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Bioassessment Tools for Stony Corals: *Monitoring Approaches and Proposed Sampling Plan for the U.S. Virgin Islands*

Final Report

Leska S. Fore
Statistical Design
136 NW 40th St.
Seattle, WA 98107

William S. Fisher
U.S. Environmental Protection Agency
Office of Research and Development
National Health and Environmental Effects Research Laboratory
Gulf Ecology Division
1 Sabine Island Drive
Gulf Breeze, FL 32561

and

Wayne S. Davis
U.S. Environmental Protection Agency
Office of Environmental Information
Environmental Analysis Division
Environmental Science Center
701 Mapes Road
Ft. Meade, MD 20755-5350

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SUMMARY

This document describes three general approaches to the design of a sampling plan for biological monitoring of coral reefs. Status assessment, trend detection and targeted monitoring each require a different approach to site selection and statistical analysis. For status assessment, coral reef stations should be sampled randomly each year to assess the current condition of the resource. For trend assessment, the initial selection of sites should be random but the same sites should be sampled on each occasion, either annually or as part of a rotating panel of sites. For the case of targeted sampling, a specific issue or situation needs to be assessed and random sampling will not be the best approach when only a small number of stations (e.g., ~ 50) can be visited each year. Instead, specific locations should be selected based on their site condition, proximity to disturbance or other features of interest.

In February 2006, EPA scientists will join biologists and managers in the USVI to test, develop, and apply coral monitoring protocols that record the coral species, the percent living tissue, and the size of each coral colony within a radial belt transect. Data collected from this collaboration are intended to resolve questions about the appropriate size of the sampling unit, the process for randomly selecting sampling units, the natural variability associated with habitat types and coastal management zones, and the association between coral metrics and human influence.

A sampling plan is proposed that addresses these questions using visits to ~200 reef stations. A second sampling design for long-term monitoring is also described that samples from coastal management zones using a rotating panel of sites over five years. New stations are selected each year for status assessment and a subset of stations are visited repeatedly to assess trend. The proposed sampling plans provides a framework for allocating sampling effort proportionally among the management questions of greatest concern and can be modified as needed.

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INTRODUCTION

Successful regional monitoring programs typically include three types of sampling approaches to 1) assess the current status of the resource, 2) detect trends over time, and 3) evaluate conditions at specific locations. Status, trend, and targeted sampling all differ in the manner in which sampling units (e.g., reef stations) are selected from among the total population of all possible sampling units. Status assessment is best accomplished with random selection of sampling locations every year. The best sampling designs for detecting trend initially select sampling locations randomly, but then re-visit the same locations each year. For targeted sampling, locations are selected based on specific criteria, such as sites with best management practices in place or sites with known sources of disturbance.

Federal and national programs often focus on assessing the status, or current condition, of a resource in order to identify regional areas with indications of degraded resource condition. States, tribes, and territories are required to provide this information to Congress (305b reporting). In contrast, states, tribes, and territories have a regional mandate to identify specific sources of degradation and develop best management practices to mitigate or minimize their effects rather than simply summarize the average regional condition. Managers at both the federal and state, or territory, level are interested in trend assessment in order to answer the question, “Is resource condition improving or declining?” This question is of interest to scientists, managers, stakeholders, and citizens. We must first answer questions about the current condition of a resources before we can move on to identify the stressors driving change and evaluate the effectiveness of policies and management decisions designed to reverse a negative trend (Brooks et al., 2006).

The only sampling design that can satisfactorily answer questions about both the overall regional condition and the condition of all the individual water bodies is a census sampling design. In a census design, all water bodies are sampled, i.e., every station on every reef. Because census sampling is too expensive, decisions must be made regarding which locations to visit each year. Status, trend, and targeted sampling designs represent different approaches to site selection and answer different types of questions. A related issue to selecting sites is the definition of what constitutes a “site.” Some water bodies can be easily recognized as discrete units, e.g., lakes; for a continuous resource, such as reefs or rivers, the individual sampling units are not as easy to define (Stevens and Olsen, 1999).

Status assessment

For assessing the overall status of a resource, the best approach is to randomly select as many sampling units from the population as possible. The primary advantage of random site selection is that any summary statistics derived from a random sample will be unbiased for the entire population, including all the sites that were *not sampled*. Although site-specific information about unsampled sites is not available, the general overall condition of sites not sampled can be inferred from the sampled sites. Evaluating sites without the expense of sampling is certainly cost-effective. This is the rationale behind the probabilistic survey sampling designs of EPA’s Environmental Monitoring and Assessment Program (EMAP; Larsen, 1997; Schimmel et al., 1999). In addition to an unbiased estimate of overall condition, the uncertainty, or variability, associated with an estimate of condition can be derived for the entire population (Urquhart et al., 1998; Schreuder et al., 2001; Yoccoz et al., 2001).

Status monitoring is designed to answer questions such as:

- *What percentage of coral colonies are composed of living tissue in the near-shore environment surrounding St. Croix?*
- *What is the total surface area of living coral surrounding St. Croix?*
- *What coral species contribute the greatest percentage to the overall coral surface area surrounding St. Croix?*

As EPA's Regional EMAP projects (R-EMAP) have been implemented in different regions, local state, tribal or territorial managers have sometimes been initially disappointed at the scale of the conclusions (Hall et al., 2000). EMAP's probabilistic sampling design was created to answer questions related to resource condition at a regional scale. EPA wants to know if the water resources in a region are getting better or worse, particularly in comparison with other large regional areas (Novitski, 1995; Larsen, 1997; Hyland et al., 2000). Overall regional condition may be useful to local managers for satisfying 305(b) reporting requirements under the Clean Water Act (Larsen, 1997; Yoder and Rankin, 1998), but a manager also needs to know *which* water bodies are degraded because management activities must then be put in place to stop or reverse the trend.

Similarly for regional trend designs, large-scale changes can be recognized more quickly, but information about individual sites is sacrificed (unless they by chance are randomly selected for sampling) because the conclusions made at the regional scale cannot be attributed to specific sites. Here is the trade-off between probabilistic sampling, i.e., the design used by EMAP, and targeted sampling, the design implemented by many states, tribes and territories. The best design, and the one recommended here, uses all three sampling approaches and provides a framework for resource managers to allocate sampling effort proportionately to their information needs.

What do we mean by a "sample"?
A note of caution regarding the word *'sample'* – its meaning changes in different contexts. In field biology, when we talk about samples of invertebrates, we mean a collection of animals from a reef transect. In this case, the population is all the invertebrates in the reef area and the sampling protocol collects a subset, or sample, of individuals present from the reef. In statistics, when we talk about sampling or sample design, we are referring to a collection of sampling units selected from a larger population. In this case, the population is all possible reef sites in the region, and the sample is the set of sites we actually visit.

Trend detection

The goal of trend monitoring is to detect a change through time should a change occur. The recommended statistical model for trend detection is a regression model in which the variable of interest, e.g., total living coral, is regressed against time. Sampling designs in which the same sites are visited each year have the greatest power to detect a trend because each site is effectively compared with itself through time (Larsen et al., 1995; Urquhart et al., 1998). In contrast, selecting a new random set of sites each year introduces a large amount of variability due to site differences and makes it more difficult to detect trend. The same sites may be visited each year, or visited in a rotating panel design, such that each site is visited every fourth or fifth year (Skalski, 1990).

Larsen et al. (2001) illustrate how to maximize the probability of detecting a trend by balancing the allocation of sampling effort among sites, replicates, and repeat visits. For trend monitoring, the most important sources of variability are site differences, year-to-year differences, interaction of site and year, and residual, or measurement error (Larsen et al., 1995). If sites are very different from each other, the most efficient design will revisit many of the same sites each year. If measurement error at each site is high, revisits to the same sites within the year will improve the precision of the monitoring design. If annual variability is high, sites should be visited less frequently, e.g., every two to five years.

Trend monitoring is designed to answer questions such as:

- *Has the percentage of live coral increased (or decreased) during the sampling period?*
- *Is coral surface area increasing (or declining)?*
- *If the percent of live coral has declined, does the intensity of decline vary according to the type of land use?*

For status and trend monitoring, most authors agree that a rotating panel design is the most efficient (Urquhart and Kincaid, 1999; Larsen et al., 2001). In this design, sites are grouped into ‘panels’ and sampled together during their particular rotation year, typically every three to five years. A different panel of sites is sampled each year. This design is more efficient than sampling the same set of sites each year because more sites can be visited through time (Larsen et al., 1995). Furthermore, because regional change typically occurs slowly over a period of years, less frequent sampling is more efficient. This design is also more effective for detecting trend than one that samples all new sites each year because a trend can be detected more quickly by comparing the same sites through time. The key point to this approach is that a panel of sites must be sampled according to the same schedule; sites must not wander from one panel to another.

Targeted monitoring

The utility of a targeted sampling approach can be imagined for a number of scenarios: to determine whether management practices are effective, to regulate specific point sources, or to refine data collection methods.

The caveat associated with selecting sites non-randomly for whatever reason is that any conclusions related to environmental condition at those sites will *only apply to those sites*, not to sites in general (Olsen et al., 1999; Hughes et al., 2000). No matter how statistically significant the trend or change observed for those sites, the conclusions cannot be extended to other sites with any known level of confidence.

In the early years of a monitoring program, targeted sampling may be the most efficient approach for classifying habitat types, testing candidate metrics against independent measures of site condition or disturbance, identifying reference sites, defining data collection and analysis protocols, and determining the level of sampling effort needed for a given level of uncertainty. But as a sampling program matures and protocols are defined, the focus of sampling should shift to include status and trend monitoring as well.

Targeted sampling may be used to answer questions such as:

- *Is the percentage of live coral lower near beaches with more frequent closures?*
- *Which coral species are most sensitive to effluent and boat traffic associated with industrial land uses?*
- *How do different stressors affect coral reefs?*
- *Which coral habitat types are most affected by industrial land uses?*

Answers to these types of questions could also be drawn out of an extensive random survey sampling if enough sites are included in the design. Typically > 200 sample sites are needed to make these types of comparisons. If four years are needed to sample 200 sites, these questions could be answered more quickly in the first couple of years with targeted sampling.

Targeted sampling and random site selection can also be designed to complement one another. Some states use random sampling to assess general regional condition at a coarse spatial scale to identify local 'hot spots,' or areas of special concern. In this approach, areas with lower (or higher) than expected biological condition are identified during the early years of a 5-year rotation. During later years, these areas of concern are targeted for more intensive sampling to evaluate specific sites, identify sources of degradation, or delineate exceptional resources within those general areas.

Probability sampling vs. 'judgment' or 'convenience' sampling

Probability sampling means that every element of the population, in our case, reef stations, has a known probability of being included in the survey sample. Sites within a similar class, e.g., fore or back reefs, would have an equal probability of selection. *Judgment* sampling refers to selection of sites based on experience or scientific criteria. *Convenience* sampling bases selection on availability of sites or ease of access. Judgment and convenience sampling can give biased estimates of resource condition. Furthermore, if sites are selected based on convenience or judgment, a significant change observed for those sites cannot be applied to sites not sampled, no matter how similar those sites may be.

BACKGROUND

Regulatory context

Both the Federal Clean Water Act (CWA, 1972) and the US Virgin Islands Water Pollution Control Act (1972) outline regulations for protection of surface waters and the biological assemblages they support. Under the CWA, two programs specifically rely on biological monitoring data in coastal marine areas: the 301(h) waiver program and the 403(c) ocean discharger program. The waiver program allows marine dischargers to defer secondary treatment if they can show the discharge does not affect biological communities. The ocean discharger program requires all dischargers to marine waters to provide an assessment of the biological community in the area of the discharge (Jameson et al., 1998). The CWA also requires states to assess and report whether surface waters are supporting designated uses. For the US Virgin Islands (USVI), surface waters are classified into three groups according to their designated uses (USVI DEP & DPNR, 2004):

Class A – Waters are for the preservation of natural phenomena requiring special conditions with existing natural conditions that shall not be changed. Class A water standards are the most stringent of the three classes because of the pristine or near pristine state of waters in this classification.

Class B – Waters are for the propagation of desirable species of marine life and for primary contact recreation.

Class C – This classification is similar to Class B, except that it has slightly less stringent water quality standards for a limited number of parameters.

Most states, tribes and territories have similar types of narrative criteria that specify the protection of aquatic life as a designated use. Phrases used above such as “preservation of natural phenomena” and “propagation of desirable species” are an example of this type of designated use. EPA’s Office of Water has developed guidance to help states, territories and tribes better characterize and more specifically define aquatic life use as part of their water quality standards (EPA, 2005).

A recent EPA guidance document lists 12 key elements in a biomonitoring program that will support the definition of biocriteria for designated uses (Table 1). These include sampling design, methods and data interpretation components. Goals for sampling in the USVI in 2006 match several of these key components. Included under sampling design components, goals for sampling in 2006 include a) identifying the spatial coverage of coral reefs, b) classifying reefs according to habitat type, and c) identifying reference sites. Under methods, most of these elements are already in place for the USVI. The indicator assemblage will be stony corals, sample collection methods are visual observations, and sample processing is simply the identification of species and coverage in the field. Data interpretation components will be addressed after returning to the lab to a) evaluate the response of proposed coral metrics to different levels of disturbance and b) calibrate metric expectations based on observations at reference sites.

Table 1. Twelve key technical elements of a bioassessment program.
Recent EPA guidance defines three components of a mature bioassessment program and describes the 12 elements that must be in place for effective long-term monitoring (from EPA, 2005).

Sampling Design Component

1. *Temporal Periodicity* of the sampling
2. *Spatial Coverage* of the sites within the area of interest
3. *Natural Classification* of the water bodies as a framework for assessment
4. *Regional Reference Condition* development
5. *Reference Sites Selection Criteria*

Methods Component

6. Number and kinds of *Indicator Assemblages*
7. Methods for *Sample Collection*
8. Methods for *Sample Processing*

Data Interpretation Component

9. Attention to *Ecological Attributes* for indicators
10. Calibration of *Biological Endpoints*
11. *Diagnostic Capability* of the indicators
12. Use of *Professional Review* of documentation and methods

Project goals

The primary purpose of this project is to assist USVI in developing assessment tools for defining biocriteria for coral reefs. This document proposes a sampling plan and survey design with adequate flexibility to address local needs as well as mesh with a larger regional design based on probabilistic survey sampling.

Coral reefs are biologically complex systems with many choices regarding which organisms to monitor (Jameson et al., 2001). As a consequence, monitoring protocols are not as well-developed for these systems compared to other water body types such as rivers or wetlands (Barbour et al., 1999; Karr and Chu, 1999). Because development of coral reef biocriteria is in its initial stages, many tasks remain. Field time for this project is limited and it may be necessary to prioritize some tasks to the exclusion of others.

Jameson et al. (2001) provide a checklist for the development of a monitoring program to support biocriteria for coral reefs. Most all the items on their checklist remain to be done for the USVI (Table 2). Some items will be addressed directly by the proposed sampling plan for 2006 (described in the next section below).

Table 2. Development steps for coral reef biocriteria.
Steps in the development of biocriteria for coral reefs and whether the step is addressed by this project (modified from Jameson et al., 2001).

Biocriteria development steps	Addressed by this project?
Step 1 Determine regional ecological expectations	
- Coral reef classification	Yes
- Reference sites representative of class categories	Yes
Step 2 Biological survey	
- Sampling along a gradient to calibrate and evaluate metric response	Yes
- Collect/compile data on biota and physical/chemical habitat	Coral data only will be compiled
Step 3 Final classification	
- Test preliminary classification	Yes
- Revise if necessary	
Step 4 Metric evaluation and index development	
- Testing and validation of metrics by coral reef class	Yes
- Evaluation of metrics for effectiveness in detecting impairment	Yes
- Aggregation of metrics into index	No
- Selection of biological endpoints	No
- Test the index for validity on another data set	No
Step 5 Biocriteria development	
- Adjustment by physical and chemical covariates	No
- Adjustment by designated aquatic life use	No
Step 6 Implementation of monitoring and assessment program	
- Determination of temporal variability of reference sites	No; need long-term monitoring data
- Balance status, trend, and targeted monitoring needs	Yes
Step 7 Management action	
- Initiate programs to preserve exceptional waters	No
- Identify causes of degradation and plan restoration	No
Step 8 Continued monitoring	
- Biological surveys to assess effectiveness of management efforts	No

Key issues to be addressed in 2006

For coral reef sampling in 2006, five primary issues will be addressed by the proposed sampling plan. Although listed in numeric order, all five tasks are part of an iterative process and must be accomplished simultaneously rather than sequentially.

1. Define the sampling unit for coral reefs.
   Data collected from an individual sampling unit need to be sufficient to characterize condition at the location but minimal in order to keep the design efficient. The more information collected at each site, the fewer sites that can be sampled. For a regional survey design and most statistical comparisons, less information from more places is better than more information from a few places. Results from coral sampling in Florida (Fore et al. 2006) indicated that a sampling unit size of 113 m^2 was probably too large and the sampling unit size has been reduced for this project to 50 m^2. To test whether this size is adequate or still too large, each radial belt transect will be divided into four equal quadrats that will be compared and evaluated for variability.

2. Define the target sampling population for coral reefs.
   For a survey design, random selection of points is required, but for coral reef monitoring, random selection often leads to locations with no corals present. Benthic maps exist and may be accurate over a large region but may not be sufficient to identify specific areas where data collection is possible.

3. Define the sampling frame for the target population.
   Once the physical population of coral reefs is defined, choices remain regarding which types of coral reefs will be sampled. This issue differs from #2 above in that the target population is coral reefs, while the sampling frame could be all coral reefs but is more likely to be some subset, such as reefs < 15 m deep or reefs of a certain type (e.g., fore reef). Thus, the sampling frame may be a subset of the target population. If so, the size of the subpopulation represented by the sampling frame must also be known in order to properly weight each station when computing regional estimates.

4. Classify coral reefs according to unique and homogeneous habitat types.
   The number of ways to classify coral reef types is potentially endless and could be based on various combinations of depth, topography, wave velocity or location relative to land forms (Mumby and Harborne, 1999; Kendall et al., 2000). The key point to remember is that classification is only needed for areas that differ in terms of their *expectations* for the coral metrics. Even if two reef types have entirely different species of coral, if the expectation based on reference condition for both locations is ~85% living coral on average, then the two reef types can be classified together and assessed in the same manner.

   Obviously, some coral metrics may be the same while others differ according to habitat type. Recent comparisons in the Florida Keys found that back reefs tended to have a lower percentage of living coral than fore or transitional reef habitat (Fisher et al., 2006; Fore et al., 2006). Rather than classify habitat types for all possible coral metrics, only those metrics that show a consistent and predictable association with human disturbance should be considered. Assigning different expectations according to habitat type is relatively straightforward. The risk associated with inadvertently combining dissimilar categories is a lack of precision for defining expectations.

5. Test the association between site condition and candidate coral metrics.
   To develop biocriteria, a clear link must be demonstrated between the monitoring tools used to summarize data (e.g., metrics) and an independent measure of site condition or human disturbance. A proposed metric might be a very logical choice from a theoretical point of view, but if it fails to indicate degradation either because it is too variable or is not strongly associated with human-induced changes, then it should not be included in the monitoring protocol. Furthermore, time and effort should not be expended to classify habitat for this potential metric or to define expectations.

   Note that the last two issues are difficult to separate in the initial stages of developing a monitoring plan. The habitat types may not differ in terms of expectations for coral metrics, if so, they should be combined. On the other hand, some habitat types may be missed in the first round of sampling and need to be added later. Often the first round of sampling is based on a ‘best guess’ as to which habitat types are relevant.

DATA COLLECTION METHODS

Study area

Of the three islands in the USVI, this project will focus first on St. Croix. Seven near-shore areas have been identified that may have different expectations for biological condition based on their geographic orientation (leeward or windward), patterns of water movement, and depth profile. Management needs may differ for these coastal management zones. Within each of the seven management zones, dominant habitat types will be identified using benthic maps created by the National Oceanic and Atmospheric Administration (NOAA, 2001).

Each of the seven coastal management zones (CMZ) has different types of human activities as well as a differing potential for reference sites (Figure 1). The zones were defined by resource managers and scientists at the USVI Division of Environmental Protection and Department of Planning and Natural Resources. Zones were defined according to the type of coral habitat observed and the type of human land use within the water, along the shore and inland. Thus, given historic land uses, expectations may be differ according to CMZ.

Moving in a clockwise direction: the west end of the island near Frederiksted has the only large public dock on the island used by cruise ships. Boat traffic is expected to increase here during coming years. The area around the pier has a history of small and large boat use which has been associated with anchor damage to reefs (Toller, 2005). Nutrient enrichment associated with untreated wastewater from anchored ships may also be a source of disturbance in this area. Located near the pier is also a small sewer overflow. The city of Frederiksted also may be expected to contribute to general disturbance. Human influence decreases further north and south from the pier. The northwest section has tourism associated with diving and fishing, but otherwise only minimal influence. In the north central area is the city of Christiansted. The harbor at Christiansted has boat traffic and 50–75 boats moored in the harbor. Urban development associated with Christiansted and a large wastewater treatment plant with discharge to the reef area represent potential non-point and point sources of disturbance.

Moving further east, Buck Island is just offshore to the northeast of the island and is designated as a National Monument that is administered by the National Park Service. This area has no human development and visitors are limited to daylight hours. Human disturbance is limited to recreational use and this area may provide reference sites for other areas on the main island. The east end of the island includes a new marine park. Sources of disturbance include run-off and sediment from many unpaved and steep roads in this zone. In the center of the south side are located a rum distillery with documented effluent, a large petroleum refinery, dredged channels for commercial docks, the airport, and the land fill. Wind is primarily from the ENE which means the western end of the south side is downstream of these disturbances during most of the year while areas to the east are nominally upstream. The southeast part of the island is used for farming.

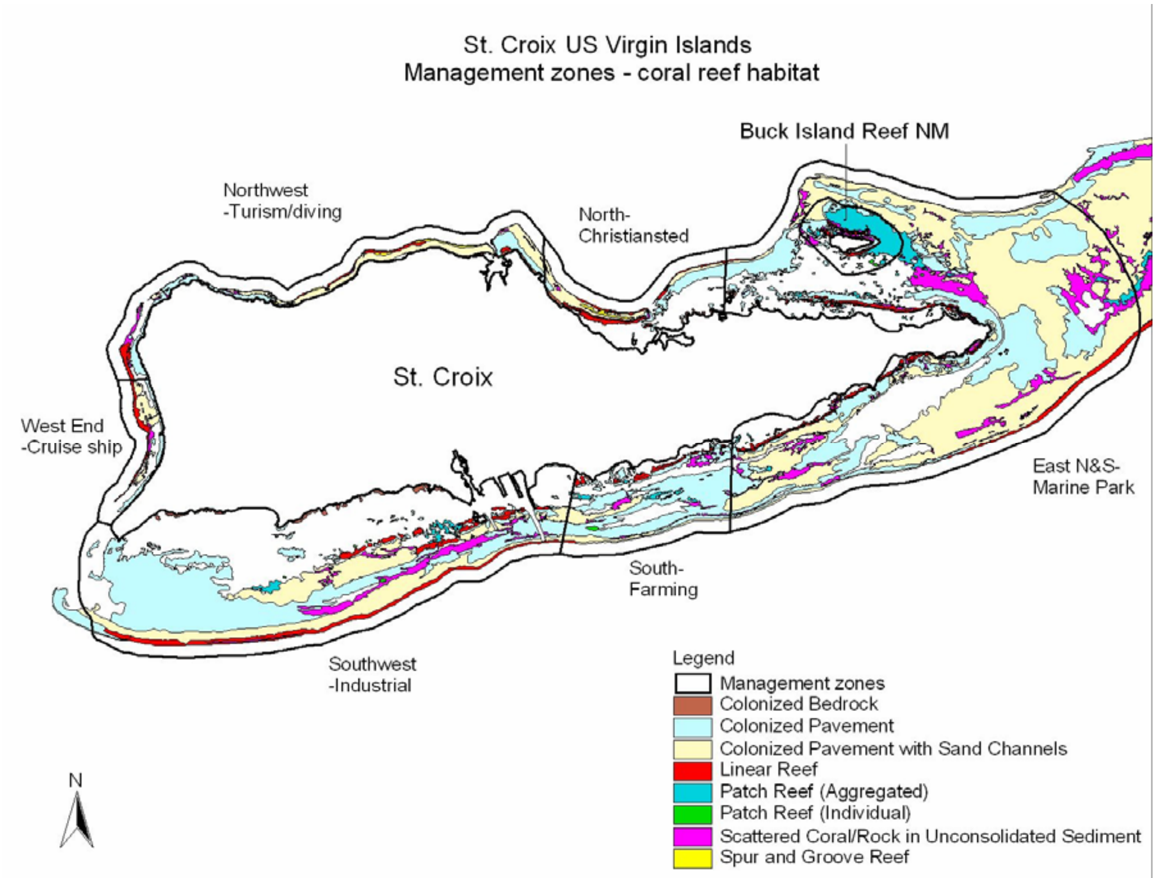

Figure 1. Map of St. Croix (USVI) showing the seven coastal management zones and the types of reef surrounding each CMZ.

Data collection

Divers will delineate a radial belt transect by marking a point on the reef and defining two circles of 3 and 5 m radii. Coral colonies within the radial belt will be identified to species, their size class noted, and the percentage of live coral recorded. The area surveyed equals 50.2 m^2. The location of each coral according to quadrat within the radial belt will be noted to later test the variability associated with different sized sampling units.

Two EPA teams of three divers each will sample for up to two weeks (10 dive days) during February 1–14, 2006 from EPA's ship the OSV BOLD. A third team of USVI divers will accompany EPA divers to learn the data collection methods, then sample locations on their own.

Data analysis

The three coral colony measures recorded in the field, species, size, and % living coral, will be used to calculate candidate metrics related to abundance and composition, physical stature, and biological condition (Table 3; Fisher et al., 2006).

Table 3. Description of candidate metrics for stony corals (from Fisher et al., 2006).

Abundance and Composition

Coral abundance – number of coral colonies in station or reef

Coral density – number of corals per m^2 sea floor surveyed in a station or reef

Species richness – number of species occurring in a reef or region

Species frequency – number of stations in a reef or region where a particular species occurs

Species diversity – number of species and their relative abundance in a reef or region

Unique species – number of rare, unique and protected coral species in a reef or region

Tolerance index – ratio of intolerant to tolerant species in a reef or region

Physical Stature

Reef surface area – total 3D surface area (m^2) of all corals in a reef

Reef volume – total volume (m^3) of corals in a reef

Reef complexity – total 3D surface area (m^2) of corals per m^2 sea floor

Reef structure – total volume (m^3) of corals per m^2 sea floor

Population structure – size-frequency distributions relating the number of colonies, quantity of coral surface area, quantity of live coral surface area, or average percent live coral of a particular species in a reef to colony size

Community structure – population structure metrics applied to all coral colonies in a reef

Biological Condition

Reef percent live coral – average percent live coral for all colonies in a reef

Reef live surface area – sum of live colony surface areas for all colonies in a reef

Reef dead surface area – sum of dead colony surface areas for all colonies in a reef

Reef live:dead surface area – ratio of live coral to dead coral surface area for all colonies in a reef

PROPOSED SAMPLING DESIGNS

The goal of Phase I sampling is to field test proposed methods and protocols for collecting and analyzing the data. Phase I uses targeted sampling to answer questions about the optimal collection area for a reef station, the repeatability of the data collection protocols, responsiveness of candidate metrics, and the appropriate classification of reefs according to habitat type. Some random sampling may be done during Phase I to test the protocols for locating random sites and placement of the transect, but most of the sampling will be at targeted sites. Habitat classification issues may be resolved in St. Croix and sampling in different habitat types may be dropped in St. Thomas and St. John. Alternatively, new habitat types may be discovered in St. John requiring additional habitat comparisons.

Once the sampling and data collection protocols are defined, the sampling approach shifts to long-term monitoring and assessment during Phase II. Phase II uses random site selection to assess status and trends of coral condition. Phase II sampling is supplemented by some targeted sampling to assess specific locations of concern. The goal of Phase II is to implement a long-term monitoring plan for the USVI. The USVI are divided into four areas of approximately equal size for Phase II sampling: 1) eastern St. Croix, 2) western St. Croix, 3) St. Thomas, and 4) St. John.

The sampling design described below is proposed as one option; however, the design is flexible and sampling effort may be allocated to answer different questions according to the situation in each CMZ. During this project, we expect knowledge to accumulate and the questions to be refined in ways that we cannot yet anticipate. Some areas may have no sources of human disturbance and only reference condition locations will be sampled. In this case, sampling effort may be reassigned to a CMZ with more complicated land use patterns.

Phase I – Field test data collection protocols

Data collection protocol. To evaluate sample unit size, radial belts will be divided into four equal-sizes quadrats and the location of each coral recorded. Exact division and careful delineation are not particularly important, so approximations can be used to save time. Using this information, different sample areas can be compared for their precision and ability to discern differences in coral condition and an optimal quadrat size defined.

Human disturbance gradient. In each of the four island areas stations will be allocated to answer questions about differences in habitat types, within reef variability, and sensitivity of coral metrics to disturbance (Table 4). A source of disturbance will be selected, e.g., for St. Croix, the industrial complex on the south shore or the cruise ship dock at Frederiksted could be used. Ten reef sampling stations will be located longitudinally away from the source parallel to shore to avoid confounding with natural differences associated with depth. To place stations longitudinally, if the disturbed area is approximately 100 m wide, for example, the stations could be located from the center of the disturbed area out toward one edge at approximately 5 m intervals for a total of 10 stations (Figure 1). To evaluate reef variability, every second station would have a matched replicate located in a similar habitat type for a total of 5 additional stations located at approximately 10 m intervals from the center of the disturbed area, that is, every other sampling point. To evaluate the response of coral metrics in different habitat types, an additional 5 stations would be sampled along the same disturbance gradient as above, but in a *different* habitat type. For this sampling design, a total of 20 stations are required: 10 along the gradient, 5 at every other location as a replicate, and 5 at every other location in a different habitat type.

Table 4. Phase I sampling.
Purpose of reef sampling, island location, number of stations sampled in each location, description of allocation and total number of stations needed.

Purpose	***Eastern St. Croix***	***Western St. Croix***	***St. Thomas***	***St. John***	***Description***
Gradient	20	20	20	20	10 along gradient; 5 in different habitat; 5 same habitat
CMZ's	9 x 4	9 x 3	9 x 4	9 x 3	9 stations in each CMZ
Total	**56**	**47**	**56**	**47**	

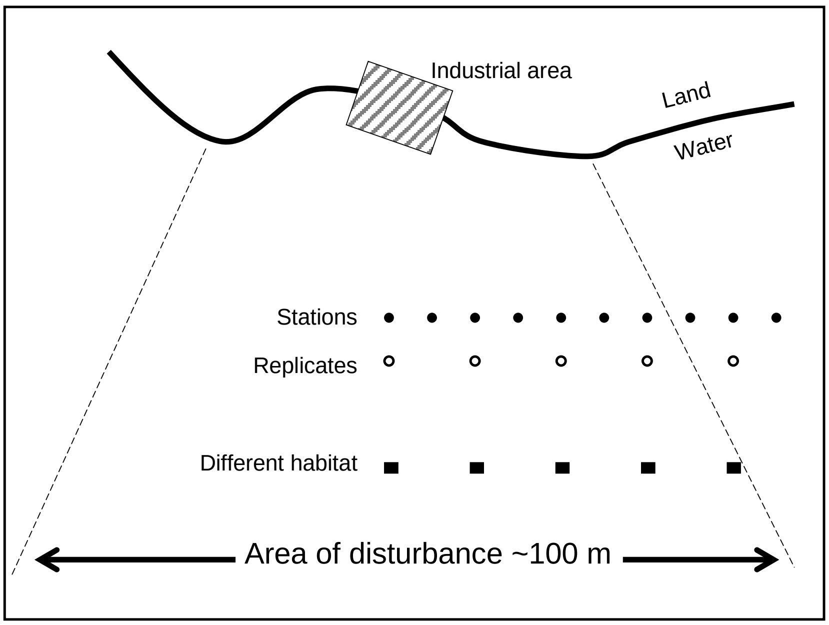

Figure 2. Schematic diagram depicting proposed sampling along a gradient of human disturbance. Shown are an industrial point source, the area of disturbance, locations of 10 sampling stations along the gradient, 5 replicate stations in a similar habitat type, and 5 stations in a different habitat type.

Coastal Management Zones. Assuming 4 coastal management zones (CMZs) in eastern St. Croix, 3 CMZs in western St. Croix, 4 in St. Thomas and 3 in St. John, 9 stations would be allocated to each CMZ to evaluate the range of coral metrics expected in undisturbed and disturbed reef areas, the influence of habitat type on coral metrics, and to establish expectations for each CMZ.

The 9 stations could be allocated within a CMZ to answer different types of questions. For example, three habitat types could be identified for sampling in a CMZ. Within each habitat type, 1 station would be located in an area with minimal human influence and a second station would be located within an area with high human influence. Three habitat types x 2 levels of human disturbance (‘low’ and ‘high’) totals 6 stations. The remaining 3 stations could be used to replicate samples in the ‘low’ disturbance areas to assess natural variability of coral candidate metrics. Alternatively, the 3 stations could be located in *moderately* disturbed locations to create a 3-point gradient to evaluate metric response to disturbance.

Additional sampling. In addition to the dive visits to specific reef stations, sampling effort will be devoted to mapping and locating reefs. This might occur before stations are located to identify dominant habitat types in the CMZ or areas with disturbance. The goal for this task is to both ground-truth the available benthic maps and test the application of a two-stage sampling approach to probabilistic sampling.

Although coral reef may be present on a map, at a smaller spatial scale, much of the reef may actually be sand and inappropriate for sampling. Random sampling based on these maps can, therefore, generate a lot of ‘misses’ which results in moving to the next random point which is time consuming. In contrast, for two-stage sampling the initial random selection (first stage) applies to a much larger area than a single point, and then second-stage sampling within that area is restricted to areas with the target habitat present. For application to the USVI, a 100 m square area could be randomly selected. In the field, the area must be visually delineated, perhaps using wands. Coral reef within the square is next mapped based on visual inspection and the location for the radial belt is determined by random sample from reef areas.

What we will know at the end of Phase I

- *The optimal size for the sampling unit.*
  Based on comparisons of coral metrics derived from different sized sampling units (¼ transect, ½ transect and full transect), we will know at the end of Phase I sampling what size area is needed to obtain a representative measure of reef condition. If coral metrics calculated from ¼ transects show the same response to human disturbance and/or have similar variance estimates to full transects, smaller may be better. In this case, rather than one radial belt of 50 m^2 area, it may be more representative to collection 2 samples of 25 m^2. If the smaller sampling units are much more variable or are not consistently associated with different levels of disturbance, we know that the larger transect area is needed.

- *How to randomly sample for status and trends.*
  At the end of two years the target habitat type for sampling will be defined and information will be available to decide between simple random sampling vs. two-stage sampling. In the first approach, random locations without reef at the exact location are abandoned for the next random location. In the second approach, an area is randomly selected, and a station is located randomly within that area by sampling a second time from among the areas with coral present. The best sampling method for status and trend monitoring will depend on the accuracy of the maps, the time needed for mapping at a location, and the frequency of missing the coral.

- *Which metrics are sensitive to human disturbance.*
  Relevant biocriteria are based on a demonstrable association between independent measures of site condition and biological response. No matter how compelling the underlying biology of a metric may be, if the metric fails to indicate human-induced degradation to the resource, it is useless as biocriteria. At the end of two years we will have information about metric

response across two gradients of disturbance in USVI as well as metric response to the types of disturbance found within each CMZ.

- *Which reef habitat types should be classified separately or together.*
  If stations within a CMZ differ more according to habitat type than by differences in human disturbance, the habitat types need to be classified separately and different expectations (biocriteria) defined for each. Habitat types with similar ranges of values for the coral metrics can be grouped together and included equally in the sampling frame for Phase II sampling.

  From the gradient analysis, we may find that coral metrics in both habitat types decline with disturbance, but one habitat type may be more sensitive or less variable than the other. In that case, future sampling would focus on the optimal habitat type. In contrast, we may find that coral metrics decline significantly in one habitat type but not in the other. In this case, we would consider the underlying biological reasons for the differences before selecting a habitat type for future monitoring.

- *Expectation for biological condition according to CMZ.*
  At the completion of Phase I, all islands and all CMZ's will have been visited and the range of condition documented for each zone by sampling in minimally disturbed and degraded locations. Some zones with minimal disturbance may have additional sampling from different habitat types instead of sampling from disturbed areas. The natural expectations given CMZ and habitat type will be defined for the proposed coral metrics.

  From replicate samples in similar habitat types we can generate variance estimates to use in calculating the statistical power of the coral reef metrics to detect change. Using statistical power analysis, we can compare the sensitivity of various sampling scenarios to detect change in reef condition. This information will be useful in determining how many stations should be sampled and how often they should be visited for Phase II status and trend monitoring.

Statistical tests for analysis of Phase I data

Sample unit size. Spearman's correlation could be used to test for association between metrics measured at ¼, ½, ¾ or full quadrats of the radial belt. If correlation with the gradient is just as high for measurements based on a single quadrat, then a smaller sampling unit may be preferred. If correlation is much higher for the full radial transect, then the sampling unit is of a good size. ANOVA can also be used to evaluate the variability of coral metrics for different sized sampling units. If variance is much higher for the smaller quadrat sizes, the full transect may be preferred.

Habitat type. An ANOVA model can be used to test for significant metric differences associated with habitat type in the different CMZ's. ANCOVA can be used to evaluate habitat differences for different habitat types along the disturbance gradients. A single metric, e.g., percent living coral, could be plotted against distance from center of disturbance for each habitat type. If the regression lines from different habitat types are offset, that result indicates the habitat types should be classified separately. If the regression lines have similar slopes and intercepts, the metric response is similar and habitats should be combined for survey sampling. Results may differ according to each coral metric; therefore, we need to know which metrics are good indicators of disturbance.

Metric sensitivity. To test metric association with human disturbance, sites located along a gradient from the center of a disturbance could be tested with Spearman's correlation. Graphical analysis can also reveal whether the response is linear across the gradient or if a sharp change is more characteristic along the gradient, potentially indicating a threshold of biological response. For minimally disturbed and degraded sites in the different CMZ's and habitat types, a paired test could be used to compare coral reef metrics.

Phase II – Implement a long-term monitoring plan

For long-term monitoring, a rotating panel design is most efficient for monitoring regional areas and a five-year rotation is often used. If we assume that a maximum of 60 stations can be visited per year, the effort could be allocated to status, trend and targeted monitoring over the five-year sampling period (Table 5). During year 1, 10 stations in each of 4 CMZs would be sampled in the eastern part of St. Croix. In addition, 10 trend monitoring and 10 targeted stations would be sampled. During year 2, The western half of St. Croix and the remaining 3 CMZs would be sampled. In each of the 3 CMZs, 10 stations would be visited. An additional 10 trend and 10 targeted stations would also be visited.

During year 3 St. Thomas would be sampled using a similar allocation of stations. In year 4 St. John would be sampled. During year 5, no status or trend stations are visited and sampling effort is reserved for targeted locations of special interest or with known concerns. During the fifth year, fewer stations are visited to allow time for data synthesis and reporting.

Table 5. Phase II proposed sampling.
Number of stations visited during each year of a 5-year rotation schedule for each island. Schedule assumes 10 trend stations visited each year and 10 status stations for each coastal management zone (CMZ). During the first year, the east side of St. Croix sampling would include 10 randomly selected trend stations, 10 randomly selected status stations from each CMZ (10 x 4 = 40), and 10 targeted stations for a total of 60 stations. The fifth year is reserved for visiting only targeted locations of special interest that may have been discovered during the random sampling.

Year	***1***	***2***	***3***	***4***	***5***
East St. Croix	10/40				
West St. Croix		10/30			
St. Thomas			10/40		
St. John				10/30	
Targeted	10	10	10	20	40
Total	**60**	**50**	**60**	**60**	**40**

What we will know at the end of Phase II

- *Status of coral condition with known level of uncertainty.*
  The condition of the coral reefs as summarized by the coral metrics would be known for the entire area represented by the sampling frame. Reef condition for the area surrounding all three islands, or for each individual island, or for an individual CMZ within an island would be known. The variability associated with the estimates would also be known such that condition across islands or CMZ's could be compared.
- *Locations of hot spots or outstanding resources.*
  Survey sampling at a broad spatial scale can identify areas with unexpected conditions, either good or bad. Poor condition might indicate a location with a land use practice that should be regulated; exceptional condition might indicate areas of particular natural value. The scale of survey sampling is coarse and while it can potentially identify general areas of concern it cannot provide detailed information regarding individual site condition or underlying causes of anomalous assessments.
- *No information for trend until the second 5-year rotation of sampling.*
  No information about trend through time would be available during the first 5 years of this panel design because revisits do not occur to a panel until the next 5 years. However, if desired, a subset of stations could be revisited each year during the first 5 years to estimate the variability associated with year-to-year differences.
- *Whether sampling allocation needs to be modified.*
  At the end of the first year or two of sampling, the design should be re-assessed to determine if agency needs are being met. For example, information from a near-pristine environment with no immediate threat of human development may not need the same intensity of sampling effort as an area with impending development, such as the western dock area of St. Croix. In this case, sampling from one CMZ could be allocated to another. For probabilistic survey sampling, sampling effort does not need to be equal in all areas, but it must be known, so that adjustments to the overall regional estimates can be made.
- *Results from of targeted sampling.*
  Results from the targeted sampling stations will depend on the particular questions to be addressed. These could be related to the effectiveness of management actions, success of best practices, or better understanding of ways existing human uses affect (or do not affect) coral reef condition.

DISCUSSION

An important point to remember is that no survey sampling design is more inherently 'correct' than any other; the best way to select sampling locations depends entirely on the questions being asked. The sampling designs described here represent one possibility and include several assumptions regarding which questions are most important. The purpose of this document is not to define a single survey sampling design, but to provide a framework for a discussion among the scientists and managers involved so that they can put together the best design to meet current needs.

Any sample survey design based on random site selection can potentially meet and mesh with EPA design goals (Paulsen et al., 1998; Olsen et al., 1999). The design proposed here assesses both status and trend using randomly selected sites. Other EPA EMAP projects select sites using the GRTS (generalized random tessellation stratified) framework which samples randomly but also yields a spatially balanced sample (Stevens and Olsen, 2004). For this survey design, very close neighbors cannot be selected as they would be for simple random sampling. For any random survey design, all possible sampling locations must be known in order to define the sampling frame. Thus, accurate maps are critical to the success of the survey design.

At the outset of developing a biomonitoring program, it can be difficult to answer all the questions at once regarding which metrics to use, where to sample, and how often to visit different areas. Resolution of some issues will be an iterative process (e.g., how to classify coral habitat), while others issues may be resolved in the first year of sampling (e.g., how large an area to measure in each station). Trend monitoring represents a big commitment over the long haul; therefore, it's important to initiate a level of sampling effort that is sustainable.

No design is ever final: management priorities may change, results from trend monitoring may indicate some areas are changing more quickly than others, and new threats to coral reefs can develop. In any of these cases, allocation of sampling effort should be reconsidered. The framework described here can guide the development of a sampling design for 2006 and for future years as well.

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