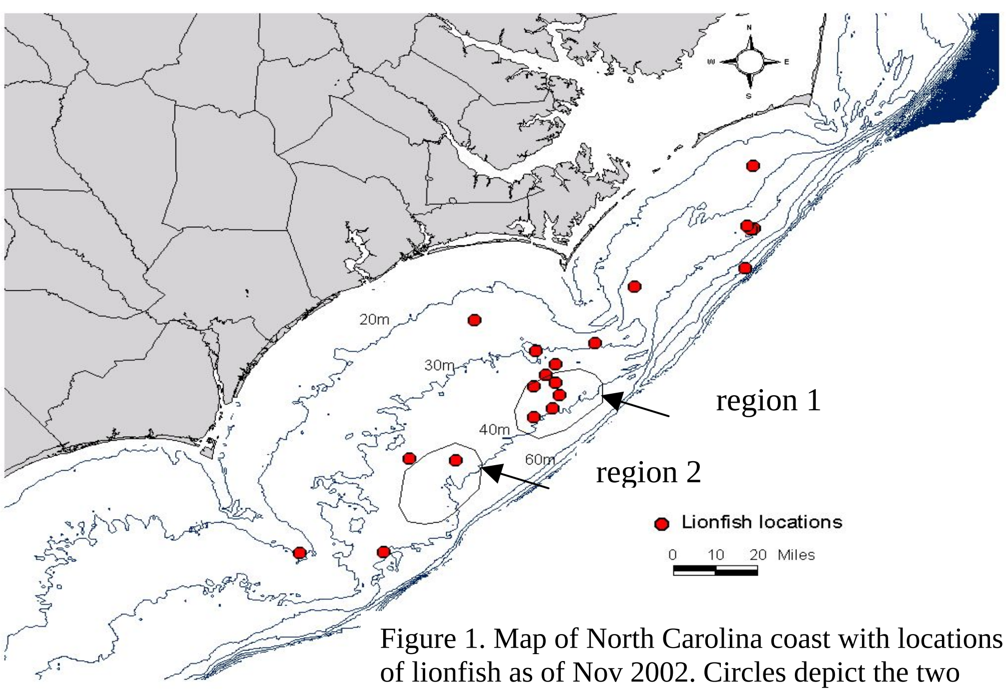

Figure 1. Map of North Carolina coast with locations of lionfish as of Nov 2002. Circles depict the two regions of study. Arrows depict rock formations of interest for higher intensity sampling.

We propose a two-year study within two general sampling regions in Onslow Bay, North Carolina: Cape Lookout and Cape Fear (Figure 1 (see circled areas)). Sampling will occur in August of 2004 and 2005. We will spend approximately 7 days of ship time at each region for a total of 14 days each summer (does not include winter sampling). Within each of these regions (circled on Figure) there is a smaller area of hard bottom habitat where lionfish have been consistently reported. We will target each of these formations at a higher resolution for the first stage of sampling (intensive sampling). Because there are no high resolution maps of these formations the scale of our sampling will be determined from side scan sonar transects that will be conducted prior to the diver surveys aboard the NOAA Beaufort Laboratory R/V Hildebrand, therefore, specific dive sites are yet to be determined. Our second stage of sampling will occur over a broader geographic area (extensive sampling) from 40 to 50 m depth within the larger regions (circled on figure 1). These sites will be randomly chosen from known hard bottom locations that are documented in the SEAMAP bottom mapping product (Moser et al. 1995) and from NOAA nautical charts. This multi-stage approach will allow us to accomplish two goals. First, we will be able to target those areas where lionfish are likely to occur and estimate their abundance in a smaller geographic area, while maximizing our potential for obtaining specimens. Specimen collections will fulfill our goal of describing lionfish population demographics, trophic role and life history characteristics. Secondly, the extensive random sampling over a larger geographic area will allow us to quantify lionfish density on hard bottom habitats without the bias imposed by the more focused non-random sampling design. The survey protocol within each site will be the same for all sites no matter which sampling design (intensive or extensive).

Two dive teams will survey each site. At least one dive team will focus solely on surveying and collecting lionfish along a linear transect while one dive team only will be conducting native fish community surveys (Obj. 2). The transect length and secchi disk visibility will be used to quantify the area surveyed in order to estimate the density of lionfish (Parker and Ross 1986, Parker et al. 1994). Our surveys will primarily be on hard bottom habitat as lionfish are found to inhabit three-dimensional structure and not sand. To maximize our search effort on the hard bottom habitat a transect line will be laid along the scarp/sand interface and visual diver observations of lionfish will be conducted from the transect and along the 'hard bottom' side to the extent of secchi disk visibility (Figure 2). All lionfish located within the extent of visibility along the 'hard bottom' side of the transect will be counted. Search time will probably not exceed 20 minutes per dive team. Each site completed will be a replicate. At each site dive teams will also collect as many lionfish as possible given the time constraints imposed by depth. Divers will use aquarium fish collecting nets or spears to capture the lionfish. Once captured or speared, lionfish will be placed in plastic bags and then to a combination mesh/canvas bags to transport to the surface.

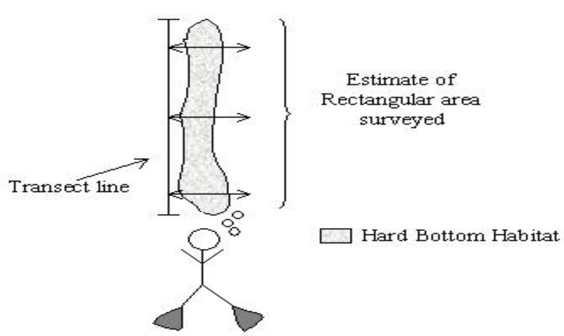

Figure 2. Illustration of the visual survey methodology (diagram not to scale).

Objective 2: Quantify native fish communities to provide baseline population estimates to better understand possible impacts by lionfish.

One dive team will exclusively conduct native fish community surveys using a stationary point count method (Samoilys and Carlos 2000). One diver in the dive team will swim along the transect and randomly stop three times, over hard bottom, and conduct a 5 minute 360º visual census documenting the species and abundance of all fishes within a 10 m radius (Samoilys and Carlos 2000). The point count method attains results not significantly different from transect methods but with improved efficiency (Samoilys and Carlos 2000). Due to time constraints we are limiting the search radius to 10 m or less, so that most fish species can be surveyed. Divers will visually census the more mobile and active species first and then focus on the smaller more cryptic, demersal, species. Each site will also be documented with digital video to have a record of each location.

Objective 3: Evaluate results of lionfish thermal tolerances with field observations and in-situ bottom water temperature collection

In the winter (January/February) of FY 05 ROV surveys will be conducted at selected sites to look for over-wintering lionfish. We expect to spend 5 days on this. As weather permits diver visual surveys will also be conducted during the winter of FY 05. Temperature sensors (HOBO Water Temp Pro, ONSET Corp) will be deployed at each site beginning in the FY 04 sampling period to be retrieved a year later in FY05. This will allow us to determine minimum winter bottom water temperatures at each site and evaluate laboratory results of lionfish thermal tolerance studies.

Objective 4: Use genetic data to characterize population structure in order to better understand the biology of North Carolina lionfish

New species invasions are often the result of relatively few members of a source population becoming established in a new locality or habitat. This is one type of 'genetic bottleneck' and as a consequence only a small portion of the overall genetic diversity found in the source population is present in the founder population, i.e. the 'founder effect' (e.g. Nei et al. 1975, Neel and Thompson 1978). The recent invasion of lionfish along the US Atlantic coast provides a rare opportunity to follow the evolution of an invasive species and study consequences of the founder effect. Genetic data gathered in such an evolution study will also allow an estimation of the effective population size and provide information on the number and nature of invasion events including the source of new North Carolina recruits and the original Atlantic population.

Lionfish will be collected at the two regions described above by divers. Additional US Atlantic coast and Indo-West Pacific (IWP) fishes will be obtained from colleagues and Aquarium fish dealers. Total genomic DNA will be extracted from muscle tissue using a PUREGENE kit (Gentra Systems, Minneapolis, MN, USA), and specific DNA loci amplified and sequenced using standard procedures (e.g. Freshwater et al. 2000, Tronchin et al. 2003). Approximately 1200 bp of *cyt-b* and 540 bp of the d-loop region will be sequenced resulting in ca. 940 synonymously mutating sites from which to establish mitochondrial haplotypes. We have already generated *cyt-b* sequences from a number of IWP lionfish, and 2 New York caught *P. volitans*, and combining these data with that from Kochzius et al. (in press) have detected multiple haplotypes (Fig. 4) and determined better primer sequences. The sequence data generated in this study will be analyzed using methods of coalescence theory (Kingman 1982, Kuhner et al. 1995) and haplotype network analyses

(Templeton et al. 1992, Templeton 1998) using TCS (Clement et al. 2000) and the LAMARC package of computer programs (http://evolution.gs.washington.edu/lamarc.html).

A. Pterois miles **B. Pterois volitans**

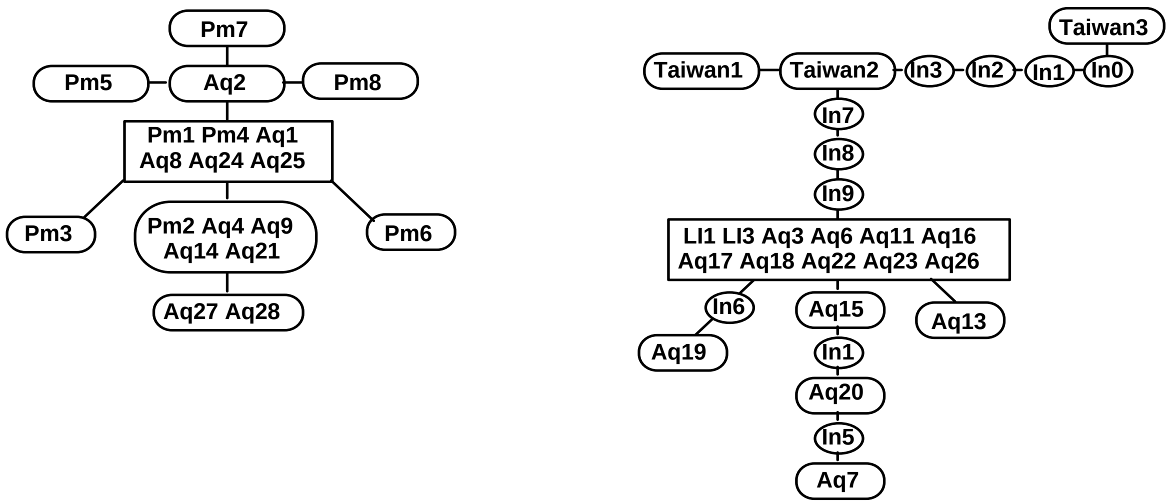

Figure 4. Mitochondrial haplotype networks for *P. miles* and *P. volitans* generated from 421 bp of *cyt-b* sequence using the TCS computer program (Pm = published *P. miles* sequences; Taiwan = published *P. volitans* sequences; Aq = sequences of aquarium fish; LI = sequences of Long Island, NY specimens; In = hypothesized intermediate haplotypes. The data set was truncated to 421 bp in order to include sequences published by Kochzius et al. (in press).

Analyses of the data generated in this study will allow us to address a number of questions concerning the basic biology of *P. volitans* within the Western Atlantic Ocean. As an example, by comparing haplotype networks of NC, other Atlantic (e.g. Florida, Georgia), and IWP populations, we will be able to estimate the extent of the genetic bottleneck that occurred during the invasion event, and begin to follow the evolution of genetic variation within the invasive population. Coalescence analyses will be used to estimate the effective population size, and haplotype and age data will be combined to determine if year cohorts are genetically distinct and if the genetic diversity of cohorts is changing over time. This will provide information on the number and nature of invasion events. Comparing sequences and haplotype frequencies of lionfish from other parts of the Atlantic with NC and IWP lionfish, we may also be able to determine a source population for the NC and Atlantic fish. Based on our limited preliminary data we can already exclude Taiwan as a probable source location (Fig. 4b). The answers to these and other questions are important for the management of invasive *P. volitans* populations and for developing strategies to limit the occurrence of marine fish introductions in the future.

Objective 5: Provide preliminary life history and ecological information for lionfish off the North Carolina coast

Our ability to predict the effect of lionfish is greatly hindered by our lack of knowledge of the basic ecology and life history in the introduced range. What do lionfish eat? How many eggs do they produce? How old are they and how fast do they grow? Without answers to these basic questions, developing any type of quantitative forecast of the abundance or ecosystem effects is impossible. Therefore to maximize the benefit gained from specimen collections, we will conduct a number of laboratory analyses to provide the first available information on the basic life history of lionfish in the introduced range.

Gonads will be used to characterize the basic reproductive biology of lionfish. Specifically, we will estimate fecundity, spawning frequency, periodicity, and seasonality. The right gonad will be preserved in Gilson's fixative for determination of fecundity following the methods given in Cailliet et al. (1986). The left gonad will be preserved in Davidson's fixative for determination of spawning frequency, periodicity, and seasonality following the methods given by Crim and Glebe (1990).

Analysis of the stomach contents will provide information regarding the diet and trophic status of lionfish. The stomach will be removed at the same time as the gonads. Collections will take place as early as possible in the morning to minimize the digestion of prey items already in the stomachs of nocturnal lionfish (Thresher 1984**)**. Collection of fishes at depth may cause regurgitation (Bowen 1992) so once sacrificed, individual specimens will be placed in plastic bags to prevent loss of regurgitated stomach contents. Mouth and gills will be examined for regurgitated prey (Parrish 1987). Gastrointestinal tracts will then be immediately fixed in 10% formalin for a minimum of seven days and then stored in 70% ethanol. Each prey item will be identified to the lowest taxonomic group possible under low magnification. Numerical and volumetric measures of stomach contents will be determined as recommended by Hyslop (1980).

Stable isotope studies will further define the trophic status of lionfish. Carbon and Nitrogen isotopes can provide insight into the sources of primary production supporting lionfish, and nitrogen isotopes are useful indices of trophic level (Thomas and Cahoon 1993). In particular, nitrogen isotopes can be used to trace ontogenetic changes in diet. In conjunction with gut content analyses, stable isotopes will provide a picture of the food web supporting lionfish in the offshore ecosystem. Portions of muscle tissue will be removed from each lionfish specimen, and stored frozen until processing for isotope analysis.

Aging of the specimens by otoliths and/or spines will provide preliminary information on the age structure of lionfish inhabiting the study sites. Because current literature does not include any information on age analysis of this species, we will extract sagittal otoliths and dorsal spines to determine the best structure to use for aging. Otoliths and spines will be sectioned in a transverse plane using a low-speed saw. Annual increments will be counted on sections of both structures using a microscope and an image analysis system. The age data may give an indication of how long lionfish have been in residence, assuming the juveniles settle to the bottom structure and do not migrate. Also, age data combined with fecundity research will be valuable in determining the age at maturity, which is an important characteristic for determining reproductive potential. Further, the age data will allow age-specific genetic analyses (described above).

Dive Operations

These dive procedures were developed for the specific purpose of conducting scientific dives as proposed by National Marine Fishery Service (NMFS) for the assessment of the status and risk posed by the invasive lionfish in North Carolina hardbottom communities.

Therefore, the environmental conditions, expected work tasks and even support vessels may be specific to the diving operations. These procedures have been developed for use by NURC/UNCW and it's visiting investigators. These procedures have proven to be safe, efficient and effective for conducting scientific research at a depth beyond 130 feet of seawater (fsw).

Detailed Dive Procedures

Diving operations will consist of an extended operation cruise (live-aboard) out of Wilmington, North Carolina. Each cruise leg will consist of a five-day excursion, weather permitting. Each leg will depart on Monday morning, returning late on Friday evening.

Operations:	*Leg 1*	*August 2 – 7, 2004*	*Onslow Bay, NC*
	Leg 2	*August 9 – 14, 2004*	*Onslow Bay, NC*
	Leg 3	*August 16 - 21, 2004*	*Onslow Bay, NC*

Prior to each operational dive all key personnel will assemble for a briefing, to include a discussion of the dive profile, objectives, personnel assignments, and other pertinent information. Each dive will consist of a bottom team of at least two divers and a minimum of one safety diver and a fully equipped standby diver.

Research divers (minimum of two) will enter the water together, either following the down line to the bottom or free drift into the study site. Planned maximum depth will be 150 fsw (45.7 meters). Bottom times during the expedition will generally be limited to 20-30 minutes (this time may be extended at the discretion of the Expedition Diving Supervisor). At the end of each dive, the divers will return to the down line and ascend or free drift off the bottom, making the necessary required decompression stops in the water column before returning to the surface.

If at any time, either Bottom Diver breathes their cylinders down to 2/3's of the starting volume (this technique of gas use calculations is also known as or referred to as "the rule of thirds" – the first 1/3 of each cylinder is allotted for use by the diver during the bottom phase of the dive, 1/3 of the cylinder is allotted for use of the diver during the ascent phase of the dive, and the final third is reserved for his/her dive partner in cases of an out of air situation or other emergency contingency), the dive is called and ascent procedures are initiated for both Bottom Divers.

Lionfish possess venomous spines and can cause painful injuries to humans. There has never been a documented case of a human fatality from a sting. Nets will be used to obtain live specimens of lionfish. Each specimen will be transferred to a plastic zip lock bag or zip lock container, sealed and then placed in a canvas tool bag to protected the specimen and prevent accidental envenomation of a Research Diver or Safety Diver during transport to the surface. Heavy weight gloves will be worn at all times while handling specimens and transferring specimens to sample bags.

Each team member will be permitted to conduct two dives a day with a minimum surface interval of three (3) hours between dives. The team size will consist of at least two divers, with the potential for a third diver whom will serve as an on-bottom safety diver. There will be an a.m. and p.m dive evolution with the possibility of each dive member conducting 10 dives total for each expedition leg. Each dive will be supported by the on-deck Diving Supervisor whom will also serve the role of Standby Diver, in the event of an on-bottom diver emergency. There will also be an in-water Safety Diver deployed at the beginning of the decompression/ascent phase of the dive. A small chase boat with operator will be underway during the dives in addition to the primary support vessel that will also be underway. A dive tender will accompany the small boat operator to assist with set-up and deployment of the surface supplied oxygen delivery system for the bottom team and to recover samples and equipment from the Safety Diver as needed.

Gas Requirements

Only NURC/UNCW approved gas mixtures and decompression tables shall be used during the operation. All gases used for diving must be of breathing quality and analyzed for oxygen percent by each individual prior to its use.

All dives will be conducted using "appropriate breathing gasses" as well as NURC/UNCW approved decompression procedures. In general, appropriate breathing gasses must have the following properties: oxygen content (PPO_2) of the "bottom mix" must not exceed 1.45 absolute atmospheres (ATA); equivalent narcotic depth (END) must not exceed 170 fsw; and the PPO_2 must not exceed 1.6 ATA during the decompression phase. For dives up to 150 fsw, the standard decompression gasses will be 100% O_2 20 fsw and 10 fsw.

NURC Dive Staff will conduct gas mixing. The Expedition Diving Supervisor will verify that cylinders are full, gas contents are correct for the planned dive and that all divers have analyzed gases to be used for each dive.

Breathing Gases

Bottom Diver Breathing Gas:
- Compressed air (21%)
- PP02 exposure at max depth of 150 fsw: 1.29 ata

In-Water Safety Diver Breathing Gas:
- Compressed Air (21%)
- PP02 exposure at max depth of 130 fsw: 1.1 ata

Decompression Breathing Gas: * (Only when required by dive plan)
- 36 % Enriched Air Nitrox
- PP02 exposure at max depth of 113 fsw: 1.6 ata

- 100 % oxygen (medical grade) (Planned for use on all dives)
- PP02 exposure at max depth of 20 fsw: 1.6 ata

Decompression Model

All dives will be conducted with the NOAA Air with O2 Decompression Tables, Hamilton Research, Ltd., (2004 July 31 rev.) to allow for pre-dive planning and gas management, excluding in-water safety and standby divers whom will use dive computers. All divers and Dive Supervisors will carry hard copies of these Decompression Schedules as reference. Each diver will be required to wear two (2) digital devices to record depth and bottom time. All divers will conduct deep decompression stops as prescribed by the tables and continue to complete all required decompression for the dive in the most efficient and conservative manner.

Single Dive Profile (Proposed)

Depth (fsw)	Stop Time	Run Time	Mix (O2)
150	25	25	21%
80	1	27	21%
40	2	31	21%
30	2	33	21%
20	5	38	100%
10	11	50	100%

Second Dive Profile after three-hour surface interval (Proposed)

Depth (fsw)	Stop Time	Run Time	Mix (O2)
150	25	25	21% (Decompress on 150/30 schedule)*
80	1	27	21%
50	1	29	21%
40	2	32	21%
30	6	38	21%
20	5	43	100%
10	17	61	100%

* NOTE: Second dive profile decompression information was calculated using Repetitive dive chart, Section D., page 53 from the NOAA Air with O2 Decompression Tables, Hamilton Research, Ltd. 2004 July 31.

Repetitive Dives

Two dives per day will be performed. A surface interval of three (3) hours will be provided for each diver**.** Appropriate decompression calculations and planning will be conducted using the NOAA Air with O2 Decompression Tables, Hamilton Research, Ltd., (2004 July 28 rev.). Repetitive dives are allowed and will be calculated utilizing the same decompression tables used to calculate the decompression obligation from the first dive by using the Repetitive dive chart, Section D., page 53. Paper backups will be laminated and carried by each diver. The minimum surface interval between dives will be three (3) hours. Repetitive dives will be conducted with the same conservatism and efficiency as the first dive.

Diver Qualifications

All divers must successfully complete a diving physical that meets current American Academy of Underwater Sciences (AAUS), NOAA Diving Program (NDP) Guidelines or equivalent medical requirements. All participants will have completed technical dive training from the National Undersea Research Center at the University of North Carolina at Wilmington or an equivalent training program prior to the start of the field expedition. All divers participating on dives that involve required decompression and the use of hyperoxic mixes for decompression will be trained to a Technical Diver Level (See IANTD Training Standards – www.iantd.com). All research divers (bottom team) must have completed at least twelve 12 dives in the past six months and at least one dive to a minimum depth of 130 fsw within 45 days of their planned dives. All divers must have also completed at least one proficiency dive within the previous 30 days period in the minimum equipment configuration to be used on the project dives. This dive may be conducted with a breathing gas of their choice, provided that they have met all other pre-dive proficiency requirements.

Divers who have not completed the prerequisite dive(s) must participate in either the pre-mission work-up dives or conduct a requalification dive(s) under NURC/UNCW staff diver supervision once at the dive site.

Classifications of Divers

Research Diver (2 minimum)
Safety Diver (1)
Standby Diver (1)

Research divers

Those divers traveling to the bottom will attempt to accomplish the mission goals as stated in the research proposal. They will dive to a maximum depth of 150 feet using compressed air (21%) for the bottom gas. All dives will involve light-duty, low task-loading activities such as photography, videography, survey and observation, instrument placement (temp-meter), sampling and specimen collection.

Safety Diver

A minimum of one fully qualified Safety Diver (non-NOAA Diver) will be prepared to assist the research divers during the decompression phases of each dive. The Expedition Diving Supervisor shall monitor dive times and dispatch the Safety Diver from the research vessel with a spare O2 decompression mix to meet the divers at their first decompression stop, transferring the stage bottle to the divers, if necessary. The Safety Diver will then ascend to a lesser depth, but within visibility of the divers at all times, staying out of decompression using a NURC/UNCW approved dive computer. Safety Diver shall begin to ascend at least 5 minutes before the expiration of the no-decompression limit. In case of failure of a dive computer and the absence of a backup computer, the Safety Diver will utilize no-decompression limits derived from the USN Air Decompression Tables. The Safety Diver will remain with the divers throughout their decompression, looking for signs of oxygen toxicity, or any other distress, and render immediate assistance if required. The Safety Diver shall breathe air throughout their dive.

Standby Diver

A fully qualified Standby Diver (non-NOAA Diver) will be located on the primary research vessel and prepared to assist divers in the event of an emergency at depth. The Expedition Diving Supervisor shall monitor dive times and watch for signs of problems, such as the appearance of a diver lift bag or sausage. If dive time indicates that the diver in distress is likely to be on or near the bottom, the Standby Diver may be deployed, wearing full technical gear, 100% oxygen stage bottles, while breathing compressed air. The Standby Diver shall remain with the distressed diver as long as necessary, within his/her own safety limits; however, as soon as possible, the Standby Diver will return to the surface, making whatever decompression stops are necessary. Because of the uncertainty of emergencies, the Standby Diver must carry and be familiar with decompression schedules for the maximum time at depth based on their air consumption rate. The Standby Diver shall remain ready for deployment within 2 minutes of notice of an emergency until notified to "stand down" by the Expedition Diving Supervisor. Such notification normally will occur once the Diving Supervisor is assured that the Safety Diver accounts for all divers.

Dive Vessel Operations

The 65-foot Research Vessel *Cape Fear* will serve as the primary dive vessel. The *R/V Cape Fear* is a fast, comfortable vessel available for research, training, and educational cruises in waters from near-shore to the continental slope. Based in Wilmington, North Carolina, the *R/V Cape Fear* operates either as a day boat or for extended operations up to five days at a time. Operations are conducted from the Chesapeake Bay to the Gulf of Mexico. The *R/V Cape Fear* has berthing for eight scientists and two crew for extended trips. The vessel is equipped with DGPS, Loran, 72-mile radar, SSB and VHF radios, a color scope fathometer and a cellular phone. The *R/V Cape Fear* is constructed of fiberglass and features six independent watertight compartments. The aft work area deck is six hundred square feet with a canopy covering 75% of the area. A water level dive platform provides easy and safe access to the water. A steering station on the aft work deck allows the vessel captain to maneuver the vessel to accommodate science operations. Hydraulic connections, an A frame, a winch, an onboard nitrox mixing station, and a small chase boat provides additional capabilities for scientific research projects. A collapsible recompression chamber, provided by the NDP will also be on board for any decompression emergencies.

The research vessel will remain in a "live-boat" mode during dive operations. The vessel captain is very experienced in supporting dive operations in both anchored and "live-boat" modes. The members of the dive team who are not actively supporting the current dive will assist the boat captain and Expedition Diving Supervisor as required.

Small Boat Support Operations

A rigid inflatable boat, 16-foot Zodiac or equivalent will be available to support all dives during all phases of the Expedition. Should a diver become separated from the line and/or buddy, the small boat will assist, as required. Additional gear in the small boat during dive operations will include; Handheld VHF marine radio (communications to the primary support vessel, food and water), one Oxygen Cylinder -100% oxygen ((for in-water oxygen breathing during decompression phase), throw rope (allows divers to maintain contact with boat during recovery phase) and a sea anchor for drift decompression.

Summary of Support Personnel and Safety Tasks

Mission Coordinator

- Responsible for overseeing all dive operations as well as diver safety
- Coordinates all mission related diving activities with Principle Investigator
- Ensures compliance with the operational dive plan
- Authority to halt diving operations if, in his opinion, the dive conditions or planned activities constitute a hazard to any member of the dive team

Expedition Diving Supervisor (1 each)

- On-deck Diving Supervisor for the day to day conduct of the dives, including mixing of the gases, inspection of the appropriate diving equipment before and after each dive and completion of the daily diving log
- Responsible for overseeing each dive evolution from start to finish, as assigned
- Will coordinate all dive operations with the Mission Coordinator
- Authority to halt diving operations if, in his opinion, the dive conditions or planned activities constitute a hazard to any member of the dive team

Safety Diver (1 each)

- Deployed from the research vessel at predetermined time during dive
- Wear twin cylinders filled with air
- Carry a stage bottle filled with O2
- Meet ascending divers at first gas switch and stop
- Avoid Decompression profile by following dive computer for multi-level dives

Standby Diver (1 each)

- Deployed from the research vessel, when and if directed by the Dive Supervisor

- Wear twin cylinders filled with compressed air
- Prepared to descend to bottom to assist divers when instructed by the Dive Supervisor

Minimum Dive Team Size (4)

- Diving Supervisor (1) * (Will be a qualified DMT, unless another DMT is available topside)
- Research Divers (2 minimum)
- Safety Diver (1)
- Standby Diver (1) **

* Note: The Expedition Diving Supervisor may participate in the daily diving activities by designating a qualified NURC/UNCW Dive Supervisor.

** Note: The Diving Supervisor will also serve in the Standby Diver role.

Diver Down-lines and Moorings

A down-line secured to anchor will be use to mark the intended dive site and can be used as a reference for the bottom team divers to descend and ascent on, as necessary. Until a down-line can be established to the dive site, or if surface currents preclude divers making it to the down-line, the Expedition Diving Supervisor may elect to deploy divers from the primary support vessel up current from the site. The divers will make a normal descent and free drift to the bottom, while attempting to locate the dive site.

Diver Communications

Signaling procedures will be used that differentiate between routine operations (i.e., drift decompression on lift bags) and life-threatening emergencies (i.e., diver entangled on bottom). In an emergency situation, a diver's slate will be attached to the lift bag prior to deployment to indicate that an emergency exists and to describe the nature of the emergency. When the Safety Diver sees the slate, but no diver beneath the bag, the slate will be retrieved and appropriate action taken.

Descent and Bottom Time

Divers will depart the surface as buddy teams and descend together, following the down-line. Descent time is flexible, but bottom time begins on leaving the surface. The buddy teams will remain together throughout the dive, always remaining within sight and easy reach of each other. If the dive team consists of three divers, then two divers will conduct the primary work activities while the third acts primarily as an on-site "on-bottom safety diver;" if there are only two divers, both will perform work activities, as required, but one of the divers will serve primarily as on-bottom safety diver. Initially and whenever possible throughout the expedition, an experienced diver will serve as the on-bottom safety diver on each team. Dives should be planned and executed in such a manner as to avoid gas shortages and in-water decompression times greater then 120 minutes. The "rule-of-thirds" (one third to get to the dive site, one-third to reach the first decompression stop, and one-third reserve) must be followed on all decompression dives.

Ascent and Decompression

Divers will depart the work site together, allowing sufficient time to reach the base of the down-line by the end of the pre-agreed upon bottom time. Then they will ascend together, following the prescribed decompression schedule. Pure oxygen (100%) will be carried by each diver in separate stage bottles. If surface sea conditions are rough, both the 20-foot and 10-foot stops can be completed at a depth of 20 feet; however, O2 exposure is reduced somewhat if some or the entire 10-fsw stop is taken at 10 fsw instead of 20 fsw. It is not considered necessary to breathe the O2 in cycles at 20 fsw, that is, no O2 break is required.

Drift Line for Decompression

Because of the likelihood of currents at the proposed dive sites, the Expedition Diving Supervisor may elect to conduct final decompression using a drift line. This surface marker will be deployed by the bottom team using a lift bag and line reel. The small boat, which will have been standing close by and carefully watching the dive times, will pull alongside the drift line bag. The Safety Diver, from the primary support vessels will enter the water with additional deco gas to ascertain that the divers are okay and oversee decompression process. The boat and divers will then drift together while decompression is completed without adverse effects from the current. A sea anchor will be deployed to reduce additional drag produced by wind and seas, while the small boat drifts with the in-water dive team. If surface sea conditions are rough, both the 20-foot and 10-foot stops can be completed at a depth of 20 feet.

The small boat will deploy a surfaced supplied oxygen deliver system via a scuba second stage. This system is designed to support 4 divers, underwater simultaneously. Divers will conduct a gas switch and remain on 100% oxygen from the surface for the remaining decompression time. The diver carried 100% oxygen stages bottles will only be used as an alternative if the bottom divers are unable to reach the surface-supplied system or if for some reason there is a failure of the delivery system.

Contingency Protocols

In order to ensure maximum safety, the following protocols have been identified and suitable responses developed:

<u>Out of gas, bottom mix</u>: Begin gas sharing with partner, abort dive, observing deco schedule during ascent.

<u>Out of gas, deco staging mix</u>: During beginning of decompression, the Safety Diver should bring one spare staging mix cylinder for each pair/trio of divers, in accordance with normal operational protocol and when in use. Any further stage gas failure would warrant gas sharing of stage mix, if required. Divers shall communicate problem to in-water Safety Diver whom shall fetch and deliver additional spare stage mix cylinder to divers.

<u>Out of gas, deco oxygen</u>: Safety Diver with extra deco gas will be with research divers during the first gas switch for decompression. Any 02 failure from research diver’s deco supply would require a Safety Diver to transport single 02 cylinders for attachment on harness and remain as gas source during completion of decompression. Any further stage gas failure would warrant gas sharing of stage mix if necessary. Divers

shall communicate problem to in-water Safety Diver whom shall fetch and deliver spare stage mix cylinder to diver.

Gas failure, source of problem unknown: Diver will reach back and close isolation valve, and then determine the cause of failure. Notify buddy of problem and abort the dive.

Aborted dive procedures: Until a depth of 150 fsw is reached and/or more than 5 minutes of run time has elapsed the bottom team can abort the dive and return directly to the surface (USN Standard Air Decompression Tables/1999). At such time the divers will be recovered by the primary support vessel and may elect to make a second drop. Divers may elect to deploy a lift bag to signal to the surface support team and dive vessels. On a repetitive dive, the abort procedure will require an additional in-water decompression stop (See Hamilton Lionfish Decompression Tables).

Omitted Decompression: If diver is on air and asymptomatic, diver will repeat all stops deeper and including the 40 fsw stop. The diver will multiply 30, 20 and 10 FSW stops by 1.5. Diver is to maximize P02. Use the most hyperoxic gas appropriate for the depth without exceeding 02 toxicity limits. If diver is symptomatic, diver will be placed on 02, hydrate, placed in the Hyperlite or evacuated to the nearest recompression facility.

Buddy team separation during deploy: If a buddy team finds themselves separated from their buddy (ies) during deployment, then the divers should abort the dive and return to the surface. Divers may elect to deploy a lift bag to signal to the surface support team and dive vessels. At such time the divers will be recovered by the primary support vessel and may elect to make a second drop.

Diver pair unable to reach down-line: If a buddy team is unable to reach the down-line during deployment, than the divers should abort the dive and return to the surface. Divers may elect to deploy a lift bag to signal to the surface support team and dive vessels. At such time the divers will be recovered by the primary support vessel and may elect to make a second drop.

Diver pair unable to locate ascent line: Remain mindful of bottom time (BT). Divers can either shoot a lift bag on a reel to the surface and begin decompression ascent on the bag line, or, if adequate gas supply is available, take an additional 5 minutes to search and extend to the next bottom time group. Be on a line beginning ascent by 5 minutes past original plan. Divers shall carry printed copies of planned decompression schedules. Decompress according to the appropriate schedule. If divers come up on the bag line, surface support will shift to the divers' location, be they drifting or stationary. In the event of loss of ascent line, divers will shoot a lift bag and commence a drifting ascent under the bag. Surface vessel will dispatch Small Boat with surface supplied oxygen delivery system. The Small Boat will maintain station near the surface bag, and deploy the second stage regulators to reach the bottom team at the twenty (20) foot stop

Diver pair ascends on line, boat gone: Expedition divers stay together upon reaching surface. Use appropriate signaling device to signal surface craft.

Divers separated, on dive site: The Bottom Divers will remain in constant contact (visual site) at all times during the dive. At no time during the dive (regardless of visibility), will the Bottom Divers be separated by more then 15 feet. Separated divers will perform a visual search for each other for one minute before returning to the base of the down-line. Once at the down-line separated divers will allow no more than four minutes to reunite. If the divers have not found one another within five minutes they will abort the dive and head to the surface using appropriate ascent techniques and decompression tables.

Divers separated, swept off dive site: Upon separation of buddy pair, unable to locate each other, the divers should independently shoot a bag to the surface and commence their own decompression. Divers shall exercise normal decompression profile, and expect to see Safety Diver in the water above them.

Diver entanglement on bottom: Divers should carry at least two knives and an additional cutting tool, either EMT scissors or a seatbelt cutter. Notify other diver (s) of problem. Evaluate the nature of entanglement and attempt to free self, or signal buddy for assistance. If separated from buddy and entangled without remedy, inflate bag to surface with penciled distress message on slate attached by snap hook to the bag. Standby Diver from primary support vessel will then enter water and search for entangled diver. The other diver, if separated and successfully decompressing on a lift bag, will be accompanied by the Small Boat. Both vessels will maintain radio contact with each other, but the primary support vessel will remain with the entangled diver and the designated Diving Supervisor will monitor the situation topside.

Given this contingency, or similar difficulties in which a pair of divers will need to assist the expedition team at the bottom, the second dive team of the day, if planned will not commence operations until the problem has been resolved and it has been deemed appropriate to make the second dive.

Diver pair swept off dive site: Divers stay together; attempt to regain position on dive site and work to ascent line to abort if necessary. If unable to return to the dive site, abort the dive and commence ascent on an inflated bag. Commence appropriate decompression schedule.

Oxygen toxicity hit: PO2 during all evolutions and decompression stops remains significantly below 1.6 ATA, and divers have been exposed to the elevated PO2 without symptoms. In the unlikely event of any VENTID symptoms, asymptomatic diver will immediately gain control of other diver and begin ascent.

No vessel overhead: Divers complete decompression and remain at surface with the buoy. Await recovery by surface craft.

Buoy/down line breakaway: Divers will shoot bags to the surface on a reel line and decompress on the line in the same manner as if unable to locate the down-line.

Change of environmental conditions during dive: In the time interval between the beginning of a dive and the completion of decompression, it is possible for environmental conditions to change sufficiently to require adjustment to the dive plan.

a. Increase in current strength

A significant increase in current strength during a dive would make it more difficult for the divers to decompress because the down-line is fixed, subjecting the decompressing divers to the full strength of the current. An option would be for “drift decompression”. This is the preferred technique.

b. Increase in surface waves and/or swells

A significant deterioration of sea conditions would make it more difficult for the divers to decompress because the down-line will rise and fall, sometimes violently, as the dive vessel strains on the line, if at anchor. Therefore, decompressing divers must take care not to hold to the down-line too tightly, especially on the shallower stops where the effect is most pronounced. In instances where there is significant movement of the down-line, divers should employ one or more lengths of "Jon line" to dampen the motion. One end of the Jon line is looped around the down-line and the other is clipped to the diver's "scooter ring." Otherwise the dive team should choose to use drifting decompression.

c. Decrease in underwater visibility

A significant decrease in visibility on the bottom would make it more difficult for the divers to work, but also might decrease the safety of the divers. Therefore, if the visibility decreases to less than 10 feet, the divers should consider terminating the dive.

d. Change of water temperature

Water temperatures at the site during the planned expedition dates are usually quite warm, with bottom temperature rarely falling below 65-70 degrees F. However, a decrease in water temperature, due to a deep-layer thermocline or to an alteration of current patterns, will affect diver comfort and, if significant, could affect safety. Divers should wear adequate thermal protection-a well-fitting wet suit and hood or a dry suit. If, during a dive, temperature decreases to an alarming degree, the divers should terminate the dive. The water temperature between 70 fsw and the surface is almost always above 70 ^{o} F, thus making the longer decompression stops quite comfortable.

<u>Hazardous marine life injury from a lionfish</u>: Remove the victim from the water as soon as possible. The wound should be soaked in nonscalding, hot water to tolerance 110 – 114 ° F (43.3 – 50C) for a period of at least 30 minutes. Include an unaffected part of the body in the hot water to “sense” temperature. Administer pain medication. Observe the diver for more severe signs of difficulty breathing or an abnormal pulse (heart) rate. Treat accordingly. Medical assistance should be obtained as quickly as possible.

Training and Workup Dives

Before commencing Expedition dives, the dive team, including Safety Divers, will participate in workup dives, reviewing and exercising all normal and emergencies procedures. Final determination of readiness to dive by any participant will be made by the Diving Safety Officer (DSO) and Expedition Diving Supervisor.

Organization, Personnel, and Responsibilities

Chain of Command (Responsible Agencies)

NURC/UNCW is responsible for the overall planning and direction of the Expedition, as well as for coordinating funding, interagency cooperation, and all other aspects of the Expedition. NURC is responsible for overseeing the diving operations. This includes conducting daily, on-site dive supervision and handling all field logistics. This includes coordinating research diving from the dive vessel, scheduling personnel rotations, procuring the breathing gases, supply diving equipment, operating the Hyperlite - Hyperbaric Stretcher, conducting on-site gas mixing and cylinder filling.

NURC/UNCW Expedition Mission Coordinator, Doug Kesling

Mr. Kesling is Training and Safety Coordinator for the National Undersea Research Center/University of North Carolina at Wilmington (NURC/UNCW). He received a B.S. in Nursing in 1985 from Wright State University, Dayton, Ohio. He was certified as an open water diver by the Professional Association of Diving Instructors (PADI) in 1977 and has since logged over 2600 dives. NAUI, PADI, and the International Association of Nitrox Divers certify him as a Diving Instructor. In addition to numerous other specialty certifications, the International Association of Nitrox and Technical Divers certified him in 2003 as a Trimix Diving Instructor. Mr. Kesling has served as the NOAA -*U.S.S. Monitor* Expedition Diving Safety Officer from 2000 - 2003.

NURC/UNCW Expedition Diving Supervisor, Jay Styron

Principle Investigator, Paula Whitfield – NOAA – NOS/NMFS

Ms. Whitfield is responsible for the overall planning and direction of the scientific objectives of the Expedition.

UNCW - *R/V Cape Fear*, Captain Dan Aspenleiter or designee

<u>Crew Roster</u>

1# Research Diver – NOS/NMFS Diver

2# Research Diver – NOS/MFS Diver

3# Research Diver – NOS/NMFS Diver

4# Research Diver – NOS/NMFS Diver

5# Diving Supervisor/DMT – NURC/UNCW Staff Diver

6# Safety Diver – NURC/UNCW Staff Diver or other designee

7# On-bottom Safety Diver – NURC/UNCW Staff Diver

8# Research Vessel Captain - UNCW

9# First Mate – Small Boat Operator -UNCW

10# Alternate Captain – 24 hour operations – UNCW

Dive Team - Proposed

NURC/UNCW: Douglas E. Kesling, Jay Styron, Glenn Taylor, Lance Horn, Tom Potts, UNCW Ken Johns, Morgan Bailey

NOS/NMFS Personnel: Paula Whitfield, Christine Addison, and Roldan Munzo

Diver Equipment

- Bottom Diver

Double Cylinders (bottom breathing mixture) - minimum 85 cu.ft. cylinders
Cylinders used for bottom phase of the dive
Cylinders will utilize a manifold with isolation valve
Nitrox Stage Cylinder (36% oxygen) – 45 cu.ft. to 71.2 cu.ft.
Cylinder use for decompression phase of the dive
Oxygen Stage Cylinder (100% oxygen) – 45 cu.ft. to 71.2 cu.ft.
Cylinder use for decompression phase of the dive

Standard Tech configuration wing and back plate/harness

- In-water Standby Diver

Double Cylinders (bottom breathing mixture) – minimum 85 cu.ft. cylinders
Cylinders used for support/ascent phase of the dive
Cylinders will utilize a manifold with isolation valve
Nitrox Stage Cylinder– 45-71.2 cu.ft.
This stage cylinder will be used for an "out of air" or "low on air" situation for the Bottom Diver before reaching 113 fsw where he/she can safely switch to the cylinder containing 36% oxygen.
Oxygen Stage Cylinder (100% oxygen) – 45-71.2 cu.ft.
This stage cylinder will be used for an "out of breathing mixture" or "low on breathing mixture" situation for the Bottom Diver during the decompression phase of the dive.

Standard Tech configuration wing and back plate/harness

Dive Assessment

During NURC/UNCW dive operations, the Expedition Diving Supervisor, or their designees, will carefully observe all activities and note the effectiveness of the methodology. Divers will complete debriefing forms following each dive, answering questions concerning all aspects of the dive, including methodology, problems, safety or equipment concerns, etc. Following the mission, the Principle Investigator or his designee will collate this information and prepare a report within 30 days.

Facilities and Equipment

Operations Equipment and Facilities

- Will be provided by NURC/UNCW

Logistics and Support

- NURC/UNCW – SAB/GOM Extended Operations Staff

Research Vessel

- Will be provided by UNCW under contract

Emergency Protocol

On-site Medical Supervision

If an emergency situation arises at-sea during the expedition, The Diving Supervisor will notify the Diver Medic (DMT) of the situation and take appropriate action to safely return the individuals to the *R/V Cape Fear*. Once the patient is stabilized on deck, the DMT will evaluate the patient and recommend appropriate treatment. The DMT will contact the NURC/UNCW Diving Medical Advisor for treatment and evacuation protocol consultation when possible.

The initial protocol for treatment will include all the following steps:

1) Perform a patient assessment and begin basic life support (BLS) if necessary,

2) Administer 100% Oxygen via a positive pressure device or demand valve and check vitals signs,

3) Conduct a five minute neurological examination and/or a secondary assessment of injuries,

4) Direct pressure to bleeding wounds, or pressure immobilization for lionfish envenemation wounds,

5) Stabilize the patient, monitor vital signs, conduct a more detailed neurological examination and prepare the patient for transfer to the Hyperlite, hyperbaric stretcher, or the shore-based hyperbaric chamber. Try to have diver keep still and protect from heat loss,

6) If patient is weak or with altered consciousness, try to start an IV with Normal Saline or Ringer's Lactate at wide-open rate (500-1000 ml/hour),

7) Place patient in the Hyperlite, hyperbaric stretcher or continue evacuation to the appropriate medical facility depending on the nature of the illness, severity, and established standing orders as outlined by the NURC/UNCW Diving Medical Advisor (DMA). Protect from overheating inside Hyperlite.

** Emergency Notifications (an Emergency Assistance Plan for emergency notification is included in this manual as Appendix A*)

Rescue and Assistance Forces

United States Coast Guard (USCG) Search and Rescue facility availability will be coordinated with the Wrightsville Beach, NC USCG station dispatch.

Divers Alert Network (DAN)

The DAN facility is available on a 24-hour basis as a backup source of support for a diving-related emergency. The DAN emergency phone number, continuously monitored, is (919) 684-8111.

Emergency Medical Treatment

Emergency oxygen resuscitator, automatic external defibrillator and a diver's first aid kit will be available at the dive site. A certified diving medical technician (DMT) for the treatment of diver decompression incidents will be available. Should an additional non-pressure-related medical facility be required, the most appropriate of the following facilities will be utilized:

- New Hanover Regional Medical Center – Wilmington, NC
- Duke University Medical Center – Durham, NC

Dr. Cobern Peterson – NURC/UNCW Medical Advisor

- (910) 791-4969 hm (910) 341-5002 beeper

Hyperbaric chamber

A NOAA approved, Hyperlite, Hyperbaric Stretcher will be placed aboard the *R/V Cape Fear* for diver related emergencies. Only NOAA approved chamber treatment tables and procedures will be used. Deviation may in extreme cases, be directed by a qualified Diving Medical Officer or Diving Physician. Prior to operation of the chamber, a pre-dive checklist will be completed.

APPENDIX A

EMERGENCY ASSISTANCE PLAN - DIVE SITE: ONSLOW BAY, N.C.
CMS/UNCW (910) 962-2301 Marine VHF 82A WWVD

EMERGENCY FIRST AID PROCEDURES

1. Remove victim from the water and place in a supine/flat position.

2. Monitor vital signs (resuscitate, if necessary)

3. Administer 100% oxygen if available.

4. If rescue squad/ambulance is unavailable transport victim to nearest medical facility without delay.

5. Provide rescue squad and/or emergency room personnel with details of accident/injury and with diving medical consultation information.

EMERGENCY MEDICAL ASSISTANCE

RESCUE SQUAD: 911 **POLICE: 911** **FIRE: 911**

U.S. Coast Guard Wrightsville Beach, N.C. (910) 256-3469 or VHF 22A

NEAREST MEDICAL FACILITY:	**DIRECTIONS:**
New Hanover Regional Medical Center 2131 South 17th Street Wilmington, N.C. 28403 Phone: (910) 343-7000	Take S. College Rd. North, turn left at 17th. Street extension. Follow 17th. Street to hospital. Follow the signs to the emergency room entrance.

MEDICAL CONSULTATION INFORMATION

NOTE: The organizations/numbers listed below will provide detailed medical consultation information for medical problems unique to diving.

1. Dr. Cobern Peterson (910) 791-4969 (hm) (910) 341-5002 (beeper)
2. Divers Alert Network (DAN) (919) 684-8111 *Call Collect*
3. Naval Diving and Salvage Training Center, Panama City, Florida (850) 234-4651

RECOMPRESSION CHAMBER INFORMATION

NEAREST RECOMPRESSION FACILITY	**RECOMPRESSION NOTES:**
F.G. Hall Hyperbaric Laboratory	Contact Duke University Medical
Duke University Medical Center	Center. Ask for the Hyperbaric
P.O. Box 3823 Erwin Road	Physician on-call. Explain situation
Durham, N.C. 27710	E.R. will arrange transportation to
(919) 684-8111 *Call Collect*	the F.G. Hall Hyperbaric Chamber