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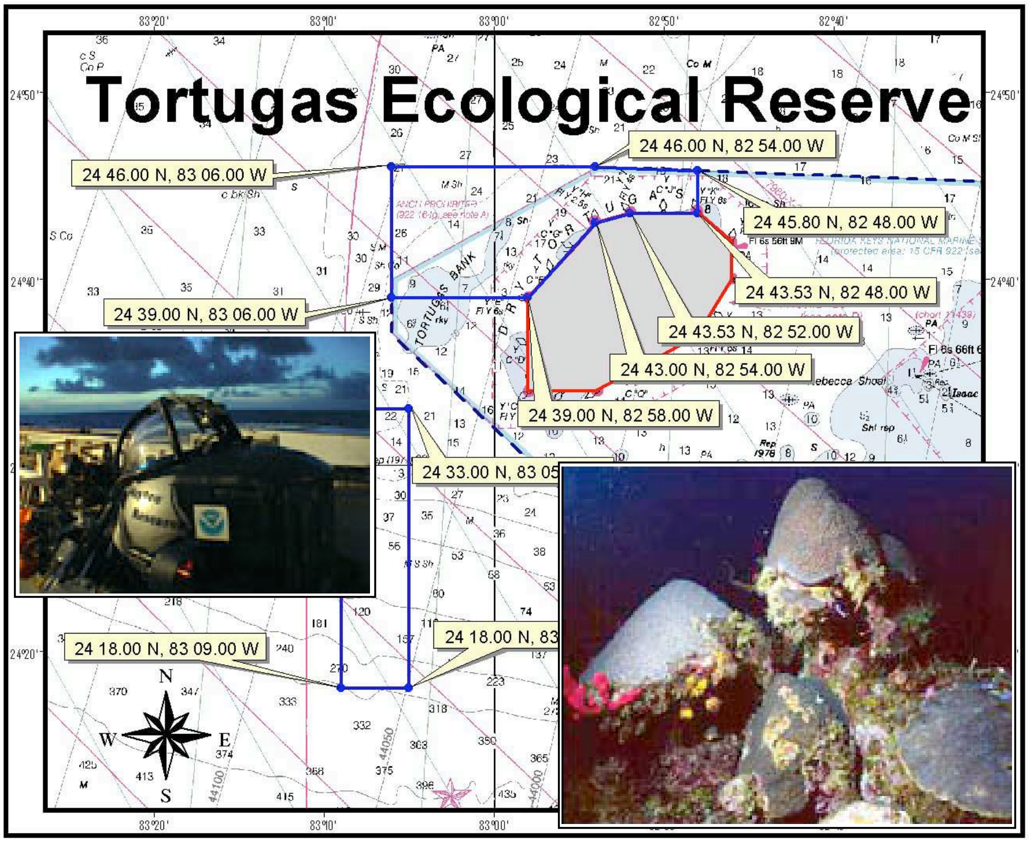

Biogeographic Analysis of the Tortugas Ecological Reserve: Examining the Refuge Effect Following Reserve Establishment

April 2004

U.S. Department of Commerce
National Oceanic and Atmospheric Administration
National Ocean Service
Office of Ocean and Coastal Resource Management
Marine Sanctuaries Division

About the Marine Sanctuaries Conservation Series

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John S. Burke, Michael L. Burton, Carolyn A. Currin, Donald W. Field, Mark S. Fonseca, Jonathan A. Hare, W. Judson Kenworthy, and Amy V. Uhrin

NOAA Beaufort Laboratory

U.S. Department of Commerce
Donald L. Evans, Secretary

National Oceanic and Atmospheric Administration
VADM Conrad C. Lautenbacher, Jr. (USN-ret.)
Under Secretary of Commerce for Oceans and Atmosphere

National Ocean Service
Richard Spinrad, Ph.D., Assistant Administrator

Marine Sanctuaries Division
Daniel J. Basta, Director

Silver Spring, Maryland
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[1] NOAA Beaufort Laboratory, 101 Pivers Island Road, Beaufort, NC 28516;
Point of Contact: mark.fonseca@noaa.gov

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ABSTRACT

Almost 120 days at sea aboard three NOAA research vessels and one fishing vessel over the past three years have supported biogeographic characterization of Tortugas Ecological Reserve (TER). This work initiated measurement of post-implementation effects of TER as a refuge for exploited species. In Tortugas South, seafloor transect surveys were conducted using divers, towed operated vehicles (TOV), remotely operated vehicles (ROV), various sonar platforms, and the *Deepworker* manned submersible. ARGOS drifter releases, satellite imagery, ichthyoplankton surveys, sea surface temperature, and diver census were combined to elucidate potential dispersal of fish spawning in this environment. Surveys are being compiled into a GIS to allow resource managers to gauge benthic resource status and distribution. Drifter studies have determined that within the ~ 30 days of larval life stage for fishes spawning at Tortugas South, larvae could reach as far downstream as Tampa Bay on the west Florida coast and Cape Canaveral on the east coast. Together with actual fish surveys and water mass delineation, this work demonstrates that the refuge status of this area endows it with tremendous downstream spillover and larval export potential for Florida reef habitats and promotes the maintenance of their fish communities.

In Tortugas North, 30 randomly selected, permanent stations were established. Five stations were assigned to each of the following six areas: within Dry Tortugas National Park, falling north of the prevailing currents (Park North); within Dry Tortugas National Park, falling south of the prevailing currents (Park South); within the Ecological Reserve falling north of the prevailing currents (Reserve North); within the Ecological Reserve falling south of the prevailing currents (Reserve South); within areas immediately adjacent to these two strata, falling north of the prevailing currents (Out North); and within areas immediately adjacent to these two strata, falling south of the prevailing currents (Out South). Intensive characterization of these sites was conducted using multiple sonar techniques, TOV, ROV, diver-based digital video collection, diver-based fish census, towed fish capture, sediment particle-size, benthic chlorophyll analyses, and stable isotope analyses of primary producers, fish, and, shellfish. In order to complement and extend information from studies focused on the coral reef, we have targeted the ecotone between the reef and adjacent, non-reef habitats as these areas are well-known in ecology for indicating changes in trophic relationships at the ecosystem scale. Such trophic changes are hypothesized to occur as top-down control of the system grows with protection of piscivorous fishes. Preliminary isotope data, in conjunction with our prior results from the west Florida shelf, suggest that the shallow water benthic habitats surrounding the coral reefs of TER will prove to be the source of a significant amount of the primary production ultimately fueling fish production throughout TER and downstream throughout the range of larval fish dispersal. Therefore, the status and influence of the previously neglected, non-reef habitat within the refuge (comprising ~70% of TER) appears to be intimately tied to the health of the coral reef community proper.

These data, collected in a biogeographic context, employing an integrated Before-After Control Impact design at multiple spatial scales, leave us poised to document and quantify the post-implementation effects of TER. Combined with the work at Tortugas South, this project represents a multi-disciplinary effort of sometimes disparate disciplines (fishery oceanography, benthic ecology, food web analysis, remote sensing/geography/landscape ecology, and resource management) and approaches (physical, biological, ecological). We expect the continuation of this effort to yield critical information for the management of TER and the evaluation of protected areas as a refuge for exploited species.

Key Words: Refuge effect, biogeography, habitat characterization, spillover, Tortugas Ecological Reserve.

BACKGROUND

Tortugas Ecological Reserve

On July 1, 2001, the nation's largest marine Ecological Reserve was designated in the Dry Tortugas. Approximately 70 miles west of Key West, Florida, Tortugas Ecological Reserve (TER) encompasses 151 square nautical miles and is composed of two separate areas: Tortugas North and Tortugas South (Figure 1). Tortugas North, located west of Dry Tortugas National Park (DTNP), covers the northern half of Tortugas Bank, Sherwood Forest, the pinnacle reefs north of the bank, and extensive low relief areas in the 15 - 40 m depth range (Figure 1). Although this area remains open to SCUBA diving (phone-in permit required), the taking of any marine life is prohibited. In addition, strict regulations have been imposed regarding vessel discharges and anchoring/mooring. Tortugas South, located to the southwest of DTNP, includes Riley's Hump, a large, ~ 30 m deepwater mount, as well as, deepwater (~ 700 m) habitats to the south (Figure 1). Recreational SCUBA diving in Tortugas South is prohibited as is the taking of any marine life. Vessels must remain in transit through this area, with the exception of Sanctuary-permitted research vessels. Based upon published information and interviews with experienced commercial fishermen, Riley's Hump has been identified as a potential spawning site for five commercially important snapper species (Lindeman et al., 2000). Other commercially important species are supported by the deepwater regions of Tortugas South, including snowy grouper, golden crab, and tilefish.

NCCOS Mission

The National Centers for Coastal Ocean Science (NCCOS), here headed by the Center for Coastal Fisheries and Habitat Research (CCFHR), and our colleagues at other NCCOS centers (Center for Coastal Monitoring and Assessment) and NOAA organizations (Coastal Services Center, National Undersea Research Center), state governments (Florida Marine Research Institute), and academia (University of South Florida, University of Virginia), are providing critical mission support to habitat characterization and marine reserve questions that are facing TER within the Florida Keys National Marine Sanctuary (FKNMS). CCFHR has researched fishery-habitat interactions in south Florida and the Keys since the early 1980's and brings a wide range of scientific expertise to bear on fisheries and habitat issues. Moreover, we are coordinating this work with the research approach and philosophy of applied studies typical of our other projects in the region - including injury recovery experiments, coral/seagrass monitoring and modeling in the FKNMS, examination of linkages among coral reefs and adjacent habitats in Puerto Rico, Essential Fish Habitat research on the contribution of deepwater primary producers to coastal fisheries, fishing gear impact studies, and long-term studies of icthyoplankton distribution, development and transport mechanisms.

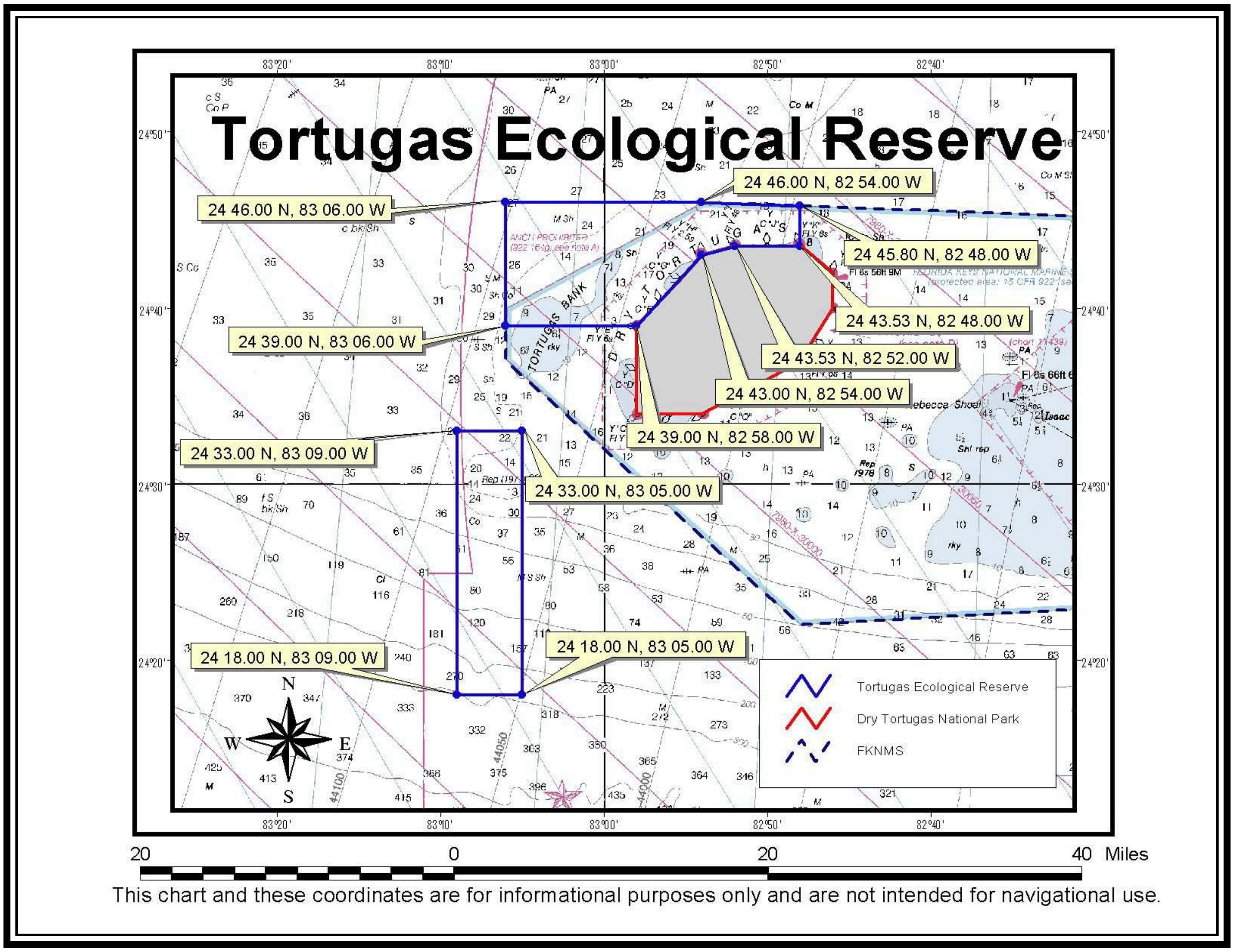

Figure 1. Boundaries of Tortugas Ecological Reserve.

Why Biogeographic Habitat Characterization?

The need for detailed habitat characterization is inextricably linked with the question of where to establish a reserve. Biogeography simply focuses attention on what ecologists have implicitly known for many years; that the geographic context of the biota not only signal the organization of ecosystem processes, but in many instances, act to control or strongly modify those processes. In other words, to examine living organisms without regard to their spatial and temporal organization at multiple scales of organization, and in association with first order environmental factors will fail to elucidate vulnerability, susceptibility, and resilience of the ecosystem. Many reef fishes leave the structure of the reef at night to forage in the adjacent sand, algal, and seagrass flats, thereby importing significant amounts of nutrients onto the reef environment, contributing to its high productivity (Meyer et al., 1983). This mass transfer also ultimately contributes to energy requirements of small grazers that cannot access the adjacent, non-coral reef resources. The adjacent seagrass beds are also significant settlement areas for post-larval reef fishes. Over-fishing of the diurnally migrating fishes and/or physical damage to the foraging/settlement environment could significantly alter TER's productivity and biological diversity. Therefore, habitat characterization is critical to determine the distribution of sessile resources that are susceptible to injury and which may be poised to rebound once any injurious activity is relaxed through implementation of the Reserve.

The Riley's Hump area of Tortugas South also provides us with a setting to expand the investigation of downstream or spillover effects of a refuge with Ecological Reserve status. Past work in this area has demonstrated that fish spawned on the west Florida shelf can provide recruits over long distances downstream. We have built upon this work through studies of larval fish distribution and life history, combined with drifter releases and study of satellite imagery to provide a synoptic assessment of the potential role that Tortugas South refuge can itself play in the maintenance of fish communities elsewhere in the Floridian province.

Lessons for MPAs

The protection afforded by the reserve status provides a unique opportunity to examine the refugia effect in a marine system on an unprecedented scale. More specifically, conducting work in TER provides a unique opportunity to compare the structure and function of a relatively undisturbed system with those elsewhere in the FKNMS and adjacent waters; an amplification of the biogeographic approach. This comparative approach has significant potential for direct application to management issues in other NOAA trust resources. Therefore, findings from this study will have significant heuristic value for consideration of marine protected areas (MPA) elsewhere in NOAA's jurisdiction.

Goals

The ultimate goal of this program is to provide a measurement of the refuge effect of TER. To achieve such an assessment, five areas of concern were identified as the focal points for research: 1) habitat characterization; 2) deep exploration and characterization; 3) biological boundaries; 4) spillover/larval export effect; and 5) effectiveness of MPA in ecosystem recovery. The specific approach for addressing each area of concern is expanded upon in the following sections. A total of eight cruises, utilizing three different NOAA ships, have been conducted in support of this research (Table 1). Details of these cruises in the form of combined progress/cruise reports can be found at CCFHR's web site [http://shrimp.ccfhrb.noaa.gov/~mfonseca/reports.html] (CCFHR, 2001).

Table 1. Completed Research Cruises.

Cruise Name	Dates	Vessel	Sea Days	# Dives
FE-00-09-BL	7/10/00 - 8/4/00	NOAA Ship *FERREL*	20	164
OT-01-01	1/4/01 - 2/13/01	NOAA Ship *OREGON II*	8	0
FE-01-07-BL	4/8/01 - 4/20/01	NOAA Ship *FERREL*	12	55
FE-01-10-BL	6/17/01 - 7/1/01	NOAA Ship *FERREL*	13	111
FE-01-11-BL	7/8/01 - 7/21/01	NOAA Ship *FERREL*	13	86
GU-01-03	7/2/01- 7/3/01	NOAA Ship *GORDON GUNTER*	2	0
	5/11/02 - 5/13/02	*F/V Alexis M* (charter)	3	1
	5/27/02 - 5/30/02	*F/V Alexis M* (charter)	4	12
	6/6/02 - 6/12/02	*F/V Alexis M* (charter)	4	9
	6/23/02- 6/26/02	*F/V Alexis M* (charter)	4	10
FE-02-14-BL	6/17/02 - 7/12/02	NOAA Ship *FERREL*	24	184
FE-02-15-FK	7/15/02 - 7/19/02	NOAA Ship *FERREL*	5	49
	7/23/02 - 7/26/02	*F/V Alexis M* (charter)	4	13
	10/20/02 - 10/23/02	*F/V Alexis M* (charter)	4	8
TOTAL DAYS AT SEA			120	
TOTAL # DIVES				702

BIOGEOGRAPHIC HABITAT CHARACTERIZATION

Physical features and processes are often the driving force behind biological phenomena (i.e., organism distributions). As a result, physical and biological processes may scale in much the same way, which comprises a biogeographic approach to elucidating refugia effects. To understand changes in the distribution and abundance of organisms as a result of the establishment of a refuge such as an Ecological Reserve, knowledge of environmental (habitat) modifications is implicit. Investigations conducted at fine-scales (high resolution) will reveal greater detail about underlying mechanisms but broad-scales (low resolution) are necessary for making generalizations. Maps created from data collected before and after Reserve implementation can then be compared for changes. In addition to map generation, the underlying data can be imported into SAS® software and habitat statistics (i.e., percent cover) can be calculated and compared between years. All these various habitat characteristics become independent variables that will be used in statistical analyses to help explain changes, if any, in faunal abundance and composition as the result of implementation of the Ecological Reserve.

Multi-scale studies offer the best approach for detecting the dynamics and patterns within a system. With this in mind, we wished to examine how high resolution ecological data of a given habitat type can be scaled to the larger spatial context of TER as a function of the survey methods employed. Four habitat survey tools of varying scale/resolution were chosen: 1) satellite imagery (10's of km), 2) aerial photography (10's of km), 3) sonar and towed video (km's), and 4) diver-based video (m's). Geospatial analyses of these data sources will be conducted to determine how each sampling tool describes the emergent features of the landscape based on the tool's inherent sampling range and resolution.

Experimental Design

2000: In the summer of 2000, a year before TER was established, the area within and outside the proposed Reserve was divided into two strata: Use and Depth. Use was broken down into the existing DTNP (Park), the proposed Reserve (not falling within the existing jurisdiction of the DTNP), and a 5 km buffer around the proposed Reserve not within the DTNP (OUT) for before/after comparisons. Within each Use category, three depth strata were arbitrarily defined as: 0 - 15 m (shallow); 15.1- 30 m (medium); and > 30.1 m (deep). The entire sample universe was broken into 1 km square grids which were randomly chosen from within each stratum for sampling. Precise sample locations from within each square km were also randomly chosen at 1 m resolution through additional sub-sampling and locating of coordinates in the field by use of survey-grade DGPS (Trimble GPS Pathfinder® Pro XR/XRS).

We soon discovered, during cruise FE-00-09-BL, that there was highly unequal representation of Depth strata among the three Use strata that would lead to an unbalanced sampling design. Specifically, depths within DTNP were too shallow to encompass all three depth strata. Decreasing the number of potential sample sites (N) within a given depth strata would greatly increase the amount of variability within that strata and prevent a statistically meaningful comparison among Use strata. After much deliberation, we chose to adopt a sampling protocol that focused on habitat interfaces (i.e., areas where coral reef meets seagrass/algal plain) using randomly selected, permanent transects, rather than concentrating on differences in depth. Our video and drop camera (video camera attached to a fixed framework and lowered from a small launch) work from 2000 detailed the extensive areas of potential sand-coral interfaces, essentially running around the entire perimeter of Tortugas Bank and DTNP.

The decision to focus sampling at the habitat interface was based upon several ecological considerations. The interface, or *ecotone*, is used in two fundamental ways in sampling designs. One approach is to use boundaries as ways to stratify sampling, thereby limiting sampling to within a certain class of conditions (e.g., habitat type) and reduce sample heterogeneity. The other approach is to focus on the boundary itself, especially when the exchange or movement of resources (propagules, migrating fauna, energy, nutrients, etc.) is of special interest. We have taken the latter approach because these boundaries are not absolute and we hypothesize that energy flow across these boundaries is critical to understanding changes among strata as a result of Reserve implementation. We pose this hypothesis because our previous work on the west

Florida shelf revealed, through stable isotope analysis of organism tissue, that the primary producers driving fish production, even fishes captured on hard bottom areas, were benthic micro- and macroalgae and the deep water seagrass, *Halophila decipiens*. This fact, taken with the observation that over 70% of TER is non-coral habitat, further strengthens the idea that the areas surrounding the coral formations are a crucial source of energy for the maintenance of the coral reef ecosystem. Finally, considering that predation is often high in low relief areas, especially at interfaces, the structure and composition of fish communities near these interfaces, along with the structure of the physical landscape, should be areas where changes resulting from Reserve implementation fast become evident, if they occur at all.

Given that we have no independent replication at the Use strata level, we adopted a Before - After Control Impact (BACI) sampling strategy (Underwood, 1991). We have the added advantage of not only the unaltered, Out strata as a comparative sample (BACI control), we have the Park as a long-term control and potential comparative sample representing a mature community, free from consumptive harvesting impacts. With the permanent transects in place and time zero data in hand ("before"), we are now poised to conduct the "after" assessments and document the efficacy of TER designation.

2001-2002: The random selection of permanent transects allowed us to stratify sampling by using previously defined Reserve, Park, and Out strata. In addition, lines were drawn through the longest axis of Tortugas Bank and DTNP, normal to the prevailing northwest-southeast currents and bisecting these features into areas facing either upstream (North) or downstream (South; Figure 2). Thus, the interface zones along both of the large reef structures in Tortugas North (Tortugas Bank and DTNP) were designated as one of six categories: 1) Out North; 2) Out South; 3) Park North; 4) Park South; 5) Reserve North; and 6) Reserve South (Figure 2).

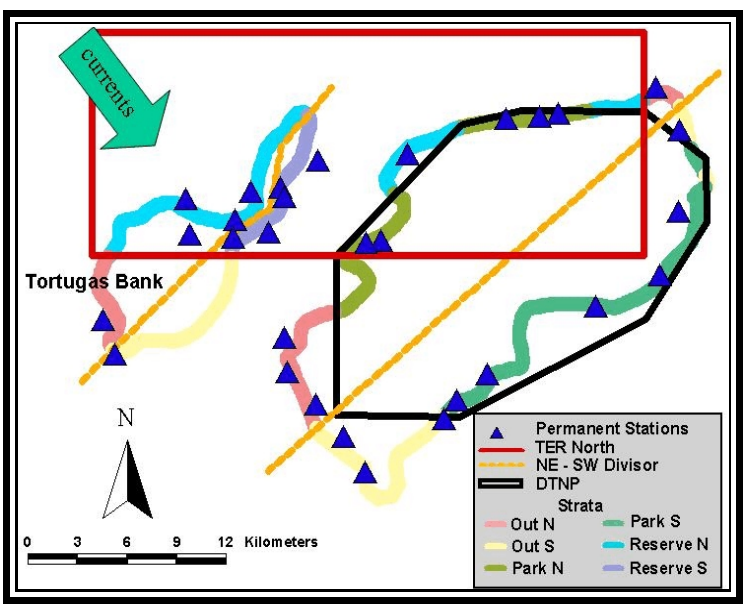

Figure 2. Location of interface strata and 30 permanent stations.

To choose the five random transects from within each of the six categories, we used ESRI's ArcInfo® software and imposed a line at roughly the 10 fathom isobath around the perimeter of the two large coral features as this roughly approximated the location of the sand-coral interface. Each line was then broken down into the six categories and random distances, 50 m apart along each line type, were selected. It was estimated that 50 m would allow for visual isolation of potentially adjacent sites, an important factor for our fish visual census method. The selection of random locations along line type was continuous across the entire landscape, even though line types were segmented among the two large coral features, yielding true randomization. Random points were spaced 50 m along segments so that visual census methods would not overlap in the event two random numbers were adjacent to each other (which did not occur).

Sampling Approach

Fine-Scale Mapping: During cruises FE-01-10-BL, FE-01-11-BL, and FE-02-14-BL (Table 1), detailed mapping of benthic composition was conducted at sub-centimeter resolution, at each permanent station. Divers were deployed at each permanent station to conduct video transects of benthic habitat and coral presence/absence surveys. In 2001, small launches navigated to the interface of each station using DGPS (Trimble GPS Pathfinder Pro XR/XRS). Coordinates for each interface had been recorded during previous mapping operations. Divers operating from the launch established semi-permanent rebar stakes at each interface. A pop float attached to the stake was released by the divers and the location of the float was recorded topside using DGPS. In 2002, when previously installed markers were not located at the specified drop point, divers searched the area for approximately five minutes. If the marker was found to be > 20 m from the drop point, a pop float was deployed at the marker and a new coordinate was recorded by personnel at the surface using DGPS. If the search revealed no marker, a temporary marker (rebar stake) was installed and removed at the end of dive activities.

Each year, divers followed transect lines beginning from the permanent/temporary marker at the interface and running 30 m out in either direction, perpendicular to the interface (sand plain vs reef). One diver used a digital video camera (SONY DCR TRV900 MiniDV Handycam® camcorder) contained in an underwater housing, to record the substrate along the length of each transect at 40 cm above the substrate. The camera unit was equipped with laser pointers and a 40 cm rod that allowed the video image to span a fixed 0.4 m^2 area. The second diver completed a coral presence/absence survey along the length of the reef transect out to 1 m on either side of the transect line (Wheaton et al., 2001). In addition, presence of lobsters was noted. Habitat cover along each transect will be determined using Point Count for Coral Reefs software (Wheaton et al., 2001). Video analysis and analysis of coral presence/absence data are currently underway at CCFHR.

Coarse-Scale Mapping:

Towed Video/Sonar: As part of our design to compare differing methods of site characterization, we conducted extensive benthic habitat mapping of the 30 stations during cruises OT-01-01, FE-01-07-BL, FE-01-10-BL, FE-01-11-BL, and FE-02-14-BL (Table 1). For

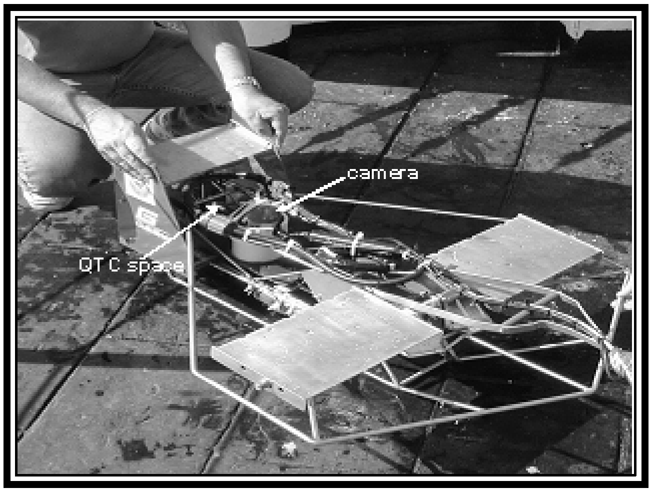

Figure 3. MiniBAT® tow body.

this work, we employed three different sampling tools. First, we used a MiniBAT® tow body housing a downward facing SeaViewer® color Sea-Drop camera to videotape the seafloor at ~ 10 cm resolution. In some locations, we also used a QTC VIEW® seabed classification system with ~ 1m resolution (Figure 3). Video was recorded onto either digital, SVHS or VHS tapes and the exact time and location along each transect was stamped onto the video using the Horita® GPT-50 GPS video titler linked to a Trimble GPS Pathfinder Pro XR/XRS. Track lines were recorded using Trimble ASPEN® software. A maximum of three parallel tracks of approximately 1 km in length and separated by a distance of ~ 200 m were made at each point, running parallel to the reef face. In order to provide a more geographically balanced survey (which facilitates geospatial analysis required under our characterization plan), additional mapping was conducted at each station by making 0.25 nautical mile "S" turns with the MiniBAT at the interface between sand and coral, running parallel to the depth contour and normal to the initial, three parallel track lines. During cruises FE-02-10-BL and FE-02-14-BL (Table 1), we mapped the stations using a sidescan sonar (Sport Scan®, Figure 4). Again, the location of the reef/sand plain interface within the vicinity of the station coordinate was determined using the ship's fathometer. A maximum of three parallel tracks (~ 500-1000 m long) were made at each station, running parallel to the reef face. On several occasions, the MiniBAT was run simultaneously with the Sport Scan as a means of video-calibrating the sonar images. On several occasions, a RoxAnn® Groundmaster seabed classification system was deployed and run simultaneously with the MiniBAT unit.

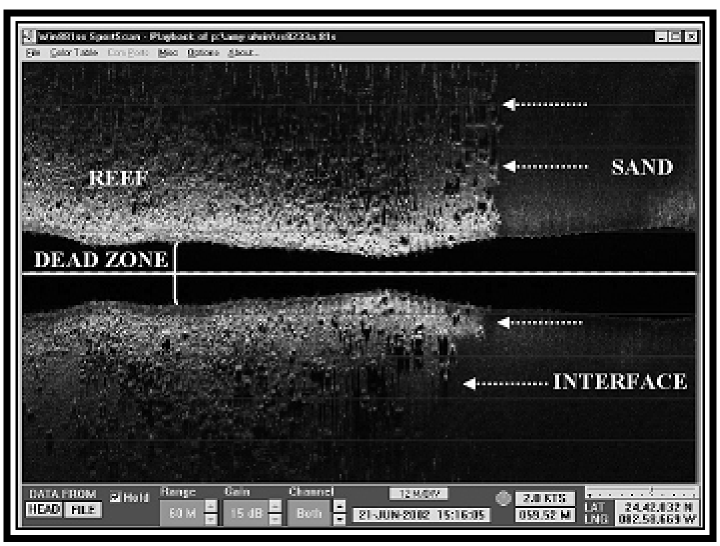

Figure 4. Sidescan sonar image of a typical interface zone. The "dead zone" is a blind spot extending out at a 40° angle from directly beneath the tow body.

Track line files generated in ASPEN were exported to Microsoft® Excel. The times and coordinates displayed on the videos correspond to the chronologic records in the ASPEN-generated Excel spreadsheet. While the video is playing, CCFHR staff record a habitat code every five seconds based upon what is viewed in the video frame at that time. The track line spreadsheet, complete with habitat classification, is converted to a text file and imported into ESRI's ArcView® software. In ArcView, the habitat codes are assigned unique color values. The color-coded track lines are then displayed on a chart of the Dry Tortugas, effectively creating a habitat map of the area (Figure 5).

In a similar fashion, QTC VIEW and RoxAnn data are being compared for errors of omission, commission, and general agreement, both to each other, and to the associated downward facing MiniBAT video. These comparisons will determine the most efficacious (of the three) sampling tool for this resolution and application. Like ASPEN, data files collected in QTC VIEW and RoxAnn also contain time and location fields. In addition, these files contain habitat classification variables. Classifications from QTC VIEW and RoxAnn are compared to each other and to video track lines (as classified above) using SAS and omission and commission computed by simple differencing. SportScan images will be calibrated with associated video transects as well. Analysis is on-going at CCFHR.

Aerial Photography: The coral, algae, and seagrass habitats of the Tortugas have been mapped only once in recent times, using color aerial photography from 1991. Due to the scale of the photography (1:48,000), and water quality conditions at the time, considerable habitat was left unmapped. In fact, GIS-based comparisons of habitat characterization of the deepwater edge of the DTNP reef system by CCFHR using sidescan sonar and underwater video, has indicated that the 1991 site characterization, which utilized aerial photography and ground truthing, underestimated coral habitats by at least 28 percent. This indicates that aerial photography is a deficient means to characterize the full extent of this reef system.

CCFHR, in cooperation with NOAA's Coastal Services Center , is undertaking a remote sensing effort aimed at updating the 1991 base map, and examining the scale and depth limitations of various remote sensing platforms. CCFHR has contracted for new aerial photography for the entire DTNP, and has acquired a 2001 IKONOS satellite image for the area around Fort Jefferson.

In 2002, three hundred random points were generated in the area surrounding DTNP for use in ground truthing activities. Using a small launch, we navigated to each point and lowered a Sea-Drop camera. Notes were made on the habitat type encountered and the depth was recorded. A total of 84 points have been visited. These observations will serve as accuracy assessment data for the aerial photography to be flown in the near future as conditions permit. In addition the new image sources will be compared to the 1991 aerial photos and in situ data collected by SCUBA divers, sidescan sonar, and underwater video, to evaluate what managers can expect from different scales of data and remote sensing platforms.

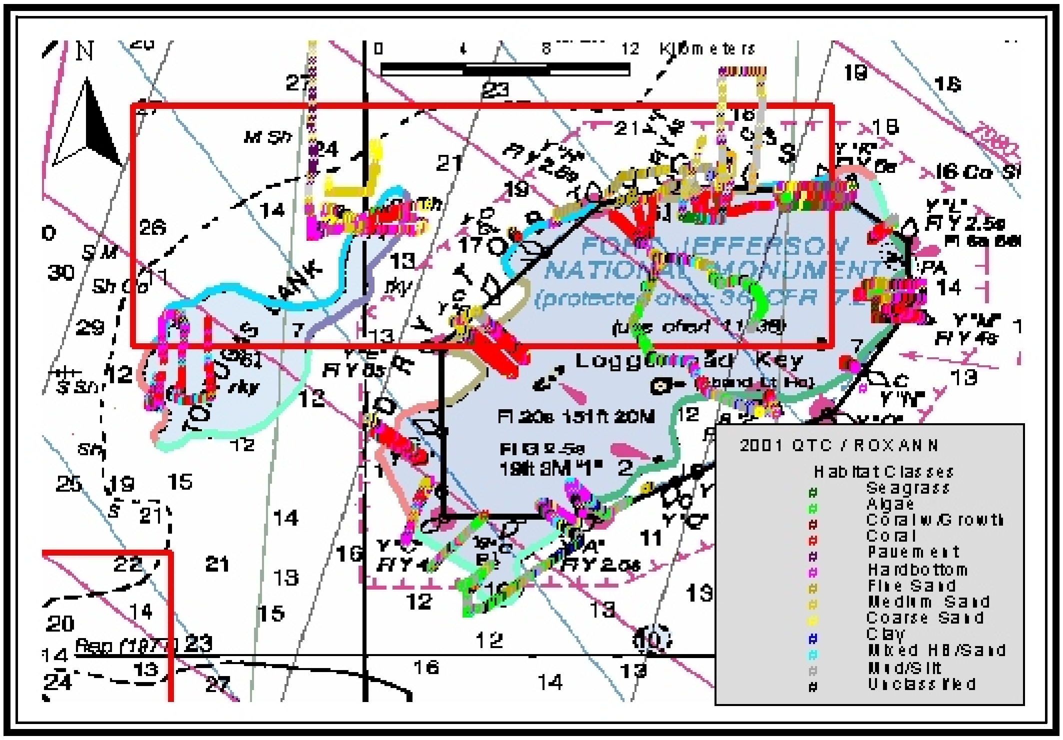

Figure 5. Track lines with habitat classification taken from 2001 RoxAnn transects.

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Sediment Characterization: Sediment particle-size is an indicator of relative wave and tidal energy at a site, and particle-size in turn influences the species composition of benthic flora and fauna found at a site. We obtained baseline data on these sediment features in order to aid our interpretation of changes in benthic communities by strata and/or over time. While conducting habitat transect videos, divers also collected four sediment cores (3 cm diameter) along the interface at each permanent station. Three of the cores were used for benthic chlorophyll analysis and the fourth for sediment particle size. Sediments at all sites sampled within TER were predominantly sandy, with the mean sand content ranging from 70 to 78% (Table 2). Park stations had a slightly greater silt content (mean = 22%) than reserve and out stations (mean = 14% and 17 %). A number of stations were sampled outside the northern boundary of TER, and sediments here were predominantly silty (Table 2). Because of the depth of all stations outside TER, these samples were obtained by using a PONAR grab sampler, rather than diver collections.

Ship's Computer Systems (*SCS*)

For each cruise utilizing the larger NOAA vessels, a copy of the Ship's Computer System data were provided to the science party. These data were recorded on 1-10 second intervals, depending on the parameter, and include vessel location, speed, heading, and water depth. Hard copies of watch logs, which includes hourly meteorological observations were also provided to the science party and remain on file at CCFHR.

Table 2. Sediment percent composition across TER.

Strata	N	Parameter	Mean Percentage	Standard Error
Out	15	gravel	7.92	1.73
Out	15	sand	78.28	1.76
Out	15	silt	13.79	1.18
Park	20	gravel	7.89	1.57
Park	20	sand	69.76	2.13
Park	20	silt	22.34	2.01
Reserve	13	gravel	7.29	2.14
Reserve	13	sand	75.77	4.19
Reserve	13	silt	16.94	2.51
northern boundary	3	gravel	0.00	0.00
northern boundary	3	sand	17.35	0.81
northern boundary	3	silt	82.65	0.81

DEEP EXPLORATION AND CHARACTERIZATION

Remotely Operated Vehicle

For the deepwater habitats at Tortugas South, depth-stratified transects (30 m increments) were mapped up to a depth of ~ 250 m using a Phantom® S2 remotely operated vehicle loaned to us by NOAA's National Undersea Research Center (NURC) in Wilmington, North Carolina and operated by NURC staff during cruise FE-00-09-BL (Table 1). Video was recorded onto Hi 8 video tapes. The ROV video was automatically titled with the time and location, updated every second. Track lines were recorded using Trimble ASPEN® software.

Video processing methods are the same as those outlined above for the MiniBAT video. Analysis of video collected with the Phantom in 2001 on Tortugas South is approximately half completed. These videos are also being assessed for their utility as a fish census tool.

Figure 6. The *Deepworker* one-person submersible.

Sustainable Seas Expeditions (*Tortugas South and Crepuscular Fish Migration)* In 2001, this project received one complete day at sea, plus follow-up work on a second day, while aboard NOAA Ship *GORDON GUNTER* (Table 1), focusing on the deepwater of Tortugas South, as well as crepuscular fish migration assessments at Tortugas North. We requested that the *Deepworker* manned submersible (Figure 6) focus on areas deeper than 240 m in Tortugas South to complement surveys we conducted in 2000 and others which we are coordinating with the USGS. In addition, we stationed the *Deepworker* in 45 m of water at an interface site to video tape and observe any crepuscular fish congregation and migration out onto adjacent non-coral habitat. We were particularly interested to determine whether there were any changes in the occurrence of crepuscular fish migration off the reef onto the surrounding flats when there are structural guideposts (i.e., boulder fields - some kind of structure sprinkled out onto the flats) as opposed to a non-structured coral/sand interface. If there are “hot spots” of migration that could be predicted from the landscape, it would allow us to make recommendations as to the level of protection that should be afforded certain features.

Approximately 2 km (linear distance) of interface habitat were video recorded by *Deepworker*, piloted by Steve Baumgartner (FKNMS), at depths of ~550 m in Tortugas South. These videos and associated track lines are stored at CCFHR and will be interpreted and plotted in the same manner as our MiniBAT and sonar data. *Deepworker*, conducted several hours of surveillance during two evenings in early July 2001, working along the coral/sand interface, stopping and observing, and then moving on. Digital video was recorded at all times. Large snapper aggregations at the reef edge were taped. The rapid migration of fish onto the sand/seagrass/algal areas repeatedly observed by the pilot were not taped as this would have warranted the use of artificial lighting that could have affected the behavior of the fish under observation. The pilot was interviewed on tape immediately after his main dive to recount the species of fish, their behavior, and impressions of the activity. The pilot’s observations revealed that this phenomenon was striking and pervasive, providing qualitative evidence for the mechanism by which primary production on the seafloor surrounding the reef may be transferred back to the reef proper (i.e., as material in fish guts or as fish tissue fluxing across this interface) further supporting this long-held contention (McFarland et al., 1979; Helfman et al., 1981; Meyer et al., 1983). Besides crepuscular massing and migration, there appeared to be a hierarchy of species movement, beginning with larger grunts and ending with smaller species.

BIOLOGICAL BOUNDARIES

The aforementioned observations of crepuscular fish massing at the ecotone and subsequent movement onto the adjacent, non-coral habitats leads us to consider the definition of habitat boundaries. The spatial organization and relief of physical boundaries, such as we are characterizing for each permanent station may play a profound role in the facilitation of fish movement between the reef and non-reef environments. For example, an abrupt, high relief interface may be less conducive to fish movement and feeding off the reef than a lower relief coral habitat that is anastomosing with sand features, eventually giving way to wholly non-coral, sandy bottom. The latter habitat could conceivably act as a stepping stone for various fish, as they move among habitats. While this is untested here, such radical variation in physical structure has been shown in other ecosystems to be strongly related to animal migration and habitat linkages (With et al., 1997). Therefore, we are focusing on a new definition of these habitats, based on biological boundaries that includes physical organization of the area and food quality in addition to organism movement.

As scientists and managers, we tend to scale experiments and establish ecological boundaries based upon our own experiences and our human perceptions of what they should be. It is especially important to avoid this pitfall when considering areas for reserve or protected area status. A prime example is the targeting of coral reefs for protection. Because reefs are highly productive, massive structures, and are linked with several commercially and economically valuable fish species, it is easy to overlook the importance of less structurally diverse habitats adjacent to reefs. In the late 1970's and early 1980's, interest in the movement patterns of reef fish, particularly migrations between resting and feeding areas, began to take hold. Numerous tropical fish species were reported to migrate from coral reefs out into the surrounding seagrass to feed during sunset hours (McFarland et al., 1979; Helfman et al., 1981; Meyer et al., 1983). The extent of this migration defines the biological boundary and it is of utmost importance to define these boundaries when proposing the physical borders of a potential reserve.

Stable Isotope Characterization of Food Webs

The importance of off-reef migration should be reflected in food web analyses. Multiple stable isotope analysis has been used to trace the sources of primary production contributing to the food web in a variety of marine environments (Fry et al., 1982; Peterson et al., 1985). In addition, stable isotopes can be used to track animal movements between habitats (Fry et al., 1999). Only a few studies have employed stable isotope analysis to identify sources of C and N supporting reef production (Yamamro et al., 1995; Heikoop et al., 1998), and used this approach to examine food webs supporting fish production in reef environments. We proposed to demonstrate the trophic linkages between secondary production of reef fishes with the primary production occurring outside the coral reef physical boundary, specifically, the transfer of benthic primary production of seagrass, macroalgae and microalgae in the benthic habitats adjacent to the reef environment to fish and shellfish occupying the reef.

We collected samples for use in a multiple stable isotope analysis of the food web supporting fish production in TER. Samples collected from within the permanent stations included primary producers (phytoplankton, benthic microalgae, benthic macroalgae, and seagrass) and secondary consumers (fish, crabs, and shrimp). Several methods of collection were employed including hook and line from the research vessel, divers armed with sling spears, beam trawls, hand collection by divers, and bucket/Niskin Bottle casts. This sampling targeted specific species from different levels of the food web in order to examine trophic relationships in the reef-interface zone.

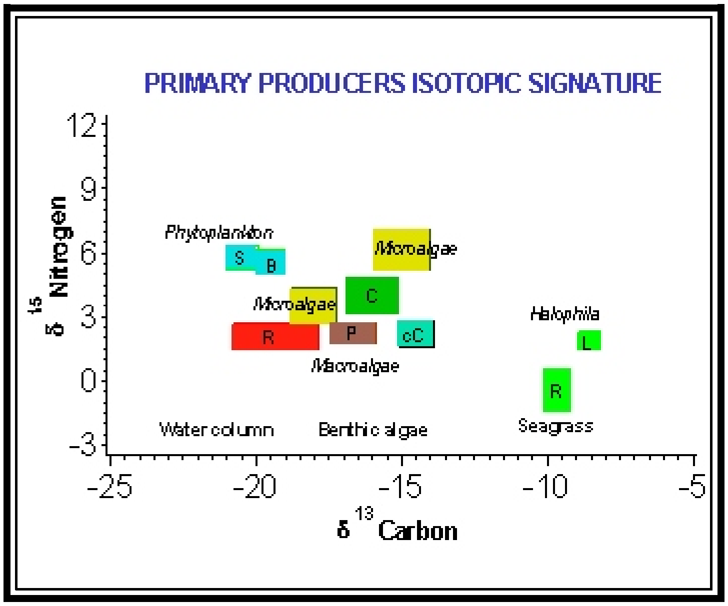

Figure 7. C and N isotope values for primary producers collected from the west Florida shelf in 1999. Boxes represent mean +/- one standard error of values for the following groups: Phytoplankton (S=surface water, B=bottom water; benthic microalgae; macroalgae (R=rhodophytes, C=chlorophytes, P=phaeophytes, cC=calcareous green algae, and *Halophila decipiens* (L=leaves, R=root/rhizomes).

A sister project on the west Florida shelf (WFS) examined the food web supporting fishery organisms associated with deepwater seagrass and associated algal communities on the sandy bottom, as well as, fish from the pelagic zone and adjacent hard bottom habitats. In Figure 7, the isotope values for various primary producers are given. Stable isotope results for fauna demonstrated that fish collected from all areas on the shelf, including pelagic and hard bottom habitats, were supported by food webs based largely upon the benthic primary producers found in the sand/seagrass community as opposed to water column constituents (Figure 8). Results from this study indicate that regions once thought to be “barren” are indeed essential fish habitat.

The number and distribution of primary producers is more complex in TER than on the WFS, and benthic algae are components of both the reef and the interface area. Coral biomass is an additional trophic resource, and we have not completed our isotopic characterization of TER corals. Nevertheless, the majority of the fish analyzed so far exhibit a C isotope signature of -16 or less, consistent with a food web based on benthic primary producers (Figure 9). Penaeid shrimp (Penaeidae), flounder (Bothidae) and gray snapper (Lutjanidae) samples exhibited the most enriched C values, consistent with a food web based in part on seagrass carbon. Some fish, such as red grouper (family Serranidae) and parrot fish (Scaridae) exhibited a wide range in C isotope values. Additional results will help us to determine whether there is a significant geographic or reserve effect on the food webs utilized by these fish.

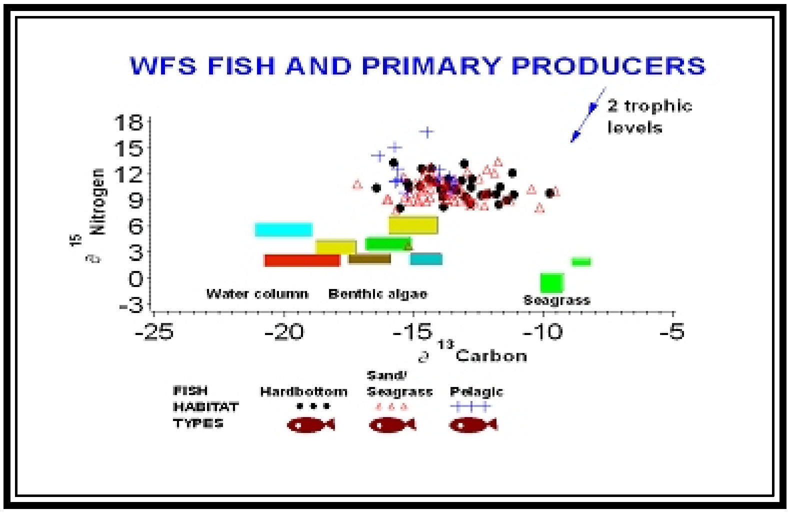

Figure 8. Comparison of stable isotope values of fish and primary producers on the west Florida shelf. Primary producers are as described in Figure 7. Fish include samples from pelagic, hardbottom, and sand/seagrass habitats. Arrows in upper right indicate the offset expected in isotope values of an animal feeding two trophic levels above primary producers. Animals are approximately one and three per mil enriched in C and N, respectively, from their food sources.

Nitrogen isotope values are helpful in determining ontogenetic changes in fishes diets, and particularly in detecting increases in trophic level. This is due to animals preferentially retaining ^{15}N, so that there is an approximate 3 per mil increase in $d^{15}N$ per trophic level. This approach can be used to help determine whether ontogenetic diet changes include a switch from herbivory to carnivory (Cocheret de la Morineire et al., 2003). Figure 10 shows an increase in nearly two trophic levels as red grouper (Family Serranid) increase in size from 25 to 70 cm. Parrotfish (Family Scaridae), however, exhibit little trophic change between 8 and 25 cm length (Figure 10). These data can help to predict the potential ecosystem effects of changes in average fish size as the result of no-take regulations.

In order to further elucidate the food web supporting fishery resources in TER, we will explore the use of fatty acids as biomarkers to distinguish among primary producers. Fatty acids have been used in a variety of environments to supplement stable isotope analysis; although, studies are few due in part to the laborious nature of the analysis. For a subset of samples, primarily reef fish, we will obtain analysis of the fatty acid composition of body tissue to determine whether this might be a useful approach.

Sources of Primary Production

Benthic chlorophyll analysis of surface sediments provides an estimate of the benthic production and microalgal food resources available at a site. Changes in benthic chlorophyll values might be due to changes in grazing pressure (top-down), or changes in light and nutrient availability (bottom-up).

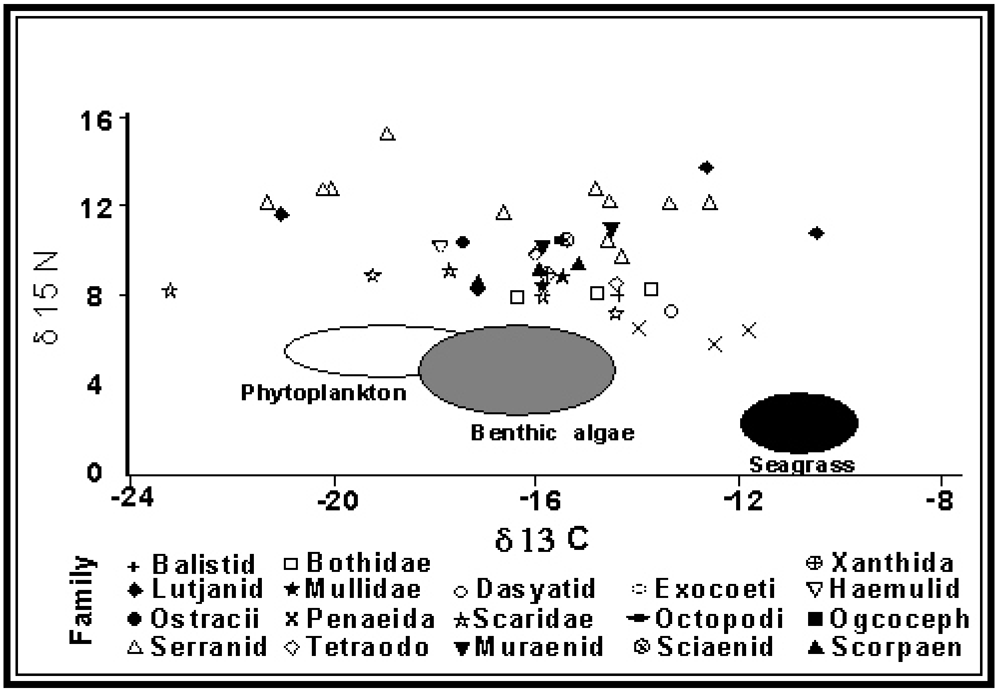

Figure 9. Carbon isotopic signatures for various fish families found in TER.

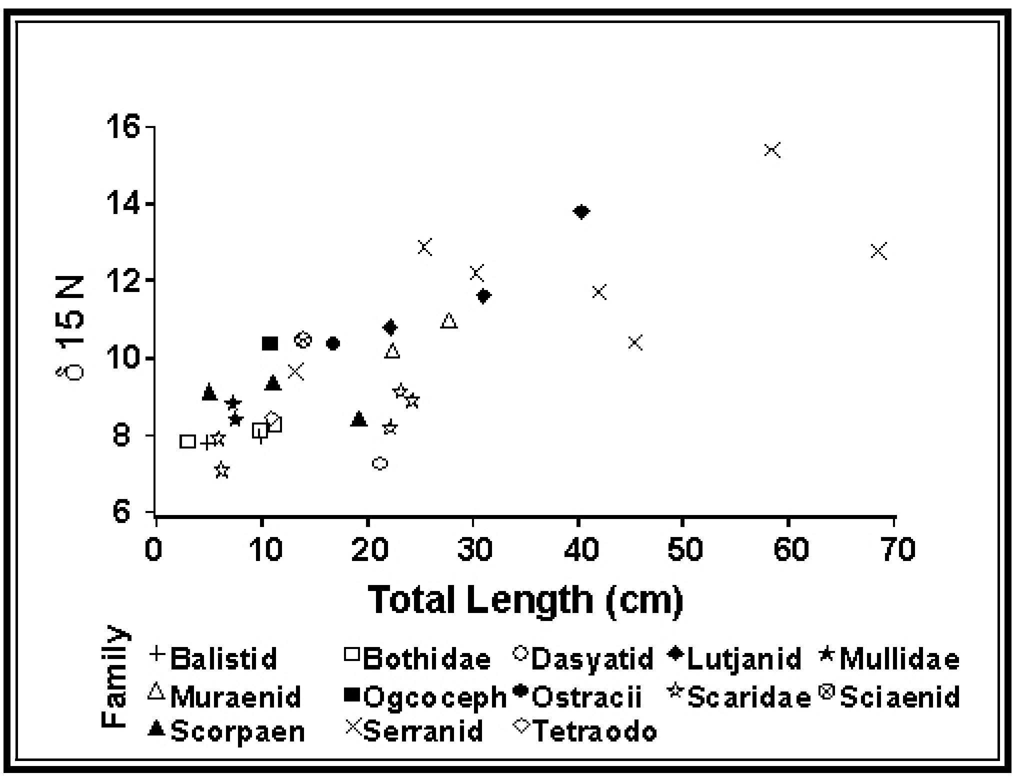

Figure 10. Nitrogen isotopic signatures (by total Length) for various fish families found in TER.

We collected samples of benthic and water column chlorophyll to estimate the biomass of benthic and planktonic microalgae. This data aids in our interpretation of stable isotope analysis of TER food webs, and also provides an additional environmental baseline in which we may be able to detect changes as a result of the imposition of TER. Most benthic chlorophyll samples were collected with small (1.1 cm diameter) syringe-cores by divers, while in deeper waters (> 35 m) surface sediments were collected by a Ponar grab sampler. Surface waters were collected by either bucket casts or with a Niskin bottle. Subsurface, or bottom water, was collected with a Niskin bottle approximately 1 m off of the bottom. We measured nutrient concentration in a subset of the water samples. These measures give us additional information on the physico-chemical nature of TER habitats. In 2000, benthic chlorophyll samples were collected from 26 stations from two depth strata from stations in the Out, Reserve, and Park strata of TER. There is a clear effect of depth on benthic chlorophyll values, as samples from shallow stations (< 12 m depth) had nearly twice the benthic microalgal biomass as stations at depths greater than 12 m. The average benthic chlorophyll from all shallow stations was 71.7 mg chl m^{-2}, while the average value from deeper stations was 41.9 mg chl^{-2}. There was not a clear effect of Reserve or Park on benthic chlorophyll values, although the highest values at both depths were from stations in the Reserve.

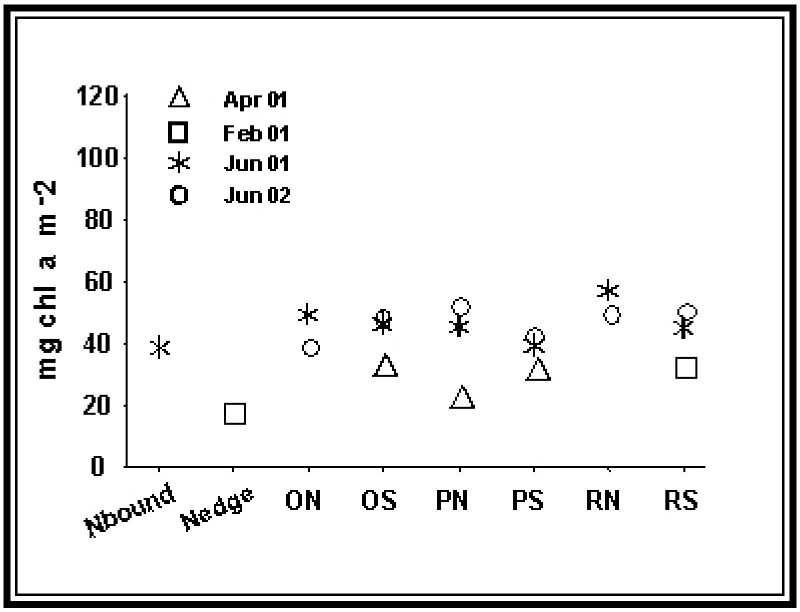

Figure 11. TER benthic chlorophyll concentrations at the 0 - 0.5 cm layer.

In 2001 and 2002, benthic chlorophyll samples were obtained from the 30 permanent stations, as well as from a few stations at the northern boundary of TER, in order to estimate benthic microalgal biomass. In 2001, several stations were sampled in February and April, while all stations were sampled during the June-July cruise. Samples were collected from within a 1 m^2 area near the midpoint of the video transect. In 2002, samples were obtained from all 30 stations in June and July. However, on this cruise samples were collected from two locations along the 30 m video transect, at the beginning of the transect at the sand-reef interface and again at the transect midpoint.

Results of this analysis are displayed in Figure 11. Benthic chlorophyll content of surficial sediments was similar between sampling locations, and there was virtually no difference in the values obtained in the June 2001 and July 2002 cruises, with an overall mean of 48 mg chlorophyll-*a* m^{-2}. However, samples collected in February and April 2001 had a lower chlorophyll-*a* content of 30 and 33 mg chlorophyll-*a* m^{-2}, respectively.

All sample locations within TER, including those in the Out, Park, and Reserve strata, had a benthic chlorophyll-*a* content in surficial sediments that equaled or exceeded that measured on the west Florida shelf. The contribution of benthic microalgae in soft sediments adjoining coral reef habitats to reef ecosystem primary productivity has been estimated in only a few studies (Uthicke and Klumpp, 1998). These studies suggest that benthic microalgal production may contribute between 25 and 35% of reef ecosystem primary production, and have emphasized the contribution of benthic microalgae in shallow reef flats and lagoons. Our results demonstrate that there is significant microalgal biomass at depths between 15 and 30 m in the soft sediments at the coral reef interface, and that this community may play an important role in the food web supporting reef organisms. Previous research has demonstrated the effects of increased nutrients and changes in temperature and irradiance on the production of benthic microalgae in reef environments (Uthicke and Klumpp, 1998). We will continue to monitor this community to determine whether the imposition of TER results in any changes in the biomass of benthic microalgae occupying soft sediments near the coral reef-sandflat interface.

SPILLOVER EFFECT/LARVAL EXPORT

One of the primary goals of any marine reserve is to preserve a vital reproductive population of fish so that recruitment of larvae into fished areas is maintained. Spillover effect is defined as the export of post-settlement fish from within reserves to adjacent, fished areas (Russ and Alcala, 1996). Riley's Hump has been identified as a potential spawning site for five commercially important snapper species (Lindeman et al., 2000). In order to assess the potential role that Tortugas South can play in the maintenance of fish communities elsewhere in the Floridian province, we embarked upon a characterization of existing snapper/grouper spawning aggregations on Riley's Hump in addition to the development of a probabilistic model of the fate of snapper/grouper larvae released over Riley's Hump.

Fish Surveys

In 2001, the top of Riley's Hump was broken into a grid composed of 34 sections, each 0.25 minutes of latitude and 0.25 minutes of longitude. Ten sections were randomly selected for visual surveys. If water depth was greater than 34 m, another section was randomly chosen. Within each section, two dive teams sampled either two or three replicate 50 m transects. One dive team worked to the east of the anchor and the other dive team worked to the west of the anchor. Direction and spacing of transects was randomly determined prior to the dive. One member of the dive team swam and counted all snapper and grouper along the transect, to the limit of visibility. The other member video recorded the transect. Visibility was estimated along the transect line in order to estimate total area (m^2) sampled.

During 2002, sampling was conducted during two trips in each of May, June, and July, during new and full moons, as well as during the October full moon. A total of 156 census transects were completed on these cruises. Data on habitat distribution was also collected using 0.5 m^2 quadrats, placed systematically every 5 m along the random transect lines. These data will be used to provide relative abundance of habitat types using the Braun-Blanquet scale. Fish census data for 2002 will be compared with 2001 data to provide a preliminary "before-after" protection comparison.

From 2001 data, ten species of grouper and seven species of snapper were observed from 44 transects at Riley's Hump. Gray snapper were the most abundant species. Preliminary examination of the data indicates that there may be spatial differences in the distribution of snappers and groupers over Riley's Hump. Further analyses are required and ongoing. These data will provide a baseline from which to evaluate changes in the number of snapper and grouper in Tortugas South over time.

Larval Export

Argos-drifter release: To predict the fate of larvae spawned over Riley's Hump, WOCE/SVP drifters were released four times during cruise FE-00-09-BL in July 2000 and three times during cruise FE-01-10-BL in June/July 2001. During each occasion, three drifters were released over Riley's Hump. This information is central to understanding the potential role of Riley's Hump as a source area for settlement stage fishes to other reef habitats.

Examination of the drifter tracks released during 2000 demonstrate that Riley's Hump can serve as a source of larvae to areas along the west Florida shelf (Figure 12), the Florida Keys and the east Florida shelf. Preliminary examination of the 2001 drifter releases shows similar variability in potential larval transport. The implications are, if spawning stock biomass of snapper and grouper increase in Tortugas South, then increased larval supply can be expected across reef and estuarine systems throughout the southeastern United States.

Ichthyoplankton: Ichthyoplankton sampling was conducted during both 2000 and 2001. A series of transects radiating from Riley's Hump were sampled. A 60-cm bongo with 333 µm mesh nets was towed obliquely from the surface to a maximum depth of 100 m at each station. CTD casts were also made at each station to collect temperature and salinity data.

Drifter tracks from both years indicated very different flow dynamics to the north and south of Riley's Hump and the larval fish collections will allow determination of the species of fish influenced by the different flow regimes. These samples are being sorted and identified.

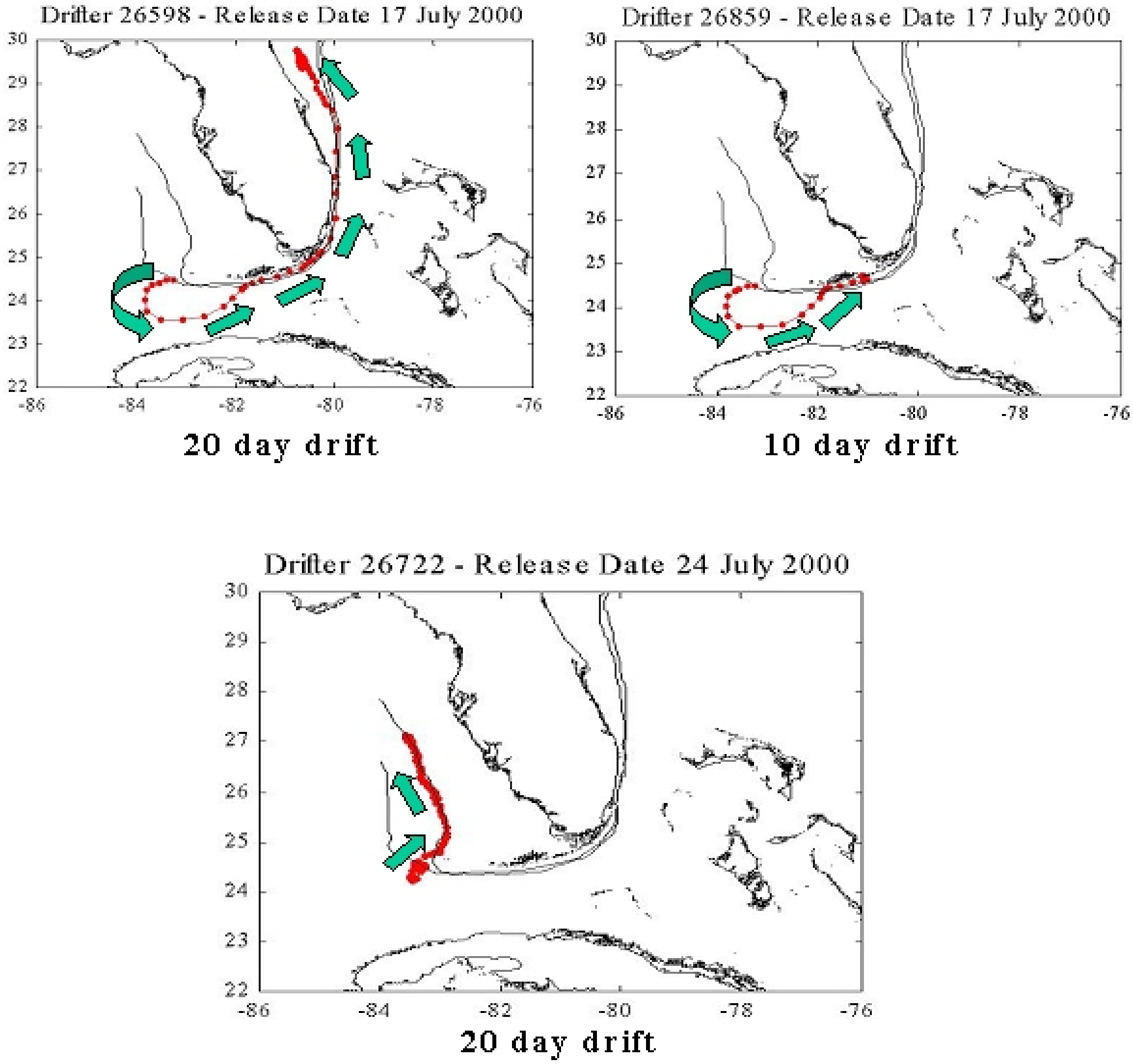

Figure 12. Drifter tracks from Year 2000 releases. Note the variability and breadth of dispersal potential.

EFFECTIVENESS OF REFUGE IN ECOSYSTEM RECOVERY

Fish Surveys

Paired band transect visual censuses were made by divers over the reef and soft bottom habitat along the 30 m transects as described above. Fish counts were made within 1 m on either side of the permanent transect. Although it is not possible to identify definitive changes in fish population dynamics after one year of protection, some interesting patterns have emerged. The numbers of fish > 20 cm total length appear to have increased in the new Reserve when compared to the Park and Open strata (Figure 13). Six fish species (representing the most abundant species in each of six important reef fish families) show an increase in number and size within the Reserve when compared to the Park and Open strata (Figure 14). In 2002, both large red and black grouper, on the order of five years old, were conspicuous parts of the fish assemblage at the reef soft bottom interface. In 2001, only large red grouper were abundant. The source of these differences could generally be attributed to the considerable natural variability of such systems, increasing grouper densities at interior reef sites, or movement with growth, of an exceptional year class of black grouper, to productive, though risky feeding habitat. The need for development of a longer-term data base is required to make effective comparisons among Use strata. Analysis of census data is ongoing at CCFHR.

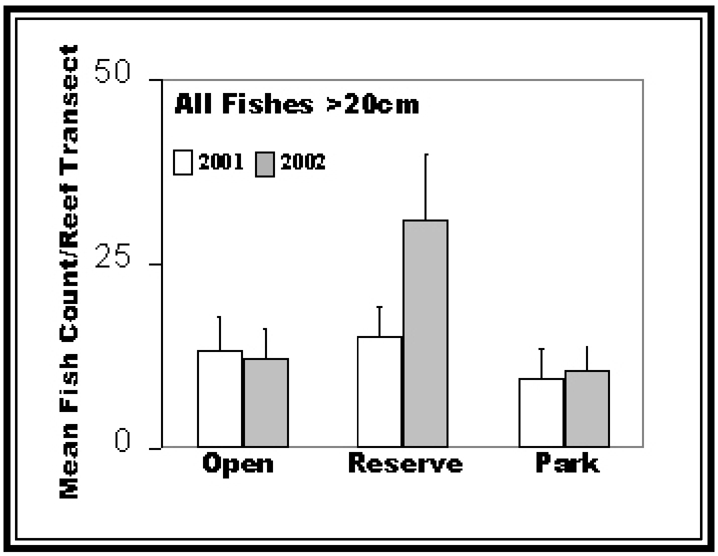

Figure 13. Mean number of fish (> 20 cm) per transect, all species combined. N = 10 for each strata per year.

Gear Impact

Historically, the waters around the Dry Tortugas region have been the principal fishing grounds for the commercial pink shrimp fishery. With the establishment of TER, 151 square nautical miles became closed to commercial fishing activities, including pink shrimp trawling. With the growing scientific concern for the effects of commercial fishing gear on benthic habitats, we designed a study that would examine the effects of trawl exclusion on the benthic habitats located in TER.

<u>Beam Trawl</u>: Along the northern boundary of Tortugas North, pairs of randomly selected coordinates were chosen for beam trawl samples. The coordinates served as starting points for the trawl tow path. One coordinate of each pair was located ~ 2 km due south of Tortugas North's northern boundary (within the Reserve), and the other, ~ 2 km due north of the boundary

(outside the Reserve). One set of coordinates spanned the eastern boundary of Tortugas North. In this case, one coordinate was located ~ 2 km due west of Tortugas North's eastern boundary (within the Reserve), and the other ~ 2 km due east of the boundary (outside the Resrve). We conducted three minute tows at each coordinate using a modified 2 m beam trawl with a 3 mm mesh cod end. Samples were sorted and initially preserved in formalin (24 h) and then transferred to ethyl alcohol. The path of each trawl was recorded using ASPEN in order to verify the location of the surveys and certify that they were in either open or closed areas.

Our faunal collections from open and protected softbottom habitat near the northern boundary of Tortugas North strongly suggest that relaxation of trawling pressure has increased benthic biomass and diversity in this area of TER. The Reserve may act as a refuge for the large pink shrimp targeted by the fishery and their density as well as biomass and diversity of smaller crustaceans was obviously higher in paired protected vs open bottom samples. Although not as obvious, differences in the fish and echinoderm assemblages between trawled and protected bottom are likely to become clear with the detailed analysis of our samples. It appears that these soft bottom communities respond quickly to relaxation of the disturbance of trawling and we hypothesize that further changes will occur over time with development of a more stable assemblage of attached invertebrates that should develop in the more physically stable parts of the shelf. We believe that an increase in fishes and other benthic animals can be assumed to be occurring in protected habitats within the Reserve. However, we do not have replicate Ecological Reserves, and that differences among the samples taken within TER versus those taken just out of the area may conceivably be an artifact of distance from the reef structure. The final interpretation of these findings will, like other aspects of the study, rely on the BACI design constraints. Moreover, whether or not the current Reserve status of TER is having a beneficial effect on the ecosystem in general and on target, long-lived reef predators will require continued assessment. Sample processing continues at CCFHR including new samples obtained in July 2003.

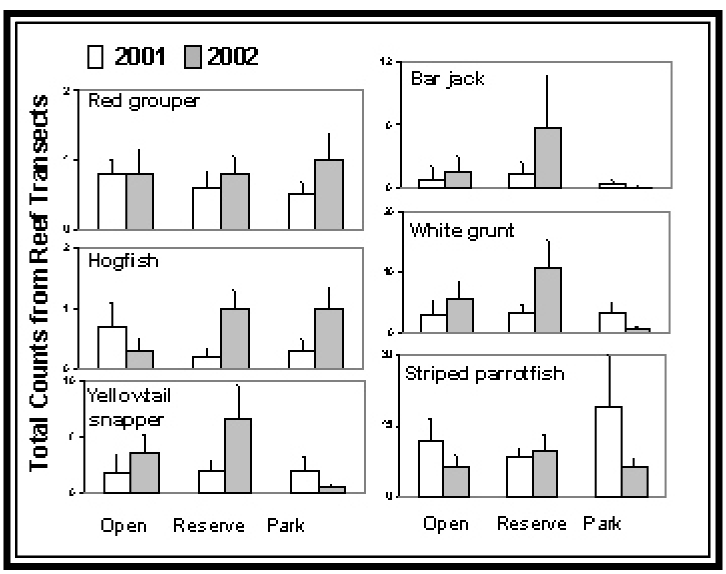

Figure 14. Total number of fish, by species, per transect for each strata per year.

Drop Camera Drifts: In conjunction with the beam trawls, drop camera and ROV drifts were made in an effort to capture a video record of trawl disturbance. Fifteen minute drifts were made at the same coordinate pairs as the beam trawls. The path of each drift was video recorded and the track was recorded using ASPEN. Trawl tracks were evidenced on several occasions. Video processing is currently underway at CCFHR using the same methods as described under Coarse-Scale Mapping.

OUTREACH

Reports

Per our agreement with NOAA's National Marine Sanctuary Program, all reports can be found at a web site for this project (CCFHR, 2001). To date, eight progress reports have been drafted in association with this work.

Open House

On July 7, 2001, the NOAA Shop FERREL was docked at Mallory Square in Key West and opened to the public. NOAA public relations, FKNMS staff, and CCFHR scientists displayed equipment, showed video, and answered the questions of ~ 200 interested members of the public. The event was featured in an article entitled, "Research boat shares Tortugas findings" in the July 7 issue of the local newspaper, The Key West Citizen.

North Carolina Seafood Festival

This work was also showcased at a CCFHR booth at the North Carolina Seafood Festival on October 6-7, 2001, in Morehead City, NC. This event, the second largest outdoor event in NC, brought at least 200 people to the booth where sampling equipment and a video regarding this project were displayed; intense question and answer dialogs with CCFHR staff was ongoing.

National Public Radio

This project was also the focus of a National Public Radio's Radio Expedition Program, hosted by Alex Chadwick, which was broadcast nationally (Figure 15). These audio files may be accessed at: http://www.npr.org/programs/re/archivesdate/index.html - select "Sanctuary for Tortugas Marine Life".

Professional Presentations/Seminars

Several presentations (oral and poster format) on this work have been given at various venues (Table 3). Audiences included research peers, graduate students, college staff, and undergraduates.

Figure 15. National Public Radio's Alex Chadwick (center, sunglasses) interviews CCFHR researchers.

Table 3. Summary of Dry Tortugas Presentations.

Venue	Location / Dates	Type / Title of Presentation
FKNMS Science Advisory Panel Meeting	Marathon, FL December 2000	Characterization of the Tortugas Ecological Reserve: I -Establishing a Baseline (oral)
NOAA Weekly Seminar Series	Silver Spring, MD February 2001	Characterization of the Tortugas Ecological Reserve: I - Establishing a Baseline and II - Measuring the Effects of Establishing a Marine Protected Area (oral)
Smith College Science Seminar Series	Northampton, MA April 2002	Characterization of the Tortugas Ecological Reserve (oral)
American Fisheries Society 132nd Annual Meeting "Turning the Tide"	Baltimore, MD August 2002	Food webs supporting fishery production at the West Florida Shelf and Tortugas Ecological Reserve: an integrated study featuring stable isotope analysis (oral)

XVII Annual Meeting of the Tidewater Chapter of the American Fisheries Society	Beaufort, NC January 2003	Fisheries at the edge, Tortugas Ecological Reserve (oral)
XVII Annual Meeting of the Tidewater Chapter of the American Fisheries Society	Beaufort, NC January 2003	Characterization of the Tortugas Ecological Reserve: A Multiple Platform Approach to Habitat Classification (poster)
XVII Annual Meeting of the Tidewater Chapter of the American Fisheries Society	Beaufort, NC January 2003	Characterization of the Tortugas Ecological Reserve: Measuring the Effects of Establishing a Marine Protected Area (poster)
Southeast Coastal Ocean Science Conference and Workshop	Charleston, SC January 2003	Marine Habitats and Protected Areas (oral)
Annual Sanctuary Research Coordinators Meeting	Charleston, SC January 2002	Characterization of the Tortugas Ecological Reserve: I -Establishing a Baseline and II -Measuring the Effects of Establishing a Marine Protected Area (oral)
Annual Sanctuary Research Coordinators Meeting	Santa Cruz, CA January 2003	Synopsis of Tortugas Research (oral) ALL THE POSTERS

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