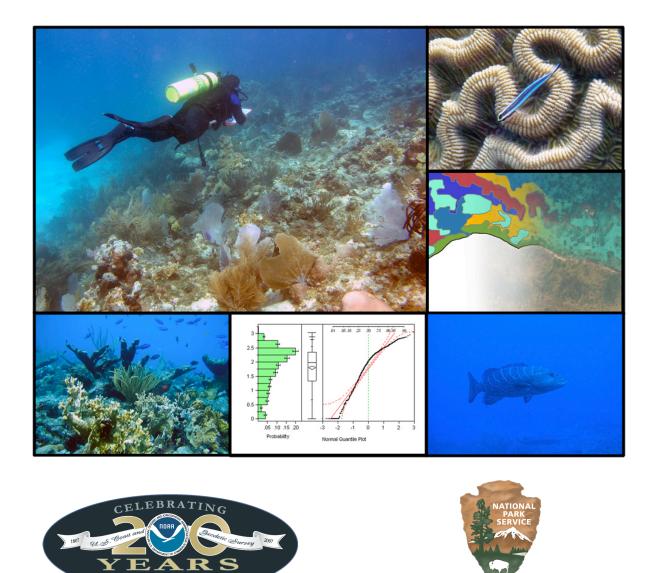
# A GUIDE TO MONITORING REEF FISH IN THE NATIONAL PARK SERVICE'S SOUTH FLORIDA / CARIBBEAN NETWORK



NOAA Technical Memorandum NOS NCCOS 39

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A Guide to Monitoring Reef Fish in the National Park Service's South Florida / Caribbean Network

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## **Executive Summary**

Reef fishes are conspicuous and essential components of coral reef ecosystems and economies of southern Florida and the United States Virgin Islands (USVI). Throughout Florida and the USVI, reef fish are under threat from a variety of anthropogenic and natural stressors including overfishing, habitat loss, and environmental changes.

The South Florida / Caribbean Network (SFCN), a unit of the National Park Service (NPS), is charged with monitoring reef fishes, among other natural and cultural resources, within six parks in the South Florida - Caribbean region (Biscayne National Park, BISC; Buck Island Reef National Monument, BUIS; Dry Tortugas National Park, DRTO; Everglades National Park, EVER; Salt River Bay National Historic Park and Ecological Preserve, SARI; Virgin Islands National Park, VIIS). Monitoring data is intended for park managers who are and will continue to be asked to make decisions to balance environmental protection, fishery sustainability and park use by visitors. The range and complexity of the issues outlined above, and the need for NPS to invest in a strategy of monitoring, modeling, and management to ensure the sustainability of its precious assets, will require strategic investment in long-term, high-precision, multispecies reef fish data that increases inherent system knowledge and reduces uncertainty.

The goal of this guide is to provide the framework for park managers and researchers to create or enhance a reef fish monitoring program within areas monitored by the SFCN. The framework is expected to be applicable to other areas as well, including the Florida Keys National Marine Sanctuary and Virgin Islands Coral Reef National Monument. The favored approach is characterized by an iterative process of data collection, dataset integration, sampling design analysis, and population and community assessment that evaluates resource risks associated with management policies. Using this model, a monitoring program can adapt its survey methods to increase accuracy and precision of survey estimates as new information becomes available, and adapt to the evolving needs and broadening responsibilities of park management.

To conduct reef fish population and community assessments, monitoring programs must collect abundance and size-frequency distribution data for distinct fish taxa. Concurrently collected data on benthic habitat and water quality are desirable as well, and can be assimilated in a survey design to improve survey performance.

The method of measurement should establish a constant search area for a sample unit (e.g. transect, fixed radius cylinder) and obtain an accurate representation of the reef fish community within the sample unit, tempered by the time required to obtain the sample. The choice of

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measurement method depends on the species or species-complex, life history stages, and habitat chosen for sampling.

Underwater visual census methods are ideal for assessing reef fishes in the Florida Keys (e.g. DRTO) and Virgin Islands (e.g. BUIS, SARI, VIIS) because of prevailing good visibility, rugose habitats, and management concerns requiring the use of non-destructive census methods. The most well known visual census methods are the belt transect and the stationary visual census. Alternative methods must be used to census fish in turbid (e.g. BISC) or deep environments or when nighttime surveys are required.

A primary consideration of a monitoring program is to delineate the target population which will be monitored. For reef fish, this can be done by selecting an ecosystem area to be surveyed. For most monitoring purposes a sample of the population is used to infer the status of the population. There are many ways to select a sample from a population, but the more information available about a population, the easier it is to devise a selection method which provides accurate and precise survey estimates. A simple random sampling design is appropriate for situations where there is no spatial structure in the variance of investigated fish metrics or little information is available. Since fish metrics are typically heterogeneous, a stratified random sampling design will sample a fish population more effectively. Maps of environmental covariates, such as benthic habitat, at the appropriate spatial scales and spatial extent can be used to effectively divide the sampled population into strata.

The main goal of sample surveys is to obtain accurate, high-precision estimates of population and community metrics at a minimum of cost. The objective of sample design analysis is to determine the appropriate number of samples required to achieve enough precision in population and community metrics (e.g., species numbers-at-size, species composition) to understand ecological processes and to make management decisions. Iterative analysis of candidate survey design performance can be used to refine survey estimates and reduce sampling cost.

The range and types of statistical analyses that will be performed to assess the status and dynamics of reef fish populations and communities in National Parks depends on the specific management questions and resource goals to be addressed. These analyses utilize the range of fundamental survey data outlined above and recommended for collection in monitoring programs. These survey data are then used to generate multiple metrics for individual species, species-complexes or life history stages to assess status and trends of reef fish over time and in relation to specific sustainability metrics.

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A single standardized monitoring protocol is not advocated because of the variability in ecological condition, size, management capability, and available data among SFCN park units. This guide is meant to serve only as a framework. Three reef fish monitoring program case studies are provided which build upon the presented framework using park-specific data sets, management concerns, and local partnerships.

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# 1 Background and Objectives

#### 1.1 Rationale

Reef fishes are conspicuous and essential components of coral reef ecosystems and economies of southern Florida (Johns *et al.* 2001; Ault *et al.* 2005a) and the United States Virgin Islands (USVI) (Hinkey *et al.* 1994; Mateo 1999, 2001). More than 500 species of reef fish including sharks, eels, flounders, gobies, puffers, groupers, parrotfishes, snappers, jacks, and damselfishes can be found in the reef ecosystems of the Florida Keys and USVI (Longley and Hildebrand 1941; Bohlke and Chaplin 1968; Clavijo *et al.* 1980; Munro 1983; Robbins and Ray 1986; Smith-Vaniz *et al.* 1995; Randall 1996; Bohnsack *et al.* 1999; Humann and DeLoach 2002).

Throughout Florida and the USVI, coral reef ecosystems are under threat from a variety of anthropogenic and natural stressors, including overfishing, habitat loss, and environmental changes. Over the past several decades, public use and conflicts over fishery resources in the two regions have increased sharply (Appeldoorn et al. 1992; Bohnsack et al. 1994; Leeworthy and Vanasse 1999), while catches from historically productive fishery stocks, especially the snapper-grouper complex, have declined (Allen and Tashiro 1976; Bohnsack et al. 1994; Ault et al. 1997, 1998, 2001, 2005a). In the Florida Keys, recent quantitative assessments of the multispecies reef fish community have shown that exploitation levels are very high, that many stocks are "overfished", and that overfishing has been clearly evident since the late 1970's (Ault et al. 1998, 2001, 2002, 2005ab). Throughout the region, there is evidence of loss of grouper spawning aggregations, reduced catch per unit effort, changed species composition of landings, and lower mean sizes and abundances of several assemblages (de Graaf and Moore 1987; Appeldoorn et al. 1992; Bohnsack et al. 1994; Ault et al. 1998, 2005b; Beets 1996ab; Garrison et al. 1998; Beets and Friedlander 1999; Beets & Rogers 2002; Beets and Muehlstein 2003). The impacts to the community are of great concern because fishing has depleted top trophic levels, shifted community structure and reduced the length and complexity of food webs that affect fishery resilience and prospects for their sustainability (Pauly et al. 2002; Ault et al. 2006). Several species, for example Goliath Grouper (Epinephelus itajara), Nassau Grouper (Epinephelus striatus) and Queen Conch (Strombus gigas), have been so depleted that they are now protected (Sadovy and Eklund 1999, Caribbean Fishery Management Council 1996).

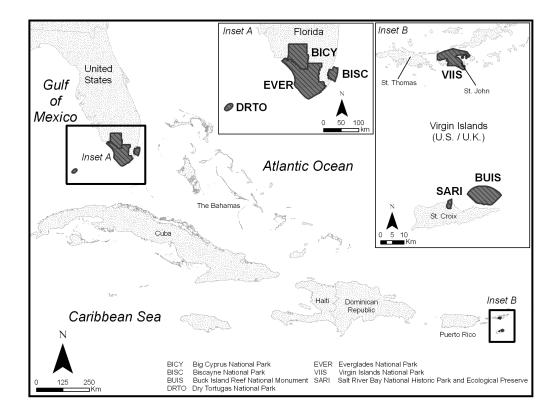
The reef fish community has also been affected by a series of other anthropogenic and natural stressors. Coastal development influences reef fish through a plethora of negative impacts

on reef fish habitats (Rogers and Beets 2001). Fish habitats are altered by dredging, boating, fishing gears, wetlands reclamation, beach renourishment, mangrove removal, and sea defense construction (Rogers *et al.* 1988; Rogers 1991; Beets 1996a; Lindeman and Snyder 1999; Rogers and Beets 2001; Wilber *et al.* 2003; Mumby *et al.* 2004; Chiappone *et al.* 2005). Additional consequences of development include changes to water quality from pollution, sedimentation, nutrient loading, freshwater inflows, and regional scale hydrodynamics (McIvor *et al.* 1994; MacDonald *et al.* 1997; Serafy *et al.* 1997; Mannoni 1999; Nemeth and Sladek-Nowlis 2001; Rogers and Beets 2001; Cowie-Haskell and Delaney 2003). These impacts may be exacerbated by intensification of hurricane activity and climate variability (Rogers *et al.* 1982; Hughes 1994; Edmunds 2002; Gardner *et al.* 2005; Pandolfi *et al.* 2005).

As coastal populations, tourism and fishing pressure continue to increase, park managers are being asked to make decisions to balance environmental protection, fishery sustainability, and visitor resource use. Balancing these conflicting uses is a complex issue, and requires an ecosystembased management (EBM) approach. EBM considers knowledge and uncertainties in biotic, abiotic, and human components of the whole ecosystem in an attempt to balance societal objectives (Christensen *et al.* 1996; Larkin 1996; Schramm and Hubert 1996; Pikitch *et al.* 2004). These broad-spectrum objectives are set within a framework created by the NPS's universal mission to protect cultural and natural resources (Organic Act of 1916, USC title 16).

#### 1.2 Management Domain

The National Park Service (NPS) through the South Florida / Caribbean Network (SFCN) is one of many administrative entities monitoring reef fishes and coral reef ecosystems in southern Florida and USVI. The SFCN is composed of four managed areas in southern Florida (Big Cypress National Park, BICY; Biscayne National Preserve, BISC; Dry Tortugas National Park, DRTO; Everglades National Park, EVER) and three in the USVI (Buck Island Reef National Monument, BUIS; Salt River Bay National Historic Park and Ecological Preserve, SARI; Virgin Islands National Park, VIIS) (**Figure 1**). Six of these areas are inhabited by reef fishes (BICY is excluded). The Virgin Islands Coral Reef National Monument (VICR) is not currently part of the SFCN, but is managed by the NPS and shares many ecological and management characteristics with VIIS, BUIS, and SARI. Information on location, date of establishment, cultural and natural resources, marine habitat area, and management zoning for each park is given on the NPS website (www.nps.gov). Site characterizations of fisheries resources and habitats have been carried out for



**Figure 1**: Map of the Caribbean region showing parks and monuments that are part of the National Park Service's South Florida / Caribbean Network.

BISC (Ault *et al.* 2001) and DRTO (Schmidt *et al.* 1999; Ault *et al.* 2002), but only habitats for SARI (Kendall *et al.* 2005).

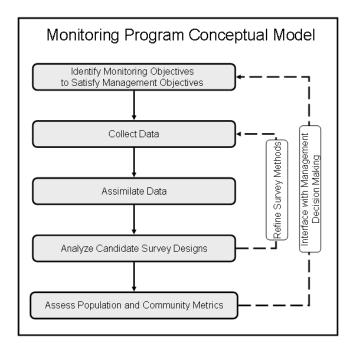
### 1.3 Objectives

The range and complexity of the issues outlined above, and the need for NPS to invest in a strategy of monitoring, modeling, and management to ensure the sustainability of its precious assets, will require strategic investment in long-term, high-precision, multispecies reef fish data that benefits EBM by increasing inherent system knowledge and reducing uncertainty. Objectives to meet the goals of park-specific EBM are to:

- Assess condition and changes in reef fish community diversity and its composition
- Assess condition and changes in the sustainability status of reef fishes under exploitation
- Assess anthropogenic impacts (e.g., fishing, sedimentation, pollution, etc.) on community dynamics
- Assess effectiveness of management strategies such as Marine Protected Areas (MPAs), fishing regulations, land and water uses, etc.
- Determine biological and physical processes that govern health and sustainability of reef fish resources

These objectives may be tailored by local resources and interested stakeholders as outlined in each park's General Management Plan, Resource Management Plan and/or Fisheries Management Plan. These plans set the management philosophy and direction for planning horizons up to 15-20 years in the future and are amended accordingly.

There is a clear need to link monitoring programs to meet management goals and objectives. Monitoring programs are comprised of an iterative process of data collection, dataset integration, design analysis, and population and community assessment that evaluates resource risks associated with management policies. **Figure 2** illustrates a conceptual model of an ideal reef fish monitoring program. The model is adaptive in two respects. First, as new information becomes available, the monitoring survey design used to collect data and assimilate data is tailored to increase accuracy and precision. Second, the monitoring process can adapt to the evolving needs and broadening responsibilities of EBM and NPS management plans. Adaptation need not be instantaneous or final. A reevaluation of the management objectives every 3-, 5- or 10-years can be sufficient to adapt to new management policies, shifted resource conditions, and a compilation of new data.



**Figure 2**: A conceptual model of an ideal reef fish monitoring program. The model illustrates the iterative process of data collection, dataset integration, design analysis, and population and community assessment that evaluates resource risks associated with management policies. Feedback loops critical to the iterative process are shown.

# 1.4 Document Organization

This guide mainly addresses the first four components of the iterative monitoring program outlined in **Figure 2**. These components are organized in the following sections of this document:

- 1. Background and Objectives
- 2. Data to be Collected and Methods of Measurement
- 3. Population to be Sampled and Selection of the Sample
- 4. Candidate Sampling Design Analysis
- 5. Population and Community Assessments

In addition, three case studies are provided that illustrate existing monitoring programs within managed areas of the SFCN. The case studies are described further in Section 6.

- 1. Case Study A: Reef Fish Monitoring in Virgin Islands National Park and Buck Island National Monument, 2001-2005 (VIIS, BUIS)
- 2. Case Study B: Monitoring Reef Fish Assemblages inside Virgin Islands National Park and around St. John, US Virgin Islands, 1988-2000 (VIIS)
- Case Study C: Assessment of Coral Reef Fishery Resources in Dry Tortugas National Park, 1999-2004 (DRTO)

# 2 Data to be Collected and Methods of Measurement

# 2.1 Data to be Collected

To conduct reef fish population and community assessments, monitoring programs must collect several types of fundamental data on reef fishes:

- Identification to the lowest possible taxonomic classification (preferably to the species level) of each individual
- Abundance and size-frequency distribution of each taxa

Concurrently collected data on benthic habitat and water quality are desirable as well and can be assimilated in a survey design to improve survey performance. Complementary data often include:

- Depth
- Substrate composition
- Benthic floral/faunal composition
- Vertical rugosity
- Benthic habitat type (based on strata of a stratified sampling design)
- Temperature
- Salinity

# 2.2 Methods of Measurement

The method of measurement chosen for a survey should establish a consistent search area for a sample unit (e.g. transect, fixed radius cylinder) and obtain an accurate representation of the reef fish community within the sample unit, tempered by the time required to obtain the sample. Consistent search areas ensure comparability among sampling units and equal (or known) probability of sample unit selection. Search methods based solely on time are not recommended if sample units are defined by area, because they invalidate the equal selection property that is fundamental to using probability-based sampling designs.

The choice of the sampling method depends on the species or species-complex, life history stages, and habitat chosen for sampling. These choices are governed by the monitoring program goals. The following sections outline methods that take these considerations into account.

#### 2.2.1 Visual Census Methods

Underwater visual census methods are ideal for assessing reef fishes in the Florida Keys and Virgin Islands because of prevailing good visibility, rugose habitats, and management concerns requiring the use of non-destructive assessment methods. The most well known visual methods are the stationary visual census and the belt transect. The stationary visual census samples all reef fish within an imaginary cylinder of fixed radius (Bohnsack and Bannerot, 1986). Belt transects sample all fish within a rectangle of fixed width and length (Brock, 1954). Belt transects may be more appropriate when sites are characterized by low visibility, highly rugose habitats, or adjacent to mangroves because they place the diver closer to fish. In addition, the belt transect may be more effective in sampling small, cryptic species (e.g. Chaenposidae, Gobiidae, Labrisomidae). Case study A collects reef fish data using the belt transect method. Stationary visual census methods may minimize measurement bias attributed to diver movements and is superior for counting pelagics (e.g. Carangidae, Scombridae, Clupidae). Examples of the stationary visual census are provided in case studies B and C. The choice of a visual census method should maximize effectiveness (minimize bias) of the census given potential fish species and habitats among sample units. Some logistical factors that could improve survey performance (more samples per unit time) and reduce diver fatigue are use of Nitrox SCUBA and "live-boating" (boat driver remains on non-anchored vessel) at dive sites.

Alternative visual census methods which do not employ SCUBA diving and thus are free of SCUBA's depth constraints utilize underwater video cameras, but they are plagued by biases associated with species selectivity and inconsistent census area. Underwater stereo-video (Harvey and Shortis 1996) and baited video cameras (Willis and Babcock 2000) are two examples.

#### 2.2.2 Non-Visual Census Methods

In general, statistical issues of capture efficiency and size-selectivity (e.g., MacLennan 1992; Gunderson 1993) are minimized using visual census methods; thus, they are typically preferred for coral reef ecosystems. However, not all fish species or individuals in the sampled area will be detected by visual methods. Alternative methods may be required in cases where: visibility is occluded by turbid waters or densely vegetated habitats; night-time sampling is most effective for target species; cryptic species are targeted; or depths exceed operational diving limits. In these situations, fish may be sampled more effectively using gear or poisons (ichthyocides) to capture fish. Two classes of gear have been used to sample reef fishes: active gear, such as trawls and seines

that are towed or pulled to capture fish within a sample unit (e.g., Robblee and DiDomenico 1991; Sedberry and Carter 1993; Serafy *et al.* 1997; Ault *et al.* 1999); and passive gear, such as gill nets, traps, and pots that are assumed to sample a fixed unit area over a given unit time (e.g., Collins and Sedberry 1991; Hickford and Schiel 1995; Pratt and Fox 2001; Watson and Munro 2004).

Morphologically or behaviorally cryptic species will likely be underestimated by visual census methods (Smith-Vaniz *et al.* in press), and active and passive gear. Cryptic species can be quantified more-effectively using ichthyocides (e.g. rotenone) (Smith-Vaniz *et al.* in press; Ackerman and Bellwood 2000), but ichthyocides negatively impact the studied assemblage.

#### 2.2.3 Measurement Biases

A sample will rarely provide an absolutely accurate measurement, but the mean of many unbiased samples will tend towards the mean of the population. A measurement bias occurs when the measurement process affects the measurements in such a way that the sample mean does not tend towards the population mean, but rather another (sometimes unknown) value. Common causes of measurement bias include inconsistent survey effort, diver behavior, species detectability, observer experience/training, and fish density. The primary reason fishery-independent data are sought for monitoring programs is because fishery data are plagued by measurement biases associated with differences in catch per unit effort. Thompson and Mapstone (1997) demonstrate observer bias can be considerable in underwater visual censuses and provide guidance for observer training to ameliorate its impact.

In general, it is assumed that the method of measurement chosen has accounted for issues in selectivity and minimizes the probability of non-detection of a species if it is present in the sampling area. Selectivity is defined as the probability that an individual will be detected (or retained) by the sampling method (gear) given that it is vulnerable (Gunderson 1993). While there is always the possibility that a species will not be detected at a site, despite being present, for most species and gear types this non-sighting probability diminishes with increasing animal size, thus demanding strategic choice of the methods of measurement. There are well-known statistical methods for correcting for size selectivity by gear or method in sampling surveys (e.g., Pope *et al.* 1975; Gunderson 1993). In addition, MacKenzie *et al.* (2002), Azuma *et al.* (1990) and Tyre *et al.* (2003) give some insights into correcting frequency of occurrence data when there are unequal selection probabilities amongst species.

# **3** Population to be Sampled and Selection of the Sample

#### 3.1 Population to be Sampled

A primary consideration of a monitoring program is to delineate the target population which will be monitored. For reef fish, this can be done by selecting an ecosystem area to be surveyed. This surveyed area in which ecological processes occur and management decisions will be made is known as the survey domain.

A distinction must be made between the target population (the population about which information is desired) and the sampled population. These populations should coincide, but sometimes due to practicality or convenience the sampled population is a restricted part of the target population. It is important to note that if a difference among these populations exists, conclusions drawn from the sample only apply to the sampled population (Cochran 1977).

Population and community assessments correspond only to fish populations within the survey domain, thus the selection and accurate demarcation of the domain is essential for meaningful fish management decisions. Although management efforts in the SFCN are directed towards the areas within park boundaries, the reef fish assemblages in these parks depend on and interact with surrounding areas over a much larger spatial-scale. To comprehensively monitor and manage the reef fish inside the parks and assess effectiveness of NPS management strategies, the survey domain should incorporate areas outside park boundaries as well.

#### 3.2 Selection of the Sample

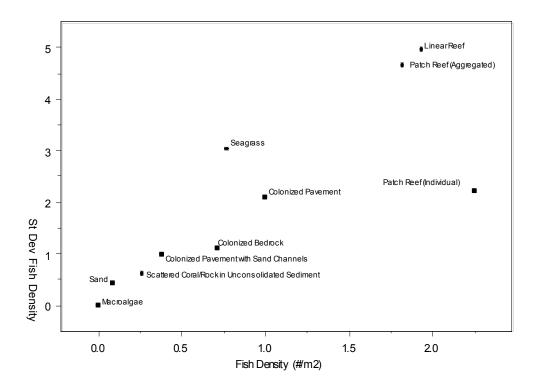
There are many ways to select a sample, but the more information available about a population, the easier it is to devise a selection method which provides accurate and precise survey estimates. Simple random sampling (SRS) is the simplest and most fundamental probability-based survey design allowing inferences to be made from sample units to the sampled population. In the previous section, we defined the sample unit for a given sampling method to have a known constant area. The complete list of all non-overlapping, independent sample units comprises the sampling frame. The SRS design considers all sample units in the sampling frame equal (i.e. all sample units have the same probability of being selected) and thus is appropriate for situations where there is no spatial structure in the variance of investigated metrics. Fish populations and communities are rarely homogenous in nature. More often, the principal metrics of reef fish show strong association with benthic habitats, depths, salinity, and other environmental covariates (Ault *et al.* 1999; Kendall *et al.* 2003, 2004) and thus are heterogeneous.

A survey design will sample a population more effectively if the corresponding variations in the spatial structure of variance within the survey domain is known and taken advantage of. A stratified random sampling design (StRS) uses the variance structure to select sample units more efficiently from the sampling frame. A StRS design may divide the survey domain into regions of relatively homogenous variance called strata and by sampling more intensively in highly-variable strata, a StRS can achieve better results than a SRS using the same sample size.

For any given metric the best criteria to use when constructing strata is the variance structure of the metric itself. As this knowledge is unknown (or else sampling would not be need), the next best criteria are variables that are highly correlated with the metric of interest. Maps of environmental covariates at the appropriate spatial scales and spatial extent are ideal, since the sampling frame is situated in a spatial framework.

The benthic habitat maps of shallow-water (depth 0 - 20 m) areas by FMRI (1988), Kendall *et al.* (2001, 2005) and Franklin *et al.* (2003) are exemplary maps of a covariate for BISC, BUIS, DRTO, SARI and VIIS. These maps classify benthic habitats that are strongly correlated with principal reef fish population and community metrics. Ault *et al.* (1999, 2005a) and case studies A and C have shown parsing the survey domain according to benthic habitat types will dramatically improve sampling efficiency compared to a SRS.

To effectively parse the survey domain into strata requires an analysis of covariance among reef fish metrics and environmental variables (e.g. benthic habitat, salinity, depth). A broad assortment of variance analysis techniques such as plots of stratum standard deviations against stratum means, Analysis of Variance (ANOVA), generalized linear models, and resampling methods can be used to investigate the variance structure of metrics. **Figure 3** is a plot of the sample standard deviation of snapper (Family: Lutjanidae) against the corresponding average sample density among 10 distinct benthic habitat types around BUIS. The graph shows that snappers were not homogeneously distributed throughout the survey domain, and that stratification according to benthic habitat can be effective in partitioning the domain into areas with differing variances. **Figure 3** also indicates that a stratification scheme employing benthic habitat type can be simplified by merging relatively similar benthic habitat types (e.g. linear reef and aggregated patch reef) into a single stratum.



**Figure 3**: A plot of the sample standard deviation of snapper (Family: Lutjanidae) against the corresponding average sample density among 10 distinct benthic habitat types around BUIS. In conjunction with a map of benthic habitat types, the information in this plot may be used to develop an efficient survey design.

Another feature of **Figure 3** is the strong relationship between stratum mean density and standard deviation. This is a common phenomenon in surveys of marine animal populations (e.g., Ault *et al.* 1999, 2003). Thus, a stratification scheme that effectively partitions the domain with respect to variance of animal density may also effectively partition the domain into areas of differing mean densities.

Commonly, a monitoring program will initially use a SRS design because of a scarcity of fish and covariate data needed to ascertain spatial relationships. As data are gathered and covariance analyses are performed, more efficient survey designs such as a StRS can be adopted. The exploration of covariance after sampling using strata differing from those actually implemented requires poststratification analysis on domains of study. Ault *et al.* (1999) use poststratification as a comparative stratification scheme analysis tool for pink shrimp in Biscayne Bay. Cochran (1977) describes the process of poststratification and corresponding computations for both SRS and StRS designs.

Although statistical techniques such as ANOVA can reveal trends and regions of relatively homogenous variance in the survey domain, they cannot be used for hypothesis tests, unless the underlying population data structure in each stratum is known and the data conforms to test-specific assumptions (e.g., homogeneity of variance, normality, independence, etc.). Goodness-of-fit tests (D'Agostino and Stephens 1986) identify suitable distributions and consequently the most appropriate tests to use if hypothesis tests are required. In some cases, applying a transformation modifies the data structure to one assumed by a particular statistical technique. Commonly used transformations are listed in Box and Cox (1964), Sokal and Rohlff (1995), and Zar (1999).

# **4 Candidate Sampling Design Analysis**

The main goal of statistical sampling surveys is to obtain accurate, high-precision estimates of population and community metrics at relatively low cost. The statistical estimation methods of survey design presume that the population of interest is finite and inhabits a finite spatial domain; consequently, these methods are well suited for application to reef fish populations and communities at ecosystem scales appropriate for resource management. Principles of statistical survey design are outlined in Kish (1967), Cochran (1977, 1983), Williams (1978), Yates (1981), Kalton (1983), Kalton and Anderson (1986), Thompson and Seber (1996), and Lohr (1999).

The objective of sample design analysis is to determine the appropriate number of samples to be taken to achieve a certain level of precision for detecting change in population and community metrics (e.g., species numbers-at-size, species composition) used to understand ecological processes and to make management decisions. The specification of a degree of precision desired is an important step in sample surveys and is the responsibility of the park managers and researchers who use monitoring data.

#### 4.1 Basic Concepts of Sampling Theory and Designs

As discussed in section 3.2, populations of coral reef fishes within an ecosystem-scale sampling domain are usually heterogeneously distributed in space rather than homogeneously distributed. In this situation, a StRS design that effectively partitions the domain into distinct strata which are internally homogenous will usually outperform other types of sampling designs (e.g., simple random, systematic, etc.). Basic concepts of StRS designs are illustrated using two population metrics, fish density  $\overline{Y}$  (number of individuals per unit area) and fish abundance Y(total number of individuals). The concepts can be applied to SRS designs as well by taking the number of strata (L) equal to one. Observations of density  $y_i$  for a given species are the number of individuals observed or captured in a standard sample unit *i*, (e.g., a belt transect of 100 m<sup>2</sup>). An estimate of the mean density in stratum  $h(\overline{Y}_h)$  is given by

$$\overline{y}_h = \frac{\sum_{i=1}^{n_h} y_{hi}}{n_h}$$
(4.1)

where  $n_h$  is the number of units sampled in stratum *h* and  $y_{hi}$  is the density in stratum *h* and sample unit *i*. An estimate of the stratum variance  $(S_h^2)$  is given by

$$s_h^2 = \frac{\sum_{i=1}^{n_h} \left( y_{hi} - \overline{y}_h \right)^2}{n_h - 1}$$
(4.2)

The estimated stratum variance is used to estimate the variance of mean density in stratum h,

$$\operatorname{var}\left[\overline{y}_{h}\right] = \left(1 - \frac{n_{h}}{N_{h}}\right) \frac{s_{h}^{2}}{n_{h}}$$

$$(4.3)$$

where  $N_h$  is the total possible sample units in stratum *h*. The quantity  $\left(1 - \frac{n_h}{N_h}\right)$  in equation (4.3) is

termed the finite population correction (FPC), where  $\frac{n_h}{N_h}$  is the sampling fraction, or the proportion of the domain of stratum *h* that is actually sampled. Note in equation (4.3) that increasing sample size  $n_h$  reduces the variance of the estimate of mean density in two ways, first by reducing the quantity  $\frac{s_h^2}{n_h}$  and second by reducing the FPC. In practice the FPC can be ignored whenever the sampling fraction is less than 5% (Cochran 1977). The resulting equations are simpler, but variance estimates are higher.

Given that  $\overline{y}_h$  represents the stratum mean number of animals per sample unit, it follows that stratum abundance is estimated by multiplying mean density by the total number of sampling units,

$$y_h = N_h \overline{y}_h \tag{4.4}$$

Variance of  $y_h$  is estimated in a similar manner,

$$\operatorname{var}\left[y_{h}\right] = N_{h}^{2} \operatorname{var}\left[\overline{y}_{h}\right]$$

$$(4.5)$$

Note that controlling the variance of stratum mean density (equation 4.3) in turn controls the variance of stratum abundance (equation 4.5).

A beneficial property of sampling design theory is that stratum estimates of population means (equation 4.1), totals (equation 4.4), and their associated variances (equations 4.3 and 4.5) are unbiased (i.e., accurate) provided that sampling is done in a random manner (Cochran 1977). The randomization procedures employed in case studies A, B, and C provide practical approaches. Sample units within a stratum were uniquely identified with respect to geographical location in a GIS. The units were then assigned a number from 1 to  $N_h$ . Specific units to be sampled within a

stratum (totaling  $n_h$ ) were selected from the complete list of  $N_h$  units using a random number procedure based on the discrete uniform probability distribution, which assigns equal selection probability to each sample unit. The procedure was repeated for each stratum in the sampling domain.

Domain-wide estimates of population means and totals are computed from the individual stratum estimates taken from samples. Mean density for the stratified survey domain is obtained by summing the weighted averages of sample strata means,

$$\overline{y}_{st} = \sum_{h=1}^{L} W_h \overline{y}_h \tag{4.6}$$

where L is the number of strata, and strata weighting factors  $(W_h)$  are given by

$$W_{h} = \frac{N_{h}}{\sum_{h=1}^{L} N_{h}} = \frac{N_{h}}{N}$$
(4.7)

where N is the total number of possible sample units in all strata. The weighting factor  $W_h$  represents the proportion of the overall survey domain (or sampling frame) contained within stratum *h*. In a SRS design  $W_h = 1$ .

The variance of  $\overline{y}_{st}$  is estiamted as

$$\operatorname{var}\left[\overline{y}_{st}\right] = \sum_{h=1}^{L} W_{h}^{2} \operatorname{var}\left[\overline{y}_{h}\right]$$
(4.8)

Domain-wide population abundance  $y_{st}$  and associated variance  $var[y_{st}]$  are obtained by summing equations (4.4) and (4.5), respectively, over all strata,

$$y_{st} = \sum_{h=1}^{L} y_h$$
 (4.9)

and

$$\operatorname{var}[y_{st}] = \sum_{h=1}^{L} \operatorname{var}[y_h]$$
(4.10)

An important point to remember about a StRS design is that the variance of the domain-wide mean or total depends on the estimates of stratum variance. If a heterogeneously distributed population were divided into strata such that all strata were homogenous (i.e.  $\sum_{h=1}^{L} \operatorname{var}\left[\overline{y}_{h}\right] = 0$ ), then population estimates would be made without error. Consequently, the basic objective of stratification is to

partition the sampling domain into sectors of homogenous variance for population metrics such as animal density. Section 3.2 describes stratification techniques.

Developing a StRS design in practice requires both a scheme for stratifying the sampling domain and a scheme for allocating sample units among strata. There are two allocation schemes commonly used for StRS designs. The first is proportional allocation, in which sample units are allocated among strata according to stratum size,

$$n_h = n \cdot W_h \tag{4.11}$$

where n is the total sample size for the survey. The second scheme is Neyman or optimal allocation in which sample units are allocated according to both stratum size and the strata standard deviations of a considered population metric (e.g. density),

$$n_h = n \cdot \left(\frac{W_h s_h}{\sum_h W_h s_h}\right) \tag{4.12}$$

Under this strategy, larger and more variable strata will receive more sampling effort, and vice versa for smaller, less variable strata.

Neyman allocation, in concert with an effective stratification scheme, can substantially reduce the variance of domain-wide population estimates (e.g., equation 4.8) compared to a simple random sampling (SRS) design of similar sample size (Cochran 1977). In contrast, reductions in estimate variance (i.e., increases in the precision of estimates) may not be achieved for a proportional allocation scheme. In theory, a SRS design with a sufficiently large sample size will be equivalent to a StRS design employing proportional allocation with respect to domain-wide estimates of population means (e.g., equation 4.6) and variances (e.g., equation 4.8). However, when sampling heterogeneous populations such as reef fishes in practice, a StRS design with proportional allocation will at least ensure that all strata will be sampled and thus provide a guard against bias in domain-wide estimates of population means and totals as discussed above. This will especially be true for surveys with relatively modest sample sizes.

#### 4.2 Sampling Design Performance Measures

Performance of sampling designs involves the trade-offs between survey costs (usually measured by sample sizes) and the precision of population estimates. Several performance measures can be computed to evaluate the efficacy of sampling designs. The most basic and perhaps most familiar performance measure is the standard error (SE) of an estimate, computed by taking the

square root of the variance of an estimate. For the case of mean density, the standard error is given by

$$SE\left[\overline{y}_{st}\right] = \sqrt{\operatorname{var}\left[\overline{y}_{st}\right]} \tag{4.13}$$

The standard error can be used to compare the performance among design types (eq. 3.1). A relative measure of precision is the coefficient of variation (CV) of mean density,

$$CV\left[\overline{y}_{st}\right] = \frac{SE\left[\overline{y}_{st}\right]}{\overline{y}_{st}}$$
(4.14)

in which the standard error is expressed as a proportion (or percentage) of the mean. A key performance measure is  $n^*$ , the estimated sample size required to achieve a specified variance in a future survey. Computation of  $n^*$  (presumed optimal allocation) is carried out for mean density using

$$n^{*} = \frac{\left(\sum_{h} w_{h} s_{h}\right)^{2}}{V + \frac{1}{N} \sum_{h} w_{h} s_{h}^{2}}$$
(4.15)

where N is the total sample units in the domain and V is the desired variance. A convenient way to express the desired variance is

$$V = \left(CV\left[\overline{y}_{st}\right] \cdot \overline{y}_{st}\right)^2 \tag{4.16}$$

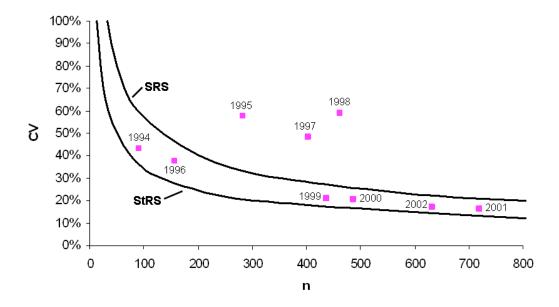
using a target CV of domain-wide mean density.

Alternatively, if the performance measure is a margin of error (d) as used in confidence intervals then

$$V = \left(\frac{d}{t}\right)^2 \tag{4.17}$$

where t is the normal deviate corresponding to the probability that the error will exceed d. This error is commonly referred to as Type I error. Case study A uses a performance measure which subsumes both Type I and Type II error rates in computations of n\*.

Aspects of design performance are illustrated in **Figure 4**, which shows performance data for estimates of black grouper density from StRS surveys in the Florida Keys coral reef ecosystem, including Biscayne National Park, during 1994-2002. Habitat-based stratification and visual sampling methods for these surveys were similar to those described in Case Study C (Ault *et al.* 



**Figure 4**: Survey design performance data for annual estimates of black grouper density in the Florida Keys coral reef ecosystem, including Biscayne National Park, during 1994-2002. Annual estimates are compared to the relationship of CV versus *n* for a stratified by benthic habitat-based survey design (StRS) and a simple random survey design (SRS). Habitat-based stratification and visual sampling methods for surveys were similar to those described in Ault *et al.* (2001).

2001). The CV-n relationship (solid line) for the StRS design was estimated using equations (4.15) and (4.16). The CV-n curve shows that gains in precision (i.e., decreases in CV) occur as n increases (i.e., as the sampling budget increases), but the gains are not limitless. For the case of black grouper, increasing n from 50 to 200 would be expected to result in a substantial decrease in CV, but increasing n from 700 to 800 would be expected to result in almost no appreciable decrease in CV.

A standard benchmark for performance of StRS designs is to compare these results with those obtained for a simple random sampling (SRS) design. The difference between a SRS design and alternate sampling design is known as the design effect. It is typically described as the ratio of the variance from the more complex design to the variance from a SRS design with the same sample units. The CV-n curve for a SRS design for black grouper was estimated by considering the whole survey domain as a single stratum. Comparing the CV-n curves for the SRS and StRS designs highlights the potential for achieving gains in precision through stratification of the domain by variables that account for spatial heterogeneity in density. Estimates of n\*, the value for n in CV-n curves, presume that sample units are allocated among strata according to a Neyman scheme. The CV-n curve for the StRS design thus represents a kind of minimum bound of CV that could be achieved in practice for a given n, because it presumes that samples are allocated on the basis of stratum size as well as stratum variance. The vertical distance between the actual CVs for black grouper density (point values by survey year) and the corresponding potential CV (CV-n curve) represents the gain in precision that could be achieved by more effective allocation of samples among strata. For the Florida Keys surveys, formal procedures of stratification, allocation, and randomization were instituted in 1999. The example of Figure 4 thus shows that achieving high precision is not simply a matter of cost, but rather the combination of effective stratification and allocation along with total sample size.

## 4.3 Composite Sampling Designs

Surveys for reef fishes will usually entail multiple target species, multiple species life stages (e.g., juvenile, adult, exploited), and multiple metrics (e.g., population abundance, community diversity). It is likely that a sampling design that performs well for one case may not perform well for other cases, requiring some sort of compromise. Obtaining a compromise from a constrained set of metrics will prove less challenging than for numerous metrics. A sensible initial step is to reduce the number of metrics to a set deemed most important and representative of other metrics.

A practical strategy for design development in this situation is illustrated in Ault *et al.* (1999) where analysis of density-habitat relationships showed that the spatial distribution of pink shrimp differed with respect to life stage (juvenile and subadult). The iterative design analysis process outlined above was applied to each life stage, and then a 'composite' stratification and allocation scheme was formulated that performed reasonably well for both life stages, although a more efficient StRS design could have been implemented for either juvenile or subadult pink shrimp. Cochran (1977) describes a process to determine a composite allocation scheme for correlated metrics when a single stratification scheme is used. The compromise is taken from the average of optimum stratum allocations among metrics. Alternative, but computationally-intensive optimization techniques are given by Chatterjee (1967), Kokan and Khan (1967), Bethel (1989) and Rahim and Currie (1993).

#### 4.4 Iterative Learning

Development of efficient (high precision, low cost) sampling designs for marine animal populations in practice usually occurs through an iterative learning process of design formulation, sampling, and performance analysis that leads to improved design formulation, sampling, and so on. The study by Ault *et al.* (1999) provides a detailed application of this iterative process to develop an efficient StRS design for a roller-frame trawl survey targeting pink shrimp in BISC. The main steps of the iterative learning process were as follows:

1) Pilot surveys were conducted in different seasons to obtain information on the temporal and spatial dynamics of the pink shrimp population in Biscayne Bay, and also to refine field sampling methods (e.g., optimal tow distance, etc.).

2) A variety of statistical methods, including some of the modeling tools described in Chapter 3, were used to identify key habitat variables influencing the spatial distribution of pink shrimp within and among seasons.

3) Alternative stratification schemes were developed based on different combinations of influential habitat variables. The design performance of these alternative schemes was evaluated using the technique of post-stratification to identify the most efficient StRS design for a future survey.

4) The refined StRS design was used to conduct a new survey, and steps 2 and 3 were repeated to further improve the sampling design.

# **5** Population and Community Assessments

The range and types of statistical analyses that will be performed to assess the status and dynamics of reef fish populations and communities in National Parks depends on the specific management questions and resource goals to be addressed. These analyses, by and large, utilize the range of fundamental survey data (abundance, size, taxonomic identification) outlined and recommended for collection in survey monitoring programs in Section 2. These survey data are then used to generate metrics for individual species and assemblages to assess status and trends of reef fish communities and populations over time and in relation to specific sustainability metrics.

## 5.1 Species and Community Metrics

#### 5.1.1 Frequency of Occurrence

Survey data relating species frequency of occurrence, i.e., the proportion of sampled sites that given species are seen, constitutes a primary index of fish community dynamics. Frequency of occurrence makes no specific reference to the actual numbers of a species at sites, but rather that they were simply observed or not. The measure can be used to assess changes in species spatial distributions over time.

#### 5.1.2 Diversity Indices

Diversity indices are measures of species composition. A large number of indices have been proposed to compute species diversity and these are outlined in the seminal works by Pielou (1969, 1977), Hurlburt (1971), Margalef (1974), Peet (1974), Legendre and Legendre (1998) and Magurran (1988).

Species richness is the simplest index and is purely the number of distinct species at sites or that are observed at all sites during a particular monitoring survey. As a fish community index, this statistic is a general measure of fish biodiversity. More complex diversity indices, such as the Shannon index (Shannon 1948), Simpson index (Simpson 1949), or Pielou's J (Pielou 1966) integrate both the number of species and the proportion of individuals in each species. A survey with many species equally represented by the same number of individuals will have a higher diversity index than a survey with fewer species or with an unequal distribution of individuals amongst species.

#### 5.1.3 Relative and Absolute Population Abundance or Biomass

Relative abundance (i.e., density =  $\frac{N_i}{a_i}$ , or numbers of animals observed or captured per unit sample area) in a sample unit (*i*) is computed as the total number of individuals ( $N_s$ ) of species *s* within a given sample area ( $a_i$ ). This quantity can be configured to represent either a relative index, or it can be related to the absolute average population size,  $\overline{N}_{abs}$ , by

$$\overline{N}_{abs} = \frac{C}{f} = \frac{N_i}{a_i} = q\overline{N}$$
(5.1)

where C is the number of fish observed (or captured) within the unit sample area, f is the nominal unit of effort (here it equals the area searched for 1 unit sample), q is the fraction of the population seen per unit sample, and  $\overline{N}$  is the average population size at the time of sampling. In this simple example, it is assumed that the design is proportional to the population (i.e., simple random sample) where all sample units have equal sampling probabilities. In a stratified survey, each of the various strata must be computed individually and weighted as discussed in section 4.1. Relative abundance has been used extensively in fisheries to characterize changes in fish population sizes for status and trends in stock assessments (Quinn and Deriso 1999, Haddon 2001, Gulland 1983) and in reef ecosystems (e.g., Bell 1983; Alcala 1988; Cole *et al.* 1990; Polunin and Roberts 1993; Dufour *et al.* 1995; Russ and Acala 1996; Friedlander and Parrish 1998 and Nagelkerken *et al.* 2000).

The total average population size (e.g., mid-year average) consisting of ages a at time t would be

$$\overline{N}(t) = \int_{ac}^{a\gamma} N(a,t) da$$
(5.2)

where  $a_c =$  minimum age at first observation (or capture) and  $a_y =$  oldest age in the population.

Population biomass is an integrated measure of the total mass (W, weight) of living biotic matter (both somatic and reproductive) for given ages at a given time. The most common procedure to estimate population biomass is to determine the relative density or abundance at a sample site, and then use species-specific allometric growth relationships to convert observations of length-at-age to weight-at-age for each individual fish (Bohnsack and Harper 1988; Ault *et al.* 1998, 2005b; Froese and Pauly, 2005). The allometric relationship between weight and length is

$$W(a,t) = \alpha L(a,t)^{\beta}$$
(5.3)

where W(a,t) is the weight of a fish at age *a* and time *t*, L(a,t) is the length of a fish at age *a* and time *t* and  $\alpha$  and  $\beta$  are coefficients of the allometric relationship. Consequently, population biomass,  $\overline{B}$ , can be calculated for a given species with

$$\overline{B}(t) = \int_{ac}^{a\gamma} N(a,t)W(a,t)da = \int_{ac}^{a\gamma} N(a,t) \left[\alpha L(a,t)^{\beta}\right] da$$
(5.4)

where parameters are as defined before. This can be done for *s* species in the reef fish community and added together for an assemblage estimate.

#### 5.1.4 Population Size-Structure

Size-structure, as derived from the sampled population, is a distributional statistic that reflects the interactions of the population-dynamic processes of individual growth, mortality and recruitment among all sizes and ages of fish in a population (Quinn and Deriso, 1999; Haddon 2001). Park managers should be interested in the status and trends of population size-structure because it provides an integrated metric of what has happened and what will happen to a fish assemblage.

Size-structure, in and of itself, is a complex measure to quantify without the aide of some summary statistic that characterizes the distribution. Ault *et al.* (1998, 2005b) have shown average length of the exploited part of a population ( $\overline{L}$ ) can be a robust indicator of community response to exploitation. This statistic is the principal stock assessment indicator variable to quantify population status (Beverton and Holt 1957; Ricker 1963; Pauly and Morgan 1987; Ault and Ehrhardt 1991; Ehrhardt and Ault 1992; Kerr and Dickie 2001). The statistic  $\overline{L}$  is a metabolic based indicator that reflects fishing mortality, because exploitation removes large individuals and species from the community (Ault et al 2002; Gislason and Rice 1998; Pauly *et al.* 1998; Kerr and Dickie 2001). Theoretically,  $\overline{L}$  at a given instant is expressed as

$$\overline{L}(t) = \frac{F(t)\int_{ac}^{a\gamma} N(a,t)L(a,t)da}{F(t)\int_{ac}^{a\gamma} N(a,t)da}$$
(5.5)

where  $a_c =$  minimum age at first capture or observation,  $a_{\gamma} =$  oldest age in the stock or population, N(a,t) = abundance for age class a, L(a,t) = length-at-age, and F(t) = instantaneous fishing mortality rate at time t. In practice, because age is unknown,  $\overline{L}$  is calculated between lengths corresponding to length at first capture and largest fish observed in the population. Case Study C describes the methods involved in calculating  $\overline{L}$  and estimating fishing mortality rates as input into calculations of sustainability benchmarks for the reef fish community in DRTO.

### 5.2 Assessing Changes to Reef Fish Metrics

An advantageous property of statistical sampling theory is that survey design estimates, such as stratum density (equation 4.1) and the variance of stratum density (equation 4.3), do not require knowledge of the underlying probability distribution (e.g., normal, gamma, etc.) of the respective population metric, (e.g., observations of stratum animal density  $y_{hj}$ ) (Cochran 1977). A second property, based on large sample theory, is that survey design estimates (e.g., population means and totals) are normally distributed due to the central limit theorem if sample size is large. These properties facilitate the analysis of survey design estimates among times or areas (e.g. monitoring density over time, assessing MPA effectiveness).

A simple, straightforward approach to performing statistical tests for differences among survey estimates for a particular time or area is via inspection of confidence intervals (CI). If the sample is relatively large (n > 100) and has a Normal distribution, the survey mean will lie within a CI bounded by

$$\overline{y}_{st} \pm t_{\alpha/k,df} SE\left(\overline{y}_{st}\right)$$
(5.6)

with a probability of  $\alpha$ , the Type I error rate, and where *t* is the critical value of Student's *t*distribution, and degrees of freedom  $df = n_h$ -1. Most commonly used reef metrics (see section 5.1) do not posses a Normal distribution which means the Type I error rate will not equal  $\alpha$ . Cochran (1977) states  $\alpha$  will be very close to what is expected if

$$n > 25 \times G_1^2 \tag{5.7}$$

where  $G_1^2$  is Fisher's measure of skewness. If a sample is too small and the population is heavily skewed, transforming the data (e.g.  $\log[y_{st} + 1], [y_{st}]^2$ ) may help.

CIs can be used to test the hypothesis that samples were drawn from the same population, as is done to assess temporal change or determine MPA effectiveness. Cochran (1977), Sokal and Rohlff (1995), and Zar (1999) describe methods using CIs to test for differences among means. A comparison of multiple CIs (e.g. a time series) requires a Bonferoni adjustment to  $\alpha$ . The Bonferoni adjustment is necessary because the true Type I error rate of simultaneous multiple tests is not  $\alpha$ , as it should be for a single test. A useful relationship stemming from equation (5.6) is that the 95% CI for a population metric is approximately twice the CV, because the value of *t* for  $\alpha = 0.05$  and df > 20 is approximately 2. Thus, for example, a StRS survey that provides a domain-wide estimate of abundance with a CV of 15% would be able to statistically detect a minimum change of 30% in population abundance between survey time periods (with a Type I error rate = 0.05).

## 5.3 Population Mortality Rate Assessments Using Size-Structure

Exploitation (or other) effects from fishing mortality could be specifically assessed by bounding the integral for Equation (5.5) to reflect the ages/sizes affected. For example, a minimum size limit  $L_c$  would constrain the solution to consider the average size ( $\overline{L}$ ) between  $L_c$  and  $L_{\gamma}$ , the minimum size limit and the maximum size observed in the catch (or seen in the visual samples) or population. In a population where fishing mortality is strictly proportional to the stock,  $\overline{L}$  of fish on the dock would be exactly equal to the  $\overline{L}$  of those fish remaining in the sea, assuming that recruitment remained constant within a finite range of population sizes.

Average size has been used by several analytical studies to assess the impacts of exploitation on reef fish populations and communities, and thus guides management decisions regarding policies to achieve sustainability of reef fish resources (Williamson *et al.* 2004; Ault *et al.* 1997, 2005a, 2005b, 2006; Nemeth 2005) [see Case Studies].

For the case where no fishing occurs, equation (5.5) in combination with fisheryindependent data, could be used to compute the natural mortality rate M, or the life-time expectation of survivorship (i.e., average maximum age in the population).

## 5.4 Population Biomass Assessments in Relation to Sustainability Benchmarks

An important measure of stock reproductive potential is population spawning biomass. One which is used more frequently in fishery management is spawning stock biomass (SSB). SSB is expressed as

$$\overline{SSB}(t) = \int_{ac}^{a\gamma} N(a,t)W(a,t)da$$
(5.10)

where  $a_m$  is the minimum age (or size) of sexual maturity. Case study C provides an example of using the SSB and the derived spawning potential (SPR) ratio to assess fishery management in DRTO. SPR is a management benchmark that measures the stock's current reproductive potential to

produce optimal yields on a sustainable basis. It is simply the ratio of spawning stock biomass from exploited and unexploited populations. Estimated SPRs can be compared to U.S. Federal standards which define 30% SPR as the overfishing threshold at which the stock is no longer sustainable at the current exploitation levels.

## 6 Case Study Foreword

The preceding five sections provide a framework for generating a standardized monitoring protocol for use in SFCN park units. The framework outlines useful methods for a monitoring program, but is not a single standardized monitoring protocol for all park units. The variability in ecological condition, size, management capability, expertise, and available data sets among park units implies different parks will have distinct management objectives and logistical constraints. Monitoring programs must be crafted for individual park units considering specific monitoring needs and abilities. One size of monitoring program does not fit all park units.

Three reef fish monitoring case studies are presented which build upon the presented monitoring framework using park-specific data sets, management concerns, and local partnerships. The case studies are offered to provide persons implementing a monitoring program with the information required to understand the pertinent: 1) management issues, 2) sampling methods, and 3) analytical methods used in monitoring reef fish in SFCN managed areas. The case studies employ distinct methodologies because they reflect differences among park needs and abilities. The case studies are similar, but utilitize different measurement methods, sampling designs, and analyses. These differences among case studies are summarized in **Table 1**.

Case study A implements a stratified random sampling design in BUIS and VIIS. Regional benthic habitat maps are used to increase survey design performance. Field work is undertaken by the NOAA Biogeography Team in cooperation with NPS. Design performance, temporal changes, and MPA effectiveness for several fish assemblages are investigated using survey data from 2001-2005. This case study is a good example of effectively utilizing a moderate amount of resources (e.g. multiple boats, dive teams) to obtain precise metrics of the community and several assemblages of special management concern.

Case study B uses the stationary visual census technique to sample at multiple, permanent, high-diversity coral reef reference sites around and in VIIS. This strategy effectively makes use of few resources to monitor constrained areas with high precision. Field work is conducted by the University of Hawaii in Hilo and NOAA Biogeography Team. Data collected from 1988-2000 are analyzed for MPA effectiveness and trends.

Case study C employs a two-stage stratified random sampling design to sample over hard bottoms in DRTO. Surveys are conducted by the University of Miami in cooperation with the NOAA Southeast Fisheries Science Center. A regional benthic habitat map and distinct management zones are used to stratify the large survey domain. Analyses of survey design

performance, sustainability status of exploited fish species, and MPA performance using 1999-2004 data are provided. This case study shows the effective use of a live-aboard dive vessel, multiple dive teams, and cluster sampling to efficiently survey a very large area (320 km<sup>2</sup>) and obtain precise metrics for fishery assessments.

**Table 1:** Summary of differences in (A) measurement methods, (B) sampling designs and (C) data analyses among case studies.

## (A) Method of Measurement

Approach	Reason(s)	Case Study - Section		
Belt transect	t (1) Increase sighting frequency of small fish, and (2) Some sites characterized by moderate visibility (>2 m), rugose benthic structure, or adjacent to mangrove prop roots			
Stationary visual census	<ul> <li>(1) Decrease measurement bias due to diver movement, and</li> <li>(2) Majority of sites have good visibility (&gt;7.5 m)</li> </ul>	B-3.4, C-2.3		

#### (B) Sampling Design

Approach	Reason(s)	Case Study - Section
Stratified random sampling design. Survey domain encompasses whole park and surrounding areas. Strata classified according to a covariate benthic habitat map.	<ol> <li>Increase survey estimate precision and reduce sampling cost compared to SRS</li> <li>Obtain representative samples</li> <li>Survey/Make inferences to whole fish community in park</li> <li>Obtain estimates of specific areas in survey domain (e.g. MPA)</li> </ol>	A-3.2
Stratified random sampling design. Survey Domain encompasses only reference sites.	Concentrate few resources into understanding references sites very well	B-3.2
Multi-stage stratified random sampling design. Strata classified according to benthic habitat map	<ul> <li>(1) Same as first, and</li> <li>(2) Cluster sample units according to mapped covariate</li> <li>(3) Sample over a large area</li> <li>(4) Increase precision by applying finite population correction on first stage of sampling</li> </ul>	C-2.2

## (C) Analysis of Data

Approach	Reason(s)	Case Study - Section
Analysis of differences in survey estimates among years and areas using confidence intervals.	Assessment of change in survey estimates among years or among areas	A-5.3, B-4.5, C-3, C-5
Analysis of size structure, average size, and spawning stock biomass	Assessment of population mortality rates and biomass sustainability benchmarks	C-4
Analysis of trend in survey estimates using Generalized Linear Model	Assessment of long-term linear trends in survey estimates among years	B-4.5, B-4.6

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# Case Study A: Monitoring Reef Fish in Buck Island Reef National Monument, Virgin Islands National Park and Virgin Islands Coral Reef National Monument 2001-2005

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## A-1 Summary

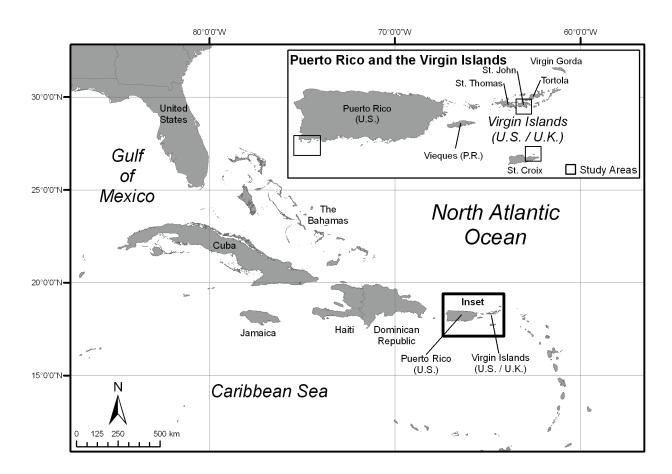
This case study describes the survey methods and analyses used by the National Oceanic and Atmospheric Administration's (NOAA) Center for Coastal Monitoring and Assessment (CCMA) Biogeography Team (BT) to monitor reef fish in Buck Island Reef National Monument (BUIS), Virgin Islands Coral Reef National Monument (VICR), and Virgin Islands National Park (VIIS). These areas are administered by the National Park Service. Two of these areas, VIIS and BUIS, are part of the South Florida / Caribbean Network (SFCN), which has been given the charge of generating long-term reef fish monitoring protocols.

The survey methods provide precise, fishery-independent, minimally-biased, multispecies and size-structured survey data, which is needed to comprehensively assess fish populations and communities. The analyses described herein are applied to: (1) Determine long-term changes in reef fish community structure, using measures of biodiversity, abundance and biomass; (2) Determine long-term changes in abundance, biomass and mean-size of selected economically and ecologically important assemblages; and (3) Compare long-term changes in fish community structure and the abundance, biomass and mean-size of selected assemblages between areas inside and outside of Marine Protected Areas (MPAs).

## **A-2 Background Information**

In 1998, the Caribbean Fisheries Management Council approached the BT to assist in the delineation of Essential Fish Habitat (EFH) in Puerto Rico and the Unites States Virgin Islands (USVI). Over the next three years, nearshore benthic marine habitat maps were developed and in 2000 BT developed a reef fish sampling protocol to gather fish data and begin determining the relationships between fish and benthic habitats. Other agencies and academic partners including but not limited to the University of Puerto Rico, USVI Department of Planning and Natural Resources, United States Geological Survey, and the NPS became interested in expanding on the EFH work. The result was the emergence of BT's Coral Reef Ecosystem Monitoring Program (CREMP), which focused around the areas of (1) St. John, USVI, (2) Buck Island, St. Croix, USVI, and (3) La Parguera, Puerto Rico (see **Figure A-1**).

The areas monitored in the USVI by the BT and which are the focus of this study include VIIS, BUIS and VICR. Two of these managed areas, VICR and BUIS, offer reef fish a high level of protection because all extractive uses and anchoring (except for limited exceptions) are prohibited



**Figure A-1**: A map of the northern Caribbean region showing Biogeography Team study areas in Puerto Rico and the US Virgin Islands.

within park boundaries. One of the goals of this case study is to assess the effectiveness of management/protection in these two protected parks.

CREMP is comprised of an iterative process of data collection, assimilation, design analysis, and population and community assessment. As data are gathered they are analyzed and results are used to refine survey methods, as needed. Data are also provided to park managers to interface with park management policies. A conceptual model, shown in **Figure A-2**, details the organization of methods which make up the CREMP strategy.

A central principle of CREMP is to concurrently characterize the reef fish community, benthic habitat, water quality, and a selection of benthic organisms requested by park managers (e.g. conch, lobster, urchins) at each sample unit. The concurrent data provide the ability to detect relationships among data at fine spatial scales. Relationships can then be integrated into an ecosystems-based management approach to reef fish management and serve the federal directive of better understanding essential reef fish habitats (NOAA 1996). Although measurements of benthic habitat, water quality, and supplemental benthic data are important components of CREMP, the methods discussed herein focus on the methods used to collect and analyze reef fish data. All methods are described online at NOAA (2006a).

CREMP was created and is maintained to explicitly satisfy three monitoring goals:

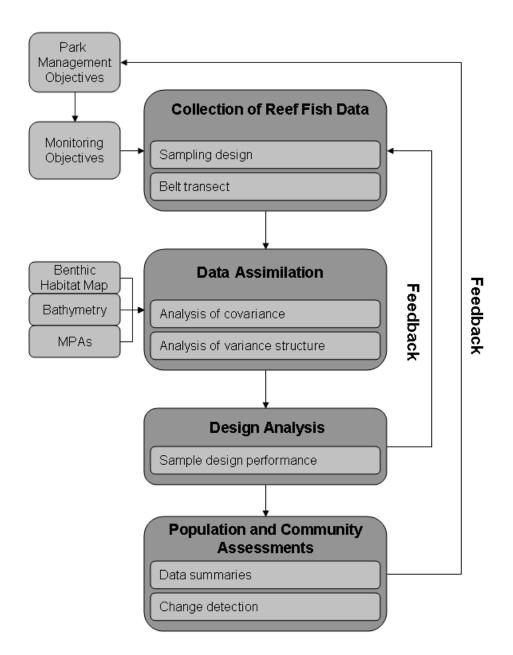
- 1. Determine long-term changes in reef fish community structure using measures of biodiversity, abundance and biomass;
- 2. Determine long-term changes in abundance, biomass, and mean-size of selected economically and ecologically important fish assemblages and species; and
- Compare long-term changes in fish community structure and the abundance, biomass, and mean-size of important fish assemblages and species between areas inside and outside of Marine Protected Areas (MPA).

The goals reflect management objectives as determined by park managers, NPS management plans, and local stakeholder needs.

## A-3 Survey Design

#### A-3.1 Population to Be Sampled

Although management efforts by the NPS are directed towards the VIIS, VICR, and BUIS, reef fishes in these parks depend on and interact with surrounding areas over a much larger spatial scale. To comprehensively monitor and manage reef fishes inside NPS parks, and determine the



**Figure A-2**: A conceptual model of the Biogeography Team Coral Reef Ecosystem Monitoring Program (CREMP). Note the feedback loops.

effectiveness of management regimes, samples were taken from areas outside park boundaries as well. The survey domains, which define the populations to be sampled in Buck Island and St. John study areas, were chosen to include as much area as possible adjacent to targeted managed areas within logistical constraints. A compilation of bathymetric data from the National Geophysical Data Center, Geophysical Data System (NGDC 2006) and multibeam data collected by BT using a hull-mounted Simrad EM 1002 multibeam sonar (NOAA 2006b) was used to determine the geographical areas within safe repetitive diving limits (i.e. 30 m). The populations to be sampled are shown by the spatial limits of strata in **Figures A-3A** and **A-3B**.

#### A-3.2 Sampling Design

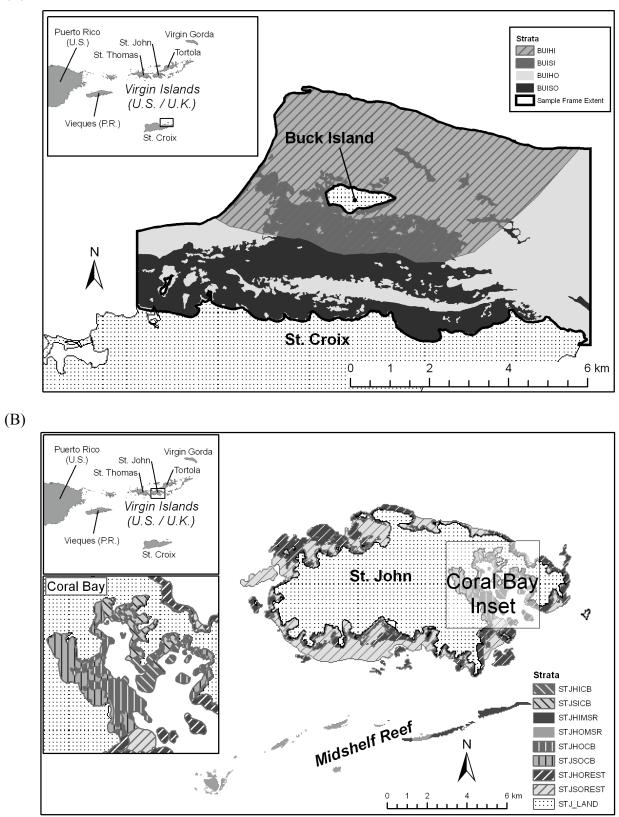
A stratified random sampling design was used to maximize the amount of information attained at a minimum of cost and allow rigorous inferences to the entire study area or internal domains designated by strata. The survey domain was partitioned into strata based on three spatial variables: benthic habitat, management zone, and geographic zone. Benthic habitat types and geographic zones were incorporated because they were covariates of reef fish and could increase sampling design efficiency. NPS managed area boundaries were used to compare different management regimes and satisfy the data requirements of the third CREMP objective.

The survey domain was divided into "hard" and "soft" benthic habitats as an effective compromise between parsimony and the results of an analysis of variance used to evaluate the covariance among representative population parameters and distinct benthic habitats. This analysis is described in Section A-5.1. Hard benthic habitat included areas characterized by bedrock, pavement, and coral reefs. Soft bottom habitats included those areas characterized by sand, seagrass, or macroalgae. Benthic habitat maps and a hierachical classification system produced by NOS (Kendall 2001) for the nearshore waters around the USVI were used as a basis for analysis and habitat designations.

The extent of classified area in the NOS maps corresponded to the majority of areas within safe repetitive diving limits, however a long reef complex south of St. John within diving limits was not classified. This complex, known as the mid-shelf reef (MSR), was classified according to previous dive surveys as a hard bottom habitat.

NPS managed areas were incorporated as a second spatial stratification level and were designated as: Inside BUIS and Outside BUIS for the Buck Island study area; and Inside VICR, Inside VIIS and Outside VIIS/VICR for the St. John study area. Areas inside BUIS and inside

(A)



**Figure A-3**: A map of the monitored (A) Buck Island and (B) St. John regions showing strata used in the stratified sampling design. Refer to **Table A-1** for information on strata.

VICR offer reef fish a high level of protection, because all extractive uses and anchoring (except for limited exceptions) are prohibited within park boundaries. Thus, BUIS and VICR were defined as MPAs. Areas inside VIIS offer reef fish an intermediate level of protection, because commercial and recreational fishing is restricted in some areas of the park. The remaining areas outside of BUIS, VICR, and VIIS and inside the survey domain are open to both commercial and recreational fishing.

Geographic zones were used as a third spatial stratification layer in St. John's sampling design. These zones were designated as: (1) the MSR, (2) Coral Bay (CB), and the remaining areas. Each zone was characterized by a distinct range of depths and water quality characteristics, which would be expected to influence the variance structure of investigated fish community and population parameters (e.g. species number, abundance, percent occurrence) and sampling cost.

Summary information for each stratum is provided in **Table A-1**. **Figures A-3A** and **A-3B** show the strata used in the Buck Island and St. John study areas, respectively. Sample units were selected for each survey by randomly choosing geographic coordinates within each stratum. A geographic information system (GIS) in the ArcGIS 9 environment (ESRI, inc.) was used to select sample units. Analyses of variance of several community and assemblage metrics were used to estimate sample size requirements and resolve the optimal sample allocation configuration among strata. A description of these analyses and corresponding results are provided in Section A-5.2.

#### A-3.3 Methods of Measurement

Reef fish data were collected using non-destructive, in situ, fishery-independent visual belt transects (adapted from Brock 1954) and stationary point counts (Bohnsack and Bannerot 1986). These methods required SCUBA gear (or snorkeling gear for shallow sites) and thus the maximum depth of samples was constrained to 30 m. Cumulative bottom time (time underwater) and the desire for monotonically decreasing (in depth) dive profiles are also constraints when using SCUBA and factors which require planning before going into the field. BT used NITROX gas to increase bottom time, planned which sites were sampled before each day of diving, and used multiple motorboats with independent dive teams to efficiently gather the maximum number of samples possible.

The average number of dives each dive team was asked to complete each day was determined as a function of the number of dive teams, days available for diving, and sample site depths. Normally bottom time per dive lasted between 20-40 minutes depending on benthic

**Table A-1**: Strata and corresponding variables used to define strata for the (A) Buck Island and (B) St. John region sampling frames.

(A)			
Strata Code	Benthic Habitat	MPA	Area (km²)
BUIHO	Hard	No	13.8
BUIHI	Hard	Yes	18.5
BUISO	Soft	No	12.3
BUISI	Soft	Yes	5.40

Ronthia			
Habitat	MPA	Geographic Zone	Area (km <sup>2</sup> )
Hard	No	Outside CB and MSR	13.8
Hard	No	CB	1.71
Hard	No	MSR	1.51
Hard	Yes	CB	0.86
Hard	Yes	MSR	1.07
Soft	No	Outside CB and MSR	15.8
Soft	No	CB	1.81
Soft	Yes	CB	0.91
	Hard Hard Hard Hard Hard Soft	HabitatMPAHardNoHardNoHardNoHardYesHardYesSoftNoSoftNo	HabitatMPAGeographic ZoneHabitatNoOutside CB and MSRHardNoCBHardNoMSRHardYesCBHardYesMSRSoftNoOutside CB and MSRSoftNoCB

substrate complexity, depth, and number of divers. Due to transport time between sample sites and time to set up and change tanks each dive required about 40-60 minutes. Typically, between 5 and 10 samples were collected by each dive team each day. A map showing sample locations and depths, such as the example shown in **Figure A-4**, was a useful tool for planning.

Divers were taken to sample units by small to medium sized motorboats using a handheld GPS unit. Boat drivers, which were usually divers as well, were required to successfully complete the requirements of the National Park Service's Motorboat Operator Certification Course. To conform to legal mandates all diving was done using NOAA/NPS scientific diver regulations (NOAA, 2006c) and standard diving procedures (buddy teams). All divers were certified as NOAA scientific divers and possessed letters of reciprocity to work in NPS managed areas. All belt divers were trained to recognize the distinguishing marks and morpholgies of different species and to accurately measure lengths underwater. Divers were encouraged to calibrate underwater length estimates before each survey with a ruler.

Although two methods were used to collect reef fish data, CREMP concentrated its efforts to collect and analyze reef fish data using the belt transect methodology, because of its ability to effectively sample multiple habitats, ease of use, and ability to work in relatively low visibility. Stationary point counts were used to allow standardized comparisons of CREMP data with historical datasets and current sampling programs that implement point counts. Important complements to the belt transect survey were the benthic habitat and water quality assessments at each sample site. These surveys provide important information to optimize future sampling designs and modeling studies and provide the requisite information for an ecosystem-based management approach. Additional data may be collected (e.g. conch, lobster, long-spined urchin, coral bleaching surveys) depending on suggestions by scientists and the needs of park managers. Only the belt transect method is described and only data collected using belt transects are analyzed herein.

The belt transect used by BT to collect reef fish data had a constant area (25 m long and 4 m wide, total 100 m<sup>2</sup>), and a constant survey time (15 min). Area and time were held constant to reduce biases associated with species detectability among habitat types and ensure the probability of sample unit selection was known.

Once on site an anchor or weighted buoy was deployed to mark the location of the sample unit. The belt transect diver and at least one other diver were deployed and descended to the seafloor. A typical diving team was represented by three divers: one performed the belt transect,

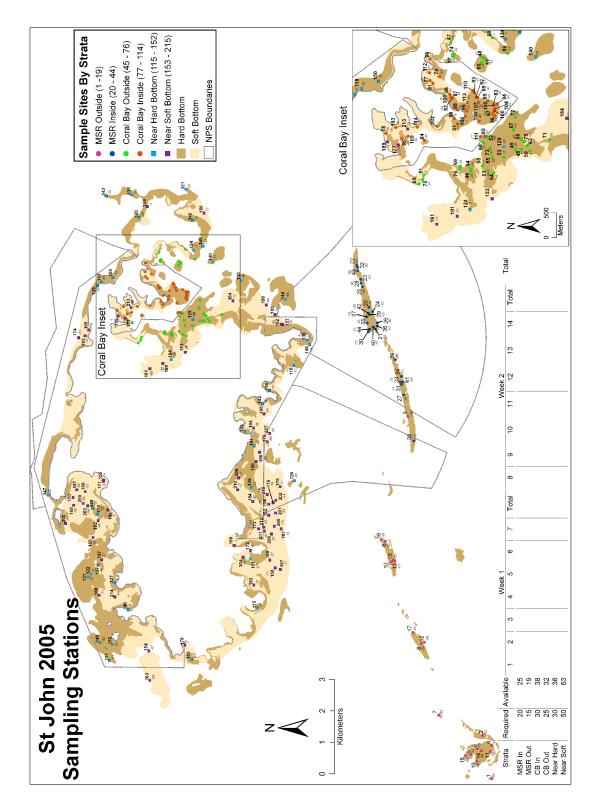


Figure A-4: Example of sample position map used in the field.

another the stationary point count, and the third the benthic habitat assessment. Whenever rough sea conditions were present, the boat driver/diver would stay on the boat. If three divers were not available for the suite of surveys, one diver would perform the belt transect and stationary point count and the second diver would perform the habitat assessment.

If a dive site's habitat characteristics were different than the corresponding stratum characteristics (i.e. a dive site was located in a strata characterized by soft benthic habitat, but the habitat within the surveys was characterized by hard habitat) the surveys were still completed, but the discrepancy was noted. If a dive site could not be reached because of obstacles, surveys were performed as close as safely possible to the unreachable original dive location and new geographic coordinates were recorded.

Once just above the seafloor, the belt transect diver fastened the end of a tape measure to the anchor or weighted buoy line or adjacent substrate, taking care not to damage fragile habitat. Then the diver proceeded away from the location where the tape was fastened in a random bearing. The diver gathered data for all fishes within two meters of either side of the transect line and all fishes towards and up to the estimated transect ending (25 m). No data were collected from the area behind the diver's position. The tape measure was unreeled as the diver swam forward and not before to minimize fish behavioral biases. Swimming speed was maintained constant such that the entire length of the transect was traversed in 15 minutes regardless of substrate type or complexity. At this relatively slow speed, all habitat types, including complex reefs were comprehensively surveyed. The diver recorded fish seen in holes, under ledges and in the water column but took care not to alter the habitat in any manner by lifting or moving structures. To identify, enumerate, or locate new individuals the diver was allowed to move off the centerline of the transect, but always remained within 2 m of the transect centerline.

As the diver progressed along the transect each new observed species (or lowest identifiable taxonomic level) was recorded and the number of individuals per species (or lowest identifiable taxonomic level) was tallied in 5 cm size class increments up to 35 cm using visual estimation of fork length. All data were entered into a standardized form (**Figure A-5**). If an individual fish was greater than 35 cm, then an estimate of the actual fork length was recorded. To decrease the total time spent writing, four letter codes were used to itemize the distinct species. The code consisted of the first two letters of the genus name followed by the first two letters of the species name (e.g. STPL = *Stegastes planifrons*). In the rare case that two species have the same four-letter code, letters are added from the species name until a difference occurs.

SAND/SEAGRASS ONLY		Name:		Station:		Method:	ΡΤ	
$\frac{\text{(Reef within 4 m of diver)}}{0 < 4 \text{ m}^2 > 4 \text{ m}^2}$		Buddy: Bearing		Date:		Time:		
0 < 4 111-2 > 4 111-2		Беанну						
fish ID	<5	5-10	10-15	15-20	20-25	25-30	30-35	>35
1								
2								
3								
4								
5								
6								
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Figure A-5: Example of fish survey data entry form.

## A-4 Data Storage and Distribution

Once data were collected in the field, each diver was responsible for entering his/her data into the Coral Reef Ecosystem Assessment and Monitoring (CREAM) database. This online database was created and is maintained by BT to store reef ecosystem monitoring data and serve it to the public. Belt transect, point count, and benthic habitat assessment data are available. All data are associated with a unique dive site ID and possess geographic coordinates to facilitate mapping. The database went online in 2005 and provides data from field studies in the USVI since 2001 and southwestern Puerto Rico since 2000. The CREAM database is publicly available on the World Wide Web (NOAA 2006d).

Researchers, coastal managers, and interested members of the public can download the entire dataset, or can query the database to provide specialized reports for specific species assemblages. The database is capable of returning biomass, richness, abundance, and/or the Shannon diversity index metrics. In addition to the survey data, a second querying function was developed to serve both fish and habitat photos that have been collected over time. All data include metadata which conform to all government standards required for data dissemination. The database is maintained on a SQLServer 2000 server, and queried by an ASP.Net web interface developed by BT. The database structure is relational, maintaining data integrity through the use of Primary and Foreign key constraints.

Data served by the CREAM database have been used by numerous institutions and include the following projects: (1) Investigations of reef fish habitat utilization patterns (Monaco *et al.* 2001; Christensen *et al.* 2003; Monaco *et al.* 2003; Kendall *et al.* 2003; Kendall *et al.* 2004; Pittman *et al.*, in review); (2) Assessment of marine reserve design (Monaco et al 2001; Monaco *et al.* 2006); (3) Validation of benthic habitat maps of Puerto Rico and the US Virgin Islands (NOAA 2001); (4) Analysis of zonation designations within the East End Marine Park of St. Croix (Ocean Conservancy); (5) Comparison with cryptic fish inventories of BUIS (USGS, University of Miami and NOVA Southeastern University); (6) Site characterization of BUIS (NOAA and NPS); (7) Evaluation of sites to observe the effects of coral bleaching on coral communities in VICR (NASA, University of the Virgin Islands); and (8) Analysis of the boundary design for BUIS and VICR (NOAA, NPS, and the University of Hawaii).

## A-5 Analyses

## A-5.1 Fish Assemblage Differences across Multiple Benthic Habitat Types

Fish data collected during 2001-2005 using belt transects were used to evaluate the covariance among representative population parameters and distinct benthic habitats. The goal of this analysis was to determine regions of homogenous variance so that these regions can be incorporated as strata in a stratified random sampling design. By parsing the survey domain into internally homogenous strata the precision of population parameter estimates can be drastically increased, which in turn drives down sampling costs.

The survey domain was initially divided among 12 distinct habitat types in each study area according to the benthic habitat types defined by Kendall *et al.* (2001). The MSR area was included as a 13<sup>th</sup> habitat type when examining the data from St. John. The covariance of a set of representative fish assemblages (listed in **Table A-2**) and habitat types were examined using nonparametric analysis of variance (ANOVA; Kruskal-Wallis test, p<0.0001) and plots of density (fish per unit area) against standard deviation of density among habitat types. Species richness (number of species) was also examined.

The spatial distributions of the representative fish assemblages were heterogeneous. Nonparametric analysis of variance indicated that habitat type explained some of the variance in all of the tested community and assemblage metrics (Table A-3). In Buck Island the highest species richness and assemblage densities were typically found in linear reef, aggregated patch reef, and individual patch reef habitats and the lowest in sand, seagrass, and scattered coral/rock in sand habitats (Figure A-6A). In St. John the highest species richness and assemblage densities were not found to be as consistently associated with habitat types as around Buck Island. Species richness, mean density of the community (all species) and mean density of groupers were highest in MSR sites, but snappers and piscivores were conspicuously absent (Figure A-6B). Aggregated patch reefs, individual patch reefs, and colonized bedrock habitats also possessed high densities for some of the tested assemblages, but this pattern was not consistent across assemblages (Figure A-6B). As in the Buck Island study area, sand and seagrass habitats were associated with low assemblage densities and species richness. The macroalgae habitat was omitted from the Buck Island analysis because too few samples were available (n=4). Samples collected in macroalgae habitat in St. John showed many similarities to samples collected in sand and seagrass habitats.

Monitored Assemblages	Species in Assemblage
Community - All Species	All observed species
Groupers	All species in the genera Mycteroperca, Cephalopholis and Epinephelus
Snappers	All species in the genus Lutjanidae
Herbivores	All species listed as herbivores (H) in FISHBASE (Froese and Pauly 2005)
Piscivores	All species listed as piscivores (P) in FISHBASE (Froese and Pauly 2005)
Cephalopholis fulvus	Cephalopholis fulvus
Stegastes planifrons	Stegastes planifrons

**Table A-2**: The list of representative reef fish assemblages, corresponding component species and aliases, and metrics used to optimize the Buck Island and St. John region monitoring programs.

**Table A-3**: The results from a nonparametric analysis of variance (Kruskal-Wallis test) for species richness, community density, and assemblage densities among 12 habitat types in the (A) Buck Island and (B) St. John study areas.

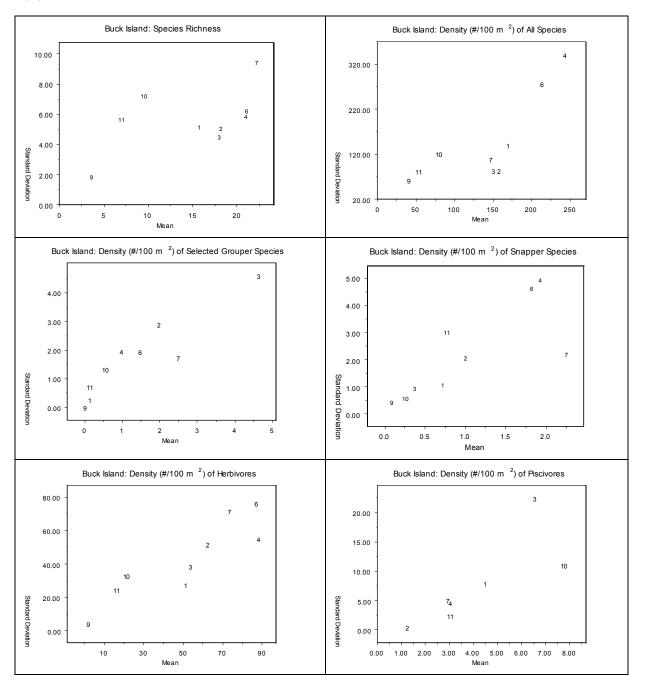
#### (A) Buck Island

Kanakal Wallia II	<b>P</b> [ <b>H</b> ] < $\chi^2_{0.05,10}$
Kruskai- w anis fi	<b>I [III]</b> × $\chi_{0.05,10}$
494.89	< 0.0001
394.21	< 0.0001
393.24	< 0.0001
81.12	< 0.0001
452.96	< 0.0001
24.36	0.0113
	394.21 393.24 81.12 452.96

#### (B) St. John

Kruskal-Wallis H	<b>Ρ</b> [ <b>H</b> ]< $\chi^2_{0.05,10}$
318.14	< 0.0001
256.01	< 0.0001
168.73	< 0.0001
31.65	0.0016
299.47	< 0.0001
22.37	0.0335
	318.14 256.01 168.73 31.65 299.47

#### (A) Buck Island



**Figure A-6**: Plots of density (fish per unit area) or species richness against variance among distinct habitat types in the (A) Buck Island and (B) St. John study areas. Benthic habitat types are defined as: 1-Colonized Bedrock, 2-Colonized Pavement, 3-Colonized Pavement with Sand Channels, 4-Linear Reef, 5-Macroalgae, 6-Aggregated Patch Reefs, 7- Individual Patch Reefs, 9-Sand, 10-Scattered Coral/Rock in Unconsolidated Sediment, 11-Seagrass and 12-MSR.

#### (B) St John

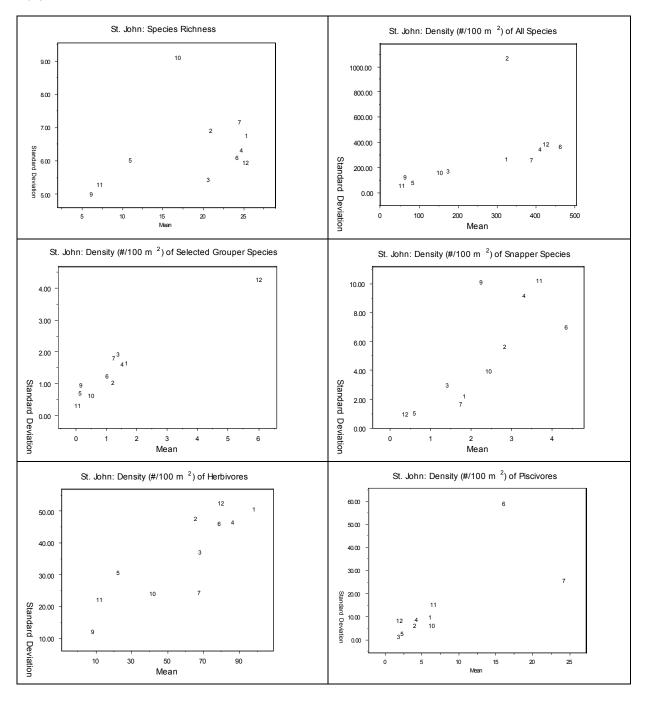


Figure A-6: (cont.)

As an effective compromise between parsimony and reduction in the variance of estimated population parameters, two mutually exclusive strata were selected to stratify the survey domain in both study areas: hard and soft bottom habitats. Hard bottom habitats subsumed colonized bedrock, colonized pavement, colonized pavement with sand channels, linear reef, aggregated and individual patch reefs, scattered coral/rock in sand, and the MSR habitats. Soft bottom habitats included sand, seagrass, and macroalgae habitats.

#### A-5.2 Optimizing the Sampling Strategy

To ensure the survey design was as efficient as possible, sample size requirements and sample allocations among strata were examined for a set of community and population metrics (listed in **Table A-2**). Sample size requirements were computed using a modified verision of the methods described by Cochran (1977) for a stratified random sampling design. The desired variance was expressed as

$$V = \left[\frac{\left(d \cdot \overline{y}_{st}\right)}{\left(Z_{\alpha/2} + Z_{\beta(1)}\right)}\right]^2 \tag{1}$$

where *d* is the desired precision of the survey mean,  $\overline{y}_{st}$  is the survey mean,  $Z_{\alpha/2}$  is the Normal deviate corresponding to the allowable probability of Type I error, and  $Z_{\beta}$  is the Normal deviate of the allowable probability of Type II error. The probabilities of error refer to the statistical errors involved in a confidence interval test to compare two surveys. The Normal deviates were set such that the confidence interval test would have a 5% probability of Type I error (deviate=1.96; reject the null when null is true) and a 20% probability of Type II error (deviate =1.64; do not reject the null when null is false) when surveys are compared and the null hypothesis is that the surveys come from the same population.

Two different analysis endpoints were used to determine sample size requirements. The first endpoint was an assessment of reef fish communities and populations in either the BUIS or VIIS among years. Hence, only data from samples collected within BUIS or VIIS were used. The second endpoint was an assessment of the difference between reef fish communities and populations inside and outside MPAs among years. To assess the difference, data from samples collected within VICR and BUIS strata, and in adjacent strata (Buck Island: BUISHO, St. John: CBO and MSRO) were used. The difference was the domain estimate inside an MPA minus the domain estimate outside and

MPA. The variance of the difference was the sum of variances inside and outside a MPA according to Cochran (1977).

The estimates of stratum means and variances were taken from 2001-2005 CREMP belt transect data and were weighted according to the sample size of each corresponding survey. Metrics which were heavily skewed so that  $n < 25 \times G_1^2$  (where  $G_1$  is Fisher;s skewness coefficient) were log[X+1] transformed, because confidence intervals would have been inappropriate (Cochran 1977).

Not all potential stratum estimates were included in sample size computations because monitoring began in different strata in each region at separate times. There was a desire to increase sampling design efficiency and some assemblages were very rare in some strata. Consequently, (1) strata characterized by soft bottom benthic habitat were omitted for assemblage and species metrics; (2) strata characterized by soft bottom benthic habitat were omitted from survey estimates of the difference among protected and unprotected domains; (3) the MSR strata was not used to define survey estimates describing community structure in St John; and (4) data from 2001 and 2002 was only used for survey estimates for VIIS in St. John because the MSR was added in 2003 and BUIS expanded greatly in 2003. **Tables A-4** and **A-5** shows the average survey means, standard errors, and sample size requirements for all community and population metrics.

Ideally desired levels of sampling precision and statistical power are used to set the most efficient sample size for a sampling design. Unfortunately, this process was not feasible for CREMP, because sample size was set by fiscal constraints (Buck Island n=120; St John n=170). Given these constraints many metrics in **Tables A-4** and **A-5** require more samples than are feasible. Although there was little control over the total sample size, there was flexibility over sample allocation among strata and allocation has a powerful influence on sampling design efficiency when multiple measurements are being taken and analyzed. To define the optimum allocation scheme for all measurements, the allocation of samples using the Neyman allocation scheme for all metrics was examined and a single allocation scheme which maximized the number of metrics that fulfilled sample size requirements was determined. The single, all-inclusive optimum allocation scheme is shown in **Table A-6**.

**Table A-4**: Estimates of principle reef fish community and assemblage metrics in (A) BUIS and (B) VIIS. Data taken from 2001-2005 belt transects.

Community or Assemblage (Metric)	Average Estimates of Raw Survey Data			Average Transfo	Sample Size Requirements		
	Mean	SE	CV	Mean	Data SE	CV	
Species Richness	15.96	0.73	0.05				96
Species Diversity	1.82	0.06	0.03				50
Community Density	158.17	18.63	0.10				639
Community Biomass	2211.09	302.56	0.13				863
Grouper (Freq. of occurrence)	0.45	0.05	0.11				570
Grouper (Density)	1.55	0.28	0.18	0.26	0.03	0.13	613
Grouper (Biomass)	164.64	44.51	0.25	1.05	0.12	0.11	602
Grouper (Mean Size)	19.61	0.8	0.04	1.29	0.02	0.01	11
Snapper (Freq. of occurrence)	0.3175	0.05	0.17				1143
Snapper (Density)	1.01	15.56	0.29	0.16	0.03	0.2	1620
Snapper (Biomass)	86.5	33.71	0.37	0.61	0.11	0.19	1499
Snapper (Mean Size)	17.09	1.6	0.09	1.17	0.06	0.05	121
Herbivore (Freq. of occurrence)	0.90	0.02	0.03				22
Herbivore (Density)	52.71	5.63	0.10	1.42	0.04	0.03	36
Herbivore (Biomass)	686.56	88.201	0.12	2.2	0.06	0.03	34
Herbivore (Mean Size)	402.4	72.02	0.17	2.04	0.07	0.03	54
Piscivore (Freq. of occurrence)	0.52	0.05	0.11				422
Piscivore (Density)	2.54	0.86	0.29	0.27	0.04	0.14	1011
Piscivore (Biomass)	432.4	151.29	0.32	1.25	0.14	0.11	578
Piscivore (Mean Size)	120.16	42.41	0.28	1.65	0.08	0.05	108
CEFU (Freq. of occurrence)	0.36	0.05	0.13				889
CEFU (Density)	1.14	0.24	0.21	0.2	0.03	0.16	1037
CEFU (Biomass)	96.8	21.23	0.21	0.82	0.11	0.14	829
CEFU (Mean Size)	19.16	0.8	0.04	1.29	0.02	0.01	11
STPL (Freq. of occurrence)	0.21	0.05	0.22				2631
STPL (Density)	1.44	0.49	0.34	0.15	0.03	0.24	1843
STPL (Biomass)	5.17	1.61	0.31	0.26	0.05	0.23	1704
STPL (Mean Size)	5.55	0.66	0.12	0.78	0.04	0.06	121

(A) BUIS

#### Table A-4: cont.

## **(B)** VIIS

Community or Assemblage (Metric)	Average Estimates of Raw Survey Data			Average Estimates of Transformed Survey Data			Sample Size Requirements
(110010)	Mean	SE	CV	Mean	SE	CV	
Species Richness	14.33	0.64	0.05				92
Species Diversity	1.6	0.07	0.04				88
Community Density	172.66	37.19	0.19				2139
Community Biomass	1318.8	236.2	0.18				1479
Grouper (Freq. of occurrence)	0.31	0.04	0.13				768
Grouper (Density)	0.70	0.13	0.19	0.14	0.02	0.15	941
Grouper (Biomass)	75.28	17.01	0.25	0.69	0.08	0.14	620
Grouper (Mean Size)	19.43	1.06	0.05	2.97	0.05	0.02	13
Snapper (Freq. of occurrence)	0.46	0.05	0.12				545
Snapper (Density)	2.59	0.72	0.25	0.28	0.04	0.15	941
Snapper (Biomass)	116.13	54.72	0.46	0.56	0.10	0.18	1470
Snapper (Mean Size)	14.95	2.00	0.13	1.01	0.04	0.05	72
Herbivore (Freq. of occurrence)	0.88	0.03	0.04				54
Herbivore (Density)	41.99	4.47	0.10	1.21	0.05	0.04	79
Herbivore (Biomass)	441.84	74.04	0.18	1.67	0.07	0.05	81
Herbivore (Mean Size)	392.4	76.72	0.16	2.02	0.07	0.03	55
Piscivore (Freq. of occurrence)	0.56	0.04	0.16				235
Piscivore (Density)	1.85	0.58	0.31	0.21	0.03	0.18	941
Piscivore (Biomass)	195.77	97.66	0.45	0.75	0.12	0.16	1180
Piscivore (Mean Size)	16.62	2.12	0.12	1.12	0.06	0.06	132
CEFU (Freq. of occurrence)	0.11	0.03	0.34				3429
CEFU (Density)	0.19	0.07	0.39	0.04	0.01	0.36	2881
CEFU (Biomass)	16.53	6.9	0.48	0.21	0.06	0.36	3763
CEFU (Mean Size)	17.34	2.14	0.13	1.22	0.06	0.06	112
STPL (Freq. of occurrence)	0.20	0.04	0.20				1844
STPL (Density)	2.26	0.87	0.39	0.16	0.04	0.25	2881
STPL (Biomass)	3.2	0.94	0.30	0.21	0.04	0.23	1673
STPL (Mean Size)	5.34	0.42	0.07	0.78	0.02	0.04	30

**Table A-5**: Estimates of the difference in principle reef fish community and assemblage metrics between reef fish (A) inside and outside BUIS and (B) inside and outside VICR. Date taken from 2003-2005 belt transects.

#### (A) BUIS

		Iı	nside B	UIS									
Community or Assemblage (Metric)	Estimates from Raw Survey Data			Estimates from Transformed Survey Data			Estimates from Raw Survey Data			Estimates from Transformed Survey Data			Sample Size
	Mean	SE	CV	Mean	SE	CV	Mean	SE	CV	Mean	SE	CV	Requirements
Species Richness	19.11	0.91	0.04				18.16	0.94	0.05				123
Species Diversity	2.08	0.08	0.04				2.16	0.07	0.03				48
Community Density	192.67	23.88	0.11				146.72	13.87	0.80				411
Community Biomass	2593.13	373.10	0.14				2575.41	516.19	0.18				1851
Grouper (Freq. of occurrence)	0.56	0.08	0.14				0.52	0.09	0.17				1380
Grouper (Density)	2.27	0.49	0.2	0.36	0.05	0.15	1.70	0.43	0.25	0.29	0.05	0.20	1370
Grouper (Biomass)	226.81	69.00	0.29	1.47	0.19	0.13	235.06	58.51	0.24	1.33	0.23	0.24	1378
Grouper (Mean Size)	18.71	0.98	0.05	1.27	0.02	0.02	23.35	1.16	0.05	1.37	0.02	0.01	20
Snapper (Freq. of occurrence)	0.32	0.07	0.25				0.37	0.08	0.23				2155
Snapper (Density)	0.98	0.32	0.34	0.17	0.04	0.26	1.33	0.56	0.39	0.20	0.05	0.27	2881
Snapper (Biomass)	102.14	43.42	0.43	0.73	0.17	0.25	110.21	59.68	0.49	0.76	0.19	0.25	2881
Snapper (Mean Size)	18.10	2.23	0.12	1.21	0.07	0.06	19.14	2.71	0.14	1.22	0.10	0.08	
Herbivore (Freq. of occurrence)	1.00	0.00	0.00				1.00	0.00	0.00				1
Herbivore (Density)	73.18	9.71	0.13	1.73	0.06	0.04	52.59	4.72	0.09	1.67	0.03	0.02	14
Herbivore (Biomass)	1101.27	171.11	0.15	2.76	0.10	0.03	761.12	130.45	0.15	2.65	0.09	0.03	53
Herbivore (Mean Size)	574.32	121.02	0.20	2.44	0.10	0.04	264.06	42.13	0.15	2.19	0.09	0.04	77

# (A) cont.

Community or Assemblage (Metric)		]	nside I	BUIS									
	Estimates from Raw Survey Data			Estimates from Transformed Survey Data			Estimates from Raw Survey Data			Estimates from Transformed Survey Data			Sample Size Requirement
	Mean	SE	CV	Mean	SE	CV	Mean	SE	CV	Mean	SE	CV	
Piscivore (Freq. of occurrence)	0.46	0.07	0.25				0.38	0.08	0.27				2043
Piscivore (Density)	2.08	0.90	0.36	0.25	0.05	0.20	2.34	1.67	0.56	0.17	0.05	0.32	23479
Piscivore (Biomass)	371.21	190.57	0.48	1.18	0.20	0.17	191.97	89.31	0.45	0.80	0.22	0.27	9977
Piscivore (Mean Size)	24.62	3.70	0.15	1.33	0.06	0.04	29.82	4.44	0.14	1.40	0.05	0.04	1021
CEFU (Freq. of occurrence)	0.46	0.08	0.18				0.39	0.8	0.22				19397
CEFU (Density)	1.76	0.42	0.24	0.29	0.05	0.18	1.15	0.35	0.31	0.20	0.05	0.25	2881
CEFU (Biomass)	143.21	37.84	0.24	1.18	0.18	0.16	153.66	45.70	0.29	0.96	0.22	0.22	2421
CEFU (Mean Size)	18.35	0.93	0.05	1.27	0.02	0.02	23.01	1.20	0.05	1.36	0.02	0.02	10
STPL (Freq. of occurrence)	0.18	0.06	0.38				0.34	0.08	0.26				5105
STPL (Density)	1.75	0.69	0.43	0.18	0.05	0.34	1.66	0.54	0.35	0.22	0.06	0.29	6516
STPL (Biomass)	6.48	2.34	0.41	0.32	0.09	0.33	7.24	2.74	0.37	0.39	0.11	0.28	7325
STPL (Mean Size)	5.78	0.85	0.15	0.32	0.09	0.33	4.66	0.74	0.15	0.72	0.05	0.07	444

## Table A-5: cont.

# **(B)** VICR

		Iı	iside V	TCR									
Community or Assemblage		tes from Raw vey Data		Estimates from Transformed Survey Data				tes from ] vey Data	Estimates from Transformed Survey Data			Sample Size	
(Metric)	Mean	SE	CV	Mean	SE	CV	Mean	SE	CV	Mean	SE	CV	Requirements
Species Richness	22.52	0.76	0.03				25.27	0.88	0.03				55
Species Diversity	2.20	0.07	0.03				1.99	0.08	0.04				74
Community Density	287.77	53.37	0.19	2.28	0.05	0.02	428.20	48.23	0.11	2.47	0.05	0.02	584
Community Biomass	3703.83	713.27	0.19	3.31	0.06	0.02	3561.00	683.90	0.17	3.35	0.05	0.02	1700
Grouper (Freq. of occurrence)	0.76	0.05	0.07				0.67	0.06	0.09				369
Grouper (Density)	3.81	0.45	0.1	0.52	0.04	0.08	3.25	0.43	0.13	0.46	0.03	0.08	196
Grouper (Biomass)	351.97	124.78	0.28	2.06	0.17	0.08	258.32	45.14	0.17	1.63	0.14	0.08	340
Grouper (Mean Size)	19.65	2.22	0.08	2.97	0.09	0.02	18.26	0.85	0.04	2.93	0.05	0.01	13
Snapper (Freq. of occurrence)	0.47	0.07	0.14				0.43	0.07	0.16				1221
Snapper (Density)	1.68	0.43	0.24	0.24	0.04	0.17	1.48	0.43	0.29	0.23	0.05	0.20	2178
Snapper (Biomass)	190.11	97.21	0.53	0.91	0.29	0.34	97.29	40.61	0.39	0.84	0.15	0.18	1470
Snapper (Mean Size)	22.04	1.82	0.07	1.33	0.02	0.02	24.47	3.21	0.12	1.35	0.04	0.03	40
Herbivore (Freq. of occurrence)	1.00	0.00	0.00				1.00	0.00	0.00				0
Herbivore (Density)	66.94	5.08	0.07	1.75	0.03	0.02	87.24	7.94	0.08	1.86	0.04	0.01	21
Herbivore (Biomass)	661.86	86.86	0.13	2.70	0.06	0.03	721.95	71.86	0.10	2.76	0.04	0.0.1	10
Herbivore (Mean Size)	7.66	0.39	0.05	0.92	0.01	0.02	8.12	0.29	0.03	0.94	0.01	0.02	5

## (B) cont.

Community or Assemblage (Metric)		Ι	nside V	/ICR									
	Estimates from Raw Survey Data			Estimates from Transformed Survey Data			Estimates from Raw Survey Data			Estimates from Transformed Survey Data			Sample Size Requirements
	Mean	SE	CV	Mean	SE	CV	Mean	SE	CV	Mean	SE	CV	
Piscivore (Freq. of occurrence)													
Piscivore (Density)	1.91	0.86	0.50	0.23	0.05	0.25	1.32	0.37	0.26	0.25	0.04	0.16	3622
Piscivore (Biomass)	332.87	146.97	0.64	0.91	0.30	0.39	608.15	232.53	0.38	1.34	0.20	0.15	6732
Piscivore (Mean Size)	26.82	5.08	0.19	1.37	0.07	0.05	32.76	4.97	0.15	1.41	0.06	0.04	1061
CEFU (Freq. of occurrence)	0.61	0.04	0.07				0.48	0.05	0.12				501
CEFU (Density)	3.05	0.42	0.14	0.42	0.03	0.09	2.08	0.41	0.19	0.31	0.04	0.13	1790
CEFU (Biomass)	148.83	20.65	0.14	1.30	0.06	0.05	112.45	18.20	0.16	1.07	0.11	0.11	487
CEFU (Mean Size)	17.05	0.80	0.04	1.24	0.02	0.02	16.82	0.69	0.04	1.23	0.01	0.01	3
STPL (Freq. of occurrence)	0.19	0.05	0.27				0.44	0.07	0.17				1611
STPL (Density)	2.55	1.00	0.39	0.19	0.05	0.30	5.49	1.69	0.31	0.42	0.08	0.19	4368
STPL (Biomass)	10.10	4.67	0.34	0.37	0.15	0.33	7.08	1.80	0.25	0.50	0.08	0.17	1180
STPL (Mean Size)	6.63	0.60	0.08	0.87	0.03	0.03	5.87	0.38	0.06	0.82	0.02	0.03	27

**Table A-6**: The optimal allocation of samples among all strata for (A) Buck Island and (B) St John study regions. The optimal allocation reflects the allocation which maximizes the number of metrics which can be tested to detect change at 10% confidence and 80% power.

(A) Buck Island

Strata ID	Unified Optimal Sample Size Allocation	Total Sample Size
BUIHO	41	
BUIHI	54	121
BUISI	18	121
BUISO	8	

(B) St John

Strata ID	Unified Optimal Sample Size Allocation	Total Sample Size
STJHOREST	38	
STJHOCB	26	
STJHOMSR	14	
STJHICB	24	170
STJHIMSR	17	170
STJSOREST	43	
STJSOCB	5	
STJSICB	3	

# A-5.3 Observations of the Reef Fish Community and Selected Assemblages during 2001-2005

The set of fish community and population metrics examined were: species richness (number of species), species diversity (Shannon diversity index), community biomass (biomass of all species), and community abundance (abundance of all species), assemblage frequency of occurrence (presence-absence), and assemblage density (number per unit area). The selection of analyzed assemblages were considered either directly related to the monitoring objectives or representative of other assemblages that could be of interest in the future (see **Table A-2**). Community metrics (e.g. richness) were examined using surveys collected over hard and soft strata during 2001-2005. Assemblage and species metrics were examined only in hard strata for the same time period.

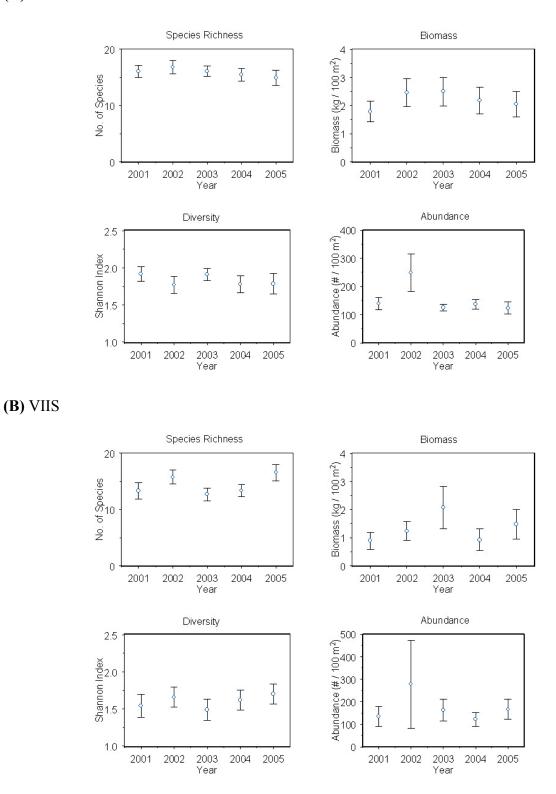
Averages for all metrics taken from within BUIS and VIIS are provided in **Table A-4**. We use the coefficient of variation as a standardized measure of precision. Higher precision is indicative of a more reliable estimate. In the context of monitoring, smaller changes can be found in a metric with higher precision than one with lower precision.

Community metrics were typically very precise (CV<0.10; **Table A-4**). Survey estimates for groupers, snappers, herbivores, and piscivores were higher, but most were moderately precise (CV<0.20; **Table A-4**). Estimates of density for *Stegastes planifrons* and *Cephalophlis fulvus* were imprecise (0.2<CV<0.5; **Table A-4**). In general, metrics ranked from highest to lowest precision were as follows: community diversity, species richness, frequency of occurrence, and density. The series of assemblages ranked from highest to lowest precision were as follows: community, herbivore, piscivore, grouper, snapper, *Cephalophlis fulvus*, and *Stegastes planifrons*.

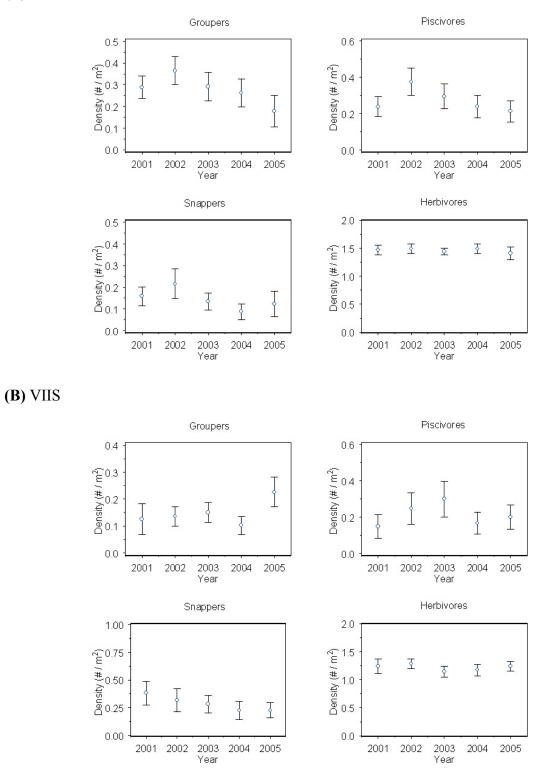
Many of the metrics examined in this study were transformed to standardize variance and create a more symmetrical distribution. This was required to generate accurate confidence intervals. The majority of survey estimates from transformed data were precise (CV < 0.10); however managers and researchers must be aware that the confidence intervals set using transformed variables are approximate to the back-transformed median not mean. If the mean of a metric changes, but the median remains the same, community shifts may not be detected. It is advisable to constantly monitor survey estimates from transformed and untransformed data simultaneously.

**Figures A-7** to **A-9** show annual survey estimates of fish community and population metrics during 2001-2005 for inside BUIS and VIIS. Confidence intervals (95%) are shown to indicate metric variability. Confidence intervals are also used to test for differences among years. The

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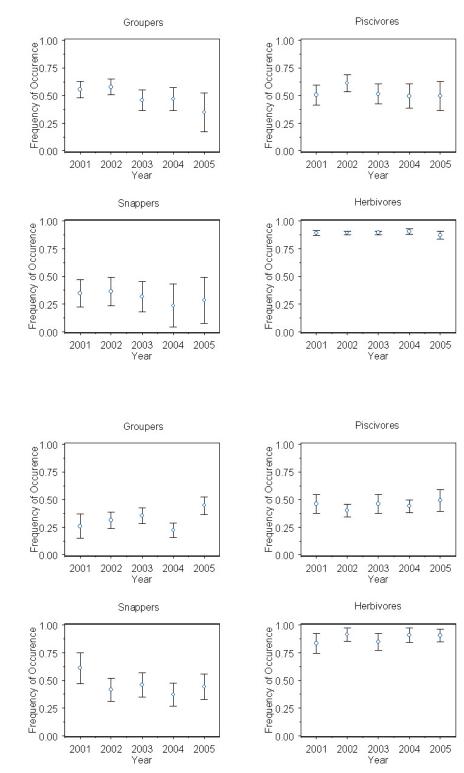
**Figure A-7**: Synoptic annual estimates of the community measures of species richness, biomass, species diversity, and density within (A) BUIS and (B) VIIS during 2001-2005. Error bars are 95% confidence intervals.



**Figure A-8**: Synoptic annual estimates of density for grouper, snapper, herbivore, and piscivore assemblages within (A) BUIS and (B) VIIS during 2001-2005. Error bars are 95% confidence intervals.



(B) VIIS



**Figure A-9**: Synoptic annual estimates of frequency of occurrence for grouper, snapper, herbivore and piscivore assemblages within (A) BUIS and (B) VIIS during 2001-2005. Error bars are 95% confidence intervals.

advantage of using confidence intervals to test for differences among years, instead of ANOVA, is its robustness to moderate violations of inherent assumptions, particularly homogeneity of variance and normality, if sample sizes are nearly equal and two-tailed hypotheses are considered (Cochran 1947; Box 1953; Srivastana 1958; Posten *et al.* 1982). In addition, confidence intervals are easily created from survey estimates of the survey mean and standard error.

The test was extended to allow multiple comparisons by incorporating a sequential Bonferroni correction. The Bonferroni correction increased the length of the confidence interval each time a survey comparison was made. Sequential comparisons were made of survey estimates in order of decreasing difference in means. Note: the confidence intervals shown in **Figures A-7** to **A-9** only represent intervals for the first comparison. Subsequent comparisons have larger intervals.

Most community measures, except density, showed little change during 2001-2005. We found significant changes in community density in BUIS (2002>2003; 2002>2004; 2002>2005) and species richness in VIIS (2003>2005). The 2002 estimate of community density in BUIS had an abnormally large confidence interval indicating a larger variation among samples than normal. This large amount of variation was not seen in estimates one year later or in other assemblage metrics estimated for 2002 in BUIS. Interestingly, the 2002 community density estimate in VIIS was also abnormally large, suggesting the increase in the density of fish was a regional phenomena.

Assemblage metrics were more variable than community metrics, but changes were found in grouper density (2002>2005), snapper density (2002>2004), piscviore density (2002>2005) in BUIS and grouper density and frequency of occurrence (2005>2004) in VIIS. Grouper, snapper, and piscivore estimates of density were all larger in 2002 than in other years, partly explaining high community density in 2002.

In addition to searching for significant changes among synoptic estimates, estimates were also examined for monotonic trends. We observed that grouper, snapper, and piscivore density decreased monotonically from 2002-2005 in BUIS and snapper density in VIIS decreased from 2001-2005. If density estimates can be considered random variables than the probabilities of a monotonic trend from 2002-2005 is  $0.125 (0.5^3)$  and 2001-2005 is  $0.0625 (0.5^4)$ .

## A-5.4 Comparison of the Reef Fish Community and Selected Assemblages inside and outside MPAs

Fish community and population metrics were compared between inside and outside of BUIS and VICR. The investigated time series was from 2003-2005. Data from 2001 and 2002 were not considered, because the survey design changed in 2002 making comparisons from before and after

2002 inconsistent. In 2002 the MSR strata was added in the St. John survey domain and the area sampled in BUIS was expanded to reflect monument expansion.

A confidence interval test similar to one used in Section 5.3 was employed to compare metrics and test for significant differences. The center of a confidence interval was the difference in synoptic annual estimates (inside BUIS/VICR minus outside BUIS/VICR) and the length of the interval was the sum of standard errors of annual estimates as shown in Cochran (1977). Confidence intervals were used to test for significant differences inside and outside BUIS/VICR and for differences among years. Sequential Bonferroni corrections were used to assess significance.

Results are shown in **Figures A-10** to **A-12**. Significant differences between inside and outside BUIS/VICR can be readily observed if a 95% confidence interval does not include zero. The confidence intervals provided in **Figures A-10** to **A-12** are only valid for the first comparison, because the confidence intervals must be widened according to the Bonferroni correction for subsequent comparisons.

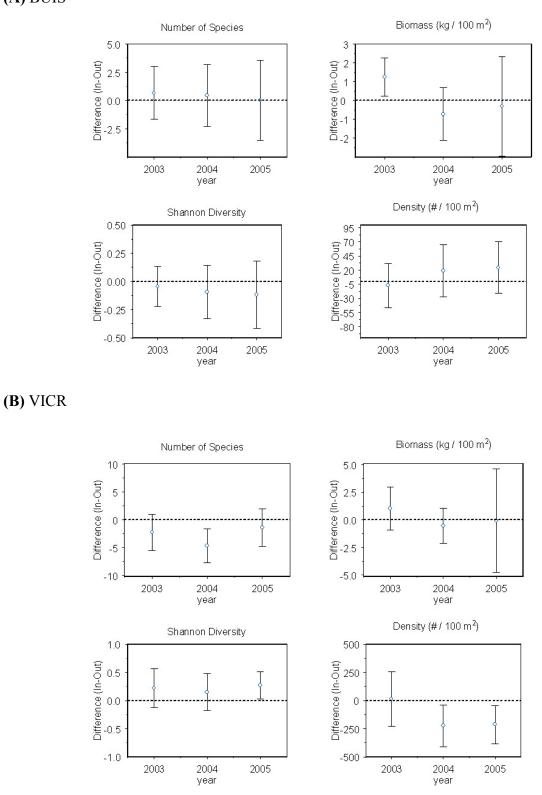
Significant differences in community biomass (2003 IN>OUT) in BUIS and number of species (2004 OUT>IN) and community density (2003 OUT>IN) in VICR were observed. These community differences were temporary suggesting the compared areas are relatively similar. Piscivore frequency of occurrence in BUIS and piscivore density, herbivore density in VICR were all significantly different in 2004 (OUT>IN). The only metric found to be significantly higher in an MPA was grouper density in VICR (2005 IN>OUT). No significant changes among years were detected using confidence intervals. Trends were not examined because the available time series was too short.

#### **A-6 Conclusions**

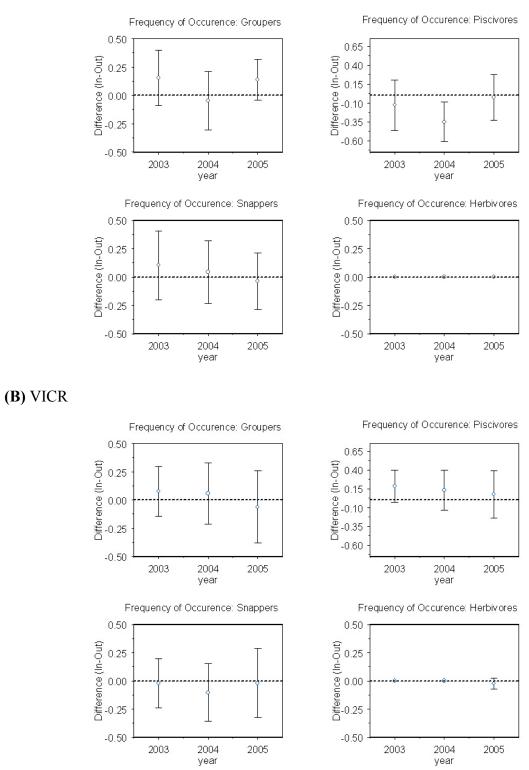
The sampling techniques described herein provide park managers and researchers with reliable unbiased estimates of common reef fish metrics. The methods used, including the belt transect, stratified random sampling design, calculation of survey estimates and confidence interval tests, are simple, efficient and require few materials and little training.

The high precision of most community metrics were sufficient to allow small changes in the reef fish community to be detected (10% change with 80% power). The lower precision of assemblage metrics require either more samples to be collected or a larger detection threshold (i.e. 20% or 30% change in metrics instead of 10%). High assemblage CV values were due to low sighting frequency. If sighting frequency could be increased, precision would increase as well.

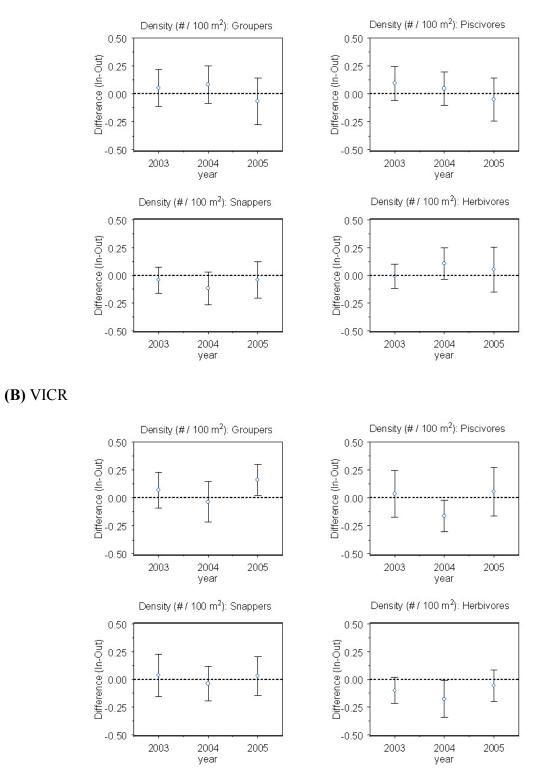
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**Figure A-10**: Synoptic annual estimates of the difference in community measures between inside and outside (A) BUIS and (B) VICR during 2003-2005. Error bars are 95% confidence intervals.



**Figure A-11**: Synoptic annual estimates of the difference in frequency of occurrence for four assemblages between inside and outside of (A) BUIS and (B) VICR during 2003-2005. Error bars are 95% confidence intervals.



**Figure A-12**: Synoptic annual estimates of the difference in density for four assemblages between inside and outside of (A) BUIS and (B) VICR during 2003-2005. Error bars are 95% confidence intervals.

Stratifying the survey domain according to sighting frequency is a strategy which may increase precision. An alternative is to examine only survey domains where sighting frequency is high, but managers would be unaware of changes in other domains.

The ability to detect covariance among common reef fish populations and benthic habitat types using simple plots of standard survey estimates proved a valuable tool. The division of survey domains into hard and soft bottom benthic habitats greatly increased the precision of metrics and hence decreased sampling costs. In future work, CREMP will examine the affect of dividing populations into juvenile and adult life stages. Corresponding survey estimates would provide managers insight into population dynamics and recruitment and would remove the effects of variable recruitment from adult estimates.

Observations of the community and assemblage metrics did not show consistent increasing or decreasing patterns. These observations do not mean the surveys were not part of an increasing or decreasing trend. Only five years of data were analyzed; many more years of data are needed before signals reflecting long-term change in reef fish populations and communities can be extracted from the noise included within a monitoring data set. The survey methods described herein are attempts decrease the noise as much as possible.

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# Case Study B: Monitoring Reef Fish Assemblages inside Virgin Islands National Park and around St. John, USVI 1988-2000

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#### **B-1 Summary**

This case study describes and explains the survey methods and analyses used by Jim Beets, Alan Friedlander, and National Park Service (NPS) / United States Geological Survey (USGS) collaborators in the Virgin Islands National Park (VIIS) to monitor reef fishes during 1988-2000. The long-term data on collected fish populations is unprecedented in duration and area of coverage for the Caribbean.

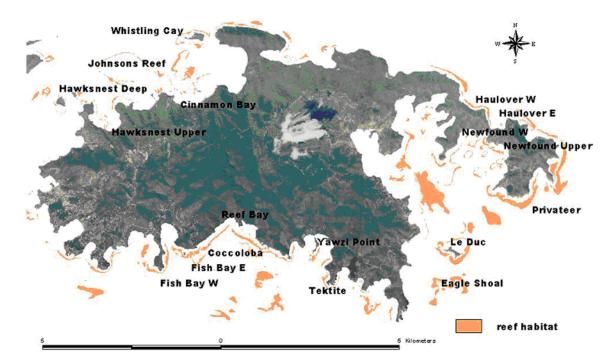
Reef fish data of common reef fish metrics (e.g. species richness, abundance and biomass) were monitored at permanent reference sites inside VIIS and on adjacent reefs around St. John, U.S. Virgin Islands. The permanent reference sites were chosen by experts to fulfill monitoring objectives. Each site was characterized by relatively high levels of live coral cover and topographic complexity.

Throughout the monitoring program survey methods evolved and the number of permanent reference sites changed due to shifting logistical constraints and park management needs. This case study focuses on data collected annually at four permanent reference sites during 1989-2000, and specific data sets of greater sampling intensity to explicitly examine the effects of different measurement collection methods and to assess sample size requirements. Statistical analyses shown in this case study are used to compare different measurement collection methods, determine sample size requirements, assess the impact of Marine Protected Areas (MPAs), and identify trends in reef fish metrics.

The purpose for this case study is to provide persons implementing a monitoring program with the information required to understand the pertinent management issues, sampling methods, and analytical methods used in monitoring reef fish in VIIS and adjacent waters around St. John.

#### **B-2 Background Information**

Monitoring projects were initiated by the National Park Service in the 1980s to provide useful data for evaluation of resources and for development of a long-term monitoring program (Boulon 1987). Starting in November 1988, two reef sites, Yawzi Point Reef and Cocoloba Reef, were sampled monthly until May 1991 (**Figure B-1**). The standard stationary point count census technique (Bohnsack and Bannerot 1986) was the method used during this period. A primary goal of this sampling effort was to document the variability in reef fish assemblage



**Figure B-1**: Map of St. John with reef habitat identified and monitoring sites labeled. Map created from NOAA/NOS data.

characteristics based on monthly samples. The results of this monitoring were described in previous reports (Beets and Friedlander 1990, Beets 1993).

Following the devastating effects of Hurricane Hugo in September 1989 (Rogers *et al.* 1991), NPS initiated reef fish sampling at 18 reef sites around St. John (**Figure B-1**). Jim Tilmant (NPS) and Dr. Joe Kimmel (representing the Florida Marine Research Institute) collaborated with Jim Beets and Alan Friedlander (representing the U.S. Virgin Islands Division of Fish and Wildlife) on this project.

One of the greatest justifications for consistent monitoring is to document the effects of natural events, such as the impact of hurricanes, and to attempt to differentiate natural fluctuations from human stresses. Storm events have devastating effects on reefs and their associated organisms. Storm intensity and frequency are quite variable, with low frequency of intense storms in some decades and several intense storms in others. Numerous storms affected the community structure of reefs around St. John during the monitoring period covered in this report, with some storms having major effects (Rogers and Beets 2001). The two largest storms passing St. John, Hurricane Hugo (1989) and Hurricane Marilyn (1995), devastated some reefs and had less influence on others (Rogers *et al.* 1991, 1997). Monitoring data allow for more critical assessment of these large disturbances and the differential effects of storms.

The survey methods and number of permanent reference sites changed during 1989-2000 due to shifting logistical constraints and park management needs. Initially, the NPS decision was to use a modified visual census technique, which was developed and used in Dry Tortugas National Park in 1987 (Kimmel 1992). Monitoring at the sites established in 1989 (originally 18 reef sites were selected with a few omitted and added among years) continued once per year during June/July, using the modified method, until 1994 (**Figure B-1**; Friedlander *et al.* 1999, Beets and Friedlander 2003).

In 1995, the standard stationary visual census technique (Bohnsack and Bannerot 1986) was employed to continue long-term monitoring at four established monitoring sites. These sites were selected based on similar reef characteristics, including depth, general reef topography (platform reefs with steeper edge habitat), spatial complexity, substrate characteristics, and coral cover.

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#### **B-3 Monitoring Strategy and Methods**

#### **B-3.1 Monitoring Objectives**

The objectives of this monitoring project were to:

- 1. Establish a baseline of information on reef fish assemblages around St. John,
- 2. Conduct sustained monitoring on representative high-diversity reefs,
- 3. Collect data on reefs with known and potential environmental degradation,
- 4. Compare fish assemblages among selected reefs, and
- 5. Determine trends in reef fish assemblages over time.

To satisfy these objectives, standard reef fish assemblage metrics, including species richness, abundance, biomass, and diversity (see **Table B-1** for descriptions), were analyzed and monitored. In addition, trophic groups, including benthic herbivores (dominated by damselfishes), mobile herbivores (dominated by parrotfishes and surgeonfishes), higher-order predators (dominated by groupers and snappers), and other predators (represented by numerous families) were investigated.

#### B-3.2 Sampling Design

To satisfy monitoring objectives, sampling was conducted at permanent reference sites with relatively high levels of live coral cover and topographic complexity (compared to reefs with low percent cover and relief) and a depth range of 1-15 m. Although monitoring was initially conducted at 18 sites (1989-1994), only four sites (Yawzi Point Reef, Tektite Reef, Newfound Bay West Reef, and Haulover Bay West Reef) have been monitored continuously during 1989-2000 (except in 1990). The four sites monitored since 1989 are the focus of this case study.

From past visual surveys, these reefs were known to have the highest species richness and biomass of reefs around St. John. The monitoring sites were located on lower forereef habitat of these fringing reefs. At Newfound Bay West Reef an additional zone (upper forereef – 'Acropora zone') was also monitored. All four reefs are of similar percent coral cover (10%-30%, greatly altered by coral diseases and storm damage in recent years, as documented for Yawzi Point reef), physical structure, and reef morphology. All reefs have a gently sloping reef platform (1-15 m) and a 'wall-like' edge, which extends sharply from the platform break to the sand zone (15-20 m). The edge zone has high topographic complexity, with numerous small to large holes. Tektite Reef is the

most developed and extensive of the four reefs, with spur and groove formations and impressive deeper forereef zone. Yawzi Point Reef has suffered the greatest impact from storm damage. Haulover Bay West Reef has a relatively narrow, but impressive, coral zone with a high density of large colonies of *Montastrea annularis*. Newfound Bay West Reef has a less developed reef platform, but well-developed edge structure. Haulover Bay Reef West and Newfound Bay Reef West had impressive upper forereef zones of *Acropora palmata*, which were devastated by the combination of white-band disease and storm damage in the 1970s and 1980s.

#### **B-3.3 Sample Selection**

Multiple samples were collected at each reference site to gather estimates of desired fish metrics (see **Table B-1** and Section B-3.1), because a complete census of each reef was not possible. Since 1989, a minimum of 18 samples were scheduled for each permanent reference site based on preliminary sample size analysis.

Sample units were restricted to reef habitat. Sampling was usually conducted from the reefsand interface to the middle portion of the reef platform, which was normally dominated by *Montastrea annularis* on lower forereef sites. Samples were divided between two reef zones (reef edge and reef platform), because data from previous years (<1989) showed differences in biomass and richness between these two zones. Reef sections for sampling were determined by divers (usually 2-4 divers) prior to sampling to avoid overlap. Reef edge was identified as a steep slope which extended from the forereef-sand interface to a change in slope, and reef platform was identified as the gradual slope from the edge or the sand interface to the next shallower zone, e.g., upper forereef [Acropora zone]).

Sample units were haphazardly selected using random kicks as described by Bohnsack and Bannerot (1986). If a haphazard sample unit occupied less than 50% hard substrate and/or reef (i.e. was greater than 50% sand), the diver moved to another haphazardly selected point on the reef.

In 1992 and 1999, sampling intensity was increased at Tektite Reef to compare point count methods (1992, n=20) and to estimate sample size requirements (1999, n=58). The data sets and corresponding analyses are discussed in Sections B-5.1 and B-5.2, respectively.

#### B-3.4 Methods of Measurement

Two 'point count' methods were used for monitoring reef fishes in sample units. The primary reef fish monitoring method used at the reference sites from 1995-2000 was the stationary

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**Table B-1:** Fish assemblage characteristics (measured and derived) examined during long-term monitoring of reef fishes in VIIS and around St. John in 1988.

#### Assemblage characteristics

Species richness - number of species per sample

Abundance – number of individuals per sample

- Biomass (g) (derived metric) weight of all individuals in the sample Biomass estimates for analysis were derived from calculated live wet weight. Live wet weight (W) was derived from the visually estimated mean fork length (FL) for each size class for each species using the relation W =  $a(FL)^b$ . Values of the fitting parameters a and b for each species were derived from Bohnsack *et al.* (1986) and the FishBase web site (http://fishbase.org/). For species not in these databases, estimates from available literature on the species or congeners were used. Biomass of all fishes recorded in all censuses was obtained by multiplying the mean live wet weight for each size class.
- Diversity (derived metric) Shannon-Weiner Diversity Index H'=S (pi ln pi), where pi is the proportion of all individuals counted that were of species i.

Benthic Herbivores - dominated by damselfishes

Mobile Herbivores - dominated by parrotfishes and surgeonfishes

Higher-order Carnivores - dominated by groupers and snappers

Other Carnivores - represented by numerous families

visual census technique ('stationary point count' - SPC) described by Bohnsack and Bannerot (1986). A single sample is conducted by a diver who settles just above the reef substrate at a haphazardly selected point. During the point count, all fish species observed are listed within a 7.5 m radius cylinder (area: 176.7 m<sup>2</sup>) for 5 min. Numbers and sizes of fishes of each species (estimated fork length placed in separate size classes) are added following the 5 min listing period. Habitat within the cylinder is briefly described, including substrate type, estimated coral cover, dominant benthic organisms, relative topographic complexity, depth, and location on the reef.

A modified 'point count' technique, developed by J. Kimmel and J. Tilmant for fish monitoring in Dry Tortugas National Park (Kimmel 1992), was used in Virgin Islands National Park from 1989 to 1994. This modification used a 5-m radius cylinder (area: 78.5 m<sup>2</sup>) and 15 min time interval with the last 5 min of the 15 min total used to search and enumerate species and individuals by swimming throughout the cylinder. Thus, this method was a 'plot count' instead of a 'point count', as described by Bohnsack and Bannerot (1986). The decision to return to the standard stationary visual census technique (Bohnsack and Bannerot 1986) in 1995 from the modified technique was made in order to standardize with investigators working elsewhere in the Caribbean (especially J. Bohnsack and colleagues working in the Florida Keys National Marine Sanctuary and Dry Tortugas National Park).

#### **B-4 Analyses**

#### B-4.1 'Point count' Methods Comparison

Methods are refined, modified, or changed as long-term monitoring progresses and as management questions change. To ensure that long-term monitoring data are suitable for analysis of trends and change, methods must be consistently applied and compared. Method changes must be tested, validated, compared, and calibrated. The change in methods of measurement used in VIIS, from a 10 m/15 min count to a 15 m/5 min count, between 1994-1995 required a methods comparison.

In 1992, a study was conducted to compare the two 'point count' methods and to evaluate the need for correction factors. The methods comparison study was conducted on July 16, 1992 on Tektite Reef, the monitoring site with the consistently greatest species richness and fish abundance. Five plots on the reef of similar topographic complexity, coral cover, and depth were selected and marked for sampling. Each plot was located between 10-12 m water depth, approximately 25 m apart. Transect lines (15 m) were laid within each plot. Four experienced fish counters conducted one sample using each method within each plot. Method, site, and time were randomly assigned. This sampling design allowed for paired comparisons between methods for each diver at a given location.

Analysis using paired t-tests for the data obtained with the two methods yielded significant differences for species richness and diversity, but not for number of individuals or biomass (**Table B-2A**). P-values were sequentially Bonferroni adjusted according to Rice (1989). Analysis by trophic group resulted in significant differences for benthic and mobile herbivores, but none for higher-order carnivores or other carnivores (**Table B-2B**). None of the eleven most abundant species were observed to have significant differences between methods (**Table B-2C**). Results of Detrended Correspondence Analysis did not show large differences between methods, but, interestingly, demonstrated considerable observer variation (**Figure B-2**).

Since analysis of the two methods yielded differences in parameter values, application of correction factors for some parameters would be appropriate for analyzing trends. Parameter values from samples at the same plot using both methods were regressed to obtain linear equations. Regression coefficients were low for all assemblage parameters and trophic groups (**Table B-3**). Except for diversity, regression analysis did not yield significant relationships for assemblage characteristics or trophic groups; therefore, strong relationships between the two methods were not observed.

#### **B-4.2 Sample Size Requirement Analysis**

In July 1999, an oversampling effort (n = 58 samples) was conducted on Tektite Reef (ca.  $13,500 \text{ m}^2$ ) in order to conduct a sample size analysis using an optimization technique developed by Bros and Cowell (1987), and provide a complete coverage for one reef among microhabitats and depths. Analyses of the later data were previously reported (Friedlander *et al.* 1999).

A species cumulation curve was used to examine the relationship between the cumulative number of species and number of samples at Tektite Reef (**Figure B-3**). The cumulative number of species reached an asymptote at 22 samples. The minimum sample size (n = 18) accounted for 96% of the total number of species sampled at Tektite Reef.

A technique developed by Bros and Cowell (1987) was used to determine the number of samples required to determine a significant difference in the means of two independent samples. The number of species, number of individuals, and biomass collected in samples were investigated. A range of differences between means (10% - 50% of the mean) were examined. A Lotus macro

**Table B-2**: Comparison of assemblage characteristics, trophic groups, and selected species between two visual census methods (15m/5 min and 10m/15 min) conducted at Tektite Reef, VIIS, on 16 July 1992. N = 20. The greatly abundant masked goby, *Coryphopterus personatus*, was not included in analysis. A table-wise sequential Bonferroni (Seq. Bon.) adjustment of p values was made to control for the overall type-I error rate for each dependent variable ( $p_i \le \alpha/(1 + k - i, Rice 1989)$ ).

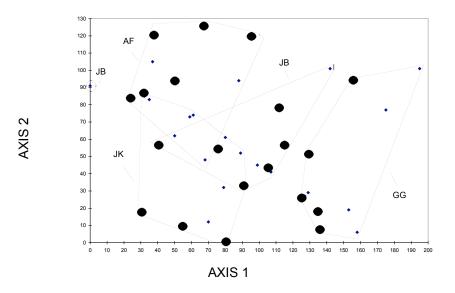
(A) Statistics for assemblage endracteristics								
Assemblage	15m	10m	Mean	Std Error	Corr.	t-Ratio	Prob >  t	Seq. Bon.
characteristics		I	Difference					
Richness	22.35	28.90	-6.55	1.1573	-0.107	-5.6597	< 0.000	Sig.
Abundance	181.45	200.00	-18.55	20.33	-0.075	-0.9124	0.3	ns
Biomass (g)	6170.28	6930.84	-760.56	1381.95	0.058	-0.5503	0.5	ns
Diversity	2.14	2.43	-0.29	0.0597	0.583	-4.8494	0.00	Sig

(A) Statistics for assemblage characteristics

Trophic group	15m	10m	Mean Diff.	Std Error	Corr.	t-Ratio	Prob >  t	Seq. Bon.
Benthic Herbivores	3.4	3.8	-0.352	0.1159	0.462	-3.354	0.00	Sig.
Mobile Herbivores	2.6	3.0	-0.403	0.1497	0.343	-2.692	0.01	Sig.
Higher-order Carnivores	1.1	1.5	-0.369	0.1839	0.219	-2.009	0.05	ns
Other Carnivores	4.7	4.8	-0.041	0.1501	-0.027	-0.270	0.79	ns

#### (C) Statistics on abundance data for 11 numerically abundant species.

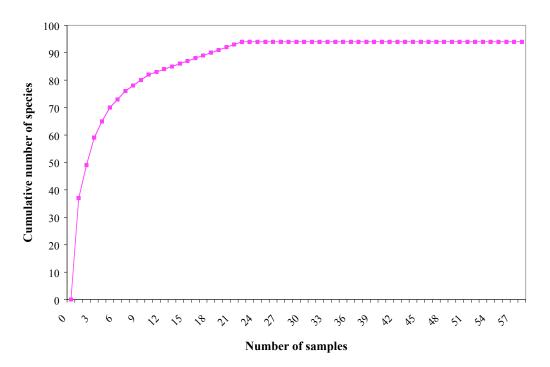
Species	15m	10m	Mean Diff.	Std Error	N	Corr.	t-Ratio	Prob >  t	Seq. Bon.
Chromis cyanea	65.0	58.9	6.15	7.93	20	0.64	0.78	0.45	ns
Stegastes planifrons	18.5	28.9	-10.42	3.42	19	0.75	-3.05	0.01	ns
Chromis multilineatus	10.3	13.1	-2.84	3.22	19	0.24	-0.88	0.39	ns
Clepticus parrai	17.8	13.3	4.50	11.41	12	-0.39	0.39	0.70	ns
Stegastes partitus	10.6	8.2	2.37	2.4	19	0.40	0.99	0.34	ns
Holocentrus rufus	8.7	7.8	0.84	2.33	19	0.48	0.36	0.72	ns
Halichoeres garnoti	5.5	7.7	-2.20	1.14	20	0.17	-1.92	0.07	ns
Haemulon flavolineatum	6.6	7.1	-0.47	1.00	19	0.89	-0.48	0.64	ns
Thalassoma bifasciatum	5.7	6.1	-0.35	1.17	20	0.21	-0.30	0.77	ns
Stegastes variabilis	3.8	7.3	-3.53	1.22	19	0.89	-2.88	0.01	ns
Scarus croicensis	3.2	6.5	-3.32	1.33	19	0.3	-2.49	0.02	ns



**Figure B-2**: Results of detrended correspondence analysis (DCA) of fish count data (4 observers; 40 samples) conducted at Tektite Reef for comparison of point count methods. Polygons denote each observer. Large circles are 10m/15min censuses; small circles represent 15m/5min censuses. Masked goby, *Coryphopterus personatus*, was not included in analysis.

**Table B-3:** Results of least-squares linear regression analyses for fish assemblage characteristics for post Hurricane Hugo (1989-1994) and post Hurricane Marilyn (1996-2000) time periods. Number of individuals and biomass were ln(x) transformed for statistical analyses.

Assemblage characteristic	Time period	Least-squares regression model	R <sup>2</sup>	F	Р
Species	1989-1994	Species = -2032.193 + 1.0337838 x (YR)	0.310	7.730	0.011
	1996-2000	Species = -496.3675 + 0.26025 x (YR)	0.026	0.483	0.496
Individuals	1989-1994	ln (individuals) = $-203.965 + 0.105$ x (YR)	0.337	9.136	0.007
	1996-2000	ln (individuals) = -104.2545 + 0.05475 x (YR)	0.060	1.103	0.307
Biomass	1989-1994	ln (biomass) = -38.59824 + 0.0202027 x (YR)	0.330	9.040	0.008
	1996-2000	$\ln (biomass) = -20.3435 + 0.011 x (YR)$	0.061	1.180	0.292



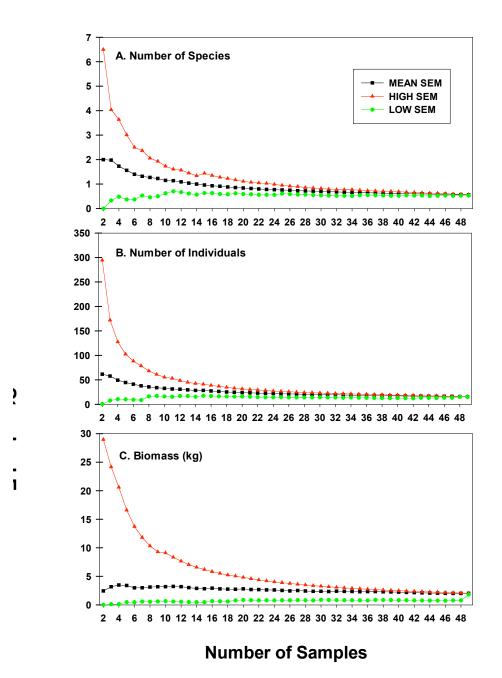
**Figure B-3**: Species cumulation curve showing the relationship between the cumulative number of species and the number of samples at Tektite Reef (Friedlander *et al.* 1999).

program written by Doug Harper of the NMFS/SEFSC/Miami Laboratory was used to conduct these analyses.

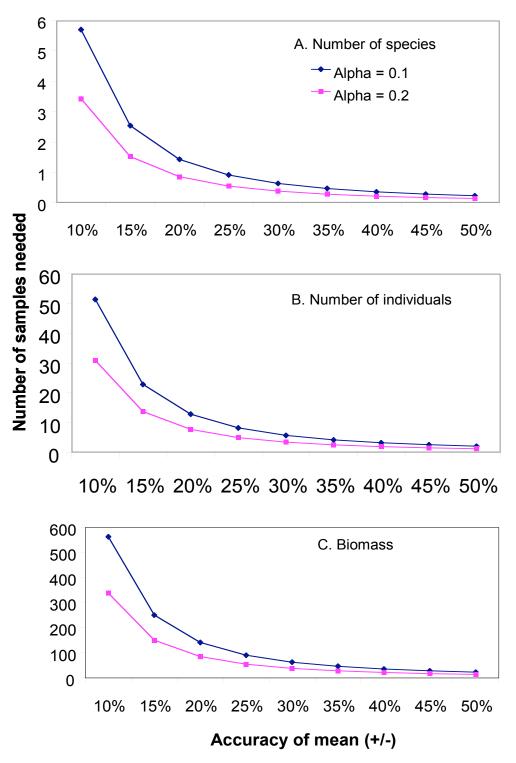
For number of species and number of individuals, standard error of the mean begins to level off and converge at approximately 11-13 samples (Figure B-4). Biomass had a much higher degree of variability and does not level off and converge until 15 to 20 samples. All analysis for number of individuals and biomass excluded masked gobies (Coryphopterus personatus) because they were ubiquitous and their large numbers (1000's) masked trends in the remainder of the fish assemblage. The estimated number of samples needed to detect various levels of change varied greatly among the three parameters (mean abundance of species, individuals, and biomass). Figure B-5 provides estimates of the number of samples needed to detect various levels of change in the mean abundance of species, individuals, and biomass (Friedlander et al. 1999) at Tektite Reef. Using a Type I error rate of 0.10, the number of samples needed to detect changes decreases rapidly with only a slight decline in precision. Less than two samples are required to detect a 20% change in number of species per survey (Figure B-5A) while more than 12 are required to detect a 20% change in number of individuals (Figure B-5B). Again, biomass is highly variable with approximately 140 samples needed to detect a 20% change (Figure B-5C). Using a Type I error rate of 0.20 substantially decreases the number of samples needed to detect change in the 10% to 20% range of precision. Less than two samples are required to detect a 15% change in number of species per survey (Figure B-5A) while 7.7 censuses are required to detect a 20% change in number of individuals (Figure B-5B). Again, biomass is highly variable with 84 samples needed to detect a 20% change (Figure B-5C).

For comparison with analyses of sample size at Tektite reef, sample size analyses were conducted among the remaining reference sites collected in 1999 as well (**Figure B-6**). Sample sizes used for analysis were the same for all reefs (n = 18). Similar results were obtained among reefs for each parameter. For species richness, 5-11 samples were required to reach an asymptote for standard error (**Figure B-6A**). Abundance required a greater number of samples among sites (7-14; **Figure B-6B**). Biomass required large sample size to adequately reduce standard error (11-22; **Figure B-6C**). Differences in estimated sample sizes were apparent among reefs, with reefs with greater parameter values generally requiring larger sample sizes.

For the four reference sites examined, two samples were needed to detect a 20% change in number of species at an alpha of 0.1 for any given site (**Figure B-7A**). The number of samples needed to detect a 20% change (at alpha = 0.1) in the number of individuals ranged from 8 samples



**Figure B-4**: Sample size optimization of data from sample size analysis project at Tektite Reef, July 1999, for number of species, number of individuals, and biomass. Relationship between standard error of the mean (SEM) and sample size. Monte Carlo simulation procedure for sample size optimization described by Bros and Cowell (1987). (Friedlander *et al.* 1999)



**Figure B-5:** Estimated number of samples needed to detect changes in the mean of data from sample size analysis project at Tektite Reef, July 1999. N = 58,  $\alpha = 0.10$  and 0.20. (Friedlander *et al.* 1999)

**Figure B-6**: Results of sample size optimization for (A) species richness, (B) number of fishes, and (C) biomass among the four reference sites, St. John, U.S. Virgin Islands. Results based on data collected in 1999. (Beets and Friedlander 2003)

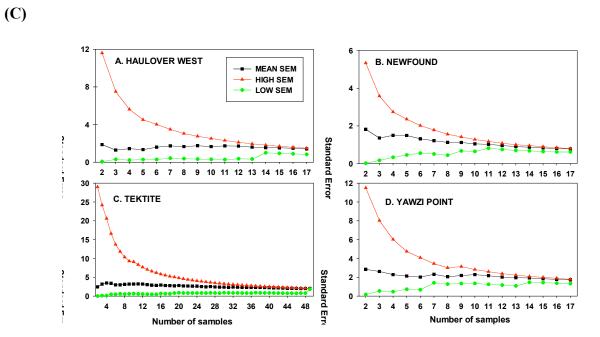
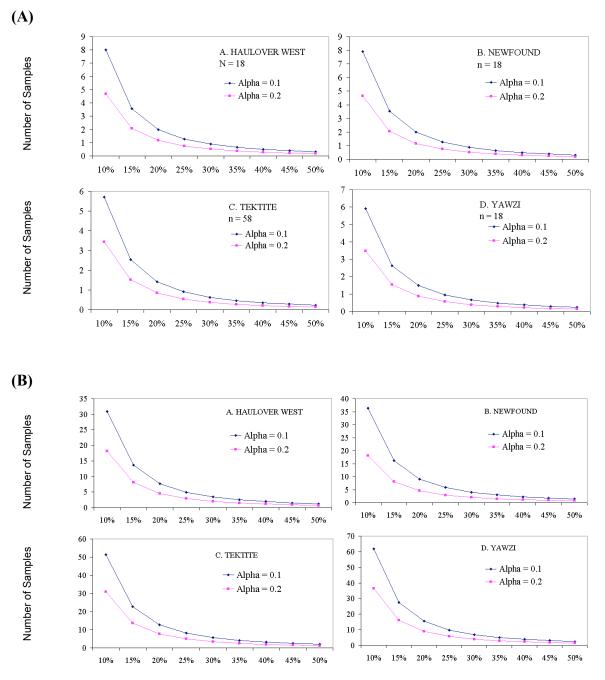


Figure B-6: cont.



**Figure B-7**: Estimated number of samples needed to detect changes in the mean for (A) species richness, (B) number of fishes, and (C) biomass among reference sites. N = 18 (except for Tektite: n = 58);  $\alpha = 0.10$  and 0.20. (Beets and Friedlander 2003).

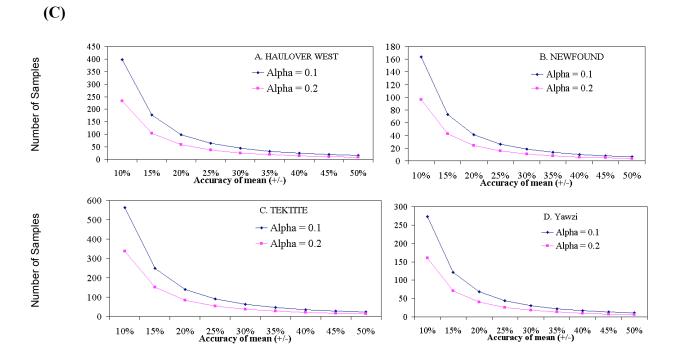


Figure B-7C: cont.

at Haulover West Reef to 15 at Yawzi Point (**Figure B-7B**). The high variance associated with total fish assemblage biomass resulted in sample sizes ranging from 41 at Newfound Bay to 140 at Tektite Reef in order to detect an accuracy within 20% of the mean (alpha = 0.1, **Figure B-7C**).

#### **B-4.3 Observer Variation**

Since observers conducted censuses in different microhabitats (Edge vs. platform) an analysis of variance (ANOVA) was used to examine if there were any differences in assemblage structure among microhabitats or observers. A Two-Way ANOVA was conducted with habitat (edge and platform) and observers (AF, JB, and JM) as fixed factors in the ANOVA. Biomass was ln(x+1) transformed. An unbalanced sample design precluded analysis of interaction terms.

For number of species, a Two-Way ANOVA with both factors fixed showed no significant difference between habitats, but a significant difference among observers was observed (**Table B-4**). Mean species counts per census were significantly greater for AF compared to JB and JM (P < 0.05). The power for the 2 way Model I ANOVA was calculated using eq. 12.43 in Zar (1999). Power is very low for detecting differences in habitat but extremely high for observer differences. This is primarily due to the ratio of the Mean Squares for each factor compared to the Residual or Error Mean Square. Therefore, there exists a very large probability of committing a Type II error (>99%) in trying to detect a difference in habitats. Number of species per census was not significantly different between JB and JM (P > 0.05). Two potential reasons for observer differences are that: 1) observers may sample different microhabitats, and 2) observer sampling bias, i.e., the inclusion or exclusion of small cryptic benthic species (e.g. gobies and blennies).

For number of individuals, a Two-Way ANOVA showed no significant difference between habitats or among observers (**Table B-4**). Mean biomass estimates were not significantly different among observers (P > 0.5) but were significantly higher on the edge compared to the platform habitat (P < 0.05; **Table B-4**).

#### **B-4.4** Assessment of Marine Protection on Reef Fish Assemblages

To compare fish assemblage characteristics inside and outside Virgin Islands National Park, data was analyzed from the period during which numerous reef sites were monitored (n = 18, 1989-1994). The selected sites were in reef habitat with greater topographic complexity than surrounding colonized pavement. Sites that did not have an analog reef either inside or outside the park were excluded from this analysis. Reef sites located inside the park that were used in analyses included: Hawksnest Bay Upper, Haulover Bay West, Fish Bay East, Yawzi Point Reef, and Tektite

**Table B-4:** (A) Results from two-way ANOVAs to investigate the effects of habitat and observers on three assemblage metrics. Habitats are edge and platform (DF=1). Observers were Alan Friedlander (AF), Jim Beets (JB), and Jeff Miller (DF=2). (B) Mean and variance values used in a pair-wise multiple comparison Bonferroni corrected t-test procedure. Underlined means are not significantly different at  $\alpha = 0.05$ . (Friedlander *et al.* 1999)

(A)			
Assemblage		Habitat	Observer
Metric			
Species Richn	less	0.442	< 0.001
Abundance		0.620	0.011
Biomass	Biomass		0.241
<b>(B)</b>			
AF	JB	JN	1
30.5 (2.7)	26.2	(2.5) 23	6.9 (3.0)

Reef (refer to **Figure B-1**). Reef sites outside the park included Haulover Bay East, Newfound Bay West, Newfound Bay Upper, and Fish Bay West. There were no significant differences in number of species (P > 0.05) or fish biomass (P > 0.05) between sites inside and outside the park (**Table B-5**). The total number of individuals was significantly greater (P = 0.002) at sites inside VIIS compared with sites outside VIIS. This is likely owing to the greater proportion of 'edge habitat', with greater topographic complexity, sampled inside the park (Haulover West, Yawzi Point, and Tektite Reef) and the associated presence of large schools of planktivores at these sites.

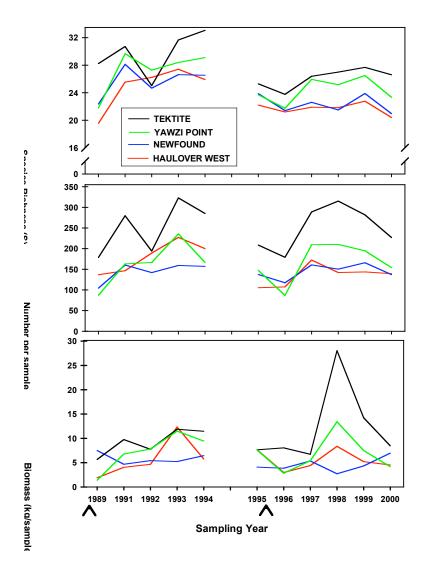
#### B-4.5 Observations of Reef Fish Temporal Variation at Reference Sites

Much temporal variation was observed for assemblage characteristics among the four reference sites (**Figure B-8**). As expected, variation in means for abundance and biomass was greater than for species richness. Generally, the sites with greater mean values (e.g., Tektite) showed greater temporal variation than the site with the lowest mean values (Haulover West). Comparison of mean values of assemblage characteristics between all reef fishes (juveniles and adults) and adults demonstrated that the adult component of the assemblage had much lower temporal variability, particularly for abundance (**Figure B-9**). This was readily apparent in comparison of standard deviation estimates for assemblage characteristics between all reef fishes and adults for data from Tektite Reef (**Figure B-10**). Standard deviation estimates were significantly smaller for adult fishes for all assemblage characteristics (t-tests values, p < 0.05). Since the adult components of the reef fish assemblages are less variable, they provide less 'noise' in analysis. Juveniles of many species are also more difficult to detect, especially from a stationary point, which is also a potential source of variability.

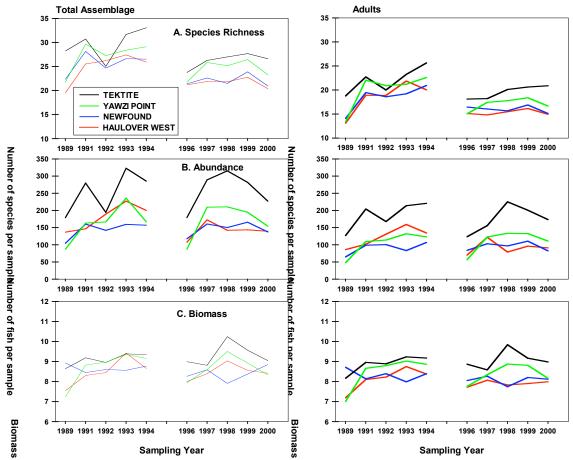
The most apparent temporal signal was due to the influence of large storm events. The Virgin Islands have been greatly influenced by numerous large storms since 1988. Data were separated into two periods (1989-1994 and 1996-2000), representing the post-storm recovery periods following the two major storms affecting St. John during the period of analysis (Hurricane Hugo, Sept. 1989; Hurricane Marilyn, Sept. 1995). Since data for 1995 were collected just prior to Hurricane Marilyn, those data were excluded from analysis. Simple least-squares linear regressions were conducted on the five years of data following each storm event. All of the assemblage characteristics analyzed (species richness, abundance, and biomass) showed statistically significant increases during the five-year period following Hurricane Hugo (1989) (**Figure B-11**). While species, number of individuals, and biomass all trended upward following Hurricane Marilyn

**Table B-5**: Comparisons of fish assemblage characteristics inside and outside of Virgin Islands National Park using 15min/5min point count data from 1989 to 1994. Values in parentheses are standard error of the mean using pooled variances. Number of individuals did not meet the parametric assumption of homogeneity of variances so a Mann-Whitney Rank Sum Test was used in place of the parametric Student's t-test. Statistical values of pooled data: t = Student t-test, U = Mann Whitney Rank Sum test.

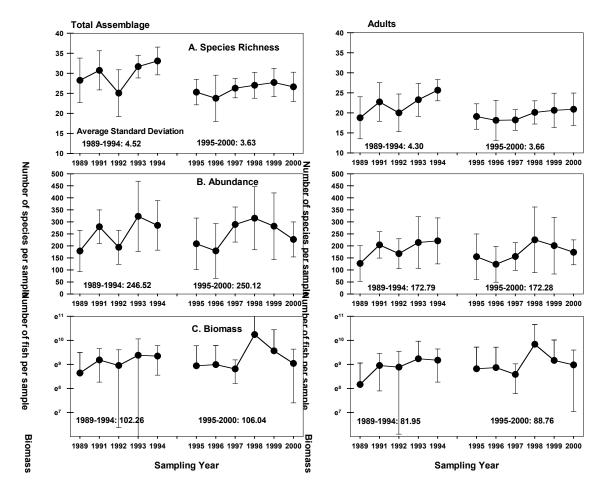
Assemblage characteristics	Inside VIIS	Outside VIIS	Statistical Value	P value
Species Richness	30.9 (0.70)	30.2 (0.80)	t = 0.714	0.476
Number of individuals	228.6 (7.5)	188.2 (8.6)	U = 9.51	0.002
Biomass (kg)	9.2 (0.7)	8.1 (0.6)	t = 1.12	0.23



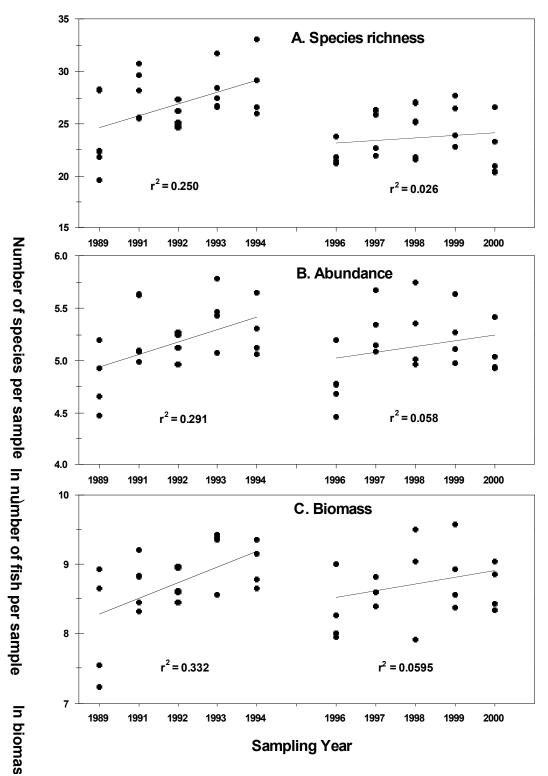
**Figure B-8**: Comparison of fish assemblage characteristics among the four reference reefs sites around St. John, US Virgin Islands. The break between 1994 and 1995 marks methods change. Arrows mark two major hurricanes. (Beets and Friedlander 2003)



**Figure B-9**: Comparison of trends of assemblage characteristic values (a. species richness, b. abundance, and c. biomass) between all reef fishes (Total Assemblage - juveniles and adults) and Adults (juveniles excluded) for the four reference sites at St. John, US Virgin Islands. Major storm events marked the beginning of each of the two periods shown in each graph (1989 – Hurricane Hugo, 1995 – Hurricane Marilyn). The change in methods occurred in 1995 (data not presented for 1995). (Beets and Friedlander 2003)



**Figure B-10**: Comparison of variance estimates (standard deviation) of assemblage characteristics (a. species richness, b. abundance, and c. biomass) between all reef fishes (Total Assemblage - juveniles and adults) and Adults (juveniles excluded) for Tektite Reef, St. John, US Virgin Islands. Average standard deviation is presented for all reef fishes and adults for both sampling periods. The change in methods, which occurred in 1995, marked the separation in sampling periods. (Beets and Friedlander 2003)



**Figure B-11:** Trends in assemblage characteristics during the five-year periods following the two major storms which affected St. John (Hurricane Hugo, Sept. 1989; Hurricane Marilyn, Sept. 1995). Average values for each of the four reference sites are represented by circles for each year. Regression lines and coefficients were obtained from linear regression analysis. Data for 1995 was excluded from these analyses.

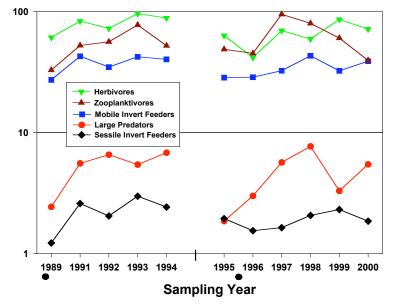
(1995), none of these trends were significant for the five year period following the storm (Figure B-11). Large storms, which passed near the Virgin Islands in 1998 and 1999, may have had a great negative impact on reef fish assemblage recovery, as lower values in assemblage characteristics were noted for 2000 (Figure B-11).

Trophic group trends were similar to overall assemblage characteristic trends, with large differences in abundance among groups (**Figure B-12**). Generally, no strong trends were apparent for any trophic group, although the influence of method change and large storm events were apparent. Trends within families presented a finer-scale perspective than did trophic groups. For example, surgeonfishes (Acanthuridae) and goatfishes (Mullidae) demonstrated no strong trends, although differences were apparent among reference sites (**Figure B-13**). Abundance values of gobies (Gobiidae) and angelfishes (Pomacanthidae) were apparently influenced by method change, with lower abundance values in both taxa following the change. Abundance values of groupers (Serranidae) and parrotfishes (Scaridae) were apparently strongly influenced by both method change and storm events. Method change and storm events were clearly confounded and difficult to assess for many groups.

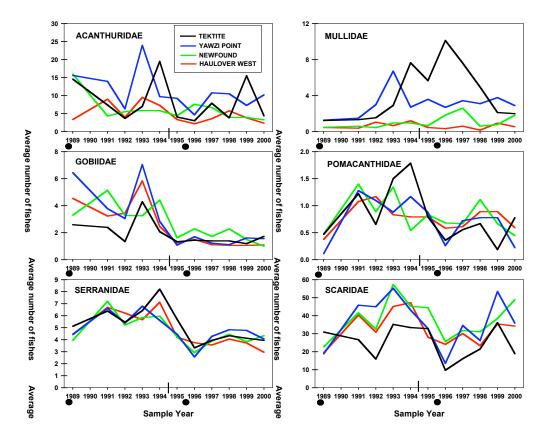
#### B-4.6 Power Analysis of Point Count Data at Reference Sites

Power analysis of monitoring data can be used to determine the duration of a monitoring program needed to identify a given trend with a known statistical power (1-Type II error) and confidence (Type I error). This analysis has the same assumptions of regression analysis (e.g., random and independent errors, homogenous variance). If these assumptions are not satisfied, trend power analysis is not recommended. We show the technique here for instances where regression is suitable to analyze monitoring data.

Analyses were conducted using the freeware program MONITOR from the USGS (USGS 2005) and by a more conservative method (Skalsky 2005). The MONITOR program provided more liberal results than output obtained from more conservative methods. For example, power values using abundance data from all permanent reference sites obtained from the MONITOR program were 0.839 (alpha = 0.05) and 0.962 (alpha = 0.1) for a 10-year monitoring period. A more conservative method (Skalsky 2005) provided much smaller power values for a 10-year monitoring period (**Table B-6**). The ability to detect small levels of change (e.g. 10% change) with sufficient power for most parameters is improbable using the conservative method.



**Figure B-12:** Abundance trends in trophic groups among the four reference sites around St. John, U.S. Virgin Islands, 1989-2000. Data were ln(x)-transformed. Method changed occurred in 1995. Black dots mark large hurricanes. (Beets and Friedlander 2003)



**Figure B-13**: Abundance trends in selected fish families among the four reference sites around St. John, U.S. Virgin Islands, 1989-2000. Vertical bar on x-axis marks the method change; black dots mark large hurricanes.

**Table B-6**: Results of power analysis for species richness and abundance data using stationary point counts collected at Tektite Reef in Virgin Islands National Park. Power values are presented for three detection levels (10%, 25%, and 50% declines) and for two monitoring periods (5, 10 years). Type I error was set at 0.10. Analysis was based on annual sampling at each site.

Metric	Decline	5 Year Monitoring	10 Year Monitoring
		Period	Period
Species Richness	10%	0.238	0.273
	25%	0.411	0.569
	50%	0.773	0.945
Abundance	10%	0.206	0.211
	25%	0.232	0.262
	50%	0.322	0.424

## **B-5 Discussion**

Management decisions should be made based on scientifically-defensible data collection and analyses, instead of perceived changes in the sample populations. Resource managers need to know the degree of accuracy associated with population estimates and statistical power of analyses in order to use monitoring data confidently. Detecting a 25% change in mean abundance may be adequate for some species or locations and insufficient for others. The number of samples taken is an important decision in any biological study because of the time and cost involved in data collection. These decisions are often based on practical as well as theoretical considerations.

The sample size (N = 18) used at Tektite Reef and other reference sites in VIIS appears adequate to detect changes in number of species and number of individuals for the entire assemblage and for many trophic guilds at a 20% level. Even with a complete census at Tektite Reef (N = 58 sample), it would not be possible to detect changes in biomass or number of individuals for some trophic guilds. The established sample size (N = 18) for reference sites is adequate to detect changes in number and biomass for some trophic guilds, such as herbivores and mobile invertebrate feeders. If trends in abundance and/or biomass and abundance of large commercially important species (snappers/groupers) are desired, then large samples and other methods must be evaluated and employed. Continued stratification by edge and platform microhabitats seems appropriate based on differences in biomass and detrended correspondence analysis results for assemblage structure.

Despite the relatively large variances associated with assemblage parameters (species richness and abundance) for individual sites, repeated annual sampling over several years (5-10 years) can detect declines with sufficient power to be useful for management purposes. Increasing alpha levels to 0.20 would improve the ability to detect these changes and is consistent with the precautionary approach to management. It is far better to make a Type I error rather than a Type II error if the sustainability of the resources is in question.

In order to increase the number of monitoring sites and number of samples at a broader spatial scale, a more intensive sampling effort will be needed. Determination of the optimal sample design for snapper/grouper surveys and juvenile fish surveys should be conducted if data on these parameters are desired. A final important point is that these results demonstrate differences due to observer bias. These samples were collected by researchers with many years of experience with fish identification, ecological methods, and, specifically, fish counting. The bias, error, and variability of

samples collected by newly trained and less experienced samplers could be quite large and could lead to problematic time series data. It is important to emphasize the need for adequate training, as well as consideration of observer bias in data analysis efforts.

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# Case Study C: Assessment of Coral Reef Fishes in Dry Tortugas National Park 1999-2004

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# **C-1** Introduction

Dry Tortugas National Park (DRTO) is a unique tropical marine environment of national significance, renowned for its productive coral reef ecosystem, diverse natural resources, broad recreational fishing opportunities, and spectacular scenic beauty. The Dry Tortugas and the Florida Keys support multibillion dollar fishing and tourism industries in south Florida, including economically-important fisheries for pink shrimp, lobster, reef fish (snapper-grouper complex), kingfish, and Spanish mackerel. Also, the Tortugas region is believed to be extremely important for coral reefs and fisheries as a source of recruitment because of its upstream location in the Florida Current that facilitates advective dispersion and transport of eggs and larvae to the rest of the Keys (Lee and Williams, 1999; Dahlgren and Sobel, 2000; Lindeman *et al.*, 2001; Ault *et al.*, 2002; Yeung and Lee, 2002; Domeier, 2004).

Over the past several decades, public use of and conflicts over fishery resources have increased sharply, while catches from historically productive snapper and grouper stocks have declined (Bohnsack *et al.* 1994; Ault *et al.* 1997, 1998, 2001). Management actions (e.g. establishing size, season, and bag limits on a number of species) implemented by the National Oceanic and Atmospheric Administration (NOAA) and the National Park Service (NPS) in the Tortugas region aim to reverse declines in important fishery and coral reef resources, including the establishment in 2001 of "no-take" marine protected areas in NOAA Sanctuary waters and a proposed research natural area (RNA) within DRTO (DOC 1996; NPS 2000; Culhane 2002). There is also broader scientific and management interest in developing a better understanding of marine reserve design and ultimate performance in rebuilding fisheries and conserving marine biodiversity (Bohnsack and Ault 1996; Bohnsack 1998). In addition, as the south Florida-Everglades restoration efforts proceed, it will be essential to have effective monitoring programs and predictive models to assess ecosystem changes.

The goal of this case study was to assess baseline conditions of coral reef fishes of DRTO during the period 1999-2004, and to evaluate the performance of marine protected areas (MPAs) in the Dry Tortugas region. This study analyzed data obtained during comprehensive fisheries-independent monitoring surveys conducted in 1999, 2000, 2002 and 2004. Data were collected using a stationary visual census method under a stratified random sampling design (Ault *et al.* 2002). Visual survey data, and additional fishery datasets, were analyzed to evaluate the status and

trends of exploited and non-exploited species in DRTO over the 1999 to 2004 time frame. These analyses are organized into three main sections:

- (1) Estimation of baseline population metrics;
- (2) Sustainability status of exploited coral reef fishes; and
- (3) MPA performance in the Dry Tortugas region.

We apply a suite of statistical and analytical methodologies previously developed to assess status and trends of coral reef fishes in DRTO and the Florida Keys coral reef ecosystem (Ault *et al.* 2001, 2002, 2003; Meester *et al.* 2001, 2004; Franklin *et al.* 2003). A key aspect of our efforts was the use of fishery-independent data to calculate the average size of fish in the exploited phase for each stock to monitor status and trends in resources. Length-based stock assessment indicators developed previously can be effectively used to quantify the condition of exploited and non-exploited populations (Ault *et al.* 2001, 2002, 2005a, 2005b).

This study also reports results from fisheries independent surveys in the Tortugas region to assess reef fish populations before and after the establishment of the Tortugas no-take marine reserves (NTMRs) in June 2001. To evaluate potential impacts of NTMRs and other factors on reef fish sustainability in the Florida Keys coral reef ecosystem, temporal changes of relatively simple population and community metrics (e.g., frequency of occurrence, abundance, size compositions, and species richness) for the Tortugas region, and within and outside NTMRs, were analyzed.

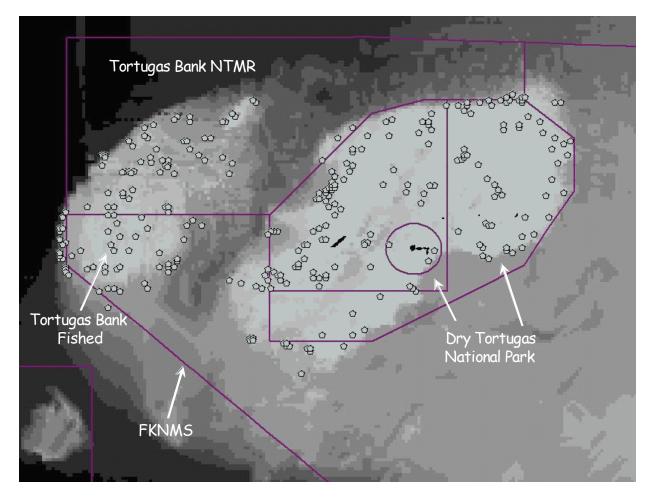
# C-2 Fishery-Independent Survey Design

### C-2.1 Population to be Sampled

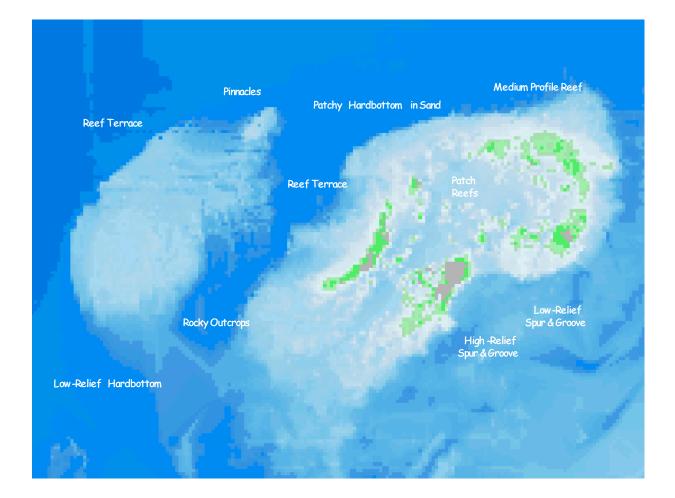
The Tortugas region is located about 113 km west of Key West, Florida, and encompasses approximately 1686 km<sup>2</sup> in two principal areas: DRTO (managed by Department of the Interior, DOI); and Tortugas Bank (NOAA, FKNMS, Department of Commerce) (**Figure C-1**). The survey domain encompassed coral reef habitats less than 33 m deep in Tortugas Bank and DRTO.

## C-2.2 Sampling Design

The survey domain was partitioned into habitat strata based on the degree of vertical relief (e.g., rugosity, complexity) and the degree of patchiness (e.g., amount of softbottom substrate interspersed among reef structures) of the hardbottom substrate (**Figure C-2**, **Table C-1**; Ault *et al.*, 2002; Franklin *et al.*, 2003). Management zones were incorporated as a second spatial stratification variable, designated as follows: Tortugas Bank NTMR -- closed to all types of fishing; Tortugas



**Figure C-1:** Dry Tortugas region spatial management boundaries. Locations of the sampled primary units during a 2004 survey are shown (open pentagons). Bathymetry is denoted by light to dark shading (white: 0-3 m; black: > 50 m).



**Figure C-2:** Spatial distribution of the 8 classified coral reef habitats in the Dry Tortugas region overlain with the 200 by 200 m primary unit sampling grid used in monitoring surveys.

**Table C-1:** (A) Habitat strata (h) characteristics and sizes in terms of primary sampling units ( $N_h$ ) and area ( $A_h$ ) for the Dry Tortugas sampling domain. (B) Habitat strata sizes for three management zones within the Dry Tortugas sampling domain; dashes denote habitats not found in a given management zone.

	Habitat	Degree of	Degree of	Domain-wide Area		
<b>Reef Habitat Classification</b>	Code Patchiness		Vertical Relief	N <sub>h</sub>	$A_h (km^2)$	
Low-relief hardbottom	LRHB	Low	Low	4,909	196.36	
Low-relief spur & groove	LRSG	Moderate	Low	296	11.84	
Patchy hardbottom in sand	PHBS	High	Low	913	36.52	
Medium profile reef	MDPR	Low	Moderate	194	7.76	
Rocky outcrops	RKOC	Moderate-High	Moderate	1164	46.56	
Reef terrace	RFTC	Low	High	422	16.88	
High-relief spur & groove	HRSG	Moderate	High	127	5.08	
Pinnacle reef	RFPN	High	High	57	2.28	
Total				8,082	323.28	

(A)

# **(B)**

Habitat		Tortugas Bank Fished		as Bank IMR	Dry Tortugas National Park		
Code	$N_h$	$A_h (km^2)$	$N_h$	$A_h (km^2)$	N <sub>h</sub>	$A_h (km^2)$	
LRHB	1,108	44.32	1,438	57.52	2,363	94.52	
LRSG		—	—	_	296	11.84	
PHBS	38	1.52	35	1.40	840	33.60	
MDPR		—	—	_	194	7.76	
RKOC	134	5.36	282	11.28	748	29.92	
RFTC	47	1.88	327	13.08	48	1.92	
HRSG		_	_	_	127	5.08	
RFPN		—	29	1.16	28	1.12	
Total	1,327	53.08	2,111	84.44	4,644	185.76	

Bank Fished -- open to all types of commercial and recreational fishing under regional regulations; and, DRTO -- open to only recreational hook-and-line fishing (**Figure C-1**). In the Tortugas region, areas open to fishing (e.g. Tortugas Bank Fished zone), allow a variety of types of legal fishing activities under regional management and represent the lowest level of resource protection in the study area. DRTO represents an intermediate level of resource protection by allowing only recreational angling. Commercial fishing has been prohibited since 1935 when it was established as a National Monument. Recreational lobster diving was prohibited in 1980. After conversion to DRTO in 1992, protection increased with the exclusion of headboats for recreational fishing in 1995. The Tortugas Bank NTMR, a no-take and no anchoring reserve, represents the highest level of resource protection. Prior to July 1 2001, Tortugas Bank was open to fishing under Gulf of Mexico Fishery Management Council and Florida Fish and Wildlife Conservation Commission regulations.

Our spatially-intensive study employed a two-stage stratified random survey design (Cochran 1977) to optimize sampling effort and to choose sampling locations. The sampling domain was overlain in a Geographical Information System (GIS) with a grid of 200 x 200 m cells (40,000 m<sup>2</sup>) which are the primary sample units. Each cell that contained reef habitat was assigned a unique number and randomly selected for sampling from a discrete uniform probability distribution to ensure that each primary unit had equal selection probability. Second-stage sample units, i.e., diver visual census locations, were then randomly positioned on appropriate habitat within each primary unit. For reef fish sampling, there are 226 non-overlapping possible 15 m diameter fish sampling stations possible within a given primary sample unit. Two second-stage units (denoted as 'diver stations') were sampled in each primary unit. Each diver station was sampled by two individual divers (i.e., a buddy pair), for a total of 4 scientific dives within each primary unit under normal operations. Because of concerns about autocorrelation and safe diving practices, each fish sampling station (i.e., second-stage unit) consisted of the average of combined stationary point estimates from two individual divers (i.e., a "buddy pair").

The principal population abundance metrics were frequency of occurrence (i.e., presenceabsence), density (number per unit area), and abundance (total number). Animal density was the primary abundance metric used for developing and optimizing the survey design (Ault *et al.* 2002, 2003). Coefficient of variation (CV) of mean density, the standard error expressed as a proportion of the mean value, was the principal measure used to evaluate survey design performance. Metrics were computed using formulae in Section C-8. **Table C-2** shows statistical sample sizes in terms of primary (n) and second-stage (nm) sample units by year, habitat, and management zone. A total of 4,092 scientific dives for more than 668 hours bottom time, including 3,234 fish survey dives, were made during 1999-2004 cruises in the Tortugas region to assess reef fishes, coral reef benthic habitats, and spiny lobsters.

A detailed analysis of stratification and allocation aspects of this sampling design was conducted during spring 2002 prior to the summer 2002 survey. A complete account of this sampling plan development is given in Ault *et al.* (2003).

This habitat-based stratification is effective because it capitalizes on the statistical covariance between fish abundance and coral reef habitat types analyzed from previous surveys (Ault *et al.* 2002, Franklin *et al.* 2003). In addition, a number of logistical factors enabled divers to quickly obtain high sample size over substantial areas at relatively low costs: (1) use of a large, live-aboard dive vessel equipped with Nitrox SCUBA; (2) "live-boating" at dive sites where the vessel never anchored, but deployed divers at specified coordinates and picked up the free-swimming groups after samples are taken; (3) utilizing highly-trained professional divemasters to oversee the complex dive operations; and, (4) conducting the annual surveys within 2-3 weeks during periods (May-June) of minimum winds.

#### C-2.3 Method of Measurement

Principal population abundance metrics were collected by standard, non-destructive, in situ, fishery-independent visual monitoring methods by highly trained and experienced divers using open circuit Nitrox SCUBA. Visual methods are ideal for assessing reef fishes in the Tortugas and Florida Keys because of prevailing good visibility and management concerns requiring the use of non-destructive assessment methods.

Reef fish data are collected by a stationary diver centered in a randomly selected circular plot (Bohnsack and Bannerot 1986). The circular plot method provides reliable quantitative estimates of species composition, abundance (density per plot), frequency of occurrence, and individual size composition for the reef fish community. Divers sample 15 m diameter circular plots (177 m<sup>2</sup>) for 5 minutes attempting to count all fish observed within each imaginary cylinder extending from the bottom to the limits of vertical visibility (usually the surface). Divers begin each sample by facing in one direction and listing all species within the field of view. When no new species are noted, new sectors of the cylinder are scanned by rotating in one direction for the 5 min period. Several complete rotations were usually made for each plot. After the initial 5 min, data are

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<b>Table C-2:</b> Reef fish survey sample sizes in terms of primary (n) and second-stage (nm) units by
habitat class and management zone for (A) 1999, (B) 2000, and (C) 2004. Habitat codes are defined
in Table 1; dashes denote habitats not found in a given management zone.

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Habitat	Tortugas Bank Fished			Tortugas Bank NTMR		Dry Tortugas National Park		Domain-wide	
Code	n	nm	n	nm	n	nm	n	nm	
LRHB	11	22	16	29	24	47	51	98	
LRSG					15	30	15	30	
PHBS	5	10	4	7	7	12	16	29	
MDPR					4	8	4	8	
RKOC	4	8	12	23	8	14	24	45	
RFTC	4	8	28	53	5	10	37	71	
HRSG		—			12	24	12	24	
RFPN		—	8	16	3	6	11	22	
Total	24	48	68	128	78	151	170	327	

## (A) 1999

## (B) 2000

Habitat		Tortugas Bank Fished		as Bank 'MR	•	ortugas 1al Park	Domai	n-wide
Code	n	nm	n	nm	n	nm	n	nm
LRHB	10	20	17	31	34	64	61	115
LRSG					5	9	5	9
PHBS	10	20	11	20	25	45	46	85
MDPR					9	17	9	17
RKOC	2	4	11	17	28	52	41	73
RFTC	0	0	17	31	7	12	24	43
HRSG					12	22	12	22
RFPN			5	10	4	7	9	17
Total	22	44	61	109	124	228	207	381

# (C<u>) 2004</u>

Habitat		as Bank hed		as Bank MR		ortugas 1al Park	Domai	n-wide
Code	n	nm	n	nm	n	nm	n	nm
LRHB	22	41	9	18	81	146	112	205
LRSG	_				14	26	14	26
PHBS	11	19	2	4	24	44	37	67
MDPR	_				23	39	23	39
RKOC	10	19	27	54	24	45	61	118
RFTC	5	9	16	32	17	33	38	74
HRSG					4	8	4	8
RFPN			9	18	7	14	16	32
Total	48	88	63	126	194	355	305	569

then collected on the abundance and minimum, mean, and maximum lengths for each species sighted. Depth, substrate composition, benthic fauna percentage cover, and vertical relief-rugosity characteristics of reef structure are recorded for each plot from the polar perspective of the centrally located observer. An all purpose tool (APT), consisting of a ruler connected perpendicularly to the end of a meter stick, is used as a reference device to reduce apparent magnification errors in fish size estimates. An innovative state-of-the-art digital laser video camera system was designed and deployed to increase the precision of both sizing and counting reef fish species. The technical methodology is being calibrated against standard divers using the visual census methods and APT meter sticks. In usual operations, divers periodically calibrate their sample radius estimates with the meter stick or fiberglass tape. Species with few individuals (e.g., Queen Angelfish Holacanthus *ciliaris*, Barracuda Sphyraena barracuda, Hogfish Lachnolaimus maximus) are counted and their size estimated immediately. Highly mobile species that are unlikely to remain in the area, such as sharks and carangids, are tabulated when first observed and then ignored. For common species (e.g., damselfishes, wrasses, etc.) one 360° rotation is made for each species by working back up the list in reverse order of recording to reduce potential bias by avoiding counting a species when they were particularly abundant or obvious. The time required to record each sample averaged 15-20 min (range 5 - 30 min), depending on the habitat.

Benthic community assessments of hard corals, octocorals, sponges, and algae were strategically integrated with the reef fish sampling effort allocations to optimize the performance and provide maximum structural coherence of both fish and habitat surveys, and to provide a quantitative basis for comparison and calibration of survey efforts that improve mapping and spatial stratifications of the survey domain.

# C-3 Estimation of Baseline Population Metrics

#### C-3.1 Methods

During the summers of 1999 and 2000, quantitative fishery-independent survey expeditions assessed the multispecies coral reef fish community and associated reef habitats in the Tortugas region. These data were used in the development of NTMRs in the Tortugas waters of FKNMS, and also used to spatially delineate a no-take Research Natural Area within DRTO. Subsequent surveys were conducted in DRTO during summers of 2002 and 2004 prior to implementation of the NTMR to obtain additional baseline data on the status of reef fish populations inside and outside the proposed boundaries of the RNA. This section documents the basic results of fishery-independent

surveys conducted in DRTO during 1999-2004. These results provide the fundamental basis for the stock assessments of Section C-4 and assessment of MPA performance in Section C-5.

Baseline population estimates were computed using samples collected within DRTO. The DRTO surveys divided among 2 spatial management zones (inside and outside the proposed RNA) (**Table C-3**). Since benthic habitat classification, digital mapping, and development of the DRTO survey design occurred concurrently with the surveys of 1999 and 2000 (Ault *et al.* 2002), abundance metrics were estimated as a composite of these two years to alleviate problems of misclassification of habitats and misallocation of samples among habitat strata. In this procedure, strata means and variance components were computed as two-year averages weighted by respective sample sizes in 1999 and 2000.

#### C-3.2 Results

Over the 1999-2004 period, we observed a total of 267 fish species in RVC surveys in the Tortugas region. Fish species richness ranged from 8 to 64 species per primary sample unit (psu) and, in general, was correlated with habitat class. Greatest reef fish species diversity (63 to 64 species per psu) was found in high rugosity habitats (reef terrace and reef pinnacles), whereas lowest diversity (8 to 11 per psu) was found in low rugosity habitats (low-relief hard-bottom and patchy hard-bottom in sand), as illustrated in Figure 4.3 for the 2004 survey.

Visual survey estimates of percent occurrence by spatial management zone are given in **Table C-4** for principal exploited and non-target reef fish species. For most species, both exploited and non-target, percent occurrence was consistent over the survey time frame with values fluctuating within 5 to 10% domain-wide and exhibiting somewhat larger fluctuations in the two management zones. Among snapper-grouper complex species, moderate increasing trends in percent occurrence were observed for black grouper and mutton snapper, and a moderate decreasing trend was observed for hogfish. Nassau grouper and goliath grouper, species under fishing moratoria in the Gulf of Mexico and South Atlantic regions, were seen very infrequently by scientific divers. Among non-target species, increasing trends in percent occurrence were observed for foureye butterflyfish (*Chaetodon capistratus*), spotted goatfish (*Pseudopeneus maculates*), and purple reeffish (*Chromis scotti*).

Visual survey estimates of population mean density by management zone and domain-wide design performance are given in **Table C-5**. Design performance improved, i.e., CV of mean density decreased, over the survey time frame for a majority of principal reef fishes (6 of 8 snapper-

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Table C-3: (A) Habitat strata (h) characteristics and sizes in terms of primary sampling units (N<sub>h</sub>) and area (A<sub>h</sub>) for the Dry Tortugas National Park (DRTO) sampling domain. (B) Habitat strata sizes for two management zones within the DRTO sampling domain.

	Habitat	Degree of	Degree of	Domain-wide Area		
Reef Habitat Classification	Code	Patchiness	Vertical Relief	N <sub>h</sub>	$A_h (km^2)$	
Low-relief hardbottom	LRHB	Low	Low	2,363	94.52	
Low-relief spur & groove	LRSG	Moderate	Low	296	11.84	
Patchy hardbottom in sand	PHBS	High	Low	840	33.60	
Medium profile reef	MDPR	Low	Moderate	194	7.76	
Rocky outcrops	RKOC	Moderate-High	Moderate	748	29.92	
Reef terrace	RFTC	Low	High	48	1.92	
High-relief spur & groove	HRSG	Moderate	High	127	5.08	
Pinnacle reef	RFPN	High	High	28	1.12	
Total						
				4.644	185.76	

(A)

(B)

Habitat	Insid	le RNA	Outsi	de RNA
Code	$N_h$	$A_{h} (km^{2})$	$N_h$	$A_h (km^2)$
LRHB	1,094	43.76	1,269	50.76
LRSG	28	1.12	268	10.72
PHBS	429	17.16	411	16.44
MDPR	36	1.44	158	6.32
RKOC	355	14.20	393	15.72
RFTC	38	1.52	10	0.40
HRSG	26	1.04	101	4.04
RFPN	19	0.76	9	0.36
Total	2,025	81.00	2,619	104.76

**Table C-4:** Visual survey estimates of percent occurrence (and standard errors in parentheses) for selected fish species during three time periods for the Dry Tortugas National Park (DRTO) sampling domain and for two spatial management zones.

		DRTO		I	nside RNA		(	Outside RNA	
Taxa	1999-2000	2002	2004	1999-2000	2002	2004	1999-2000	2002	2004
<b>Snapper-Grouper Complex</b> Groupers (Serranidae)									
Goliath Grouper (Epinephelus itajara)	1.1 (1.1)	7.1 (2.7)	1.1 (0.5)	0.1 (0.1)	7.1 (4.0)	2.5 (1.2)	1.9 (1.9)	7.1 (3.6)	0.0 (0.0)
Red Grouper ( <i>E. morio</i> )	62.6 (4.2)	57.9 (4.8)	54.5 (3.7)	53.2 (7.1)	50.4 (8.4)	61.6 (5.1)	69.8 (5.1)	63.7 (5.6)	49.1 (5.2)
Nassau Grouper ( <i>E. striatus</i> )	1.5 (1.1)	1.1 (1.0)	0.5 (0.4)	2.0 (2.0)	0.2 (0.2)	0.1 (0.1)	1.0 (1.0)	1.7 (1.7)	0.8 (0.6)
Black Grouper ( <i>Mycteroperca bonaci</i> ) Snappers (Lutjanidae)	27.6 (3.8)	36.7 (4.7)	36.8 (3.3)	28.2 (6.6)	29.2 (6.0)	34.4 (4.9)	27.2 (4.3)	42.5 (6.8)	38.6 (4.4)
Mutton Snapper (Lutjanus analis)	17.1 (3.8)	26.3 (6.2)	27.4 (3.3)	16.9 (7.1)	24.3 (6.8)	26.2 (4.7)	17.2 (4.0)	27.9 (9.7)	28.3 (4.7)
Gray Snapper (L. griseus)	17.9 (3.7)	14.0 (5.0)	14.2 (2.8)	22.6 (6.8)	15.2 (4.3)	16.9 (3.9)	14.3 (4.1)	13.1 (8.3)	12.2 (3.9)
Yellowtail Snapper ( <i>Ocyurus chrysurus</i> ) Wrasses (Labridae)	82.4 (3.5)	78.2 (4.1)	79.5 (2.7)	85.1 (5.2)	85.9 (5.1)	76.2 (4.3)	80.4 (4.8)	72.2 (6.0)	82.1 (3.5)
Hogfish ( <i>Lachnolaimus maximus</i> ) Grunts (Haemulidae)	52.1 (4.5)	39.4 (4.0)	41.5 (3.6)	48.2 (7.5)	42.3 (5.7)	45.1 (4.7)	55.1 (5.4)	37.2 (5.6)	38.7 (5.3)
White Grunt ( <i>Haemulon plumieri</i> )	86.4 (2.9)	73.2 (3.5)	76.8 (3.2)	86.1 (4.6)	71.6 (6.3)	76.7 (4.3)	86.6 (3.7)	74.4 (3.9)	76.9 (4.5)
Bluestriped Grunt ( <i>H. sciurus</i> )	5.8 (2.0)	6.6 (3.0)	8.3 (1.9)	3.0 (1.6)	5.4 (2.3)	13.4 (3.5)	8.0 (3.3)	7.5 (5.1)	4.3 (1.9)
Non-Target Fishes Surgeonfishes (Acanthuridae)									
Ocean Surgeon (Acanthurus bahianus)	43.9 (4.9)	39.8 (4.8)	49.0 (3.9)	40.0 (8.4)	35.9 (7.1)	54.1 (5.3)	47.0 (5.8)	42.9 (6.6)	45.0 (5.6)
Blue Tang ( <i>A. coeruleus</i> )	72.3 (4.4)	75.6 (5.2)	74.9 (3.3)	78.7 (7.0)	78.1 (8.0)	78.1 (4.5)	67.4 (5.6)	73.7 (6.9)	72.5 (4.7)

#### Table C-4 (cont.)

		DRTO		]	Inside RNA		(	<b>Outside RNA</b>				
Taxa	1999-2000	2002	2004	1999-2000	2002	2004	1999-2000	2002	2004			
Butterflyfishes												
(Chaetodontidae)												
Foureye Butterflyfish	23.3 (3.6)	27.0 (6.3)	34.5 (3.0)	16.1 (4.9)	16.7 (7.7)	24.9 (3.9)	28.9 (5.2)	35.0 (9.4)	41.9 (4.4)			
(Chaetodon capistratus)	25.5 (5.0)	27.0 (0.5)	54.5 (5.0)	10.1 (4.9)	10.7 (7.7)	24.9 (3.9)	20.9 (5.2)	55.0 (7.4)	+1.9 (+.+)			
Spotfin Butterflyfish	50.1 (4.8)	39.7 (4.3)	47.5 (3.3)	51.3 (8.6)	41.3 (6.6)	45.4 (5.2)	49.1 (5.3)	38.4 (5.5)	49.1 (4.2)			
(C. ocellatus)	20.1 (1.0)	59.7 (1.5)	17.0 (5.5)	01.0 (0.0)	11.5 (0.0)	10.1 (0.2)	19.17 (0.07)	50.1(5.5)	19.1 (1.2)			
Goatfishes (Mullidae)												
Spotted Goatfish	47.5 (4.6)	41.5 (4.2)	65.1 (3.5)	52.1 (7.5)	38.4 (6.4)	67.7 (4.7)	43.9 (5.8)	43.8 (5.5)	63.1 (4.9)			
(Psuedupeneus maculatus)	()	()							()			
Angelfishes												
(Pomacanthidae)												
Blue Angelfish	45.4 (4.8)	53.4 (5.1)	46.1 (3.5)	53.1 (8.6)	49.3 (8.9)	43.5 (4.3)	39.4 (5.2)	56.5 (6.0)	48.1 (5.3)			
(Holocanthus bermudensis)	× /	. ,		. ,	× /	× /						
Gray Angelfish ( <i>Pomacanthus arcuatus</i> )	42.4 (4.7)	42.5 (4.5)	47.5 (3.3)	39.1 (8.3)	38.5 (7.6)	48.2 (4.2)	44.9 (5.4)	45.6 (5.4)	47.1 (4.9)			
(Pomacaninus arcuaius) Damselfishes												
(Pomacentridae)												
Purple Reeffish												
(Chromis scotti)	25.4 (4.4)	54.7 (5.3)	53.5 (3.5)	23.9 (7.7)	48.7 (7.6)	48.0 (5.4)	26.6 (5.0)	59.3 (7.4)	57.8 (4.5)			
Bicolor Damselfish												
(Stegastes partitus)	58.4 (4.8)	67.8 (4.4)	58.7 (3.7)	61.2 (7.9)	54.5 (6.8)	57.4 (5.0)	56.2 (6.0)	78.1 (5.7)	59.7 (5.4)			
Cocoa Damselfish												
(S. variabilis)	91.5 (2.2)	89.0 (3.5)	97.3 (0.9 )	93.2 (2.7)	85.7 (6.2)	99.5 (0.5)	90.2 (3.4)	91.5 (3.9)	95.7 (1.6)			
Parrotfishes (Scaridae)												
Striped Parrotfish									054(10)			
(Scarus iseri)	89.6 (2.8)	84.1 (3.3)	93.3 (1.8)	95.8 (3.2)	89.4 (5.3)	90.7 (3.6)	84.7 (4.3)	80.0 (4.1)	95.4 (1.8)			
Redband Parrotfish		(0, 0, (1, 0))										
(Sparisoma aurofrenatum)	80.9 (4.2)	69.3 (4.9)	85.4 (2.2)	78.3 (7.4)	65.7 (6.5)	81.2 (3.8)	83.0 (4.6)	72.1 (7.0)	88.6 (2.6)			
Stoplight Parrotfish	50 1 (4 7)	52 4 (5 5)	$(5 \ 4 \ (2 \ 2))$	50.0 (0.1)		(7, (1, 0))		(0,0,(7,0))	(20(12))			
(Sparisoma viride)	58.1 (4.7)	53.4 (5.5)	65.4 (3.2)	59.9 (8.1)	44.7 (7.3)	67.6 (4.8)	56.7 (5.5)	60.0 (7.9)	63.8 (4.3)			

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**Table C-5:** Visual survey estimates of population mean density D (and standard errors in parentheses) for selected fish species during three time periods for Dry Tortugas National Park (DRTO) sampling domain and for two spatial management zones. The design performance measure CV (%) of mean density is also provided for the DRTO sampling domain. Density unit is number per 177 m<sup>2</sup>.

Taxa	1999-2000 D (SE)	CV	DRTO 2002 D (SE)	) CV	2004 D (SE)	CV	1999-2000 D (SE)	Inside RNA 2002 D (SE)	2004 D (SE)	1999-2000 D (SE)	Outside RNA 2002 D (SE)	2004 D (SE)
	D (SE)	01	D (SE)	01	D (SE)		D (61)	D (01)	D (61)	D (SL)	D (01)	D (61)
Exploited	0.62 (0.06)	0.2	0.68 (0.08)	11.9	0.54 (0.04)	75	0.56 (0.09)	0.51 (0.08)	0.50 (0.06)	0.67 (0.07)	0.82 (0.13)	0.50 (0.06)
Red Grouper	· · · ·	9.2	· · · ·		0.54 (0.04)	7.5	( )	( )	0.59 (0.06)	~ /		0.50 (0.06)
Black Grouper	0.22 (0.03)	15.6	0.60 (0.12)	20.7	0.47 (0.06)	12.0	0.19 (0.04)	0.53 (0.19)	0.41 (0.08	0.24 (0.05)	0.66 (0.17)	0.52 (0.08)
Mutton Snapper	0.14 (0.05)	34.8	0.34 (0.14)	39.5	0.31 (0.05)	17.5	0.18 (0.11)	0.22 (0.11)	0.30 (0.09)	0.12 (0.03)	0.44 (0.23)	0.31 (0.07)
Gray Snapper	1.14 (0.47)	40.8	0.95 (0.36)	38.2	6.05 (4.96)	81.9	1.30 (0.78)	1.14 (0.51)	12.3 (11.4)	1.02 (0.57)	0.80 (0.51)	1.26 (0.46)
Yellowtail Snapper	5.09 (0.64)	12.6	9.40 (2.48)	26.4	12.4 (1.41)	11.4	4.75 (0.94)	13.7 (5.43)	11.7 (2.42)	5.34 (0.88)	6.09 (1.31)	12.9 (1.67)
Hogfish	0.60 (0.09)	14.3	0.53 (0.07)	13.5	0.45 (0.05)	11.2	0.51 (0.11)	0.61 (0.11)	0.49 (0.07)	0.67 (0.13)	0.47 (0.09)	0.43 (0.07)
White Grunt	7.10 (1.26)	17.8	4.69 (0.82)	17.6	7.24 (1.77)	24.4	5.60 (1.66)	3.06 (0.65)	6.67 (2.31)	8.26 (1.83)	5.95 (1.37)	7.68 (2.58)
Bluestriped Grunt	0.16 (0.10)	64.5	0.60 (0.49)	82.2	0.74 (0.40)	54.5	0.06 (0.06)	0.22 (0.11)	1.37 (0.91)	0.23 (0.18)	0.90 (0.87)	0.24 (0.11)
Non-Target												
Ocean Surgeon	1.00 (0.18)	18.4	0.70 (0.15)	21.6	1.03 (0.13)	12.5	0.93 (0.30)	0.66 (0.25)	1.08 (0.16)	1.06 (0.23)	0.74 (0.19)	0.99 (0.19)
Blue Tang	1.92 (0.27)	13.9	3.65 (0.38)	10.5	3.67 (0.41)	11.1	1.94 (0.38)	4.32 (0.73)	3.90 (0.40)	1.90 (0.37)	3.13 (0.38)	3.49 (0.65)
Foureye Butterflyfish	0.33 (0.07)	20.2	0.52 (0.14)	26.7	0.49 (0.06)	12.8	0.25 (0.11)	0.34 (0.20)	0.30 (0.06)	0.40 (0.08)	0.66 (0.19)	0.64 (0.10)
Spotfin Butterflyfish	0.64 (0.08)	12.1	0.86 (0.22)	25.3	0.64 (0.06)	9.5	0.59 (0.13)	0.99 (0.46)	0.58 (0.07)	0.67 (0.09)	0.75 (0.14)	0.68 (0.09)
Spotted Goatfish	0.53 (0.07)	13.6	0.61 (0.08)	13.7	1.51 (0.21)	13.9	0.57 (0.10)	0.74 (0.16)	1.37 (0.22)	0.50 (0.10)	0.50 (0.08)	1.62 (0.33)
Blue Angelfish	0.56 (0.08)	15.1	0.91 (0.15)	16.4	0.66 (0.07)	11.1	0.65 (0.15)	0.78 (0.22)	0.81 (0.13)	0.49 (0.10)	1.02 (0.21)	0.54 (0.08)
Gray Angelfish	0.45 (0.06)	13.4	0.58 (0.08)	13.8	0.96 (0.33)	34.2	0.43 (0.11)	0.58 (0.13)	0.72 (0.11)	0.46 (0.07)	0.58 (0.10)	1.15 (0.58)
Purple Reeffish	1.73 (0.52)	30.2	10.9 (3.63)	33.4	7.07 (1.01)	14.3	1.48 (0.85)	6.22 (2.76)	7.01 (1.41)	1.93 (0.66)	14.5 (6.08)	7.12 (1.43)
Bicolor Damselfish	3.38 (0.62)	18.2	7.30 (1.81)	24.7	4.30 (0.45)	10.6	2.47 (0.72)	2.83 (0.71)	4.09 (0.59)	4.08 (0.94)	10.8 (3.16)	4.46 (0.67)
Cocoa Damselfish	4.88 (0.33)	6.8	5.98 (0.82)	13.7	4.99 (0.25)	5.1	5.72 (0.61)	5.78 (0.89)	5.23 (0.31)	4.23 (0.35)	6.13 (1.28)	4.80 (0.39)
Striped Parrotfish	10.1 (1.98)	19.5	11.7 (1.97)	16.9	11.1 (0.55)	4.9	13.0 (4.45)	14.1 (4.28)	12.1 (0.92)	7.88 (0.69)	9.79 (1.15)	10.3 (0.66)
Redband Parrotfish	2.87 (0.58)	20.1	2.59 (0.43)	16.5	4.72 (1.62)	34.4	2.34 (0.44)	2.94 (0.78)	2.92 (0.29)	3.27 (0.96)	2.33 (0.46)	6.12 (2.87)
Stoplight Parrotfish	1.07 (0.13)	12.4	1.33 (0.22)	16.6	1.87 (0.24)	12.8	1.26 (0.25)	1.35 (0.39)	2.34 (0.44)	0.93 (0.14)	1.31 (0.25)	1.50 (0.25)

grouper species, 9 of 13 non-target species). Among the eight snapper-grouper species analyzed, CVs were below 15% in 4 cases and ranged from 15 to 25% in 2 cases for the 2004 survey. High CVs were estimated in 2004 for gray snapper (*Lutjanus griseus*) and bluestriped grunt (*Haemulon sciurus*), two species with relatively low frequency of occurrence (**Table C-4**), i.e., high frequencies of zero observation samples. Among non-target species, CVs were below 15% in 11 of 13 cases. A CV of 15% enables statistical detection of a 30% or more change in a population metric from one time period to another.

Among snapper-grouper species, red grouper (*Epinephelus morio*) and white grunt (*Haemulon plumierii*) exhibited fairly stable domain-wide densities over the survey time period (**Table C-5**). Consistent with estimates of percent occurrence, increases in density over time were observed for black grouper (*Mycteroperca bonaci*) and mutton snapper (*Lutjanus analis*), and a slight decrease was observed for hogfish (*Lachnolaimus maximus*). Density also increased for yellowtail snapper (*Ocyurus chrysurus*). These trends in domain-wide density were also observed in the two management zones in most cases. Mean densities appeared to increase for gray snapper and bluestriped grunt, but estimates for these species were quite variable. Eight of 13 non-target species exhibited either stable or fluctuating trends in domain-wide density over time, and five species (Blue tang *Acanthurus coeruleus*, foureye butterflyfish, spotted goatfish, purple reeffish, stoplight parrotfish *Sparisoma viride*) showed increases in density from 1999-2000 to 2004.

Further insights to time trends in population densities for snapper-grouper species are provided in **Table C-6**, which provides life stage-specific density estimates. Observed increases in population densities for yellowtail snapper and black grouper were also apparent for both juvenile and adult life stages. The increase in population density of mutton snapper seems to have occurred in the adult life stage only, whereas the decrease in population density for hogfish seems to have mainly occurred in the juvenile life stage. Interestingly, stable trends in population density for red grouper corresponded with an increase in adult density and a decrease in juvenile density.

#### C-3.3 Conclusions

Reef fish surveys conducted during the 1999-2004 time period provide a robust baseline for population abundance metrics prior to implementation of the no-take RNA in the Park. Our survey approach of estimating abundance for both pre-exploited and exploited life stages of reef fishes will facilitate separating the effects of reductions in fishing from fluctuations in recruitment in future

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**Table C-6:** Survey estimates of mean density (and standard errors in parentheses) for juvenile, adult, and exploited life stages of principal snapper-grouper species during three time periods for the Dry Tortugas National Park (DRTO) sampling domain and for two spatial management zones. Density unit is number per 177 m<sup>2</sup>.

			DRTO						Inside R	NA					Outside	RNA		
	1999-	2000	2002		2004		1999-	2000	200	2	2004		1999-	2000	200	2	2004	1
Taxa	D	SE	D	SE	D	SE	D	SE	D	SE	D	SE	D	SE	D	SE	D	SE
Juveniles																		
Red Grouper	0.43	0.05	0.46	0.07	0.26	0.03	0.30	0.07	0.34	0.07	0.25	0.04	0.52	0.06	0.55	0.10	0.26	0.04
Black Grouper	0.20	0.03	0.46	0.11	0.33	0.04	0.19	0.04	0.43	0.17	0.28	0.06	0.21	0.04	0.48	0.14	0.37	0.06
Mutton Snapper	0.03	0.02	0.07	0.07	0.02	0.02	0.03	0.02	0.00	0.00	0.01	0.01	0.04	0.02	0.12	0.12	0.03	0.03
Gray Snapper	0.64	0.36	0.19	0.14	1.90	1.48	0.80	0.67	0.08	0.02	3.65	3.38	0.51	0.39	0.28	0.25	0.55	0.33
Yellowtail Snapper	3.87	0.56	5.12	0.85	7.41	1.02	3.68	0.82	5.38	1.35	6.71	1.70	4.02	0.77	4.91	1.08	7.96	1.24
Hogfish	0.19	0.04	0.05	0.02	0.05	0.01	0.12	0.04	0.07	0.04	0.03	0.01	0.25	0.07	0.03	0.02	0.06	0.02
White Grunt	4.90	1.09	3.33	0.67	3.73	0.77	3.35	1.41	1.61	0.34	3.69	1.22	6.11	1.59	4.65	1.16	3.76	0.98
Bluestriped Grunt	0.07	0.03	0.39	0.31	0.47	0.30	0.02	0.01	0.17	0.09	0.90	0.68	0.10	0.06	0.56	0.54	0.15	0.08
Adults																		
Red Grouper	0.19	0.04	0.23	0.05	0.28	0.03	0.26	0.07	0.18	0.04	0.34	0.04	0.14	0.03	0.26	0.08	0.24	0.04
Black Grouper	0.02	0.02	0.14	0.03	0.15	0.02	0.003	0.003	0.10	0.04	0.14	0.03	0.03	0.03	0.18	0.05	0.15	0.03
Mutton Snapper	0.11	0.04	0.27	0.08	0.29	0.05	0.15	0.10	0.22	0.07	0.29	0.09	0.08	0.02	0.31	0.12	0.28	0.06
Gray Snapper	0.50	0.19	0.76	0.28	4.15	3.48	0.50	0.16	1.06	0.50	8.60	7.98	0.51	0.31	0.53	0.31	0.71	0.25
Yellowtail Snapper	1.21	0.28	4.28	1.90	4.98	0.69	1.07	0.40	8.30	4.31	4.99	1.09	1.32	0.39	1.17	0.47	4.97	0.89
Hogfish	0.41	0.06	0.48	0.07	0.41	0.05	0.39	0.09	0.54	0.10	0.46	0.06	0.43	0.09	0.43	0.09	0.37	0.06
White Grunt	2.19	0.54	1.36	0.36	3.51	1.26	2.25	0.87	1.44	0.49	2.98	1.23	2.15	0.67	1.30	0.51	3.92	2.01
Bluestriped Grunt	0.09	0.07	0.21	0.19	0.26	0.12	0.04	0.03	0.04	0.03	0.48	0.25	0.13	0.13	0.34	0.33	0.10	0.08
Exploited Phase																		
Red Grouper	0.15	0.03	0.15	0.04	0.22	0.03	0.22	0.07	0.12	0.04	0.27	0.04	0.10	0.02	0.16	0.05	0.17	0.03
Black Grouper	0.02	0.02	0.14	0.03	0.15	0.02	0.003	0.003	0.10	0.04	0.14	0.03	0.03	0.03	0.18	0.05	0.15	0.03
Mutton Snapper	0.10	0.04	0.22	0.06	0.23	0.04	0.14	0.10	0.18	0.05	0.26	0.07	0.07	0.02	0.25	0.09	0.22	0.05
Gray Snapper	0.45	0.18	0.73	0.27	4.15	3.48	0.45	0.15	1.05	0.50	8.59	7.98	0.45	0.30	0.48	0.28	0.71	0.25
Yellowtail Snapper	0.59	0.18	1.03	0.50	1.94	0.37	0.47	0.27	2.07	1.14	1.91	0.62	0.69	0.25	0.22	0.13	1.97	0.45
Hogfish	0.21	0.04	0.29	0.06	0.30	0.03	0.22	0.05	0.38	0.10	0.1333	0.06	0.20	0.05	0.21	0.07	0.25	0.04
White Grunt	2.52	0.59	1.44	0.37	4.32	1.57	2.70	0.94	1.56	0.52	4.04	1.97	2.39	0.75	1.33	0.51	4.54	2.32
Bluestriped Grunt	0.14	0.10	0.26	0.20	0.49	0.30	0.05	0.03	0.13	0.08	0.97	0.68	0.21	0.18	0.35	0.35	0.12	0.08

analyses of NTMR performance. Recent efforts to refine benthic habitat maps for DRTO should lead to improvements in the accuracy and precision of abundance estimates in future surveys.

# C-4 Status and Trends of Exploited Coral Reef Fishes

Our research focus has been to quantify the reef fish community response to exploitation in the Florida Keys (Ault et al. 1998, 2005b). The principal stock assessment indicator variable to quantify population status is average length ( $\overline{L}$ ) of the exploited part of the population, which is a metabolic-based indicator that is highly correlated with population size (Beverton and Holt 1957; Ricker 1963; Pauly and Morgan 1987; Ault and Ehrhardt 1991; Ehrhardt and Ault 1992; Kerr and Dickie 2001). For exploited species,  $\overline{L}$  reflects the rate of fishing mortality. Because body size is broadly correlated with trophic level, large individuals and species are often top predators. Biomass declines of these animals are usually the most marked community response to exploitation (Ault et al. 1998; Pauly et al. 1998; Gislason and Rice 1998; Kerr and Dickie 2001). Previous studies have shown that the  $\overline{L}$  estimator of mortality rate is unbiased under conditions of constant annual recruitment to the exploited stock (Ehrhardt and Ault 1992; Quinn and Deriso 1999). However, resulting mortality estimates may exhibit positive bias under conditions of an increasing trend in recruitment. In the Florida Keys, recruitment of the most severely depleted stocks may be increasing at present in response to a series of recent management actions for the snapper-grouper complex, including gear restrictions, minimum size and bag limits, and spatial closures (Ault et al. 2005a).

We evaluate average size as an estimator of exploitation status for Dry Tortugas and Florida reef fishes. Our objective is to apply the  $\overline{L}$  estimator of mortality simultaneously to a suite of reef fish species under the same nominal fishing effort as a first-order indicator of the community response to exploitation.

## C-4.1 Methods

**Table C-7** provides life history parameters for Florida reef fishes taken from Ault *et al.* (1998, 2005b) and Claro *et al.* (2001). Natural mortality rate (M) was estimated from lifespan, applying the procedure of Alagaraga (1984). Total instantaneous mortality rate (Z) was estimated using the method of Ehrhardt and Ault (1992), which is based on length at first capture ( $L_c$ ), maximum length in the stock ( $L_{\lambda}$ ), and average length in the exploited stock ( $\overline{L}$ ), in conjunction

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				Input	Parameter		Estimated Parameters							
Species	$\mathbf{a}_{\lambda}(\mathbf{y})$	$\mathbf{K}(\mathbf{y}^{-1})$	${f L}_{\infty}$ (mm)	<b>a</b> <sub>0</sub> (y)	$\mathbf{W}_{\infty}$ (kg)	L <sub>c</sub> (mm)	L <sub>m</sub> (mm)	$L_{\lambda}$ (mm)	Lbar (mm)	<b>M</b> (y <sup>-1</sup> )	<b>F</b> (y <sup>-1</sup> )	SPR (%)	F/F <sub>msy</sub>	B/B <sub>ms</sub>
Groupers														
Rock Hind	12	0.167	499	-2.50	2.48	200	329	455	289.3	0.250	0.175	33.2	0.70	1.54
Graysby	15	0.130	415	-0.94	1.14	200	165	362	234.3	0.200	0.511	24.9	2.56	0.53
Coney	17	0.145	699	-1.08	1.50	200	185	648	_	0.176	-			
Red hind	11	0.200	471	-2.40	1.75	180	341	439	267.6	0.272	0.116	47.2	0.43	1.41
Goliath	37	0.054	2394	-3.62	244.9	600	979	2126	1823.0	0.081	0.0	10.0	0.0	3.72
Red	17	0.153	938	-0.10	11.9	500	434	869	584.4	0.176	0.480	23.5	2.72	0.50
Nassau	17	0.153	938	-0.10	11.9	600	483	869	600.0	0.176	1.000	15.7	5.67	0.27
Black	33	0.170	1306	-0.30	41.4	600	801	1301	737.1	0.091	0.624	2.0	6.87	0.05
Yellowmouth	17	0.063	882	-9.03	15.7	500	470	710	610.0	0.176	0.0	5.0	0.0	1.82
Gag	23	0.149	1187	-0.80	25.1	600	684	1074	713.2	0.130	0.457	15.7	3.50	0.41
Scamp	21	0.126	1000	-1.36	19.3	500	489	940	500.0	0.143	1.000	2.0	7.01	0.02
Tiger	26	0.110	740	-1.88	6.38	500	460	705	_	0.115	_			
Yellowfin	15	0.170	860	0.00	15.7	300	529	792	739.5	0.200	0.0	5.0	0.0	2.24
Snappers														
Mutton	14	0.129	939	-0.74	14.1	400	276	798	502.5	0.214	0.338	33.4	1.58	0.74
Schoolmaster	12	0.180	570	0.00	3.28	250	145	504	344.7	0.250	0.144	61.0	0.57	1.34
Blackfin	9	0.084	730	-2.90	2.41	220	230	459	_	0.333	_			
Gray	12	0.136	722	-0.86	5.25	250	230	596	282.6	0.250	1.58	6.8	6.35	0.18
Dog	12	0.100	854	-2.00	10.2	300	229	643	496.4	0.250	0.0	10.0	0.0	2.57
Mahogany	10	0.097	618	-1.73	3.18	230	130	419	_	0.300	_			
Lane	10	0.097	618	-1.73	3.24	200	206	418	225.7	0.300	1.176	9.3	3.92	0.24
Silk	9	0.092	781	-2.31	9.28	190	304	504	_	0.333	_			
Yellowtail	14	0.209	455	-0.71	1.24	250	199	433	270.6	0.214	1.865	13.2	8.71	0.27
Vermilion	14	0.144	650	-0.24	3.40	230	273	566	_	0.214	_			
Wrasses														
Hogfish	23	0.080	913	-1.78	14.1	300	249	786	394.1	0.130	0.309	17.3	2.37	0.46
Grunts														
Porkfish	7	0.440	397	-0.35	1.72	200	231	381	260.0	0.428	0.567	28.0	1.32	0.77
Margate	10	0.174	753	-0.45	8.57	200	428	630	355.8	0.300	0.052	75.2	0.17	3.60
Tomtate	11	0.091	442	-2.09	1.89	150	131	307	172.0	0.272	0.871	17.1	3.20	0.41
French	12	0.179	294	0.00	0.57	160	177	260	197.1	0.250	0.398	27.6	1.59	0.66
Cottonwick	9	0.320	350	-0.50	0.82	160	207	333	225.5	0.333	0.257	42.6	0.77	1.24
White	8	0.186	512	-0.78	3.06	170	180	411	206.6	0.375	1.176	9.9	3.14	0.28
Bluestriped	8	0.300	413	0.00	1.36	180	205	375	231.9	0.375	0.671	20.3	1.79	0.55

**Table C-7:** Life history input parameters and estimated population parameters for Florida reef fishes (see text for description of symbols).

with the Bertalanffy growth parameters K and  $L_{\infty}$ . Estimates of Z were computed using an iterative numerical algorithm (LBAR; Ault *et al.* 1996; FAO 2003), and annual estimates of fishing mortality rate (F) were obtained by subtracting M from Z. All input values are given in **Table C-7**.

Theoretically,  $\overline{L}$  in year t is expressed as

$$\overline{L}(t) = \frac{F(t)\int_{a_c}^{a_\lambda} N(a,t)L(a,t)da}{F(t)\int_{a_c}^{a_\lambda} N(a,t)da}$$
(1)

where  $a_c$  is the minimum age at first capture,  $a_{\lambda}$  the oldest age in the stock, N(a,t) the abundance for age class a, L(a,t) the length at age, and F(t) is the instantaneous fishing mortality rate at time t. In practice,  $\overline{L}$  is usually estimated in the length range  $L_c - L_{\lambda}$ . Estimates of average length and the corresponding variances were obtained from fishery-independent length composition data, applying standard statistical procedures (Sokal and Rohlf 1981). Nonnormality of length observations was corrected by log-transformation.

A numerical cohort-structured model, REEFS (Reef Fish Equilibrium Exploitation Fishery Simulator; Ault and Olson 1996; Ault *et al.* 1998) was used to conduct simulation analysis of uncertainty properties of F estimates based on average size, and to compute several fishery management reference points of stock status, or "sustainability benchmarks", including yield-per-recruit (YPR), spawning potential ratio (SPR), and limit control rules. The benchmarks used to evaluate sustainable exploitation in terms of a limit control rule were:  $F_{msy}$  (F generating maximum sustainable yield, MSY);  $B_{msy}$  (population biomass at MSY); and SPR (spawning potential ratio; Mace 1997; Restrepo and Powers 1999). We define  $F_{msy}$  as F = M. The REEFS models the age-size distribution of the population from larvae to mature adults to maximum sizeage using a number of population dynamic functions to regulate birth, growth, and survivorship processes, including selection and harvest by the fishery (see Ault *et al.* 1998).

Since population biomass B(a,t) is the product of numbers-at-age times weight-at-age, yield in weight  $Y_w$  from a species was calculated as

$$Y_{w} = (F, L_{c}, t) = F(t) \int_{L_{c}}^{L_{\lambda}} B(L|a, t) dL = F(t) \int_{L_{c}}^{L_{\lambda}} N(L|a, t) W(L|a, t) dL$$
(2)

Spawning stock biomass (SSB), a measure of stock reproductive potential, was obtained by integrating over individuals in the population between the minimum size of sexual maturity  $(L_m)$  and the maximum size  $(L_\lambda)$ ,

$$SSB(t) = \int_{L_m}^{L_\lambda} B(L|a,t) dL$$
(3)

Spawning potential ratio (SPR) is a management benchmark that measures the stock's current reproductive potential to produce optimal yields on a sustainable basis,

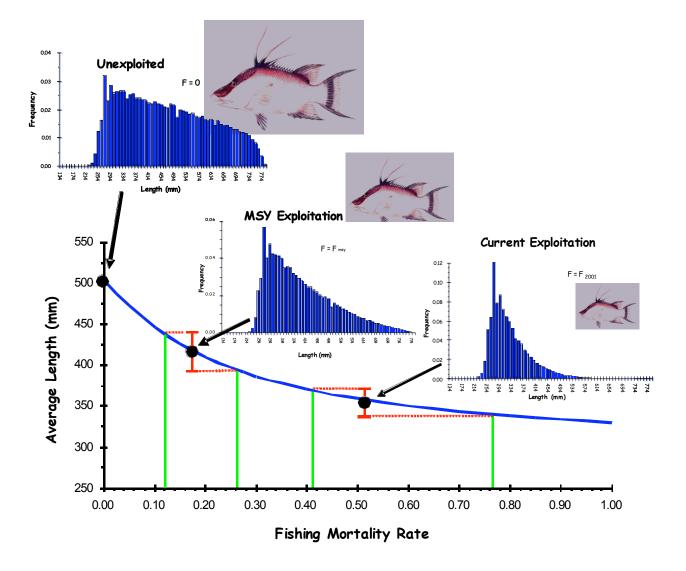
$$SPR = \frac{SSB_{exploited}}{SSB_{un exploited}}$$
(4)

Estimated SPRs are compared to U.S. Federal standards which define 30% SPR as the overfishing threshold at which the stock is no longer sustainable at the current exploitation level.

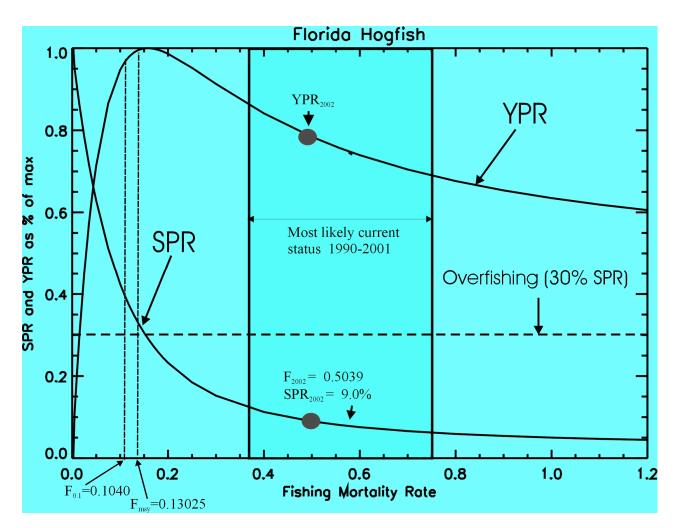
#### C-4.2 Results

The analytical relationship between  $\overline{L}$  and F, some aspects of uncertainty in average length mortality estimates, and the expected stochastic population length compositions at three levels of fishing mortality (F = 0;  $F = F_{msy}$ ;  $F = F_{2001}$ ) are depicted for hogfish in **Figure C-3**. Although the 95% confidence interval (CI) of  $\overline{L}$  is larger at  $F_{msy}$  than at  $F_{2001}$ , the corresponding CI of F is higher at  $F_{2001}$  owing to the non-linear relationship between  $\overline{L}$  and F. The non-linear relationship also results in asymmetric CIs of F that are more pronounced at higher exploitation rates. The  $\overline{L}$  method thus has high statistical power for discerning between sustainable and overfished levels of F, but has low power for discerning between overfished and severely overfished levels of F.

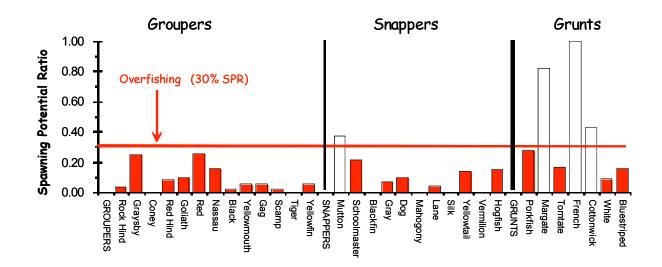
 $\overline{L}$  mortality estimates, based on fishery-independent size composition data collected in 2004 from Dry Tortugas region, and management benchmarks for 31 species of reef fish are provided in Table C-7. For some species (e.g., coney *Cephalophlus fulvus*, goliath grouper *Epinephelus itajara*, tiger grouper *Mycteroperca tigris*, yellowfin grouper *Mycteroperca venenosa*), it was difficult to obtain reliable mortality estimates due to low sample sizes. The theoretical relationships among various fishery sustainability decision metrics are shown for hogfish in **Figure C-4**. Estimates of SPR for Tortugas reef fishes are graphed in **Figure C-5**. In



**Figure C-3**: Relationship of *Lbar* in the exploited phase and fishing mortality F for hogfish, and the variation in F estimates (dotted horizontal bars) resulting from variation in *Lbar* (dashed vertical bars). Insets show representative population length frequency compositions at F0, Fmsy, and F2001.



**Figure C-4**: Theoretical relationship of the fishery sustainability decision metrics spawning potential ratio (SPR) and yield-per-recruit (YPR) to fishing mortality rate (F) for hogfish. Graph shows position of maximum sustainable yield (MSY) and Fmsy that are used to compute limit control rules under the precautionary approach to fishery management.



**Figure C-5:** Spawning potential ratio (SPR) analysis for species in the snapper-grouper complex from the Dry Tortugas National Park (DRTO) for the period 1999-2004. Dark bars indicate overfished stocks and open bars indicate stocks that are above the 30% SPR standard.

general, the majority of species in the snapper-grouper complex for which estimates could be made are below the 30% SPR standard. A comparison of DRTO to the broader Tortugas region shows that SPR estimates are fairly similar (**Figure C-5**).

Values of the  $F/F_{msy}$  ratio plotted against the  $B/B_{msy}$  ratio (**Figure C-6**) suggest that most species of the snapper–grouper complex experience overfishing (F-ratio >1, B-ratio <1; Restrepo and Powers 1999) and have been subject to unsustainable rates of exploitation in recent years. Overfishing appears most severe for long-lived, slow-growing fish (cf. **Table C-7**).

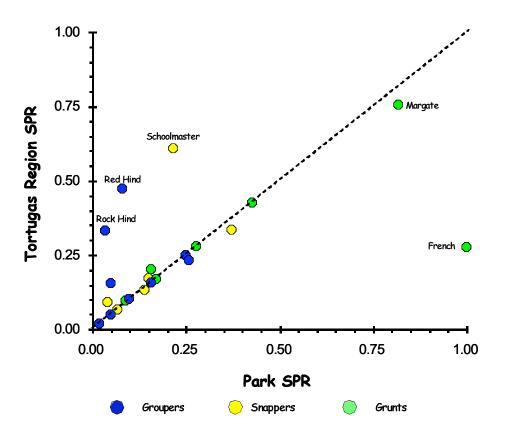
SPR estimates for Florida Keys reef fishes in comparison to those from Dry Tortugas are also shown in **Figure C-7**. The overall pattern of exploitation appears to be similar between the two regions. It appears that snapper SPRs are marginally higher in the Dry Tortugas.

Of particular concern are the populations of long-lived fishes like black grouper, which live more than several decades and reach relatively large sizes (cf. **Table C-7**). These long-lived, slow growing fishes tend to be exceptionally sensitive to even modest exploitation rates, and as exploitation increases there is a significant truncation of the older, mature size classes in a process known as "juvenescense", i.e., making the population younger through excessive fishing mortality (**Figure C-8**).

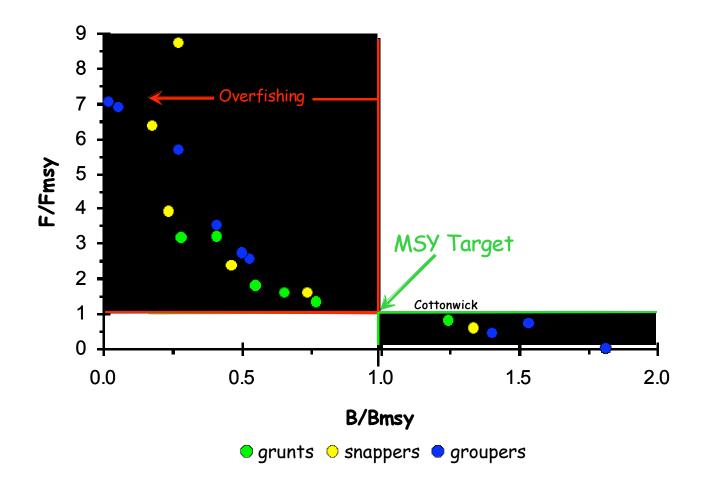
Ecological interpretation of the plot of SPR dependent on M/K (Figure C-9) suggests that the greater proportion of those fishes experiencing non-sustainable rates of exploitation (SPR < 30%) are those with relatively low M/K values. Fish with M/K values greater than 1.5 could be expected to recover in sustainable population sizes in 5-15 years. However, for those with M/K values less than 1.5, recovery for stocks with SPRs less than 30% may be expected to take 2-3 decades or more.

#### C-4.3 Discussion

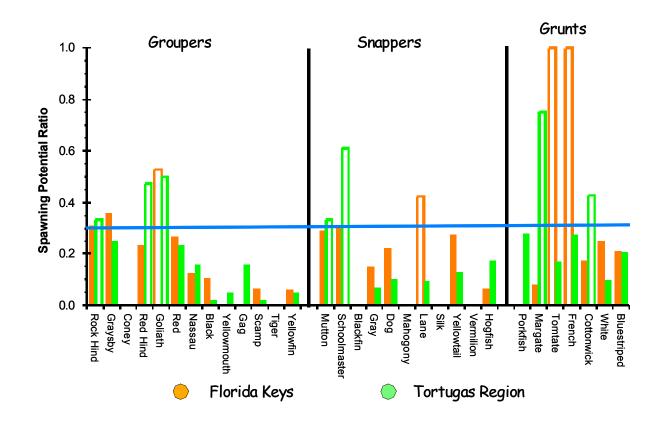
The *L* method for estimating total mortality exhibits relatively robust properties for assessing exploitation impacts on the Florida coral reef fish community (Ehrhardt and Ault 1992; Quinn and Deriso 1999; Ault *et al.* 1998, 2001, 2002, 2005b). The use of  $\overline{L}$  as an estimator of fishing mortality, and therefore indirectly as an indicator of exploitation, has several practical advantages: (1) relatively simple data requirements (i.e. accurate and precise information on the age-and-size relationship, length frequency compositions for the exploited stock, and estimates of M); (2) the method applies to both fishery-independent and -dependent data, and therefore



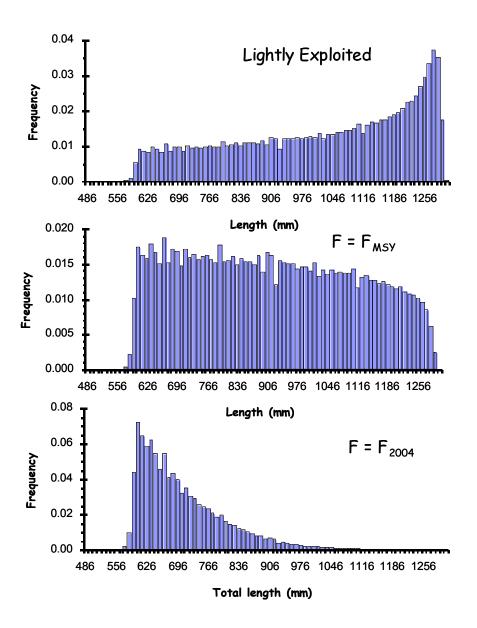
**Figure C-6**: Comparison of spawning potential ratio (SPR) for reef fish in the exploited snappergrouper complex in Dry Tortugas National Park (DRTO) versus the Tortugas Region domain (Park and Tortugas Bank). Perfect agreement between estimates follows the dotted line. Note the relative high degree of correlation between regional estimates.



**Figure C-7**: Plot of F/Fmsy ratio against B/Bmsy ratio for fishes in the snapper-grouper complex in the Dry Tortugas region for 2004 (blue - groupers; yellow - snappers and wrasses; green - grunts).



**Figure C-8:** Comparative SPR analysis for exploited reef fishes between the Dry Tortugas region (green) and Florida Keys (orange) for the period 2000-2002. Dark bars indicate overfished stocks, open bars indicate stocks that are above the 30% SPR standard (blue horizontal line).



**Figure C-9**: Process of "juvenesecense" of a black grouper population when: (upper panel) lightly exploited; (middle panel) exploited at MSY; and, (lower panel) current exploitation level in the Florida Keys.

may help to resolve incongruencies that exist among individual data sets; (3) relatively simple computational requirements for the  $\overline{L}$  algorithm, because length-to-age transformation is not required, unlike most contemporaneous age-based mortality estimators (Quinn and Deriso 1999); and (4) the method might be used to generate a "community control rule" indicator of exploitation status, when used in the context of synoptic fishery-independent survey data.

The principal fleet targeting the reef fish complex of approximately 50 species is recreational, and the principal gear is hook and line, which is relatively non-selective (Ault *et al.* 2005a). Because many of the species co-occur in similar habitats, capture probability for most species on any given trip at any location is greater than zero. Therefore, nominal fishing effort in the Florida Keys affects the snapper–grouper complex as a whole, but acts differentially on individual species, depending on their life history characteristics. The impact of exploitation was more severe for the slow-growing, long-lived groupers and hogfish than for other species. Because of these factors, management to build sustainable fisheries may need to consider the entire reef-fish complex and perhaps invoke a spatial context to interventions.

### C-5 MPA Performance in the Dry Tortugas Region

An extensive literature has touted the use of 'no-take' marine reserves (NTMRs -- areas protected from all extractive uses) as the means to reverse declining trends in tropical coral reef ecosystems (Polunin 1990, 2002; Roberts and Polunin 1991; DeMartini 1993; Bohnsack and Ault 1996; Roberts 1997; Allison *et al.* 1998; Guenette *et al.* 1998; Bohnsack *et al.* 2004; Gell and Roberts 2003; Halpern and Warner 2002, 2003; Hastings and Botsford 2003; Lubchenco *et al.* 2003; Willis *et al.* 2003; Hooker and Gerber 2004; Meester *et al.* 2001, 2004; Ault *et al.* 2002, 2005a; Mangel and Levin 2005).

In the Florida Keys, increased fishing pressure from rapid regional human population growth and environmental changes associated with coastal development have raised concerns about fisheries sustainability and persistence of the coral reef ecosystem (Porter and Porter, 2001; Ault *et al.* 2005a; Pandolfi *et al.* 2005). In response to declining trends in reef fishery catches, a series of regional federal and state management regulations were imposed, including recreational bag limits, minimum size limits, commercial quotas and trip limits, seasonal closures, gear restrictions, limited commercial entry, closed fisheries, species moratoria, game fish status, and restrictions on sale and possession. These regulations were implemented to stabilize catches, protect spawning stock biomass, and reduce fishing mortality rates. Despite the

bevy of regulations imposed in the Florida Keys, recent fishery assessments indicate that, for example, black grouper spawning stock biomass was less than 10% of its historical size (Ault et al., 2005b).

In recent years, new ecosystem-based management measures have been enacted in the Florida Keys, including the 1997 implementation of a network of 23 NTMRs by the Florida Keys National Marine Sanctuary (FKNMS, NOAA, www.fknms.noaa.gov). In July 2001, the Florida Keys network was expanded to become the largest in North America with the implementation of two NTMRs in the Dry Tortugas region covering about 566 km<sup>2</sup>.

#### C-5.1 Methods

Our statistical analyses focused on changes between baseline years 1999 and 2000 (before) and 2004 (after), when synoptic surveys were conducted in both Tortugas Bank and DRTO. Statistical analysis of change was evaluated using a community metric, species richness, and two population metrics: frequency of occurrence and abundance. Statistical estimation procedures followed Cochran (1977) for a two-stage stratified random sampling design. In these procedures, strata means and variances of a given metric are weighted by strata sizes, i.e.,

 $W_h = N_h / \sum_h N_h$ , to obtain overall means and variances for either specific management zones, or for the entire Tortugas domain. Species richness was estimated on a primary sample unit basis (i.e., the number of unique species observed within a primary unit by the group of divers) to ensure a sufficient search area for obtaining reliable estimates. In this case, the statistical sample size was *n*, the number of sampled primary units. Both frequency of occurrence and abundance were estimated by species on a second-stage unit basis, the standard approach for two-stage designs (Cochran, 1977), where the number of second-stage units *nm* was the statistical sample size. Since benthic habitat classification, digital mapping, and development of the Tortugas survey design occurred concurrently with the baseline surveys of 1999 and 2000 (Ault et al. 2002), each population and community metric was estimated as a composite of the two baseline years to alleviate problems of misclassification of habitats and misallocation of samples among habitat strata. In this procedure, strata means and variance components were computed as twoyear averages weighted by respective sample sizes in 1999 and 2000.

Species chosen for detailed analyses reflected the range of population dynamic processes (growth and survivorship) for relatively abundant exploited and non-exploited components of the reef fish community. Statistical tests for differences among estimates of mean density, total

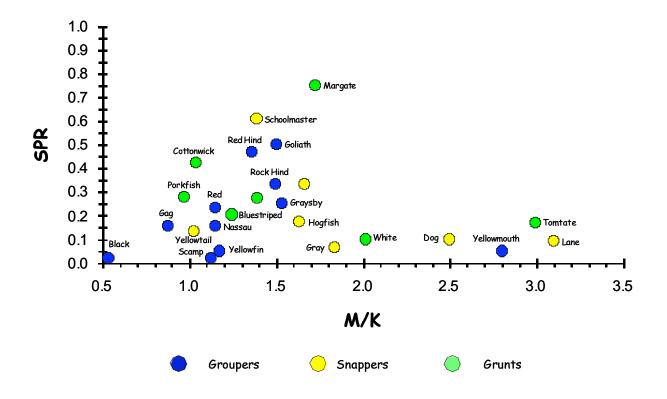
abundance, and mean proportion of samples for the sampling design configuration were conducted by inspection of confidence intervals (CI) using Bonferroni adjustments (Cochran, 1977). Detection of change was defined as the ability to discriminate between the 95% CI of mean responses between the two time periods. The Bonferoni CI t-test was used because it is more suited to sample design statistics and does not require homogenous variance between two distributions to test differences in the mean responses. Changes in length compositions between time periods were evaluated using standard two sample Chi-square tests (Agresti 1996). The absolute ability to detect changes was thus determined by the precision of the survey estimates (e.g., standard error).

#### C-5.2 Results

For the Tortugas sampling domain, we detected no change in mean species richness (mean number of species per psu) between the 1999-2000 ( $37.1 \pm 0.7$  SE) baseline and 2004 ( $38.1 \pm 0.5$  SE), even though we could have detected a change > 1.4 species (i.e., approximately 2 SE). We found similar results for selected taxa. For example, mean richness for species of exploited snappers and groupers was  $7.8 \pm 0.2$  for both 1999-2000 and 2004 survey periods. Species richness (diversity) of the snapper-grouper complex was also related to reef rugosity, in that it was highest on reef terrace and pinnacle habitats found on the northwestern Tortugas Bank and western DRTO, and also in medium profile reef in the northwestern portion of the Park (**Figure C-10**). It was lowest in low-relief hardbottom and patchy hardbottom in sand habitats.

The relatively stable community structure shown for richness was also reflected in domain-wide estimates of frequency of occurrence or sighting frequency. Although there were minor changes in ranks between years, only four of the top 50 species (of 267 total) for the 2004 survey were not among the top 50 species for the 1999-2000 surveys (**Table C-8**). The top 50 species included 11 (of 55 total) species from the exploited snapper-grouper complex.

Estimates of frequency of occurrence and abundance for representative species of principal families are given in **Tables C-9** and **C-10**, respectively. Analyses of change between 1999-2000 and 2004 using black grouper as an example are illustrated. Domain-wide percent occurrence for black grouper increased from 19.5% in 1999-2000 to 28.8% in 2004 (**Table C-9**; p<0.01), along with a concomitant abundance increase of 124% (**Table C-10A**; p<0.001). Detection of temporal change in abundance was facilitated by a decrease in the survey coefficient of variation (CV=SE/Mean) from 14.5% to 10.3%. The increase in domain-wide abundance was accompanied by a shift in the length composition between 1999-2000 and 2004



**Figure C-10**: Plot of estimated spawning potential ratio (SPR) dependent on M/K (natural mortality rate divided by the growth rate) for exploited groupers (blue), snappers and wrasses (yellow), and grunts (green) from the Dry Tortugas.

				rrence Rank	
Common name	Scientific name	Family	2004	1999-2000	Change
Bluehead	Thalassoma bifasciatum	Labridae	1	1	=
Striped parrotfish	Scarus iseri	Scaridae	2	2	=
Cocoa damselfish	Stegastes variabilis	Pomacentridae	3	3	=
Redband parrotfish	Sparisoma aurofrenatum	Scaridae	4	5	+
Yellowhead wrasse	Halichoeres garnoti	Labridae	5	10	+
Blue tang	Acanthurus coeruleus	Acanthuridae	6	8	+
Bicolor damselfish	Stegastes partitus	Pomacentridae	7	11	+
Spotted goatfish	Pseudupeneus maculatus	Mullidae	8	20	+
White grunt	Haemulon plumieri	Haemulidae	9	4	-
Slippery dick	Halichoeres bivittatus	Labridae	10	7	-
Yellowtail snapper	Ocyurus chrysurus	Lutjanidae	11	9	-
Saucereye porgy	Calamus calamus	Sparidae	12	6	-
Stoplight parrotfish	Sparisoma viride	Scaridae	13	15	+
Bridled goby	Coryphopterus glaucofraenum	Gobiidae	14	12	-
Red grouper	Epinephelus morio	Serranidae	15	13	-
Purple reeffish	Chromis scotti	Pomacentridae	16	28	+
Ocean surgeon	Acanthurus bahianus	Acanthuridae	17	18	+
Blue angelfish	Holacanthus bermudensis	Pomacanthidae	18	16	-
Spotfin butterflyfish	Chaetodon ocellatus	Chaetodontidae	19	17	-
Butter hamlet	Hypoplectrus unicolor	Serranidae	20	29	-
Greenblotch parrotfish	Sparisoma atomarium	Scaridae	21	24	+
Masked goby	Coryphopterus personatus	Gobiidae	22	25	+
Blue hamlet	Hypoplectrus gemma	Serranidae	23	38	+
Yellowhead jawfish	Opistognathus aurifrons	Opistognathidae	24	21	-
Gray angelfish	Pomacanthus arcuatus	Pomacanthidae	25	23	-
Hogfish	Lachnolaimus maximus	Labridae	26	19	-
Foureye butterflyfish	Chaetodon capistratus	Chaetodontidae	27	32	+
Clown wrasse	Halichoeres maculipinna	Labridae	28	26	-
Threespot damselfish	Stegastes planifrons	Pomacentridae	29	30	+
Beaugregory	Stegastes leucostictus	Pomacentridae	30	34	+
Harlequin bass	Serranus tigrinus	Serranidae	31	27	-
Saddled blenny	Malacoctenus triangulatus	Labrisomidae	32	14	-
Barred hamlet	Hypoplectrus puella	Serranidae	33	31	-
Neon goby	Elacatinus oceanops	Gobiidae	34	22	-
Graysby	Cephalophilis cruentatus	Serranidae	35	37	+
Black grouper	<i>Mycteroperca bonaci</i>	Serranidae	36	44	+
Blue chromis	Chromis cyanea	Pomacentridae	37	45	+
Mutton snapper	Lutjanus analis	Lutjanidae	38	52	+
Tobaccofish	Seranus tabacarius	Serranidae	39	46	+
Bar jack	Caranx ruber	Carangidae	40	40	=
Queen angelfish	Holacanthus ciliaris	Pomacanthidae	40	43	+
Great barracuda	Sphyraena barracuda	Sphyraenidae	42	49	+
Sharpnose puffer		Tetraodontidae	42	39	Т
Spanish hogfish	Canthigaster rostrata Bodianus rufus	Labridae	43 44	39 47	+
Tomtate	Haemulon aurolineatum	Haemulidae	44 45	47	Ť
		Scaridae	45 46	41 61	-+
Princess parrotfish	Scarus taeniopterus				+
Reef butterflyfish	Chaetodon sedentarius	Chaetodontidae Haemulidae	47	36	-
French grunt	Haemulon flavolineatum		48	50	+
Cero	Scomberomorus regalis	Scombridae	49 50	117	+
Bucktooth parrotfish	Sparisoma radians	Scaridae	50	101	+

**Table C-8:** Ranked top 50 reef fish species in terms of percent occurrence for 2004 compared to 1999-2000. Common names in bold denote species in the exploited snapper-grouper complex.

**Table C-9:** Domain-wide estimates of percent occurrence for representative exploited and non-target fish species for baseline years 1999-2000 and the 2004 survey. Statistically significant change between baseline years and 2004: ns - not significant; \* - p<0.05; \*\* - p<0.01; \*\*\* - p<0.001.

	Percent Occ	urrence (SE)	
Taxa	1999-2000	2004	Change
Snapper-Grouper Complex			
Groupers (Serranidae)			
Goliath grouper (Epinephelus itajara)	0.5 (0.4)	1.3 (0.5)	ns
Red grouper (E. morio)	67.0 (3.3)	62.8 (3.1)	ns
Nassau grouper (E. striatus)	1.0 (0.6)	0.3 (0.2)	ns
Black grouper (Mycteroperca bonaci)	19.5 (2.5)	28.8 (2.4)	**
Snappers (Lutjanidae)			
Mutton snapper (Lutjanus analis)	14.8 (2.4)	25.8 (3.0)	***
Gray snapper (L. griseus)	17.3 (2.5)	12.2 (1.5)	*
Yellowtail snapper (Ocyurus chrysurus)	74.7 (3.2)	68.1 (3.1)	*
Wrasses (Labridae)			
Hogfish (Lachnolaimus maximus)	52.8 (3.5)	42.6 (3.0)	**
Grunts (Haemulidae)			
White grunt (Haemulon plumieri)	82.0 (2.7)	71.5 (2.7)	***
Bluestriped grunt (H. sciurus)	6.4 (1.7)	7.7 (1.2)	ns
Non-Target Fishes			
Surgeonfishes (Acanthuridae)			
Ocean surgeon (Acanthurus bahianus)	54.9 (3.3)	60.3 (2.7)	ns
Blue tang (A. coeruleus)	76.4 (3.1)	80.9 (2.2)	ns
Butterflyfishes (Chaetodontidae)			
Foureye butterflyfish (Chaetodon capistratus)	34.0 (3.3)	42.3 (2.8)	*
Spotfin butterflyfish (C. ocellatus)	56.4 (3.4)	49.9 (3.0)	ns
Goatfishes (Mullidae)			
Spotted goatfish (Psuedupeneus maculatus)	50.7 (3.6)	71.7 (2.2)	***
Angelfishes (Pomacanthidae)			
Blue angelfish (Holocanthus bermudensis)	57.9 (3.2)	55.9 (2.7)	ns
Gray angelfish (Pomacanthus arcuatus)	45.5 (3.3)	43.9 (2.8)	ns
Damselfishes (Pomacentridae)			
Purple reeffish (Chromis scotti)	37.2 (3.4)	62.2 (3.1)	***
Bicolor damselfish (Stegastes partitus)	72.7 (2.9)	72.6 (2.3)	ns
Cocoa damselfish (S. variabilis)	87.7 (2.3)	90.0 (2.0)	ns
Parrotfishes (Scaridae)			
Striped parrotfish (Scarus iseri)	88.4 (2.4)	94.3 (1.3)	*
Redband parrotfish (Sparisoma aurofrenatum)	80.8 (2.9)	86.9 (1.9)	*
Stoplight parrotfish (Sparisoma viride)	59.3 (3.5)	64.5 (3.3)	ns

**Table C-10:** (A) Domain-wide estimates of abundance (and associated coefficient of variation CV) and changes between baseline years 1999-2000 and 2004 for representative exploited and non-target fish species. (B) Population abundance changes between 1999-2000 and 2004 within management zones in the Dry Tortugas region. Statistically significant change between baseline years and 2004: ns - not significant; \* - p<0.05; \*\* - p<0.01; \*\*\* - p<0.001.

	1999-2000		2004			
Tama	Abundance	CV	Abundance		Char	~ ~
Taxa	(millions)	(%)	(millions)	(%)	Chan	ge
Snapper-Grouper Complex						
Red grouper	1.260	6.8	1.237	6.5	-2%	ns
Black grouper	0.277	14.5	0.622	10.3	+124%	***
Mutton snapper	0.216	21.2	0.452	13.2	+109%	***
Gray snapper	3.714	54.3	5.155	74.0	+39%	ns
Yellowtail snapper	8.257	13.0	23.169	27.2	+181%	*
Hogfish	1.121	10.7	0.910	12.0	-19%	ns
White grunt	9.317	15.5	9.644	21.6	+4%	ns
Bluestriped grunt	0.330	47.0	0.854	42.0	+159%	ns
Non-Target Fishes						
Ocean surgeon	2.045	13.3	2.275	8.0	+11%	ns
Blue tang	3.474	9.7	5.747	7.8	+65%	***
Foureye butterflyfish	0.960	10.8	1.083	7.5	+13%	ns
Spotfin butterflyfish	1.315	7.5	1.256	6.8	-5%	ns
Spotted goatfish	1.076	10.7	3.204	9.8	+198%	***
Blue angelfish	1.555	8.0	1.525	6.8	-2%	ns
Gray angelfish	0.868	9.2	1.588	27.2	+83%	ns
Purple reeffish	11.518	17.8	20.219	13.0	+76%	***
Bicolor damselfish	12.914	10.4	17.269	7.8	+34%	**
Cocoa damselfish	7.654	5.9	7.384	4.9	-4%	ns
Striped parrotfish	16.117	18.3	22.290	10.1	+38%	*
Redband parrotfish	4.565	16.2	7.096	23.3	+56%	ns
Stoplight parrotfish	1.936	9.7	3.012	10.3	+56%	***

**(A)** 

### Table C-10: (cont.)

(B) \_\_\_\_\_

Taxa	Tortugas Bank Fished		Tortugas Bank NTMR		Dry Tortugas National Park	
<b>Snapper-Grouper Complex</b> Red grouper	-43%	*	+38%	*	-9%	•
0 1				*		ns ***
Black grouper	+84%	ns	+120%	**	+128	***
Mutton snapper	-45%	ns	+303%	* *	+142%	* * *
Gray snapper	-96%	ns	-51%	ns	+270%	ns
Yellowtail snapper	-19%	ns	+367%	ns	+132%	***
Hogfish	-27%	ns	+6%	ns	-25%	ns
White grunt	+7%	ns	+24%	ns	+2%	ns
Bluestriped grunt	+50%	ns	+13%	ns	+242%	ns
Non-Target Fishes						
Ocean surgeon	+2%	ns	+75%	**	-9%	ns
Blue tang	+13%	ns	+28%	ns	+99%	***
Foureye butterflyfish	+86%	*	-18%	ns	+32%	ns
Spotfin butterflyfish	+35%	ns	-31%	*	0%	ns
Spotted goatfish	+133%	**	+326%	***	+175%	***
Blue angelfish	-18%	ns	-20%	ns	+31%	*
Gray angelfish	-24%	ns	+58%	ns	+120%	ns
Purple reeffish	+31%	ns	+42%	ns	+263%	***
Bicolor damselfish	+6%	ns	+73%	**	+17%	ns
Cocoa damselfish	-28%	ns	-21%	ns	+6%	ns
Striped parrotfish	+51%	ns	+127%	*	+9%	ns
Redband parrotfish	+121%	***	+26%	ns	+56%	ns
Stoplight parrotfish	+9%	ns	+26%	ns	+84%	***

towards a higher proportion of exploited-phase animals in the black grouper population (**Figure C-11A**; Chi-square p<0.001 for lengths > 30 cm).

Abundance estimates for black grouper showed increasing trends in all three management zones over the survey time period, but statistically significant increases were detected only in Tortugas Bank NTMR and DRTO (**Table C-10B**). In 2004, black grouper population size structure appeared to expand in the Bank NTMR and Park areas, but was highly truncated above the minimum legal size in the Bank fished area. Changes in length compositions within management zones paralleled changes in abundance (**Figure C-11B, Table C-10**), with a higher proportion of exploited phased animals in the Bank NTMR (p<0.001). No change in length composition was detected in the Tortugas Bank Fished area.

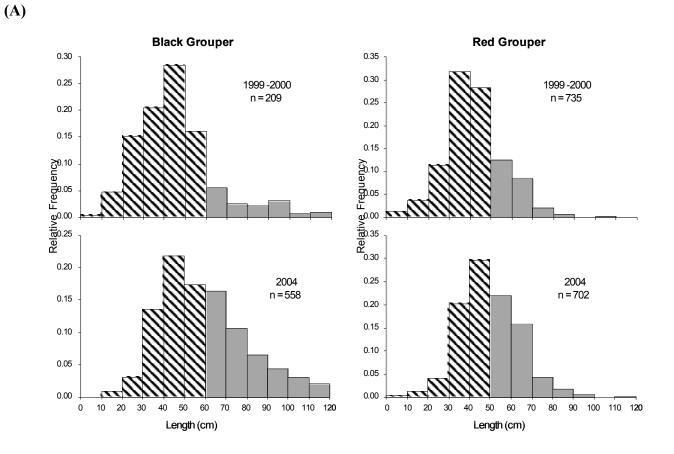
Significant increases in domain-wide occurrence and abundance were also detected for mutton snapper, corresponding with significant increases in abundance in the Tortugas Bank NTMR and DRTO management zones. In general, trends in occurrence mirrored those for abundance for species with relatively small population sizes.

No change in either occurrence or abundance for red grouper was detected domain-wide; however, a significant decrease in abundance in the Tortugas Bank fished area and a significant increase in Tortugas Bank NTMR was detected. Similar to black grouper, increases in the population proportion of larger (older) individuals for red grouper (**Figure C-10A**; Chi-square p<0.001 for lengths > 30 cm) was noted.

A marginal decrease in domain-wide occurrence for yellowtail snapper was detected; on the other hand, a domain-wide increase in abundance corresponding with a significant increase in the Park was also detected. Evidently, more fish were seen at fewer sites, however, the observed decline in percent occurrence probably had little biological significance. As a result, abundance may be a more indicative metric of population change. This disparity between occurrence and abundance was also observed for other schooling species: gray snapper, hogfish, and white grunt.

Domain-wide occurrences of goliath grouper and Nassau grouper, two species under fishing moratoria, remained low over the survey period. We observed goliath grouper in one primary sampling unit in 1999, two units in 2000, and in 10 primary sampling units in 2004 (seven in the Park and three in the Bank NTMR) which is perhaps encouraging for its recovery, but was not a statistically significant change in frequency of occurrence.

Among species not targeted by exploitation, domain-wide increases in both occurrence and abundance were detected for spotted goatfish, purple reeffish, and striped parrotfish (*Scarus iseri*).



**Figure C-11:** (A) Domain-wide comparisons of length compositions for black grouper (left panels) and red grouper (right panel) between 1999-2000 (top) and 2004 (bottom) surveys. (B) Comparison of the 3 spatial zones for black grouper for 2004. Hatched bars are pre-exploited phase and shaded bars are exploited phase animals. Number of length observations are given on each panel.

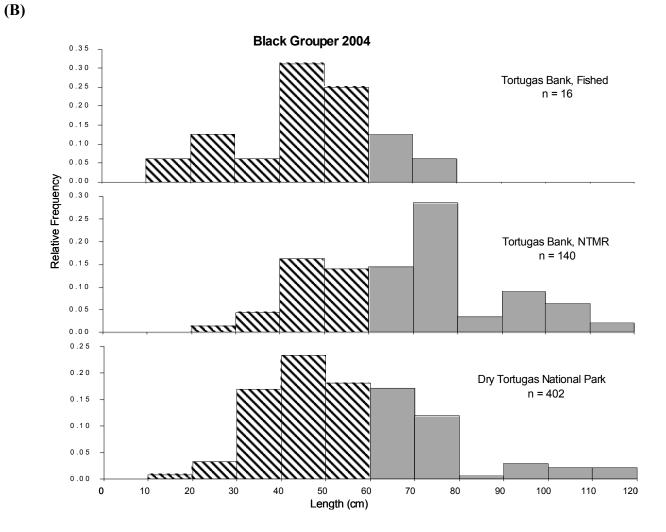


Figure C-11: (Cont.)

On the other hand, we detected increases in domain-wide occurrence but no changes in abundance for foureye butterflyfish and redband parrotfish (*Sparisoma aurofrenatum*). For blue tang, bicolor damselfish (*Stegastes partitus*), and stoplight parrotfish, no changes were detected in domain-wide occurrence but we detected increases in domain-wide abundance. Domain-wide increases in spotted goatfish corresponded to significant increases in abundance in all three management zones. Domain-wide increased abundances in the Park. Increases in domain-wide abundance of bicolor damselfish and striped parrotfish were accompanied by significant abundance increases in Tortugas Bank NTMR. In several cases, management zone changes in abundance were detected but these did not correspond to domain-wide changes.

An unexpected occurrence in the 2004 survey, based on our previous cruises, was the sighting of large (>2000 fish) schools of large (> 9 kg) permit (*Trachinotus falcatus*) at 8 primary sampling unit locations. The timing and schooling behavior of these mature permit suggests that these may have been spawning aggregations. Seven of the 8 schools were sighted on Tortugas Bank, and these were either inside or just outside the Bank NTMR.

### C-5.3 Discussion

The Tortugas region represents a de facto adaptive management experiment in which three discrete, contiguous areas are being managed under different levels of resource protection. The Tortugas Bank Fished zone operates under regional fishing regulations and is less protected than DTNP which is open only to recreational angling. Finally, the Tortugas Bank NTMR (North Ecological Reserve), protected from all extraction, is the most protected. Determining the efficacy of the suite of management approaches is one of Florida's most critical resource management problems and a unique challenge for science-based resource management.

A number of authors have pointed out that detection of changes in population abundance and biomass in response to any fishery management action has often suffered from lack of rigor in the design of both fishery-dependent and fishery-independent surveys (e.g., Hurlbert, 1984; Stewart-Oaten *et al.*, 1986; Underwood 1990, 1993; Willis *et al.*, 2003; Hilborn *et al.*, 2004; Sale *et al.*, 2005). Relative to traditional fishery-dependent approaches, quantitative assessments of NTMRs present their own unique challenges because there are no catches to examine from closed areas and data must be spatially-explicit. In addition, data must be collected that reflects community dynamics, not just exploited species dynamics, to evaluate the performance of ecosystem-based management. These principles were the impetus for our survey sampling approach in the Tortugas region.

The fisheries-independent RVC surveys provided fairly precise estimates of species richness and frequency of occurrence. However, while also a precise measure, abundance was a metric more indicative of population change because it tracked population variability at both low and high population sizes. In general, our population detection limits for changes in abundance ranged between 15% to 30%, i.e., twice the measured CV. In some cases it was difficult to obtain precise estimates of abundance. For example, low sighting frequency coupled with relatively high abundance at few sites yielded high CVs for gray snapper. Overall, CI t-tests were found to be a conservative application of statistical methods because they required detection of differences in mean abundance with respect to each time period. The method became less robust as the size of the spatial unit (e.g., management zone, habitat type, etc.) decreased.

The impacts of management actions on population biomass could take years to occur and then be detected (e.g., Beverton and Holt, 1957). However, signs of recovery in the Tortugas reef fish community over a relatively short time after implementation of NTMRs was observed. We have shown that metrics of the reef fish community (e.g., richness and species composition) were very stable over the study time period. However, for a representative suite of 21 reef fishes, increases in domain-wide abundance for three exploited species (black grouper, mutton snapper, yellowtail snapper) and six non-target species (blue tang, spotted goatfish, purple reeffish, bicolor damselfish, striped parrotfish, and stoplight parrotfish) were detected. No decreases in domain-wide abundance were detected for any of the species analyzed.

The observed contrasts where abundance changes occurred between exploited and non-target species suggests that spatial protection may have been an important contributing factor in region-wide changes. Abundance increases were detected for non-target species in all three management zones, but only one species, *Chaetodon ocellatus*, decreased, and that occurred in the Bank NTMR. For exploited species, significant abundance increases were confined to the Bank NTMR and DRTO, while the only significant abundance decrease occurred in the Bank Fished area. Moreover, significant shifts in length compositions towards larger animals for black grouper and red grouper were found. In addition, in the Bank Fished area, black grouper size frequency distributions showed continued truncation of fish larger than the legal minimum size limit consistent with continued fishing pressure. Similar responses to spatial protection have been observed in the region for heavily exploited spiny lobster and mutton snapper (Davis and Dodrill, 1980; Cox and Hunt, 2005; Burton *et al.*, 2005).

However, our results also suggest that the population increases observed in the Bank NTMR and Park could have been augmented by co-occurring regional fishery management actions or favorable environmental conditions. Increases in abundance of larger animals would also be expected in response

to traditional management measures such as bag limits and size limits. For example, minimum size limits for black grouper have been increased from 18" (45.7 cm) established in 1985 to 20" (50.8 cm) in 1990, and 22" (55.9 cm) for recreational fishers and 24" (61.0 cm) for commercial fishers in 1999. The last regulation brought the minimum size up to the minimum size of sexual maturity (Ault *et al.* 2005b). Generally, abundance changes in non-target species would not be expected to be in direct response to fishery management policy. Increases in non-target species abundance suggests that the environment plays an important role and may have contributed to good recruitment events in recent years. Random variability in year class strengths or the passing of several hurricanes in the late-1990s may also have influenced recruitment for both exploited and non-target reef fishes. In reality, it is likely that many of the factors interact.

Similar observations of fish population recovery, have been made in other coral reef ecosystems but usually over longer time frames (c.f., Halpern and Warner 2002; Russ *et al.* 2004; Alcala *et al.* 2005). According to populations dynamics theory, not enough time has elapsed since implementation of the Tortugas NTMR to fully explain the findings. It is thus highly likely that not all the observed changes were a direct response to NTMR implementation. Furthermore, potential impacts on reef fish community dynamics are complex and may be influenced by shifts in composition, trophic cascades promulgated by predator-prey responses and habitat competition. The next research challenge will be to develop and refine methods for improved understanding of the relative contributions of NTMRs, various fishery management actions, community interactions, and environmental factors in terms of achieving the goal of building sustainable fisheries.

As this rebuilding process and reef ecosystem responds to management actions over the next several decades, a continued concern will be balancing fishing with resource protection. A particular concern is the likely continued growth in demand from the recreational fleet and its fishing power due to technological improvements. Although failure to adequately control fishing mortality can have potentially detrimental consequences for the stocks and the economy (Steele and Hoagland 2003), removal of units of fishing effort once they have been established will be difficult, i.e., the "ratchet" effect (Ludwig *et al.*, 1993). In the long run, a precautionary ecosystem-based approach to management using multiple control methods offers promise for providing fishery sustainability and persistence of the Florida Keys coral reef ecosystem. As noted by Stefansson and Rosenberg (2005), combining catch controls with large closed areas may be the most effective system of reducing risk of stock collapse while maintaining short and long-term economic performance and buffering uncertainty.

# C-6 References

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C-7 Computational Formulae
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Symbol	Definition	Computational Formula
Domain Var	riables	
h	Stratum subscript ( $h = 1,, \lambda$ )	
i	Primary stage sample unit subscript ( $i = 1,, N_h$ )	
j	Second-stage sample unit subscript ( $j = 1,, M_{hi}$ )	
A	Area of entire survey domain	$A = \sum_{h=1}^{\lambda} A_h$
$A_h$	Area of stratum <i>h</i>	$A = \sum_{h=1}^{\lambda} A_h$ $A_h = \sum_{i=1}^{N_h} a_{hi}$
$a_{hi}$	Area of <i>i</i> th primary stage unit in stratum <i>h</i>	$a_{hi} = \sum_{j=1}^{M_{hi}} a_{hij}$
$a_{_{hij}}$	Area of <i>j</i> th second-stage unit in primary unit $i$ in stratum $h$	
$N_h$	Total number of primary units <i>i</i> in stratum <i>h</i>	$N_h = \frac{A_h}{a_{hi}}$
${M}_{\scriptscriptstyle hi}$	Total number of second-stage units $j$ in primary unit $i$ in stratum $h$	$M_{hi} = \frac{a_{hi}}{a_{hij}}$
$N_h M_h$	Total number of possible second-stage units $j$ in stratum $h$	
$n_h$	Number of sampled primary units <i>i</i> in stratum <i>h</i>	
$m_{_{hi}}$	Number of sampled second-stage units $j$ in primary unit $i$ in stratum $h$	
$n_h m_h$	Total number of second-stage units $j$ sampled in stratum $h$	
$W_h$	Stratum <i>h</i> weighting factor	$w_h = \frac{N_h M_h}{\sum_h N_h M_h}$

## **Computational Formulae** (cont.)

Symbol	Definition	Computational Formula
Animal Den	sity	
$D_{hij}$	Density (individuals per m <sup>2</sup> ) at sampled second-stage unit <i>j</i> in primary unit <i>i</i> in stratum <i>h</i>	
$\overline{D}_{hi}$	Mean density in primary unit <i>i</i> in stratum <i>h</i>	$\overline{D}_{hi} = \frac{1}{m_{hi}} \sum_{j} D_{hij}$
$\overline{\overline{D}}_h$	Mean density in stratum <i>h</i>	$\overline{\overline{D}}_{h} = \frac{1}{n_{h}} \sum_{i} \overline{D}_{hi}$
$\overline{\overline{D}}_{st}$	Domain-wide mean density for a stratified random survey	$\overline{\overline{D}}_{st} = \sum_{h} w_h \overline{\overline{D}}_h$
$s_{1h}^{2}$	Sample variance among primary units $i$ in stratum $h$	$s_{1h}^{2} = \frac{\sum_{i}^{n} \left( \overline{D}_{hi} - \overline{\overline{D}}_{h} \right)}{n_{h} - 1}$
$S_{2h}^{2}$	Sample variance among second-stage units $j$ in stratum $h$	$s_{2h}^{2} = \frac{1}{n_{h}} \sum_{i} \left[ \frac{\sum_{i} \left( D_{hij} - \overline{D}_{hi} \right)^{2}}{m_{hi} - 1} \right]$
$\operatorname{var}\overline{\overline{D}}_h$	Variance of mean density in stratum <i>h</i>	$\operatorname{var}\left[\overline{\overline{D}}_{h}\right] = \frac{\left(1 - \frac{n_{h}}{N_{h}}\right)}{n_{h}} s_{1h}^{2} + \frac{\frac{n_{h}}{N_{h}} \left(1 - \frac{m_{h}}{M_{h}}\right)}{n_{h} m_{h}} s_{2h}^{2}$
$\operatorname{var}\overline{\overline{D}}_{st}$	Variance of domain-wide mean density	$\operatorname{var}\overline{\overline{D}}_{st} = \sum_{h} w_{h}^{2} \operatorname{var}\overline{\overline{D}}_{h}$
$SE\overline{\overline{D}}_{st}$	Standard error of domain-wide mean density	$SE\left[\overline{\overline{D}}_{st}\right] = \sqrt{\operatorname{var}\left[\overline{\overline{D}}_{st}\right]}$

<b>Computational Formulae</b>	(cont.)
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Symbol	Definition	Computational Formula
Frequency o	of Occurrence	
$p_{hij}$	Proportion occurrence for second-stage unit $j$ within primary unit $i$ in stratum $h$	
$\overline{p}_{hi}$	Mean proportion in primary unit <i>i</i> in stratum <i>h</i>	$\overline{p}_{hi} = \frac{1}{m_{hi}} \sum_{j} p_{hij}$
$\overline{\overline{p}}_h$	Mean proportion in stratum <i>h</i>	$\overline{\overline{p}}_{h} = \frac{1}{n_{h}} \sum_{i} \overline{p}_{hi}$
$\overline{\overline{P}}_{st}$	Domain-wide mean proportion for a stratified random survey	$\overline{\overline{p}}_{st} = \sum_{h} w_{h} \overline{\overline{p}}_{h}$
$S_{1h}^{2}$	Sample variance among primary units $i$ in stratum $h$	$s_{1h}^{2} = \frac{\sum_{l} \left(\overline{p}_{hi} - \overline{p}_{h}\right)^{2}}{n_{h} - 1}$
$S_{2h}^{2}$	Sample variance among second-stage units $j$ in stratum $h$	$s_{2h}^{2} = \frac{1}{n_{h}} \left[ \sum_{i} \left( \frac{m_{hi}}{m_{hi} - 1} \right) (\overline{p}_{hi}) (1 - \overline{p}_{hi}) \right]$
$\operatorname{var}\left[\overline{\overline{p}}_{h}\right]$	Variance of mean proportion in stratum <i>h</i>	$\operatorname{var}\left[\overline{\overline{p}}_{h}\right] = \frac{\left(1 - \frac{n_{h}}{N_{h}}\right)}{n_{h}} s_{1h}^{2} + \frac{\frac{n_{h}}{N_{h}}\left(1 - \frac{m_{h}}{M_{h}}\right)}{n_{h}m_{h}} s_{2h}^{2}}$ $\operatorname{var}\left[\overline{\overline{p}}_{st}\right] = \sum_{h} w_{h}^{2} \operatorname{var}\left[\overline{\overline{p}}_{h}\right]$
$\operatorname{var}\left[\overline{\overline{p}}_{st}\right]$	Variance of domain-wide mean proportion	$\operatorname{var}\left[\overline{\overline{p}}_{st}\right] = \sum_{h} w_{h}^{2} \operatorname{var}\left[\overline{\overline{p}}_{h}\right]$

Definition	Computational Formula
bundance (number of animals) in stratum h	$\hat{Y}_{h} = \left(\overline{D}_{h}\right) N_{h} M_{h} \left(T_{h}\right)$
main-wide abundance for a stratified random survey	$\hat{Y}_{st} = \sum_{h} \hat{Y}_{h}$
riance of abundance in stratum <i>h</i>	$\operatorname{var} \hat{Y}_h = \operatorname{var} \overline{\overline{D}} (N_h M_h)^2 (T_h)^2$
riance of domain-wide abundance	$\operatorname{var}\left[\hat{Y}_{st}\right] = \sum_{h} \operatorname{var}\left[\hat{Y}_{h}\right]$
1	undance (number of animals) in stratum <i>h</i> main-wide abundance for a stratified random survey riance of abundance in stratum <i>h</i>

### **Computational Formulae** (cont.)