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Bioassessment Tools for Stony Corals: *Statistical Evaluation of Candidate Metrics in the Florida Keys*

Final Report

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ABSTRACT

Measurements of coral reef condition were collected from stations in the Florida Keys National Marine Sanctuary and Dry Tortugas National Park during 2003-04. Four assessment endpoints of reef condition were derived from transect censuses and measurements of stony corals: total surface area of all corals in the reef transect (TSA), percent live coral averaged across all colonies in the reef transect (%LC), the sum of live surface areas for all colonies in a reef transect (LSA), and percent live surface area within the reef transect (%LSA). TSA and LSA were highly correlated; both measures were dominated by a few very large species of coral. Repeat samples were collected within stations, within reefs at different stations, and at the same stations during different years. For all measures, much of the variance was associated with different stations within reefs. Some stations near Key West had much lower values for %LSA. Some of the lowest values for %LC were associated with back reef habitats. For all measures, back reef habitat was most variable. Principal components analysis identified unique species assemblages associated with back and transitional reef habitats.

Variance estimates were derived from repeat samples to determine the minimum detectable differences (MDD) in mean values of coral metrics that would represent a statistically significant change in reef condition. MDD values were derived for a sampling design with repeat visits to the same stations and a second design with repeat visits to different stations. For TSA and LSA, 20 or more of the same stations should be sampled each year to detect a reasonable level of change. For %LC and %LSA, the same or different stations could be sampled each year to provide the same level of sensitivity to change and 10–20 stations are probably adequate. Strong agreement in coral metrics for opposite halves of the belt transect suggested that a smaller area could be sampled at each station.

ACKNOWLEDGMENTS

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INTRODUCTION

Protection and management of natural resources depend foremost on the development of a reliable and meaningful method to assess resource condition (Olsen et al. 1999). The primary purpose for monitoring natural resources is to determine whether their condition has improved, declined, or remains unchanged. Ward et al. (1986) provide a general framework describing the steps needed to design an effective monitoring program (Table 1). The five key components of a mature program are: 1) clearly stated program goals, 2) statistical design criteria, 3) a statistical sampling plan, 4) detailed written protocols for data collection and analysis, and 5) an information reporting procedure.

The current analysis focuses primarily on their Step 2 by evaluating the sensitivity and potential of four candidate coral metrics to detect change in reef condition. The four coral metrics described in this study are considered “candidate” metrics because they have yet to be tested for a response to human disturbance. “Assessment endpoints,” or “attributes,” or “measurements” of the coral assemblage are typically not labeled as metrics in the accepted terminology of biocriteria until they demonstrate a correlation with disturbance (Jameson et al., 2001). High variability associated with candidate coral metrics translates into a lower probability of detecting changes through time because observed differences must exceed the range of natural variability typically observed. Coral metrics that are less variable are more precise, and relatively smaller changes can be detected for these measures. Evaluation of the variance structure for different coral metrics provides a foundation for selecting among different sampling scenarios that differ according to the number of stations visited each year, the frequency of sampling, or the type of data collected.

Statistical design criteria include definition of the sampling unit, delineation of the sample population, and selection of sampling protocols and measures. Because reefs are a continuous resource, no obvious method exists to objectively divide the resource into discrete sampling units. For population censuses, for example, the household or individual taxpayer is used as the sampling unit. For coral, the size of the sampling unit must be defined somewhat arbitrarily but in a way that reliably characterizes the sampling location. The sample population was defined as stations within reefs; however, different strata could be defined as subpopulations within the larger sampling frame, e.g., fore, back or transitional habitats. The sampling protocols measured the total surface area of coral in a reef transect, the percent live coral on each colony, the live surface area in the reef transect, and the percentage of live surface area (see Fisher et al. 2006 for details).

The goal of this analysis was to evaluate the variability associated with the sampling protocols and candidate coral metrics and apply the results to two sampling design options for assessing coral reef condition through time. The two sampling designs were based on repeat visits to the same stations each year and visits to new stations each year. The two sampling designs require different statistical tests to determine whether change has occurred through time. More complex survey designs than these exist, but the two statistical models presented here provide a starting point for judging the relative merits of different sampling scenarios for the selected coral metrics (Skalski 1990, Larsen et al. 2001).

Table 1. Steps in the design of an effective natural resource monitoring program. Relevant examples for coral reef monitoring are show for each step along with specific items within each step (modified from Ward et al., 1986).

Step and description
<u>Step 1: Evaluate information expectations</u>
Identify resource condition targets (healthy living coral)
Define management goals (e.g., identify stressors)
Explicitly define monitoring goals (i.e., as statistical hypotheses)
<u>Step 2: Define statistical design criteria</u>
Define sampling unit (e.g., radial belt transect on reef)
Define the ‘population’ to be sampled (e.g., stations within the Florida Keys)
Evaluate variability of sampling protocols and measures (e.g., %LC, LSA, %LSA.)
Select appropriate statistical test (e.g., two-sample *t* test)
<u>Step 3: Design monitoring plan</u>
Identify what to measure (e.g., stony corals)
Identify where to sample (e.g., randomly selected stations)
Define how frequently to sample (e.g., every year, every five years)
<u>Step 4: Develop operating plans and procedures</u>
Field sampling and analysis procedures
Data analysis procedures
Quality control
Data management
<u>Step 5: Develop information reporting procedures</u>
Identify target audience
Define report structure, format and content
Determine frequency and distribution of reports

METHODS

Data collection

Coral reef sampling stations were located in the Florida Keys National Marine Sanctuary and Dry Tortugas National Park (Figure 1). During 2003, data were collected from 14 station visits to five reefs (Table 2). During 2004, 35 station visits were made to 10 reefs. Some sampling stations were also visited in the Dry Tortugas during 2004, but those data were unavailable for this analysis. Back, fore and transitional reef habitat types were visited on most reefs during 2004. During both years, some stations were sampled more than once (duplicate sampling). During 2003, some belt transects were divided into two parts (sections) and data from each were kept separate.

At each station, corals were surveyed using radial belt transects which were delineated by the area between two circles with radii of 8 m and 10 m measured from permanent stakes (for additional details on sampling or coral measures see Fisher et al. 2006). The radial belt encompassed 113.1 m^2. Within the radial belt transect, each stony coral colony was identified to species, volume of the colony was estimated, and the percent live coral noted. From these data, four candidate coral metrics were calculated for each station: total reef surface area (TSA) measured in m^2, average percentage live coral for all colonies (%LC), live surface area (LSA) measured in m^2, and the percentage of the total reef surface area covered in live coral (%LSA; Appendices I, II and III).

Total surface area in the reef transect (TSA) was derived from estimates of coral volume. Size classes were defined as cubes, and the size class assigned to a colony was based on the smallest size cube that could contain the colony. Colonies are not cube-shaped, so this method represents an approximation. Estimates of surface area were based on the surface area of the five sides of the cube (excluding the sixth side that faced the substrate). In 2003, corals were graded into five volumetric size classes (1, 10, 50, 100 or > 100 L), but this did not sufficiently separate larger corals and differences between 50 L and 100 L size classes were difficult to discern. In the second year, six size classes were used: 1, 10, 100, 500, 2500 and > 2500 L.

Percent live coral (%LC) was graded into seven categories in 2003 (0, 0-20, 20-40, 40-60, 60-80, 80-100, and 100%); the values used in calculations were the midpoint of the ranges. In 2004, %LC was graded into six categories (0, 1-25, 26-50, 51-75, 76-99, and 100%). For each station-visit, the colony values of %LC were averaged to provide a station value.

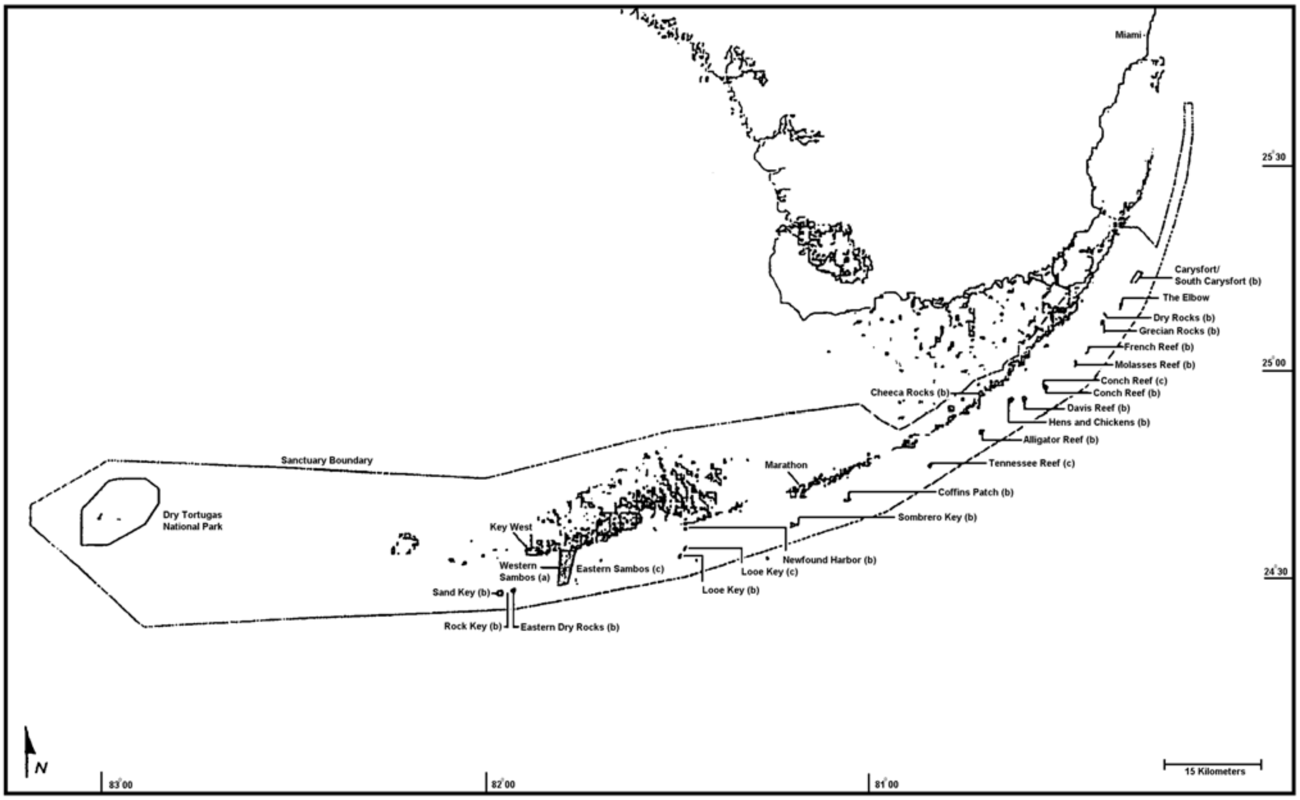

Figure 1. Reef locations in the Florida Keys National Marine Sanctuary. Source: NOAA Technical Memorandum NMFS-SEFSC-427.

Table 2. Number of stations surveyed in each habitat type.
Zone name, reef code, reef name, and the number of samples collected in back, fore and transitional reef habitat for 2004 data (2003 samples noted in parentheses). Some repeat samples were duplicate samples of the same station and other replicates were from different stations within the same reef.

Zone	Reef	Reef name	Back	Fore	Trans	Unknown type
Dry Tortugas	BK	Bird/Bush Key				(4)
	LR	Loggerhead Reef				(5)
Key West	ED	Eastern Dry Rocks	1	1 (1)	1	
	RK	Rock Key	1	1		
	SK	Sand Key	1	2 (2)	2 (1)	
	WS	Western Sambo	1	4 (1)	1	
Lower Keys	ES	Eastern Sambo	1	1	3	
	LK	Looe Key	1	1	1	
Middle Keys	AR	Alligator Reef		1	1	
	SR	Sombrero Reef		1	1	
Upper Keys	CR	Carysfort Reef	1	3	1	
	MR	Molasses Reef		1	1	

The live surface area (LSA) for an individual colony was calculated as the product of total surface area (TSA) and percent living tissue (%LC) divided by 100 for each individual colony. LSA for each station-visit equaled the sum of the colony values and measured in m^2. The percent living surface area (%LSA) was calculated as LSA divided by TSA multiplied by 100%.

To summarize, TSA was the total surface area of coral in the reef transect. %LC was the average percent live coral for all colonies in the reef transect such that small and large colonies contributed equally to the station average. LSA was the sum of the live surface area of all the individual colonies found within the reef transect. % LSA was the percentage live surface area in the radial belt. For the last two metrics, large colonies contributed more to the final percentage than did smaller colonies.

Reconciliation of sampling protocols

Year 2003 was a pilot year for data collection and methods were better defined in 2004. A subset of coral species were measured in 2003 when the methods were being tested. In 2004, all the stony corals were measured with the exception of two species. *Millepora alcicornis* was excluded because it is predominantly an encrusting rather than reef-forming species (Table 3). *Siderastrea radians* was excluded because colonies were small and occurred in clusters, which made it difficult to discern individual colonies. In addition, *S. radians* occur flush to the sea floor and provide no structural relief for habitat. In addition, from 2003 to 2004, size classes and coverage classes changed. Nonetheless, because estimates of section differences and annual differences could only be obtained using the 2003 data, an effort was made to make the data sets as similar as possible. A section was defined as half the belt transect. Variability was estimated for neighboring sections using the 2003 data because data from the two halves of the transect were only identified separately during that year. Replicate samples collected at different stations during 2003 and 2004 were used to evaluate differences associated with measurement error. More stations were visited during 2004 and sufficient data were collected to evaluate the influence of reef habitat type and reef location on candidate coral metrics. Repeat visits to five stations in 2003 and 2004 were using to estimate annual variance.

Table 3. Coral species code, genera and species name.
Species code, genus, species and whether the taxon was excluded from sampling in 2003 or 2004.

Species Code	Genus	Species	Excluded?
ACER	*Acropora*	*cervicornis*	
APAL	*Acropora*	*palmata*	
AGAR	*Agaricia*	*agaricites*	
AFRA	*Agaricia*	*fragilis*	2003
ALAM	*Agaricia*	*larmarckii*	2003
CNAT	*Colpophyllia*	*natans*	
DSTO	*Dichocoenia*	*stokesii*	
DCLI	*Diploria*	*clivosa*	
DLAB	*Diploria*	*labyrinthyformis*	
DSTR	*Diploria*	*strigosa*	
MDEC	*Madracis*	*decactis*	2003
MFOR	*Madracis*	*formosa*	2003
MMIR	*Madracis*	*mirabilis*	2003
MAD	*Madracis*		2003
MMEA	*Meandrina*	*meandrina*	
MALC	*Millepora*	*alcicornis*	2004
MCOM	*Millepora*	*complanata*	
MANN	*Montastrea*	*annularis*	
MCAV	*Montastrea*	*cavernosa*	
MFAV	*Montastrea*	*faveolata*	
MFRA	*Montastrea*	*franksii*	
MALI	*Mycetophellia*	*aliciae*	
MDAN	*Mycetophellia*	*danaana*	
MFER	*Mycetophellia*	*ferox*	
MLAM	*Mycetophellia*	*larmarckiana*	
MYCET	*Mycetophellia*		
PAST	*Porites*	*astreoides*	
PPOR	*Porites*	*porites*	
SRAD	*Siderastrea*	*radians*	2004
SSID	*Siderastrea*	*siderea*	
SIDsp	*Siderastrea*		
SBOU	*Solenastrea*	*bournoni*	
SMIC	*Stephanocoenia*	*michelini*	

For all analyses of 2003 data, changes were made to match the 2004 protocols where possible. Two species excluded in 2004 were excluded from the 2003 data (*Millepora alcicornis* and *Siderastrea radians*). Size classes also changed from 2003 to 2004. In 2003, five size classes were used: 1, 10, 50, 100, and >100 L. In 2004, the 50L size was not used and three new large size classes were added which yielded size classes of 1, 10, 100, 500, 2500, and >2500 L. For the size class >100 L a value of 200 L was used; for the size class >2500 L a value of 5000 L. To make the 2003 data more similar to the 2004 data, the 50 L size class was combined with the 100 L size class. From 2003 to 2004, %LC cover categories were reduced from seven to six categories. For the 2003 data, %LC categories were modified to match the method used in 2004 (0, 1-25, 26-50, 51-75, 76-99, and 100%).

For most of the analyses involving the 2004 data, no changes were made to the data or calculations of the coral metrics. An exception was the comparison of the 2003 and 2004 data to evaluate changes through time. To make the two data sets as similar as possible, coral taxa excluded from the sampling in 2003 were also eliminated from 2004 data for the annual comparison only. In addition, the three largest size classes were collapsed to the largest size class recorded in 2003 (>100 L) to make the candidate coral metrics more comparable for the annual comparison only.

Data analysis
A variety of replicate and duplicate samples were available to answer questions about patterns of variance for the candidate coral metrics. Unfortunately, individual estimates of variance were derived from different sets of stations because a limited number of repeat samples were available. For the purposes of this analysis, “replicate” samples refer to repeat samples taken within a reef while “duplicate” samples are repeat censuses of the same station within a reef.

Sampling designs also differed from 2003 to 2004. Half belt transects (sections) were sampled in 2003 only and these data were used to estimate variance associated with neighboring sections from the opposite side of the belt transect. Duplicate samples were collected in both years and these data were used to estimate variance associated with same-day revisits. Data from 2004 were used to evaluate geographic differences in coral metrics associated with location along the archipelago. Data from 2004 were used to compare coral metrics for different reef habitat types (back, fore, and transitional). One station was eliminated from the analysis of variance for 2004; station WS04C had an artificially high value for %LC and %LSA because dead *A. palmata* and *M. complanata* were accidentally excluded from the survey.

Data were used to address three questions:

- *What is the optimal size for a coral sampling unit?*
- *What are the sources of variance for the candidate coral metrics and what are their relative contributions to the total variance?*
- *How much change in coral condition could we detect through time for a given sampling effort?*

Optimal sample size. To answer the first question, data from 2003 were used to compare %LC, LSA and %LSA values from opposite sides of the radial belt. All 10 stations from 2003 had coral survey data kept separate for each side of the belt (section) or two replicate samples for each station. In some cases, the same section was surveyed more than once by different divers. In these cases, %LC, LSA and %LSA were calculated for each diver and averaged for the section.

Sources of variance. Sources of variance for %LC, LSA, and %LSA that could be evaluated from the available data included differences within reefs (stations), differences associated with habitat type (fore, back, or transitional), microhabitat differences (neighboring sections), and measurement error (duplicate samples). Back reef was defined as the area between the reef crest and the shore, excluding sandy lagoons. Fore reef included the area from the reef crest that begins to slope down into deeper water toward the bank or shelf. Transitional reef was the area sloping away from the fore reef into deeper water; transitional reef is also referred to as the deep fore-reef.

An ANOVA model was used to estimate variance associated with opposite sections of the radial transect where the 10 stations represented a single factor and the two replicate sections at each station were used to estimate variance. A second ANOVA model was used to estimate variability associated with 2003 duplicate samples using two stations with three duplicate samples each. For 2004, a third ANOVA model was used to estimate variability associated with 29 stations and three duplicates at three of those stations for 2004. One duplicate sample for 2004 was eliminated (WS04C) because dead *A. palmata* and *M. complanata* were inadvertently left out of the survey. A fourth ANOVA model was used to estimate variance associate with annual duplicate sampling at five stations sampled in 2003 and 2004. Each of these four ANOVA models was applied separately to the four candidate coral metrics. Because the estimates of variance were derived from small sample sizes as well as different study areas sampled at different times, the estimates should only be considered approximate at best.

Because replicate samples within reefs were associated with different reef habitat types, that source of variability could not be quantified for these data. Graphical analysis also revealed that although coral metrics differed by reef habitat types, the differences were not consistent across reefs. Additional analysis of habitat type was pursued using an exploratory multivariate technique, principal components analysis (PCA). The total surface area of each species at each station was used in PCA to evaluate patterns associated with reef habitat type.

Power analysis. Statistical power is defined as the probability of detecting a change when a change truly occurs (Peterman 1990). Statistical power is a function of the number of samples collected, the variance of the measure of interest, and the level of acceptable uncertainty (i.e., probability of a Type I or Type II error).

Variance estimates were used to calculate the minimum detectable difference (MDD) for various sampling scenarios for TSA %LC, LSA, and %LSA. For each candidate coral metric, the statistical power was calculated to detect a change based on visits to 10, 20, 30, and 50 reef stations each year. The statistical power of each of the four coral metrics was evaluated separately. Two statistical models were used for each measure: a two-sample *t* test based on two sets of stations sampled in different years and a paired *t* test based on repeat samples from the same stations in different years.

Two sample t test. “Two sample” refers to the fact that different reefs are visited on each occasion, and two (different) samples are collected. Two variance estimates were available to use for this model. During 2003, 10 stations were surveyed and during 2004, 29 stations were surveyed. Variance estimates were selcted from 2004 data because more stations were sampled and because the sampling protocol used was more robust.

MDD was calculated using the following equation (Zar, 1984):

$$\text{MDD} \geq \sqrt{\frac{2s^2}{n}}\left(t_{\alpha(1),\nu} + t_{\beta(1),\nu}\right),$$

Where s^2 = station variance for 2004,

n = the number of stations surveyed,

$t_{\alpha(1),\,\nu}$ = the t value for an α of 0.1 for a 1-sided test,

$t_{\beta(1),\,\nu}$ = the t value for β of 0.1 for a 1-sided test, and

$\nu = 2n - 2$.

α and β were set to 0.1 because, in the context of resource monitoring, we are just as concerned that we might miss a change (Type II error, β) as we are that we might incorrectly conclude a change has occurred (Type I error, α; Dayton 1998, Di Stefano 2001, Yoccoz et al. 2001). A 1-sided test was selected because we are primarily concerned with loss of coral through time.

Paired t test. A "paired" test refers to the fact that each station is paired with itself for the second sampling event and the same stations are visited during each visit. This model is based on a one-sample *t* test in which the one sample is composed of the *differences* between each station during the first and second visit. If the average differences are significantly greater (or less) than zero, a change in condition would be indicated. Variance was calculated for the *differences* between each coral metric observed at five stations during two different years.

The equation for MDD was very similar to the equation above:

$$\text{MDD} \geq \sqrt{\frac{s_d^2}{n}}\left(t_{\alpha(1),\nu} + t_{\beta(1),\nu}\right),$$

Where s_d^2 = the variance of the *differences* between stations for repeat visits,

n = the number of stations surveyed,

$t_{\alpha(1),\,\nu}$ = the t value for an α of 0.1 for a 1-sided test,

$t_{\beta(1),\,\nu}$ = the t value for β of 0.1 for a 1-sided test, and

$\nu = n - 1$.

RESULTS

During 2003, 23 coral taxa were identified to species and two were identified to genus (*Mycetophellia* and *Siderastrea*). A total of 28 coral species were identified and recorded in 2004. Species within the genus *Madracis* were sometimes identified only to genus.

For the 2004 data, LSA was highly correlated with TSA (r = 0.92; Figure 2). One exception to this relationship was SK01, which had several large colonies of *Montastrea cavernosa* and *M. faveolata*, and *Acropora palmata* with a high percentage of dead coral. In 2004, LSA ranged from 2.3–121.4 m^2 and TSA ranged from 5.3–206.1 m^2. When LSA was plotted against TSA for each coral species, the correlation was again high (r = 0.95; Figure 3) with the exception of one species, *A. palmata* which had a higher percentage of dead coral than other species. Because TSA and LSA were so highly correlated, subsequent analysis focused on LSA alone.

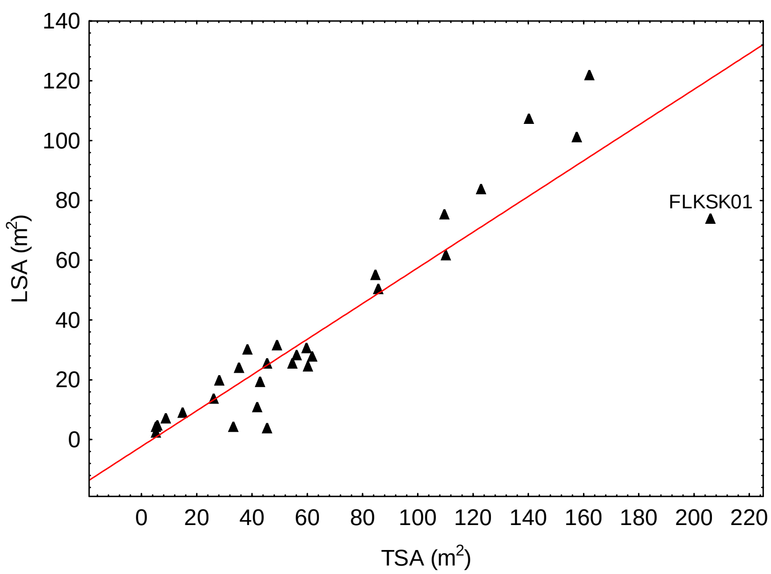

Figure 2. LSA was highly correlated with the TSA for 2004 stations measured using a 113 m^2 radial belt transect (N = 29; r^2 = 0.84; Pearson's r = 0.92, $p < 0.01$).

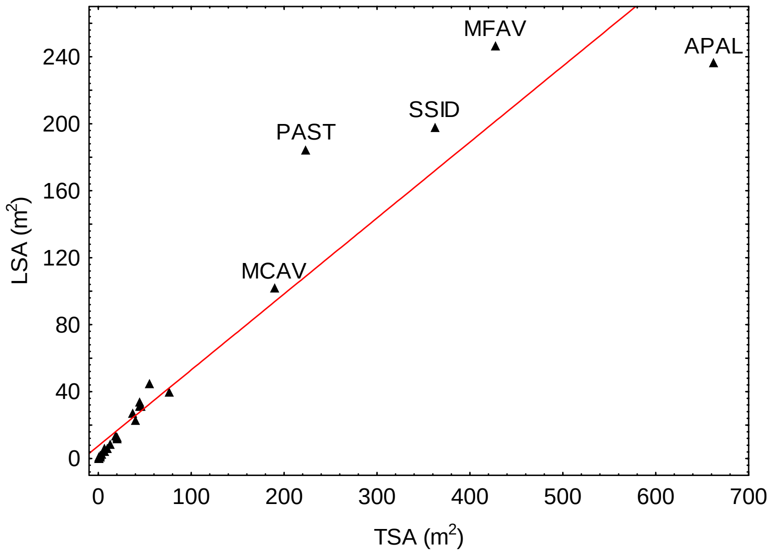

Figure 3. LSA was highly correlated with the TSA for species summed across all stations ($r^2 = 0.90$; Pearson's $r = 0.95$, $p < 0.1$, $N = 29$ taxa).

Influence of geographic gradient and reef habitat type

%LC, LSA, and %LSA showed different patterns across stations when stations were ordered along a geographic gradient from Key West to the ENE toward Carysfort Key (Figure 4). %LC did not show an association with the geographic gradient. Station values for %LC ranged from 62–94% with the exception of two stations, both of which were located in back reef habitat. RK03 and ES03 had much lower values of 28 and 26%. Duplicate values at station WS04 showed a much higher variability for %LC than did duplicates at ES01 or CR02 stations.

LSA also failed to show an association with geographic gradient (see Figure 4), nor were the outliers consistently associated with a particular type of reef habitat. In contrast, %LSA indicated that a higher percentage of the areal coverage of the reef tended to be alive for stations located further to the ENE. Some stations closer to Key West had a lower %LSA. In addition, some of the lowest values for %LSA were associated with back reef habitat. For both LSA and %LSA, the variance associated with duplicate samples was higher at station WS04 than at either ES01 or CR02 stations.

Reef habitat types differed in average depth with transitional reefs deepest, followed by fore reefs and back reefs which were the most shallow (Figure 5). Coral metrics also differed according to reef type (Figure 6). For some stations, %LC was much lower on back reefs. For several back reefs, %LSA was much lower; in addition, %LSA was slightly lower in fore reef habitat than in transitional habitat.

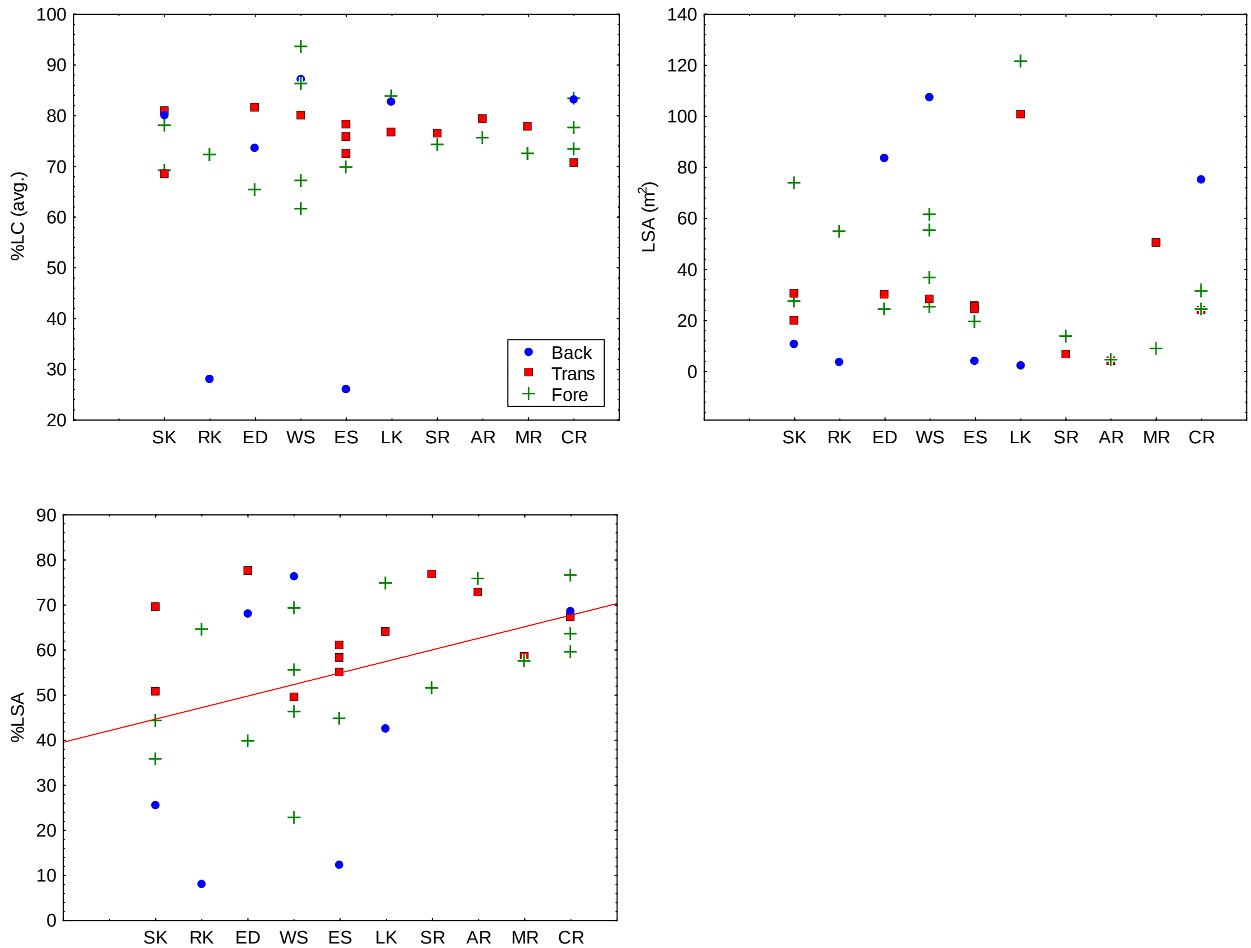

Figure 4. Shown for each reef are values for %LC, LSA, and %LSA observed in 2004. Reef habitat types are noted. Reefs are arrayed from left to right in order from Key West toward the ENE to Carysfort Key. Three duplicate samples were collected on WS (fore reef habitat), ES (transitional), and CR (fore), N = 35.

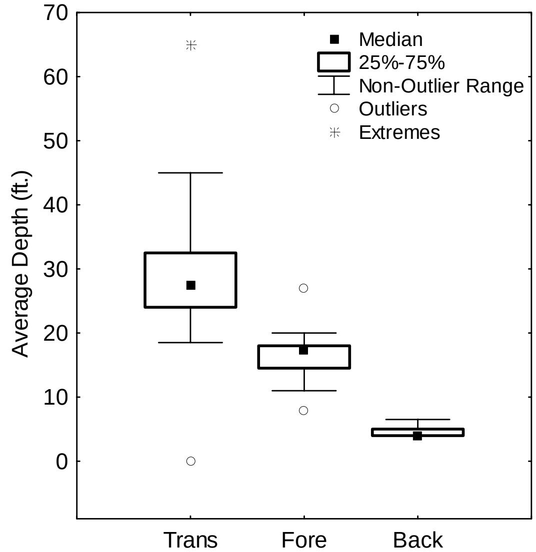

Figure 5. Transitional reef habitat tended to be deepest, followed by fore reef, and back reef was the most shallow (2004 data, N = 35).

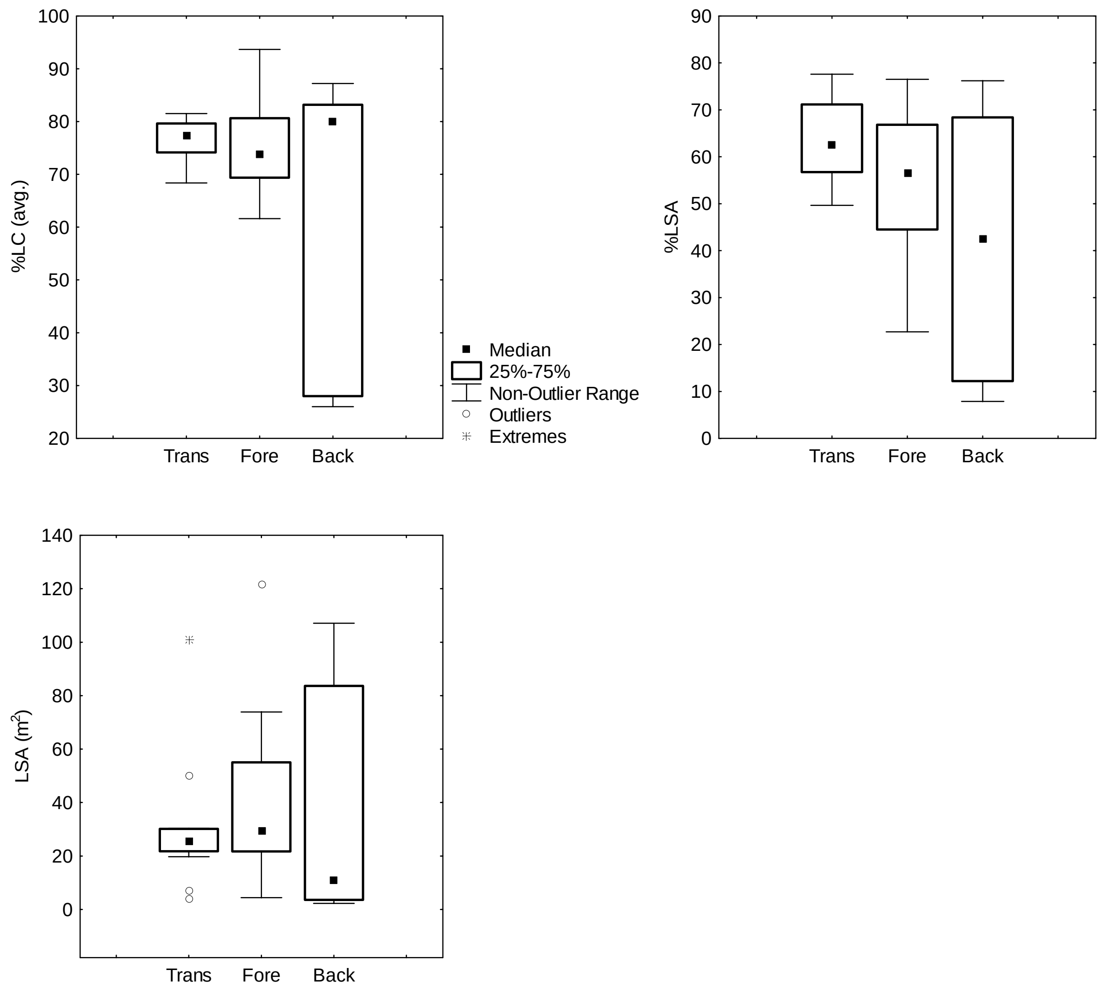

Figure 6. Average %LC values were similar in different habitat types. %LSA was higher on transitional reef habitats and %LSA was higher on fore reef than back reef habitat (upper panel). In contrast, LSA was lower in transitional habitat and highest in some back reef habitats (lower panel). Back reef habitats were more variable for all three coral metrics.

Species assemblages associated with different reef habitat types

Principal components analysis (PCA) based on the total surface area (TSA) of every species at each station identified the species assemblages associated with different reef habitat types. As a check that PCA could successfully identify similar coral assemblages, duplicate samples from the same station were compared. The three duplicates from three stations plotted very closely together when station visits were plotted for the first two factors from the PCA (Figure 7). For the same plot, when stations were identified by reef types, stations in back reef habitat plotted closely together (Figure 8). Some transitional habitat stations plotted close together. Fore reef habitat stations were also grouped, but some stations were also spread among other stations that were characterized as back or transitional habitats. Correlation between the factors and each coral species indicated that *Acropora palamata* was the primary species characterizing back reef habitat. The transitional habitat stations in the upper right quadrant of Figure 8 were characterized by *Diploria clivosa*, *D. strigosa*, *Dichocoenia stokesii*, *Madracis decactis*, *Meandrina meandrina*, and *Solenastrea bournoni*. Stations in fore habitat and some stations in transitional habitat were characterized by a mix of several species with *Montastrea faveolata* and *M. franksii* being the most highly negatively correlated with PCA factor 1.

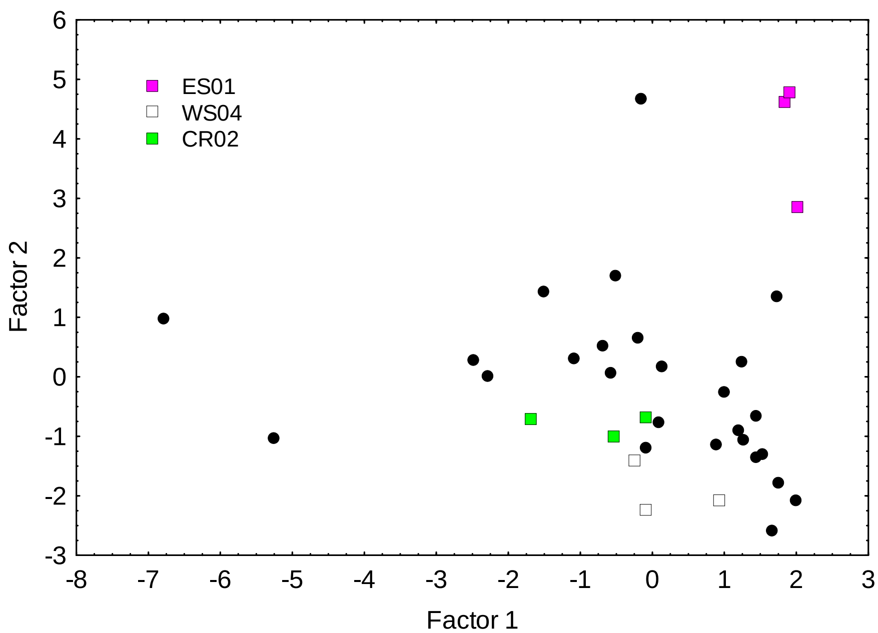

Figure 7. Factors 1 and 2 from principal components analysis for data collected in 2004. Each point represents a station visit. Three stations with duplicate samples were also included in the PCA and are indicated on the graph. Duplicates from the same station plotted close together.

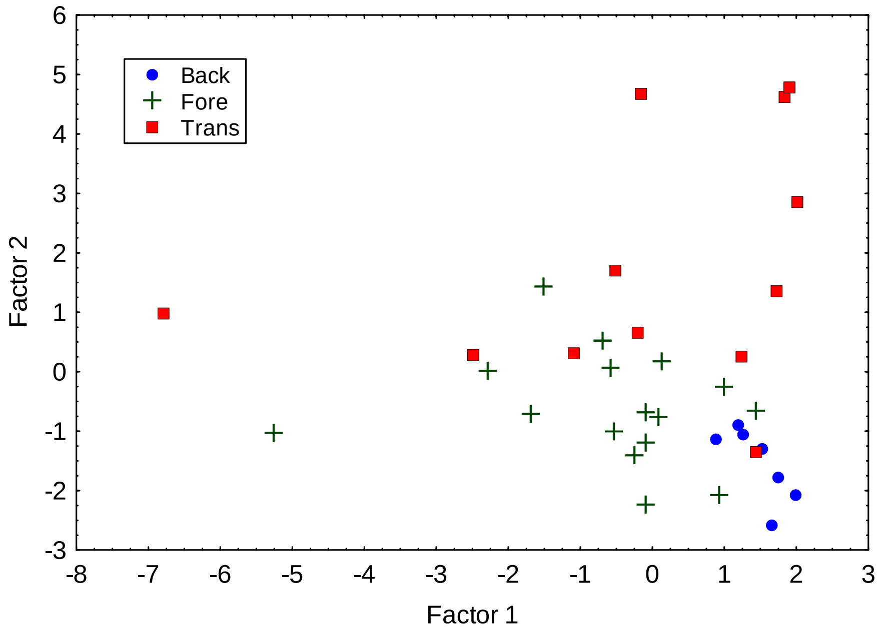

Figure 8. Factors 1 and 2 from PCA for data collected in 2004. Each point represents a station visit. Back, fore, and transitional reef habitat types are indicated.

Sources of variance

Patterns of variance differed according to the candidate coral metric being considered (Table 4). As for previous comparisons, TSA and LSA showed similar patterns and only LSA is reported here. For LSA, the greatest percentage of the total variability was associated with differences in reefs. Relatively smaller percentages of the total variance were due to year, duplicates and sections (Figure 9).

For %LC, the total overall variance was dominated by differences associated with duplicate samples and year differences. These results were somewhat difficult to interpret because duplicate variance estimates were derived from only three stations and two of the stations had nearly identical values for all three duplicates while one station (WS04) had very different values for duplicate samples. The latter sample greatly influenced the estimate of variance due to duplicate sampling. For %LSA, variance associated with duplicate sampling represented a large proportion of the total variance, again due primarily to the very different values observed at WS04.

Table 4. Variance estimates for TSA, %LC, LSA and %LSA.
Estimates of variance for different sources of variability and four coral candidate metrics. Variance estimates were derived from different data sets and ANOVA models. Shown are year of data collection, variance estimates for TSA, %LC, LSA and %LSA, number of stations from which the estimates were derived, number of replicate sections, and number of duplicate samples (repeat measure of the same station). WS04C was left out of the calculations for 2004 because dead *A. palmata* and *M. complanata* were left out of the survey.

Source	Year	TSA	%LC	LSA	%LSA	Stations	Reps	Dupes
Sections	2003	94.73	16.79	27.73	50.06	10	2 each	
Same day reps	2003	66.20	2.64	13.66	23.50	2		3 each
Same day reps	2004	315.99	83.63	67.94	142.11	3		2-3 each
Stations	2004	2361.28	98.23	932.99	198.28	29		
Annual	2003–04	250.82	89.36	38.96	19.72	5		2 each

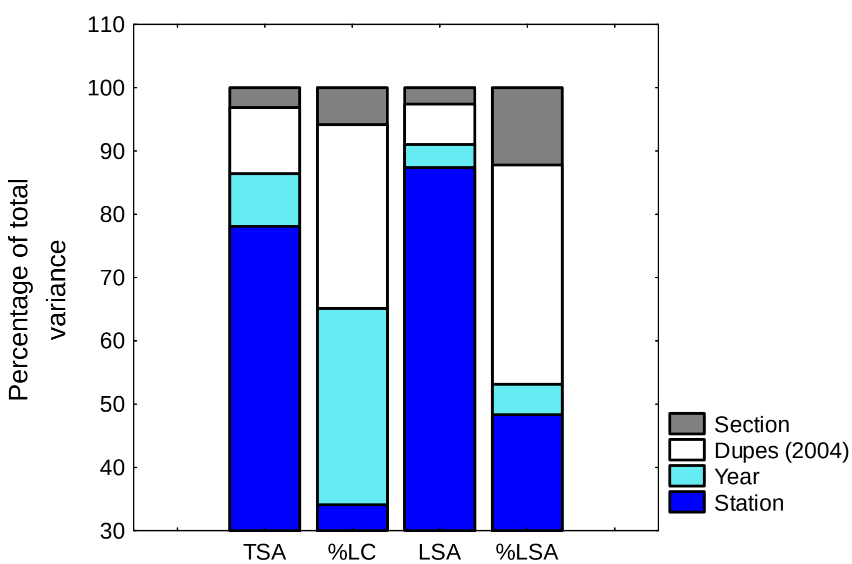

Figure 9. Relative contribution to the overall variance of candidate coral metrics due to section differences (2003 data), same-day duplicate sampling (2004 data), year differences (2003 and 2004), and station differences (2004 data).

No measurable variance could be associated with habitat type for any of the candidate coral metrics because the differences were not consistent across reefs. For example, for LSA at stations SK, RK ES, and LK much lower values were recorded in back reef than in other habitat types. In contrast, at ED, WS and CR, the highest values for LSA were found in back reef habitat.

Patterns observed for the variability analysis represent a statistical approach to what can be seen in Figure 4. Taking %LC as an example, reefs were fairly similar in values of %LC and that pattern is reflected in the very low percentage of the total variance associated with reef. In contrast, the values for duplicate samples on reef WS extended beyond the range of values for most other reefs and that pattern is reflected in the high percentage of the variance associated with duplicate sampling.

Annual differences could only be assessed from visits to five stations during 2003 and 2004 (Figure 10). %LC was higher at all five stations during 2003. Observed differences in %LC may have been due to differences in the sampling protocol. The inclusion of small colonies in 2003 may have results in higher values for %LC particularly if smaller coral colonies are younger and tend to be healthier. Agreement between stations for both LSA and %LSA was very good.

Power analysis

A key point to consider when interpreting the results of the power analysis, is that the ranges of values for TSA, %LC, LSA, and %LSA were much smaller for these five stations than for the larger set of 29 reefs sampled in 2004. For example, LSA in 2004 ranged from 2.3–121.4 m^2, but the highest value observed for the five repeat sites was 45.4 (see Figure 5; Table 5).

As expected, smaller differences in coral metrics would be statistically significant for a paired model than for the two-sample model (Table 6). This difference is expected because pairing a station with itself eliminates the variance associated with different stations. Differences in MDD for the two sampling designs were more extreme for TSA and LSA than for %LC or %LSA (Figure 11). Both TSA and LSA were too variable to detect a change for a design based on 10 stations using a two-sample test. The MDD subtracted from the mean of the observed values went below zero. For both %LC and %LSA, the MDD was small enough that the difference between the observed mean and the change in the mean that would indicate a significant change was also relatively small. For these candidate coral metrics, the power to detect change was high and several significant increments of change could be detected before the mean values declined to zero.

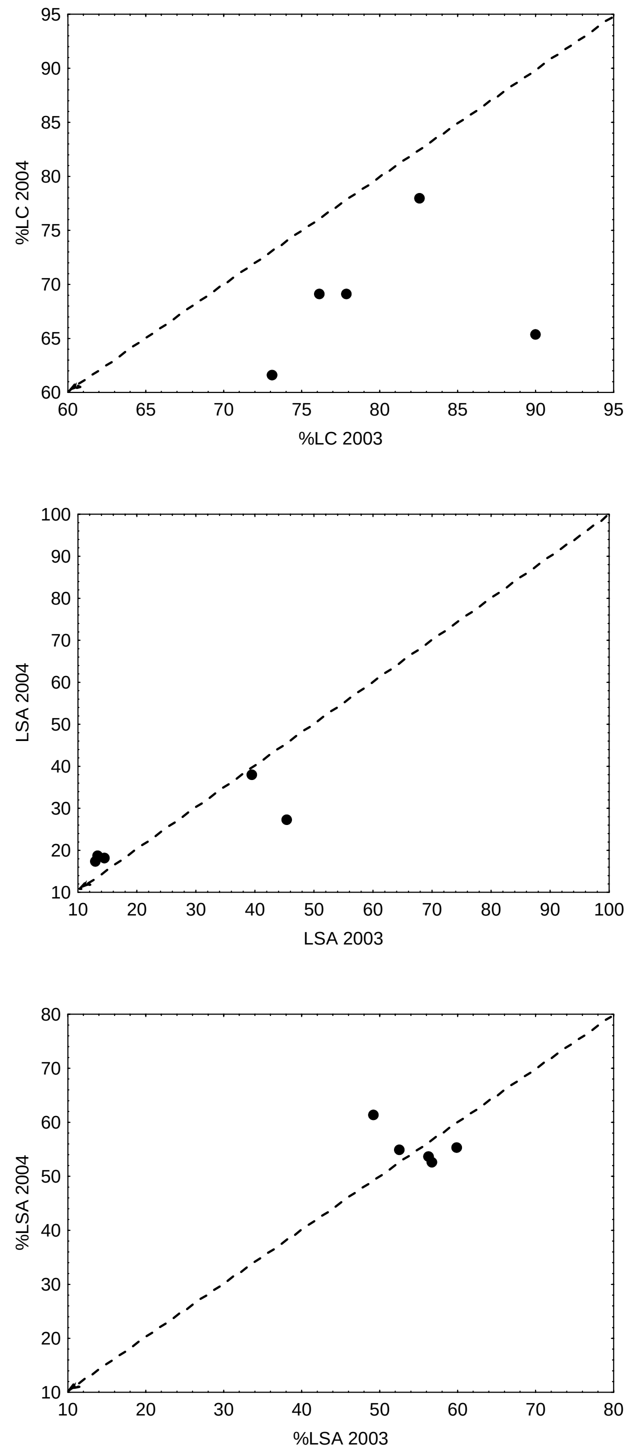

Figure 10. Comparison of %LC, LSA, and %LSA for five stations sampled in both 2003 and 2004. Dotted lines indicate the line of perfect agreement. The ranges of the axes match the range of values observed from the larger 2004 data set of 29 stations to provide perspective on differences observed in 2003 vs. 2004.

For TSA and LSA, the paired test was much more likely to detect a change through time. For %LC and %LSA, the paired test was also more sensitive, but the difference in statistical power was not that much greater than for the two-sample test. The primary advantage associated with a two-sample test is that twice as many sites can be visited during the same time period.

Table 5. %LC, LSA, and %LSA for both 2003 and 2004.
Values observed for %LC, LSA and %LSA during each year, the difference between years, and below, the mean and variance of the differences for each coral metric.

	%LC			LSA			%LSA		
Station	**2004**	**2003**	***d***	**2004**	**2003**	***d***	**2004**	**2003**	***d***
ED01	65.3	90.0	-24.7	18.0	14.6	3.3	52.6	56.8	-4.3
SK01	69.1	77.9	-8.8	37.8	39.5	-1.7	53.5	56.4	-2.9
SK02	77.9	82.6	-4.7	27.2	45.4	-18.2	61.3	49.3	12.0
SK03	69.1	76.1	-7.1	17.3	13.0	4.3	55.2	59.9	-4.7
WS03	61.6	73.1	-11.5	18.6	13.5	5.2	54.7	52.5	2.2
Average (*d*)			**-11.4**			**-1.4**			**0.5**
Variance (*d*)			**61.9**			**94.9**			**49.0**

Table 6. MDD for two-sample t test and paired t test.
MDD for four candidate coral metrics for 10, 20, 30 and 50 stations sampled during each time period. As an example, for %LC and 10 different stations sampled per visit, a decline of 11.8% would likely represent a statistically significant change; if the same 10 stations are sampled on both occasions, a decline of 6.9% would likely represent a significant change ($\alpha = \beta = 0.1$).

Number of stations	**TSA**		**%LC**		**LSA**		**%LSA**	
	2 sample	**Paired**	**2 sample**	**Paired**	**2 sample**	**Paired**	**2 sample**	**Paired**
Mean	*45.0*	*45.0*	*74.3*	*74.3*	*24.5*	*24.5*	*55.2*	*55.2*
10	57.8	21.5	11.8	6.9	36.3	8.5	16.8	6.1
20	40.1	14.6	8.2	4.7	25.2	5.8	11.6	4.2
30	32.5	11.8	6.6	3.8	20.4	4.7	9.4	3.4
50	25.1	9.0	5.1	2.9	15.8	3.6	7.3	2.6

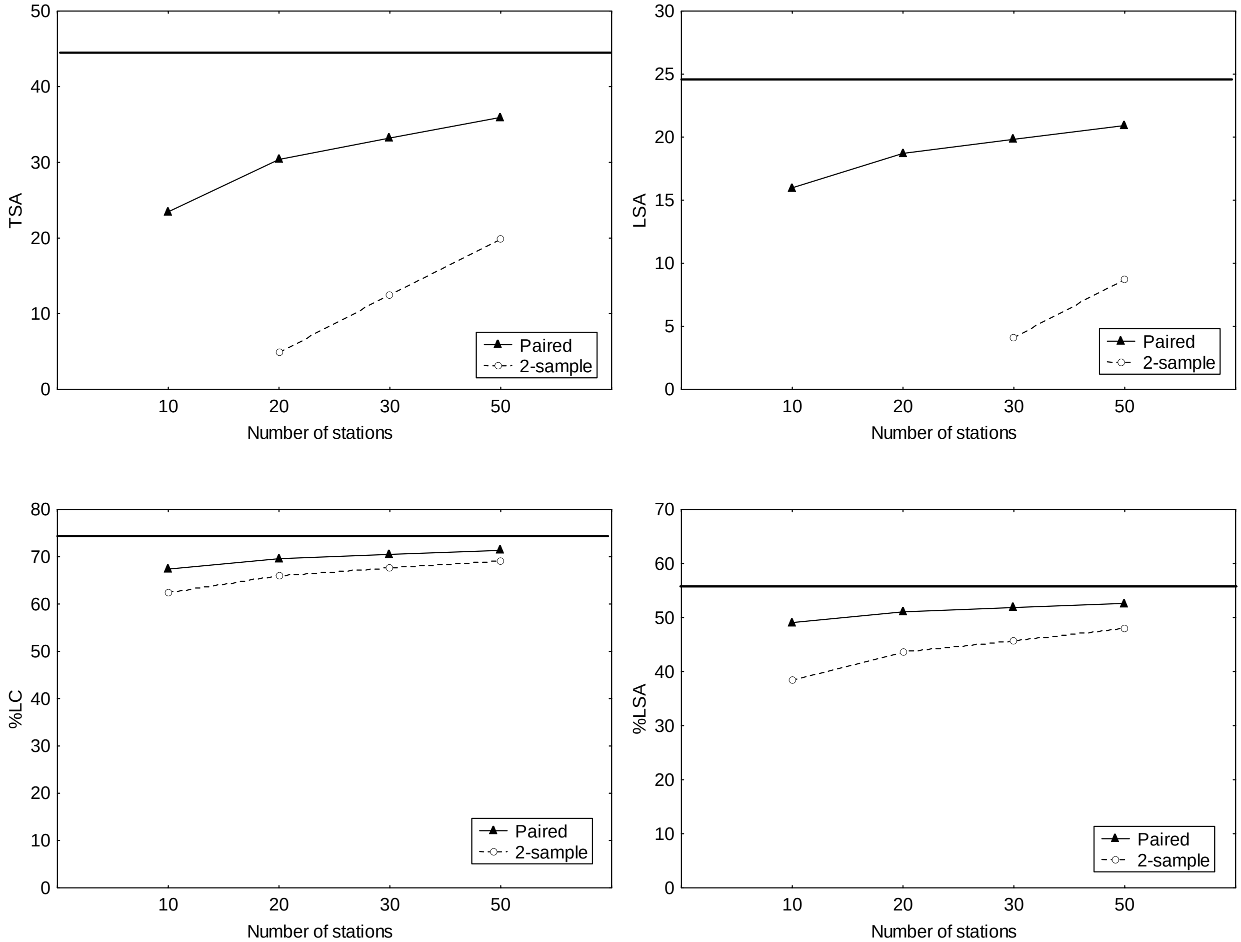

Figure 11. Power curves for candidate coral metrics. Shown in each panel are the mean value for five stations visited in both 2003 and 2004 (solid upper line), the mean value that would be statistically significant for 10, 20, 30 or 50 of the same stations sampled each year (paired test), and the mean value that would be significant for different stations sampled each year (2-sample test).

DISCUSSION

Stations sampled for this study represented a very large geographic area from Carysfort Key at the northeastern end of the Florida Keys, south and west past Key West to the Dry Tortugas. Although the survey design used to collect data for this study was not ideal for evaluating patterns of variance, the data were not collected with this specific purpose in mind. The primary purpose of the original project was to refine and test sample survey protocols to determine how coral should be censused and how best to summarize information for each colony and the reef transect. As part of that study, some replicate and duplicate samples were collected. From these pilot data, the relative contribution of the different sources of variance to the overall estimates of reef condition could be evaluated; however, the limited number of replicate samples and the fact that variance estimates for different sources were made from different stations should be kept well in mind. In other words, the results from this analysis are likely reliable for relative comparisons, but the exact values for variance may not be accurate. Because coral reef data are expensive to collect, many data sets must serve more than one purpose. Although more replicate samples would yield more robust conclusions, the data available for the Florida Keys can, nonetheless, provide valuable insight into the best approaches for coral reef monitoring and protection.

The four candidate coral metrics summarized different aspects of reef condition and also behaved quite differently in terms of their variance structure and their association with natural features. Coral species contributed unequally to the coral metrics due to large differences in size. Five species contributed disproportionately to LSA calculations and swamped the contributions of other smaller colonies. These species were *Acropora palmata*, *Montastrea cavernosa* and *M. faveolata*, *Siderastrea siderea*, and *Porites astreoides*. Similarly, %LC and %LSA summarized two very different aspects of reef condition. %LC treated all colonies equally, averaging across colonies irrespective of size. In contrast, %LSA measured live tissue on the reef surface as a function of area while ignoring the identity of individual coral species.

TSA and LSA tracked each other closely for all analysis and a reasonable conclusion seems to be that the living surface area depends heavily on the amount of coral surface area available. Patterns of variance for %LC and %LSA were also somewhat similar to each other. These results indicate that the most efficient sampling designs for these two sets of variables may be different.

TSA and LSA varied locally throughout the Florida Keys; in contrast, %LC was much less variable from reef to reef. %LSA showed marked differences from reef to reef, with the lowest values observed around Key West on fore and back reef habitat. If the observed differences are associated with human land use, %LSA may be a strong indicator of reef condition. Many of the highest observed values for TSA and LSA were associated with stations near Key West, though high values were also observed on reefs near the northeast end of the sampled area.

Because the candidate coral metrics summarize different aspects of reef condition, they may also be sensitive to different aspects of human disturbance. LSA may be a better indicator of physical damage while %LC may be a better indicator of global stressors such as increasing ocean temperatures. %LC and %LSA may also be sensitive to local stressors such as nutrient enrichment or sediment associated with terrestrial land use practices.

Natural sources of variability
Three natural sources of variability could be assessed with these data: geographic gradient, reef habitat type, and depth. %LC showed very little association with geographic gradient with most stations showing similar values. An exception was two stations with much lower %LC, both of which were located on back reef. LSA and TSA showed a broader range of values from reef to reef with the largest differences observed around Key West. %LSA showed the strongest association with geographic gradient in that there was a trend toward greater %LSA in the more northeastern reefs.

For all four candidate coral metrics, stations on the same reef were very different. It was not generally true that stations within reefs were more similar than stations on different reefs. Lack of replicates within reefs meant that the effects of different locations within a reef could not be separated from different habitat types within a reef.

Although stations within reefs varied according to habitat type, the differences were not consistent across reefs, e.g., back reefs were not consistently lower for %LSA. The only consistent pattern was that back reefs were the most variable and fore reef habitat was slightly more variable than transitional habitat for all candidate metrics.

Depth and habitat type were strongly associated with each other: transitional habitat was deepest, followed by fore reef habitat and then back reef habitat. Whether the reef habitat type or the depth was the more important factor underlying observed differences could not be determined from the data. Depth may be an inherent feature of the type of reef habitat. Given coral dependence on light, depth may also be a factor in determining which coral were associated with different types of habitat.

To further explore patterns in the coral assemblage associated with reef habitat type, an exploratory technique (PCA) was used to identify which coral species were more typical of the different habitat types. Back and transitional habitats had a small subset of coral species that characterized the assemblage. These results further support the idea that reefs differ by habitat type and any survey design developed to assess reef condition should consider either stratifying on habitat type or only assessing one reef habitat. Creating strata for different subpopulations can greatly reduce the variance associated with the final estimates of reef condition when the strata differ for whatever reason, either due to underlying natural differences or the influence of human disturbance.

Variance structure
ANOVA was used in two different ways for this study, both to compare the relative contribution of different sources of variance to the total variance of each candidate coral metric as well as to estimate the actual variance values which were then used to calculate the MDD. Unfortunately, the estimates of variance associated with different reef sections, duplicate samples, annual differences and station differences were all derived from different stations. A better design would estimate each source of variance using the same locations. The difficulty associated with estimating variance from different reefs is that the individual stations may be more variable in general (regardless of the reason) and, therefore, the particular aspect of variance being estimated from those locations may be inflated. Although the actual numbers derived for variance from this study may be approximate,

several general conclusions could be drawn from the data that can guide the design of future surveys.

During 2003, data from half-sections of the each belt transect were kept separate. Calculations from the two halves of the belt transect showed high agreement for all four coral metrics and the variance estimates associated with differences from one side of the transect to the other were relatively small. The close agreement suggest that the sampling unit (i.e., the belt transect) could be smaller. Smaller sampling units translate into less field time at each station and provide the opportunity to visit more sites. When surveying large areas, a small amount of information collected from more locations is often preferred to highly precise information about fewer locations because larger sample sizes yield more accurate estimates of resource condition.

Variance analysis formalized the observations first summarized in simple graphs of the candidate coral metrics. For TSA and LSA, the greatest differences were associated with different stations. Neighboring sections and duplicates varied little and, furthermore, stations varied little from year to year. In contrast, %LC varied little from station to station, most of its variability was associated with duplicate sampling and annual differences. However, although these sources of variability represented a large percentage, their actual value was still quite low. This translated into a high precision and a good ability for %LC to potentially detect even very small changes in reef condition. %LSA had the greatest proportion of its variability associated with duplicate sampling; unfortunately, this result was driven almost entirely by the high variability in duplicates found at one station (WS). %LSA for different years at five other stations showed high agreement which translated into a good ability of %LSA to detect a change through time for the MDD analysis.

Duplicate samples at stations represented the measurement error associated with the sampling protocol. Duplicate same-day samples ideally should yield the same values because coral condition does not change within such a short period of time. For coral surveys, measurement error could be due to differences in crews, difficulty assigning size category classes or coverage classes of live coral, or missed coral. The small number of reefs with duplicate samples made it difficult to draw robust conclusions regarding the magnitude of the measurement error, particularly because the three stations with duplicates in 2004 showed such markedly different patterns. Two stations showed very consistent values for all coral metrics, but a third, WS, showed very divergent values for duplicates. With a small data set, it's difficult to know which stations would be more typical. To better answer this question and estimate the measurement error more accurately, duplicate samples from 10-20 stations would be needed.

The data set used to estimate year to year variability was quite small (five stations) and included only two years. A more reliable data set would have repeat samples from 20-30 stations sampled for three to five years. As a consequence, actual variance estimates are most certainly approximate; however, MDD values derived from the variance estimates provide information about the relative sensitivity of the different measures. For example, TSA and LSA need a paired sampling approach to detect change through time because the observed values varied greatly from station to station. In contrast, %LC could be successfully monitored using different stations each year. For this analysis only two time periods were considered, but the approach could be expanded to a trend model based on multiple years of sampling when better estimates of annual variability become available (Urquhart et al. 1998).

The two statistical models used in this analysis demonstrated that a much smaller change can be detected when a site is paired with itself through time than when new sites are sampled each year. This difference in sampling design parallels a larger issue which is the trade-off between the assessment of status and trends. To assess the general, overall status of a large regional resource, we are more confident when the assessment is based on visits to many locations. In contrast, the most sensitive design for detecting trend through time visits the same locations each year. Allocation of resources depends on which aspect of monitoring is more important for resource protection.

CONCLUSIONS

The field sampling protocol used to estimate candidate coral metrics provided reliable, repeatable estimates of coral condition. In addition, all coral metrics had good statistical precision for detecting change through time. The physical area sampled at each station may actually be larger than needed to assess a station. Association between geographic location and some coral metrics may indicate a natural gradient or a difference in coral condition which should be considered when designing a sample survey for a large geographic area. Reef habitat type was associated with different species assemblages as well as much of the variability within a reef. Differences within reefs should be considered when designing a monitoring plan by either stratifying on homogeneous habitat types or by sampling only in habitats that are easy to identify. All four candidate coral metrics had adequate statistical precision to detect change through time based on a reasonable number of stations sampled. The candidate metrics quantified very different aspects of coral reef condition; because of this difference, their variance structure also differed. Consequently, a single sampling design may not be optimal for all measures. Development of a long-term monitoring plan for a resource as extensive and complex as coral reefs is an iterative process. Results from this study support the implementation of these field collection protocols and coral metrics to monitor and protect coral reefs.

RECOMMENDATIONS

- ***Connect measures of reef condition to stressors or measures of site condition.***
  A good indicator is characterized by three features: 1) it represents an aspect of the biota that is biologically meaningful, 2) it correlates with independently derived measures of site condition or stressors, and 3) it has adequate statistical precision to detect a change should a change occur (Fore 2003). The first and third features have been demonstrated for these candidate coral metrics, but the second test remains. A strong relationship between indicators and stressors also must be established before any assessment based on coral condition can be interpreted. TSA and LSA were more variable across stations than %LC and %LSA. Whether these differences reflect underlying natural variability or human disturbance would influence the choice of sampling design.

- ***Reconsider the size of the sampling unit.***
  Because coral reefs are continuous resources, dividing them into discrete sampling units is somewhat arbitrary (Stevens and Olsen 1999). On the one hand, sampling units should be as small as possible to minimize time spent in the field collecting data. On the other hand, enough data should be collected to adequately characterize the sampling location and minimize measurement error associated with microhabitat differences. The close agreement for these candidate coral metrics from opposite sides of the radial belt transect suggests that a smaller sampling area may be adequate. A simple way to decide would be to continue with the radial belt transect method as is, but divide the radial belt into four parts in the field and note the quadrant for each coral surveyed. The variance from four quarters, two halves, and duplicates could be compared for each coral metric and the optimal sampling unit size determined.

- ***Recognize habitat types in future survey designs.***
  Much of the variance in these four coral metrics was associated with different habitat types although differences could also be more simply explained by differences in locations on the reef; the two potential sources of variance could not be distinguished for these data. It was clear, however, that back reef habitat was much more variable than other habitat types for all candidate coral metrics. Could back reef habitat be further divided into subcategories? The key point for managing this source of variance is to continue to distinguish between habitat types when collecting data, but also to consider only sampling the habitat type that is the least variable or, alternatively, the best indicator of reef condition.

- ***Revisit at least 10 of the stations.***
  Variability through time is one of the most difficult aspects of variance to assess but critical for designing long-term monitoring plans (Larsen et al. 2001). If possible, at least 10 stations should be revisited soon and in subsequent years.

- ***Design experiments with a balanced design.***
  Any future survey design is likely to have replicate sampling included or imposed by the funding agency. A balanced design provides much more reliable variance estimates and is characterized by equal sampling of stations for each aspect of variance being assessed. For

example, if variance of habitat type and variance of stations within reefs are to be assessed, the design should include the same number of replicates at each station and the same locations should be used to evaluate both. A specific example might select 10 reefs and sample two stations each in back, fore and transitional reef areas. Selecting site locations that are nearby eliminates underlying natural sources of variability that the sampler may be unaware of. In general, a better design would have two replicates from 10 stations rather than three replicates from six stations.

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APPENDIX A
CANDIDATE CORAL METRICS BY SECTION (2003)

Study area, station name, section, initial of diver, and values for coral reef measures collected in 2003. For sections with duplicate samples, average values are shown.

Study Area	Station	Section	Diver	TSA	%LC	LSA	%LSA
Dry Tortugas	BK06	NS	BQ	23.2	87.7	15.6	67.4
Dry Tortugas	BK06	SN	BQ	20.7	89.1	16.0	77.6
Dry Tortugas	BK06	NS	JC	21.6	84.0	14.1	65.1
Dry Tortugas	BK06	SN	JC	19.4	86.9	16.2	83.5
Dry Tortugas	BK06	NS	MP	34.1	81.6	19.1	56.1
Dry Tortugas	BK06	SN	MP	28.1	87.3	20.7	73.6
Dry Tortugas	LR05	NS	BQ	15.3	90.9	13.5	88.3
Dry Tortugas	LR05	SN	BQ	12.9	87.0	8.7	67.1
Dry Tortugas	LR05	NS	JC	20.1	88.1	16.3	81.2
Dry Tortugas	LR05	SN	JC	7.7	93.3	5.6	72.4
Dry Tortugas	LR05	NS	MP	19.1	87.8	14.5	75.7
Dry Tortugas	LR05	SN	MP	9.6	92.5	5.9	61.9

APPENDIX B
CANDIDATE CORAL METRICS FOR STATIONS (2003)

Study area, station name, and values for coral reef measures collected in 2003.

Study Area	Station	TSA	%LC	LSA	%LSA
Dry Tortugas	BK06	43.9	88.4	31.7	72.2
Dry Tortugas	BK06	41.0	85.4	30.3	73.8
Dry Tortugas	BK06	62.2	84.2	39.8	64.0
Dry Tortugas	BK07	39.6	84.5	30.0	75.7
Dry Tortugas	LR05	28.2	88.9	22.2	78.6
Dry Tortugas	LR05	27.7	90.3	21.8	78.8
Dry Tortugas	LR05	28.7	90.0	20.4	71.1
Dry Tortugas	LR06	37.6	87.0	30.1	80.3
Dry Tortugas	LR07	28.4	89.5	21.4	75.2
Key West	ED01	25.8	90.0	14.6	56.8
Key West	SK01	70.0	77.9	39.5	56.4
Key West	SK02	92.1	82.6	45.4	49.3
Key West	SK03	21.7	76.1	13.0	59.9
Key West	WS03	25.6	73.1	13.5	52.5

APPENDIX C
CANDIDATE CORAL METRICS FOR STATIONS (2004)

Study area, station name, duplicate sample identification, and values for coral reef measures collected in 2004.

Study area	Station	Dupe	TSA	%LC	LSA	%LSA
Middle Keys	AR01		5.6	79.3	4.1	72.8
Middle Keys	AR02		5.8	75.6	4.4	75.7
Upper Keys	CR01		35.4	70.7	23.8	67.2
Upper Keys	CR02	A	31.6	83.3	24.2	76.5
Upper Keys	CR02	B	53.4	73.3	31.9	59.6
Upper Keys	CR02	C	49.3	77.5	31.3	63.5
Upper Keys	CR03		110.1	83.2	75.3	68.4
Key West	ED01		60.7	65.3	24.1	39.7
Key West	ED03		123.0	73.5	83.7	68.0
Key West	ED04		38.5	81.5	29.9	77.6
Lower Keys	ES01	A	41.8	75.8	25.5	60.9
Lower Keys	ES01	B	45.9	72.5	25.3	55.1
Lower Keys	ES01	C	41.5	78.2	24.2	58.3
Lower Keys	ES02		43.2	69.7	19.3	44.7
Lower Keys	ES03		33.4	26.0	4.1	12.2
Lower Keys	LK01		157.8	76.7	100.9	63.9
Lower Keys	LK02		162.3	83.8	121.4	74.8
Lower Keys	LK03		5.3	82.6	2.3	42.4
Upper Keys	MR01		85.7	77.8	50.2	58.5
Upper Keys	MR02		15.2	72.4	8.8	57.5
Key West	RK02		85.2	72.2	54.9	64.4
Key West	RK03		45.9	28.0	3.6	7.9
Key West	SK01		206.1	69.1	73.9	35.8
Key West	SK02		62.0	77.9	27.4	44.3
Key West	SK03		59.9	68.4	30.4	50.7
Key West	SK04		28.4	80.9	19.8	69.5
Key West	SK05		42.4	80.0	10.8	25.5
Middle Keys	SR01		8.9	76.4	6.8	76.7
Middle Keys	SR02		26.5	74.3	13.7	51.6
Key West	WS02		140.6	87.2	107.1	76.2
Key West	WS03		54.8	61.6	25.4	46.3
Key West	WS04	A	110.4	93.7	61.2	55.5
Key West	WS04	B	161.3	67.2	36.6	22.7
Key West	WS04	C	79.7	86.2	55.1	69.2
Key West	WS05		56.6	80.0	28.1	49.6