MESOAMERICAN BARRIER REEF SYSTEMS PROJECT (MBRS)

MANUAL OF METHODS FOR THE MBRS SYNOPTIC MONITORING PROGRAM

Selected Methods for Monitoring Physical and Biological Parameters for Use in the Mesoamerican Region

P.C. Almada-Villela, P.F. Sale, G. Gold-Bouchot and B. Kjerfve

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Project Coordinating Unit
Coastal Resources Multi-Complex Building
Princess Margaret Drive
P.O. Box 93
Belize City Belize
Tel: (501) 223-3895; 223-4561
Fax: (501) 223-4513
Email: mbrs@btl.net
Website: http://www.mbrs.org.bz

CONSERVATION AND SUSTAINABLE USE OF THE MESOAMERICAN BARRIER REEF SYSTEMS PROJECT (MBRS)

Belize – Guatemala – Honduras - Mexico

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PO Box 93

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LIST OF ACRONYMS AND ABBREVIATIONS USED

ADCP	Acoustic Doppler Current Profiling Meter
AGRRA	Atlantic and Gulf Rapid Reef Assessment
AIMS	Australian Institute of Marine Science, Australia
ASK	Amigos de Sian Ka'an, Mexico
BFD	Belize Fisheries Department, Belize
BICA	Bay Island Conservation Foundation, Honduras
CARICOMP	Caribbean Coastal and Marine Productivity
CCAD	Comisión Centroamericana de Ambiente y Desarrollo
CCO	Cuerpos de Conservación Omoa, Honduras
CEMA	Centro de Estudios del Mar y Acuacultura, Guatemala
CICESE	Centro de Investigación Científica y de Estudios Superiores de Ensenada, Mexico
CINVESTAV	Centro de Investigación y de Estudios Avanzados del IPN, Mexico
CONANP	Comisión Nacional de Áreas Naturales Protegidas, Mexico
CONAP	Comisión Nacional de Áreas Protegidas, Guatemala
CPACC	Caribbean Planning for Adaptation to Climate Change
CTD	Conductivity-Temperature-Depth
CZMA/I	Coastal Zone Management Authority and Institute, Belize
Dbh	Diameter at breast height
DiBio	Dirección de Biodiversidad, Honduras
ECOSUR	El Colegio de la Frontera Sur, Unidad Chetumal, Mexico
FHAC	Fundación Hondureña para los Arrecifes Coralinos, Honduras
FUNDAECO	Fundación para el Desarrollo y la Conservación, Guatemala
GMT	Greenwich Mean Time
GPS	Global Positioning System
GR	Green Reef, Belize
HCMR	Hol Chan Marine Reserve, Belize
IAEA	International Atomic Energy Agency, Monaco
INWEH	International Network for Water, Environment and Health
JD	Julian Date
MAFC	Ministry of Agriculture, Fisheries and Cooperatives, Belize
MARN	Ministerio de Ambiente y Recursos Naturales, Guatemala
MBRS	Mesoamerican Barrier Reef Systems Project
MPA	Marine Protected Area

MSL	Mean Sea Level
NCORE	National Center for Caribbean Coral Reef Research, USA
NGO	Non Governmental Organization
NMS	National Meteorological Service, Belize
NOAA	National Oceanographic and Atmospheric Administration, USA
OBS	Optical Back-scatter System
OCC	Oficina de Cambio Climático, SERNA, Honduras
PA	Protected Area
PCQM	Point-Centered Quarter Method for Mangroves
PCU	Project Coordinating Unit
PROARCA	Proyecto Ambiental Regional para Centroamérica
REEF	Reef Environmental Education Foundation, USA
REIS	Regional Environmental Information System
SEMARNAT	Secretaría de Medio Ambiente y Recursos Naturales, Mexico
SERNA	Secretaría de Recursos Naturales, Honduras
SICA	Sistema de la Integración Centroamericana
SM	Secretaría de Marina, Mexico
SMP	Synoptic Monitoring Program
SS	Strategic Site
SWCMR	Southwater Caye Marine Reserve, Belize
TS	Transboundary Site
TWGs	Technical Working Groups
UB	University of Belize
UCR	Universidad de Costa Rica, Costa Rica
UCSC	University of California at Santa Cruz, USA
UGAMPC	Unidad de Gestión Ambiental Municipalidad de Puerto Cortés, Honduras
UNIPESCA	Unidad de Manejo de la Pesca y Acuicultura, Guatemala
UNU	United Nations University
UQROO	Universidad de Quintana Roo, Mexico
USAC	Universidad de San Carlos de Guatemala, Guatemala
USC	University of South Carolina, USA
USGS	United States Geological Survey, USA
WB	World Bank
WCS	Wildlife Conservation Society
WWF	World Wildlife Fund

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PREFACE

The Mesoamerican Barrier Reef System (MBRS) is a complex, large marine ecosystem, rich in biodiversity which extends over the countries of Belize, Guatemala, Honduras and Mexico. Approximately 1 million people from multiple social and ethnic backgrounds, benefit from these valuable resources through activities related to fisheries, tourism and coastal development, among others. Such activities continue to increase in the region, placing varying degrees of pressure on the natural ecosystems in the MBRS, which include coral reefs, and its associated ecosystems. Examples of latter comprise seagrass beds, mangroves, coastal lagoons and rivers.

Given the importance of the coral reefs and coastal ecosystems, it is necessary to assess their health with the purpose of improving the management of these coastal and marine resources throughout the region.

Designing a suitable monitoring program that would accommodate the various degrees of complexity encountered in the MBRS Region, in terms of technical, social and cultural needs, is an enormous challenge, not only because of the physical size of the project area but also because there are currently several worthwhile monitoring efforts at several levels in the region. However, because many of these programs are being conducted using a diversity of methodologies, it has not been easy to compare results among programs or among countries.

The MBRS Regional Synoptic Monitoring Program (SMP) was therefore designed as a regional multi-level methodology to monitor changes in ecosystem health for management purposes. Some of the answers required for management, however, require information in different time lines: short, medium and long-term. The SMP is thus attempting to provide advice consistent with these time lines.

We further acknowledge that several useful protocols for the region already exist, such as CARICOMP and AGRRA, which have also been designed specifically for this region. In the interest of avoiding duplication of efforts and optimizing resources, we have meticulously reviewed these protocols, as well as several others from the region and elsewhere, with the intention of selecting the best currently available methods to cover those attributes of ecosystem health for the MBRS, identified during the various technical consultations for the SMP. Utilizing aspects of existing methodologies also provides the opportunity for closer collaboration between other programs and groups already working in the MBRS.

The main SMP Technical Consultations include the First MBRS Meeting of the SMP Technical Working Group in Tegucigalpa, Honduras (August, 2001); the Expert Meeting on the SMP in Cancún, México (May, 2002), which was attended by over 35 national and regional scientists, covering the areas of coral reef ecology and associated ecosystems, marine pollution and physical oceanography/models; and finally, the Second MBRS Meeting of the SMP Technical Working Group, which took place in Flores, Petén, Guatemala (June, 2002).

We hope that this ***Manual of Methods for the MBRS Synoptic Monitoring Program*** will be found valuable by those who will use it. We would appreciate being informed of any problems encountered in its use, as well as any suggestions on how to improve it. Please send any comments to the SMP Regional Coordinator, at the MBRS Project Coordinating Unit (PCU).

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This ***Manual of Methods for the MBRS Synoptic Monitoring Program*** was compiled and edited by Patricia C. Almada-Villela, Regional Coordinator of the Synoptic Monitoring Program at the MBRS PCU, who also led the activities and teams that worked on the three core themes of the SMP. These teams were: Peter Sale from the University of Windsor, Canada, who led the Consortium UNU-INWEH that carried out the regional study on Coral Reef Ecology and Associated Ecosystems; Gerardo Gold-Bouchot, from CINVESTAV, Mexico conducted the regional studies for Marine Pollution; and Björn Kjerfve from the University of South Carolina, USA and his team are conducting the regional study for Physical Oceanography / Models. At the time of preparation of this manual, Björn Kjerfve and his group were developing the Nested 3-D Oceanographic Model for the MBRS Region on behalf of the MBRS Project.

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1. INTRODUCTION

The Mesoamerican Barrier Reef System (MBRS), extending from Isla Contoy on the north of the Yucatan Peninsula to the Bay Islands of Honduras, includes the second longest barrier reef in the world (Maps 1.1 and 1.2). It is approximately 1,000 km long and spans over four countries and two trans-boundary areas: Chetumal Bay, between Belize and Mexico; and the Gulf of Honduras, between Belize, Guatemala and Honduras. The MBRS is unique in the Western Hemisphere due to its length, composition of reef types, and diverse assemblage of corals and related species. The MBRS contributes to the stabilization and protection of coastal landscapes, maintenance of coastal water quality, and serves as breeding and feeding grounds for marine mammals, reptiles, fish and invertebrates, many of which are of commercial importance. The MBRS is also of immense socio-economic significance providing employment and a source of income to an estimated one million people living in adjacent coastal areas.

The goal of the Mesoamerican Barrier Reef System Project is to enhance protection of the unique and vulnerable marine ecosystems comprising the MBRS, and to assist the countries of Mexico, Belize, Guatemala and Honduras to strengthen and coordinate national policies, regulations, and institutional arrangements for the conservation and sustainable use of this global public resource. The Project is part of a long-term Program to safeguard the integrity and continued productivity of the MBRS. The MBRS initiative is being actively promoted by a variety of donors and partners in the region and within the context of the Mesoamerican Biological Corridor Program.

The objectives of the MBRS Program, agreed to by the four participating countries, are to: a) strengthen Marine Protected Areas (MPAs); b) develop and implement a Regional Environmental Monitoring and Information System that will provide a synoptic view of the health of the MBRS and facilitate dissemination of these findings throughout the region; c) promote measures which will serve to reduce non-sustainable patterns of economic exploitation of the MBRS, focusing initially on the fisheries and tourism sectors; d) increase local and national capacity for environmental management through education, information sharing and training; and e) facilitate the strengthening and coordinating of national policies, regulations, and institutional arrangements for marine ecosystem conservation and sustainable use. The second objective, Regional Environmental Monitoring and Information System, is in turn divided into two sub-components: i) the Creation and Implementation of a Distributed Regional Environmental Information System (REIS) and ii) the Establishment of a Synoptic Monitoring Program (SMP).

The MBRS Project is funded by the Global Environmental Facility, is implemented by the World Bank, and is executed by the CCAD-SICA on behalf of the governments of Belize, Guatemala, Honduras and Mexico.

Background to the Synoptic Monitoring Program (SMP)

The Mesoamerican Region is under increasing pressure from a variety of anthropogenic sources such as coastal and tourism development; pollution from point and non-point sources, such as from excess nutrients from agricultural run-off, coastal aquaculture, shrimp farming and domestic waste on the reef; over-fishing; increased tourism activities such as boating and golfing (which results in run-off of herbicides) and other inappropriate use of its resources.

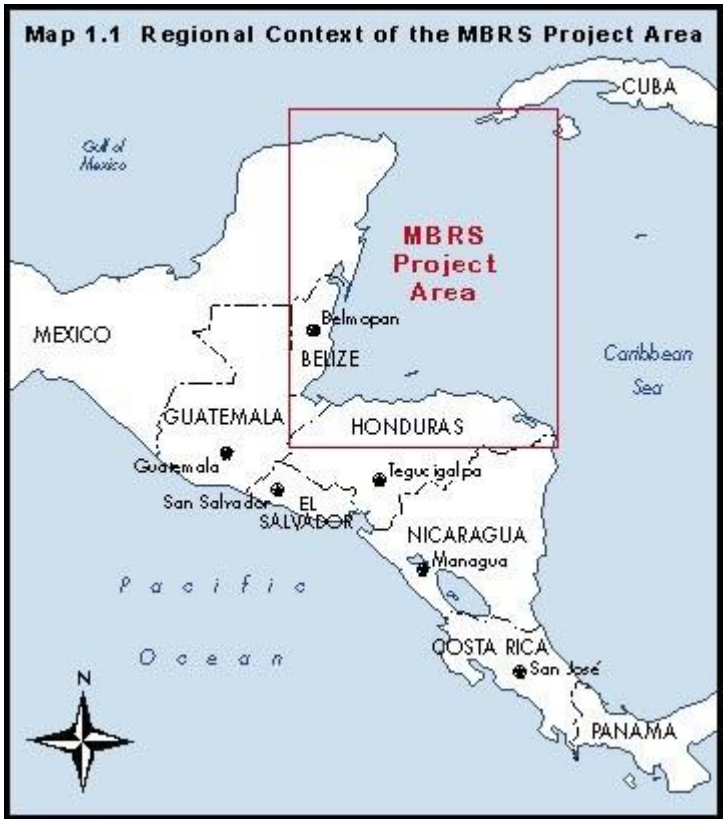
Additionally, it is subjected to periodic apparently natural phenomena including episodes of warmer temperature, flooding (with resulting sedimentation), bleaching, outbreaks of disease, storms and hurricanes. Some of the human activities may be aggravating the impacts of these natural events, resulting in an inability of the ecosystems to recover as rapidly as they might have done under natural circumstances. It has therefore become increasingly important to measure the 'health' of the various ecosystems in the MBRS in order to establish, as far as possible, the nature and extent of changes, the causes of those changes, and the potential solutions.

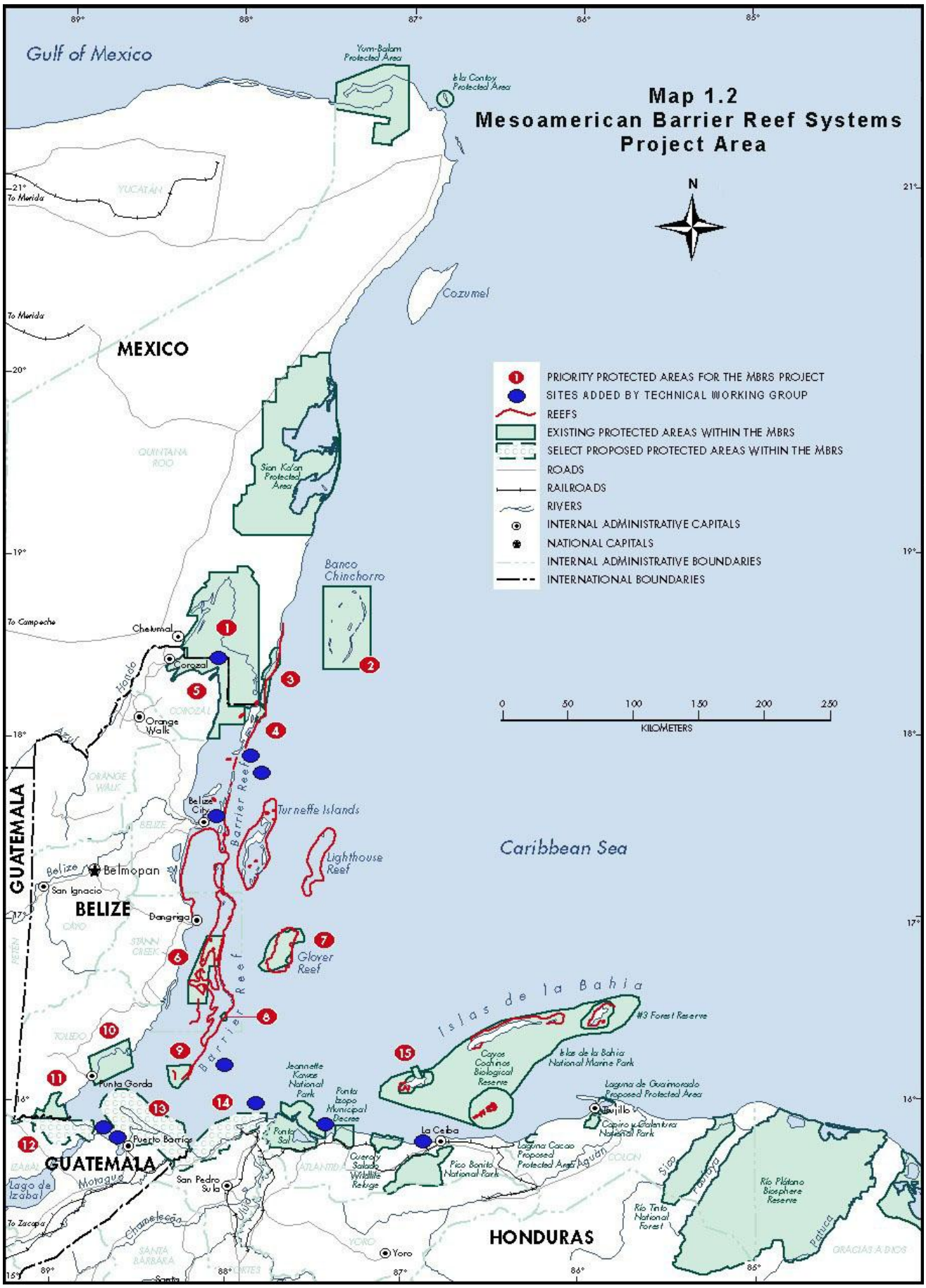
The Synoptic Monitoring Program (SMP) has been developed to try to answer some of the questions on the health of the reef and its associated ecosystems, in order to assist in the management of this unique and shared resource.

The SMP evolved through a consultative process, in which nationals from Belize, Guatemala, Honduras and Mexico, as well as regional and international scientists and field biologists were involved. The main consultations included Technical Working Groups, with specialists from each MBRS participating country, an Expert Meeting and specialist consultations.

In addition, there were three key regional consultancies: Coral Reef Ecology and Associated Ecosystems; Marine Pollution and Physical Oceanography/Models, which were supported by National Consultancies (except for the section of Coral Reef Ecology for Mexico). At the time of preparation of this manual, the regional and national Consultancies for Physical Oceanography were still ongoing. Under this consultancy, A nested 3-D MBRS Ocean Model is being developed under these consultancies as a regional tool, to assist in our understanding of the pattern of currents, and the dispersion of biological components such as propagules, coral and fish eggs, larvae, nutrients and pollutants.

Finally, there have been numerous consultations with scientists and coral reef workers in the four countries, the Caribbean region and elsewhere. The SMP has certainly benefited from this collective experience.




Map 1.2
Mesoamerican Barrier Reef Systems
Project Area
N
Gulf of Mexico
Yum-Balam Protected Area
Isla Contoy Protected Area
YUCATAN
To Merida
MEXICO
Cozumel
QUINTANA ROO
Sian Ka'an Protected Area
Banco Chinchorro
To Campeche
Chetumal
Corozal
Orange Walk
Belize City
Belize
Belmopan
San Ignacio
BELIZE
Dangriga
Barrier Reef
Turneffe Islands
Lighthouse Reef
Glover Reef
Caribbean Sea
GUATEMALA
Punta Gorda
Puerto Barrios
Jeannette Kawas National Park
Punta Izopo Municipal Decree
Punta Sal
La Ceiba
Islas de la Bahia
#3 Forest Reserve
Cayos Cochinos Biological Reserve
Islas de la Bahia National Marine Park
Laguna de Guaimoreto Proposed Protected Area
Trujillo
Capiro y Calentura National Park
Laguna Cacao Proposed Protected Area
Cuero y Salado Wildlife Refuge
Pico Bonito National Park
Rio Tinto National Forest
Rio Plátano Biosphere Reserve
HONDURAS
Lago de Izabal
Motagua
San Pedro Sula
Chamelecón
Ulua
Aguán
Yoro
Santa Barbara
To Zacapa
PRIORITY PROTECTED AREAS FOR THE MBRS PROJECT
SITES ADDED BY TECHNICAL WORKING GROUP
REEFS
EXISTING PROTECTED AREAS WITHIN THE MBRS
SELECT PROPOSED PROTECTED AREAS WITHIN THE MBRS
ROADS
RAILROADS
RIVERS
INTERNAL ADMINISTRATIVE CAPITALS
NATIONAL CAPITALS
INTERNAL ADMINISTRATIVE BOUNDARIES
INTERNATIONAL BOUNDARIES
0 50 100 150 200 250
KILOMETERS

1.1 OBJECTIVES OF THE SYNOPTIC MONITORING PROGRAM

The MBRS Synoptic Monitoring Program (SMP) is a long-term regional endeavor involving the countries of Belize, Guatemala, Honduras and Mexico, to gather data and information on the health of the coral reefs and various key associated ecosystems and species in the Mesoamerican Region, in the short, medium and long-term, in order to provide a sound basis for their management.

Development of the Synoptic Monitoring Program
The SMP utilizes a multidisciplinary approach to assist in the understanding of some of the key processes that are taking place in the MBRS Region, in order to provide advice useful for managers. The SMP has been designed as a long-term monitoring effort that includes physical and biological components, as reflected in its core themes:

a) Coral Reef Ecology and Associated Ecosystems, which include: mangroves, seagrasses and other coastal wetlands;

b) Marine Pollution: from land and marine sources; and

c) Physical Oceanography: understanding the ocean circulation and the complex gyres in the MBRS Region and the reef lagoon.

The SMP has therefore been developed as a framework for:

- Regional synoptic measurements of the health of the coral reef and its associated ecosystems through time
- A regional environmental database that will facilitate assessments of the entire region
- Provision of information and support to management decisions throughout the region.
- Improvements to the understanding of ecosystem processes.
- Provision of opportunities for individuals or groups in the region to conduct monitoring activities that will result in raised awareness of environmental issues in the coastal and marine environments in the region.

How will it be done?

- By harmonizing and standardizing the monitoring methodologies currently being used in the MBRS Region
- By utilizing monitoring methodologies that are simple and accessible to a large number of people in the region
- With the collaboration of the MBRS Support Agencies that will be the main actors in the Synoptic Monitoring Program

1.2 THE ROLE OF THE SMP SUPPORT AGENCIES

The MBRS is a large marine ecosystem complex that requires a multidisciplinary approach. A great deal of organization is also required to successfully undertake a regional monitoring program such as the SMP. In order to assemble a reliable and strong regional monitoring team, we have enlisted the collaboration of the SMP Support Agencies, which are a mixture of government agencies, NGOs, academia, and civil associations interested in one or several of the objectives and/or themes of the SMP. Their assistance is vital to the SMP as they are the main providers of the human resources for the actual monitoring program. They can also aid the SMP as sources of data and information or by offering logistic support. Thus, the potential roles of the Support Agencies are as follows:

Sources of Data and Information

- Provision of data relevant for the MBRS as available within the Support Agency

- Assistance in obtaining data or literature relevant to the SMP from other institutions
- Provision of maps and satellite images for geographic areas relevant to the MBRS
- Support with the analyses of data and/or images

Providers of Logistic Support

- Assistance in organizing field work
- Use of equipment and boats available to the Support Agency in order to carry out the SMP. The equipment may be for diving, laboratory analyses or data processing
- Use of vehicle for local transport to the monitoring sites
- Use of trained personnel, such as field staff, guides, etc.
- Use of living quarters for SMP personnel during field visits

Providers of Monitoring Expertise

- Use of trained personnel to form the monitoring teams for the MBRS
- Support in training trainers so that they may continue training others in their countries. Such training may be in species identification, use of laboratory equipment, data analyses, interpretation of results, safety in the field, first aid or other related fields
- Validation of monitoring data
- Data analyses

Mutual cooperation among SMP Support Agencies will lead to better understanding of the processes involved in the health of the MBRS. In order to cover the key sites and habitats in the MBRS, the SMP needs to count on the decided and enthusiastic collaboration of its Support Agencies in the region. Given the enormous nature of this undertaking, the collaboration of these Support Agencies is crucial.

1.3 DEVELOPMENT OF THE SMP METHODOLOGY

The lack of a suitable harmonized and standardized methodology to measure changes in the health of coral reef ecosystems has been one of the main difficulties in understanding the complex interactions occurring in such environments. Such limitation has also been a major impediment in the comparison of results between different programs.

There is a great deal of interest in developing suitable monitoring methodologies that can be utilized over large geographical areas, not only at MBRS but also in the larger Caribbean Region and elsewhere. To this effect, there have been several regional efforts in the past that have produced useful protocols that are applicable to the MBRS Region. Such worthwhile protocols include those developed by the Atlantic and Gulf Rapid Reef Assessment (AGRRA, 1999 and 2002), the Caribbean Coastal and Marine Productivity (CARICOMP, 2001), the Caribbean Planning for Adaptation to Climate Change (CPACC, 2000) and the Mexican Monitoring Protocol for Marine Protected Areas in Mexico, currently being developed (García Salgado, in prep.). The Long-term Monitoring Program of the Australian Institute of Marine Sciences, which holds an impressive amount of data spanning over 10 years, is probably the best example of a current monitoring program in that it is large-scale and sustained over many years (Halford and Thompson, 1994; Bass and Miller, 1998; Page *et al*. 2001). Other efforts have focused on assessments that can be conducted very simply at the community level; such protocols include REEFCHECK and REEF, both of which have achieved an increasing degree of success.

This manual presents the chosen methodologies for the MBRS Synoptic Monitoring Program at this moment in time, and reflects the identified needs for a regional SMP and the availability of resources, both in human and economic terms. It should therefore be viewed as a dynamic document that will continue to grow as we further our understanding of monitoring methods and as we develop new approaches, technologies and capacities to measure changes in environmental health.

Each section is autonomous to allow the user to easily focus on the task at hand; thus, the coral and mangrove sections are independent and self-contained. Recognizing the value of existing methodologies, we have made use of some of them giving full credit to the original sources.

The SMP Monitoring Manual has been designed to respond to the management needs of the MBRS Region. We recognize that different questions require different levels of monitoring intensity, data and detail and that no single methodology necessarily addresses all aspects deemed relevant. We also recognize that some Sites are more easily accessed, or are considered of higher priority to monitor than others, and could be monitored several times a year. These more frequently monitored Sites will provide more detailed information on temporal changes, and can be monitored in ways that require more frequent access to the Site. We further recognize that there will be occasions when disturbances happen in particular places, e.g. due to storms, ship groundings, outbreak of coral diseases, when it will be important to carry out a rapid assessment of the severity of the impact, and that such rapid assessment capability should also be a component of the SMP. It is therefore recommended that the MBRS SMP be carried out using four different levels of monitoring that will be termed Monitoring Categories. Thus:

1.4 THE SMP MONITORING CATEGORIES

- **Category 1:** This is the baseline level of monitoring and so it utilizes the smallest set of parameters of all the categories. It is expected therefore that more Sites can be monitored in this category and also that baseline surveys can be conducted more frequently than those for Category 2 and Category 3 Sites. For instance Category 1 monitoring at Sites that are located within easy reach of field teams (e.g., Calabash Caye) can therefore receive a high level of

monitoring effort. Category 1 will include the set of **core** parameters **plus** the **specific** parameters for each particular ecosystem. Category 1 sampling should occur at least one a year but may occur up to **six times a year,** if resources allow it for particular Sites.

- **Category 2:** Medium-term analyses of all Category 1 parameters plus an additional set of **selected** parameters. This monitoring will occur **four times per year** at a subset of sites termed Category 2 Sites. These were chosen as of highest priority for monitoring. At Category 2 sites, additional parameters will be measured to track changes in such things as coral mortality, and water quality over shorter time scales.

- **Category 3:** This monitoring will occur **once annually**, at selected Sites in the region. It will include the **full** suite of parameters (**Categories 1 plus 2**), **plus** a selection of measurements over varying depths (corals and seagrasses) or increased spatial cover and productivity (seagrasses and mangroves) that can be measured simply and inexpensively. It will provide data for long-term (e.g. 5 to 15 years) analyses of trends in coral cover, fish recruitment, and spatial extent of seagrass beds, among other indices of environmental 'health'.

- **MBRS Rapid Assessment Method:** This monitoring is used to assess the effects of specific disturbances, such as hurricanes, flooding, oil spills, or other disturbances, and provide immediate management advice concerning the severity of the event. This Category will use a subset of the Category 1 and 2 methods as appropriate to measure impacts of the particular disturbance that has occurred. This rapid assessment method will take place at sites impacted by the disturbance, whether or not those sites are included among the regularly monitored Category 1 or 2 Sites.

Data and information from all Categories above will provide management information in the long-term as we continue to accrue knowledge on the ecosystems and species in the MBRS Region.

1.5 APPLICATIONS TO MANAGEMENT

It is intended that the SMP will serve to measure effects of management actions taken, whether these actions are taken in response to detected changes in 'health', or are taken in an effort to mitigate or eliminate impacts of anticipated pressures.

The applications to management that we envisage include the following:

1. Establishing much needed baseline data on the condition of key reef, mangrove and seagrass sites; and on the status of pollution and oceanographic conditions, on which to build a picture of the health of the MBRS Region, with particular attention to potential transboundary issues affecting its sustainable management.

2. Establishing the extent and patterns of decline in key ecosystem components; e.g. corals, fish, seagrasses, and identifying processes causing those trends.

3. Measuring the impacts of anthropogenic activities within the region, e.g. impacts of coastal development such as shrimp farms, agricultural run-off, tourism activities, over-fishing, sedimentation, extraction of resources, etc.

4. Recording the impacts of natural events, e.g. hurricanes, flooding, bleaching, and disease.

5. Establishing a regional database on environmental resources in the MBRS region, including ground-truthing of habitat maps.

6. Demonstrating linkages between land-based activities and coastal health, providing the necessary baseline information on suitable management actions, and monitoring the effectiveness of those actions.

7. Guiding the development of management plans and zonation, and monitoring the effectiveness once those policies are implemented.

8. Identifying new potential sites for MPAs while assessing the ecological effectiveness of existing ones.

2. THE MBRS SYNOPTIC MONITORING PROGRAM

This manual focuses on methodologies considered to be the best group of techniques to address the needs of the MBRS. We feel confident that such methods will allow us to cover the main issues relating to the SMP's core themes (coral reefs and associated ecosystems; marine pollution and physical oceanography). The manual has been divided into Sections that reflect this basic philosophy. Other taxonomic groups were identified at the Technical Working Groups and Expert Meeting, including aquatic mammals (cetaceans, and manatees), coastal and marine birds, reptiles (marine turtles and crocodiles), crustaceans (Penaeids, lobster), mollusks (conch) and holothurians. Similarly, it was suggested that the SMP be expanded to include some fisheries data such as standing stock, density, sizes, population structure, that could yield useful information on the status of commercially important species of finfish. Such expanded monitoring effort is outside the initial scope of the SMP and would require considerably more effort and resources.

It is hoped that it may be possible to expand the SMP to include more ecosystems or taxonomic groups in the future. Agencies or NGOs monitoring different taxonomic groups and ecosystems will have the opportunity of participating in this regional effort through the establishment of data sharing agreements.

2.1 MONITORING PARAMETERS

It was agreed at the First and Second Technical Working Groups (August 2001 and June 2002) and at the Expert Meeting (May 2002) that water quality assessment is important throughout the region. We propose a set of core physical-chemical parameters to be measured at all sites on every monitoring visit, as well as suites of parameters to be measured at each site that will depend on the nature of the habitat present there. Further details are given under the relevant Chapter.

Parameters to be Measured for the Various Categories
The number of parameters, or spatial coverage, will increase from Category 1 to Category 3 Sites in a cumulative manner. All Sites will be monitored once annually and coincidentally for a suite of parameters according to the Category assigned to them. The parameters will remain the same at all times and for each Category. Rapid Assessment Monitoring (RAM) will use a subset of appropriate measurements that will vary depending on the nature of the event that triggers the RA mobilization. Table 2.1 contains a summary of the procedure for the standardized SMP. Definitions for Locations, Ecosystems, Habitats and Sites are given in Section 2.2 (*Terminology to Define Sites for the SMP)*.

Parameters to be measured comprise four types of data: a) Site description, b) Meta data; c) Physical data; and d) Specific parameters. Site description is also covered under Section 2.2. Meta data comprises data such as the date and time of data collection, the person responsible for data collection. Physical data is core data collected **at every Site and at every visit** and comprises measurements of parameters such as water and air temperature, salinity, pH, dissolved oxygen, weather conditions, sea state, light, turbidity, sedimentation, nutrients and depth range. Specific parameters comprise the detailed measurements for the ecosystem being studied. Further details of these can be found in Sections 3.1, 3.2 and 3.3.

The SMP Monitoring Schedule
There are numerous variables in tropical environments, such as rainy and dry seasons, seasonal species abundance and distributions and variable weather conditions. It is therefore imperative that the SMP be based on a monitoring schedule that is uniform across Locations. Monitoring surveys in the SMP will therefore be conducted at an agreed time and surveys will be constrained to a short time interval, occurring at the same time interval in each successive year (Table 2.1). Please note that monitoring for Physical Oceanography/Models will mostly be conducted automatically and continuously with the aid of some oceanographic instruments. This monitoring schedule, therefore, does not apply to such measurements.

Table 2.1 Table of Standardized Procedures for the MBRS-SMP for Coral Reefs, Seagrasses, Mangroves and Pollution. Full descriptions are included under each relevant Chapter

A: Coral Reef Sites

Category	Sites	Parameters	Frequency	Time Window
C_1	All Sites	**Set of core parameters** Video panoramic view or manta tow. **Specific parameters for coral communities:** • Percentage algal cover • Percentage coral cover • Abundance of selected fish and invertebrate species • Coral mortality index and condition	Minimum of once a year at all Sites A subset of Sites to receive more intensive monitoring	June 1-July 31 for annual surveys All year
C_2	Specific Category 2 Sites	As for Category 1, plus: • Coral recruitment • Algal production • Water chemistry • Sedimentation	Once every 3 months	March September December (middle 2 weeks of each listed month)
C_3	Specific Category 3 Sites	As Category 2, plus: • Increase in spatial coverage	June 1 - July 31	Once a year; at the same time in each successive year

B: Seagrass Sites

Category	Site	Parameters	Frequency	Time Window
C_1	All Sites	**Set of core parameters** **Specific parameters for seagrasses** • Percentage seagrass cover • Abundance • Species composition • Standing crop & biomass	Twice per year	June and December
C_2	Specific Category 2 Sites	As Category 1 plus: • Increased seasonality (frequency) • Spatial coverage • Growth (new leaf biomass)	Y1: Every 3 months Y2: Every 6 months	March September December
C_3	Selected Category 3 Sites	As Category 2 plus: • Productivity • Leaf area index • C:N:P content	Once per year	June1-July 31

C: Mangrove Sites

Category	Site	Parameters	Frequency	Time Window
C_1	All Sites	**Set of core parameters** **Specific parameters for Mangroves:** • Forest characterization/zonation • Establishment of plots • Trunk dbh and height • Community description	Once per year	June 1-July 31

		• Abundance and percentage cover **Seedlings and saplings (growth):** • Establishment of subplots • Growth		
C_2	Subset of Sites	As for Category 1 plus: • Increase in spatial cover and biomass	2 x year (dry and wet seasons)	June and December
C_3	Subset of Sites	As Category 2 plus: • Leaf area index • Productivity (*Rhizophora mangle* only) • Litter fall collection • Nutrients	**Leaf litter:** Y1: monthly; Y2: every 3 months, to eventually reduce to 2 x year **Nutrients:** Every 3-6 Months	June1-July 31 March June September December

D: Rapid Assessments

Rapid Assessment	Any Sites following a perceived disturbance	**As for Category 1, plus specific impact to be assessed, e.g.:** • Physical damage (broken corals as % of coral cover) • Sediment load • Debris from storms or flooding • Oil spills	Intermittent. A rapid response to a disturbance	Within 1 week of event

E: Pollution Sites

Category	Site	Parameters	Frequency	Time Window
C_1	All Pollution Sites	**Set of core parameters** **Specific parameters for pollution:** • Cholinesterase activity • PAH metabolites • Organochlorine pesticides • Bioaccumulation studies	Year 1: monthly **Sediments:** once a year Year 2 and after: Seasonal* (rainy & dry, cold fronts)	July 1 – Aug 30
C_2	Specific Category 2 Sites	As for Category 1 plus: • Increased seasonality (frequency) • Spatial coverage • Temporal and spatial variation	Year 1: monthly **Sediments:** once a year Year 2 and after: Seasonal* (rainy & dry, cold fronts)	July 1 – Aug 30 April – May
C_3	TS plus selected Sites, e.g. MPAs	As for Category 2 plus: • Increased seasonality (frequency) • Spatial coverage • Temporal and spatial variation • PAH analysis on sediments and other biomarkers e.g. vitellogenin in plasma and histopathology (to be sent away for analysis in certified laboratories).	Years 1 to 3: monthly **Sediments:** once a year. Year 4 and after: Seasonal* (rainy and dry seasons, cold fronts)	**Wet:** July 1 – Aug 30 **Dry:** Apr – May **Cold Fronts:** Dec – Jan October* - Peak period for freshwater discharge

**** = Rainy and dry seasons may vary slightly along the latitudinal range in the MBRS***

Table 2.2 shows examples of potential combinations for monitoring Categories and Locations. It will be helpful for Support Agencies to develop their own matrices with their Locations and Sites.

Table 2.2 Example of SMP Monitoring Locations and their Corresponding Monitoring Category

Monitoring Categories	Monitoring Locations											
	Location 'A'			Location 'B'			Location 'C'			Location 'n'		
	C	M	S	C	M	S	C	M	S	C	M	S
C_1									√	√		√
C_2				√	√	√		√			√	
C_3	√	√	√				√					

C = Coral reefs; M = Mangroves; S = Seagrasses

2.2 SELECTION OF THE SMP PRIORITY MONITORING LOCATIONS

There are large variations in the size, types and diversity of ecosystems and habitats throughout the MBRS region. The currently identified Monitoring Locations for the SMP were selected by members of the SMP Monitoring Technical Working Group in Tegucigalpa, Honduras in 2001. The selection process took into account ecosystems present in the locations as well as their relative importance in terms of the three SMP Core Themes and include a mixture of MPAs and strategic sites, chosen because of their proximity to the MBRS Transboundary Areas. Monitoring at these Locations must include at least two replicate monitoring Sites, but many will require more than that because they are extensive and contain several habitats. Site selection needs to be carefully done.

During the revision of this manual, it was evident that the two MBRS Transboundary Areas should be specifically included in the list of Monitoring Locations for the SMP. Therefore, Chetumal Bay and the Gulf of Honduras, including the areas of Bahía de Amatique and all the main rivers feeing into it, have been dully added to Table 2.3. The new total number of Monitoring Locations for the SMP is thus 25.

Selection of Monitoring Locations by the Support Agencies
Each SMP Support Agency will be able to decide which SMP Priority Locations and/or Sites they will monitor and at what Category or level of effort. However, a concerted effort **needs to** be made so that all SMP Locations **are actually** monitored. Prior to the launch of the Synoptic Monitoring Program, each MBRS Support Agency will submit to the MBRS-PCU, a list of Locations and Sites for which it will have monitoring responsibility and which Monitoring Categories they will use. These Sites must be **permanent** monitoring Locations for the SMP, or at least be fixed, so that while they can move a Site from Category 1 to Category 2 or Category 3, they cannot easily do the reverse. An example of such potential combinations is included in Table 2.4.

Table 2.3 Priority Monitoring Locations Identified for the MBRS Synoptic Monitoring Program

Country	Monitoring Locations	Status
Belize	Bacalar Chico	MPA*
Belize	Corozal Bay	MPA*
Belize	South Water Caye	MPA*
Belize	Glover's Reef	MPA*
Belize	Gladden Spit	MPA*
Belize	Sapodilla Caye	MPA*
Belize	Port Honduras	MPA*
Belize	Belize River	SS
Belize	Hol Chan	MPA
Belize	Caye Caulker	MPA
Belize	Sarstoon-Temash	MPA*
Guatemala	Río Sarstún	MPA*
Guatemala	Punta de Manabique	MPA*
Guatemala	Río Dulce	SS
Guatemala	Bahía Santo Tomás	SS
Honduras	Puerto Cortés	SS
Honduras	Omoa-Baracoa	MPA*
Honduras	Turtle Harbor	MPA*
Honduras	Tela	SS
Honduras	La Ceiba	SS
Mexico	Santuario del Manatí	MPA*
Mexico	Banco Chinchorro	MPA*
Mexico	Arrecife de Xcalak	MPA*
Mexico-Belize	Bahía de Chetumal	TS
Belize-Guatemala-Honduras	Golfo de Honduras	TS

SS = Strategic Site; * = MBRS priority MPA; TS = Transboundary Site

Table 2.4 Examples of Potential Selection of SMP Monitoring Locations by the Support Agencies and the Monitoring Category

Support Agencies	Number of Monitoring Locations		
	Category 1	Category 2	Category 3
Support Agency 'A'	**6**	**4**	**3**
Support Agency 'B'	**10**	**5**	**2**
Support Agency 'C'	**3**	**2**	**1**
Support Agency 'D'	**4**	**2**	**0**
Support Agency 'n'	**2**	**0**	**0**

The list of SMP Locations listed in Table 2.3 can, and indeed should, be expanded to include specific Locations and/or Sites of interest to the Support Agencies and which easily fit into the SMP. We will however, suggest that the selection of any new Locations be made in consultation with the PCU and other relevant agencies to enhance ecosystem representation in the SMP. Locations that have already been highlighted during the preparation of the SMP and discussions with participants of the four countries during the Training Course in Monitoring Methodologies in Belize, November 2002 are included in Table 2.5. We encourage the inclusion of these Locations as part of the SMP in any future monitoring efforts in the region.

Table 2.5 List of Potential Locations to Receive Priority for Inclusion in the SMP

Country	Recommended Locations	Status
Belize	Gallows Point	SS
Belize	Goffs Caye	SS
Belize	Caye Chapel	SS
Belize	Ragged Caye	MPA
Belize	Lighthouse Reef	MPA
Belize	Turneffe Atoll	MPA
Belize	Laughing Bird Caye	MPA
Honduras	Cayos Cochinos	MPA
Honduras	Roatán	MPA
Honduras	Guanaja	MPA
Honduras	Río Aguán	SS
Honduras	Biósfera de Río Platano	PA
Honduras	Laguna de Caratasca	PA
Mexico	Isla Contoy	MPA
Mexico	Cancún	MPA
Mexico	Puerto Morelos	MPA
Mexico	Cozumel	MPA
Mexico	Sian Ka'an	MPA
Mexico	Akumal	SS
Mexico	Majahual	SS

SS = Strategic Site; * = MBRS priority MPA; PA = Protected Area

The SMP Monitoring Teams

Initially, the SMP Monitoring Teams will largely consist of a mixture of members from the SMP Support Agencies in the four countries. Consequently, it is very likely that the level of monitoring experience will vary among members. We suggest, therefore, that the SMP be conducted at two different levels: a) **General** – suitable for field personnel, and b) **Advanced** – suitable for researchers handling data analyses and interpretation of results.

In addition, there will be **Monitoring Coordinators** in each country who will be responsible for supervising the Monitoring Teams and activities *in situ*. They will also have responsibility for validating the field data sheets and for liaising directly with the PCU.

It will be important to establish a management structure for the monitoring program that will facilitate communication among individuals and agencies engaged in monitoring in different parts of the region. This structure will facilitate the making of decisions on procedures, on methodological questions, and so on, and will be a vital factor in maintaining the integrity and uniformity of the monitoring program. During the first year, it will be a sound strategy to use the data collected as pilot data to ensure that decisions on replication and on field methods have been correctly made.

Terminology to Define Sites for the SMP

The SMP will follow the basic terminology and procedures of Woodley (1999), except that it will use '**Location**' instead of '**Area**'. Thus, each '**Location**' will have one or more '**Ecosystems'** which in turn will have one or more '**Habitats**' to be monitored; within each '**Habitat**' there will be a number of potential '**Sites**'. At each selected '**Site**', monitoring activities will be carried out. This terminology will be applicable to all habitats in the SMP, i.e. coral reefs, mangroves and seagrasses.

Ideally, Sites should be selected using a formal stratified random design as there are important statistical advantages that derive from this. However, it is recognized that many Locations will already have some degree of management or may be subjected to management in the future. In these cases, and in order to avoid duplication of efforts between projects or programs, every effort should be made to include those specific Sites that are in use by ongoing monitoring projects, or are of particular interest to local management for other reasons. Such strategic decisions will require the full collaboration of all parties involved.

Selection of Monitoring Sites

For the purposes of the SMP, LOCATIONS contain ECOSYSTEMS, within which SITES are selected for monitoring. A LOCATION might be ~10-100 km in extent, while a **SITE** (~0.2 km scale) is the area readily accessible from the anchored boat. The SITE's geographic location is the GPS reading where the boat is anchored, and it is important to return to this location on each monitoring visit. SITES are **permanent** monitoring locations. When LOCATIONS include reef ecosystems, the natural patchiness of the reef environment makes Site selection more complex. We recognize that reefs vary greatly in size, complexity, depth, profile, and coverage per km of coastline throughout the MBRS region. What follows are our recommended procedures for selecting Sites, however we fully understand that it may be necessary to modify these procedures to accommodate the special conditions at each Location. It is vital for the success of the SMP that these procedures are followed as closely as possible, and that all modifications to them are carefully noted when the data are compiled. Figure 2.1 shows a diagrammatic representation of a theoretical Monitoring Location, with Ecosystems, Habitats and Sites.

Site Selection in Reef Environments

Where a Location contains reef ecosystem, an effort should be made to establish two or more Sites in each of the three reef Habitats to be monitored:

1. Shallow, back-reef (leeward) habitats in 1-5m depth
2. Shallow fore-reef (windward) habitats in 1-5m depth
3. Deep fore-reef habitats in 8-15m depth.

Each monitoring Site will include a single Habitat. Judgment must be used in deciding whether two Sites of each Habitat type is sufficient to characterize that Location. This will depend on how extensive the reef environment is at that Location. Remember that the distribution of Sites at the Location should sample the Location representatively. If the Location is a large protected area, with extensive reef environments throughout, it would not be adequately monitored by a couple of Sites in one small corner.

If the extent and/or number of reefs (e.g.: fringing, patch, barrier) is limited it may not be possible to establish more than two Sites in each Habitat, and it may even be that one or more of the reef

Habitats is not present. However, if the extent and/or number of reefs is large, then they should be subdivided or “stratified” and representative examples randomly selected from each subdivision (e.g. the apples, pears, and bananas approach). Use the best, locally available sources of information (benthic maps, aerial photographs, charts, local knowledge, reconnaissance by manta tow-board (see Appendix 1 for details) and/or videography) to obtain a panoramic perspective of the reef. You may also use secondary reef characteristics such as size, depth, and position relative to land. Once reefs are stratified, the idea is to select Sites randomly (unbiased). Give each reef within a subdivision a number and use a random method to select which ones to assess. If there are no clear bases for making subdivisions (e.g.: a continuous bank barrier or fringing reef several kilometers long), then Sites should be located using a numbered grid (each square should be 200 x 200 m) superimposed on delineated reef zones and randomly selecting squares (Sites). When choosing Sites, avoid hardgrounds, pavements and other habitats that lack a framework constructed of reef-building corals, and remember that shallow and deep fore-reef Sites will usually be co-located.

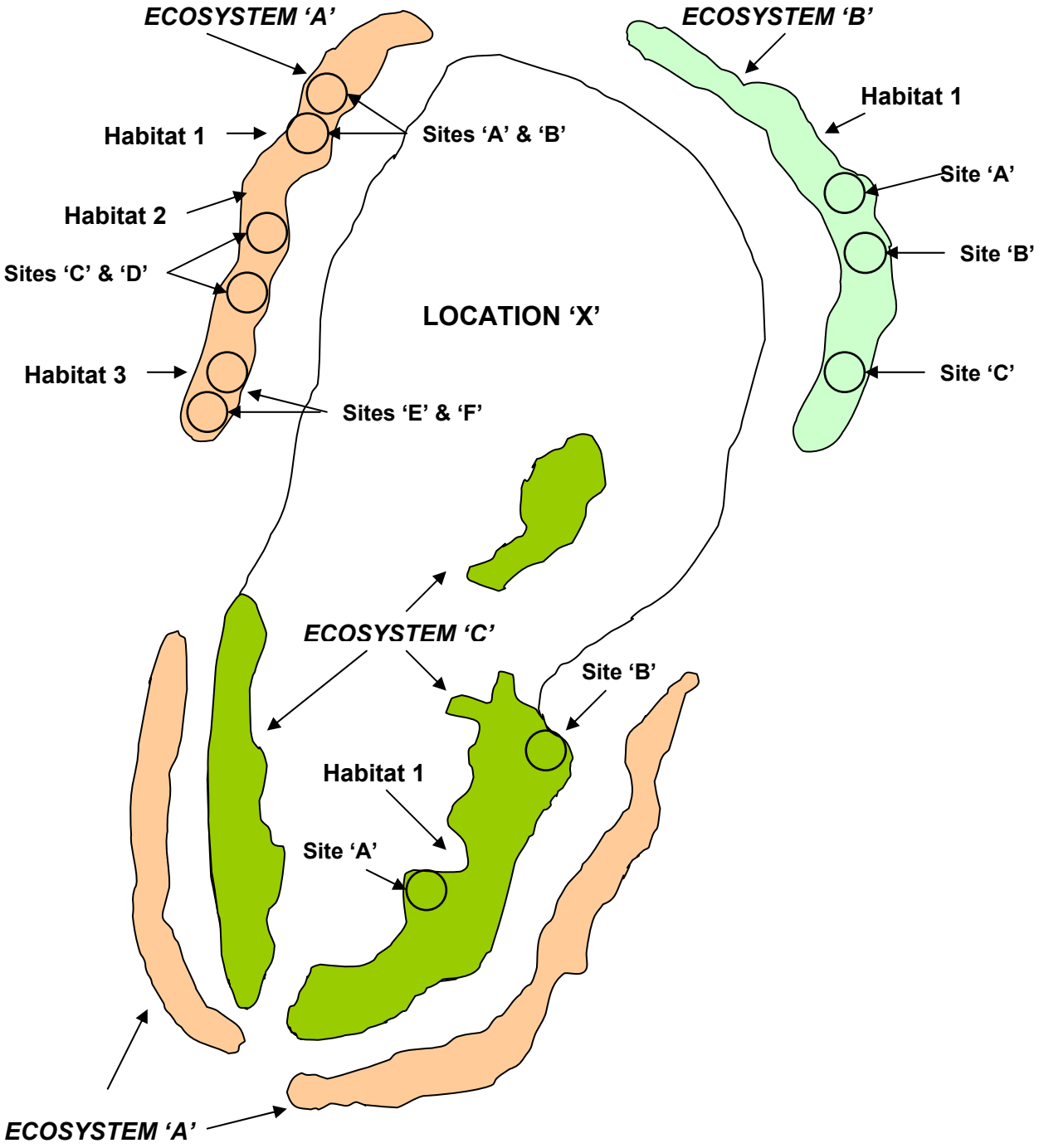


Figure 2.1 Diagram of a SMP Monitoring Location, its Ecosystems, Habitats and Sites. Ecosystem 'A' = Coral reefs; Ecosystem 'B' = Seagrass bed;' Ecosystem 'C' = Mangrove forest. Please note that monitoring will occur in 3 habitats for coral reefs (see Section on Site Selection).

Depending on the methods and resources available, Sites that are selected will generally fall into one of three groups:

1. Unbiased- chosen based on a random sampling strategy (the preferred method);
2. Strategic- chosen with local knowledge because they are threatened, suspected to be degraded, or in particularly good condition, or because they are currently being monitored through another program.
3. Representative- chosen with local knowledge to be representative of reefs in that area.

For regional comparisons, it is best to have Sites that are chosen randomly (1) Sites that are chosen strategically should be clearly flagged as such.

Site Selection for Pollution Monitoring
Within the SMP, monitoring of pollutants in the MBRS Transboundary Sites (TS), Bahía de Chetumal and the Golfo de Honduras, have received the highest level of priority as they are a) representative of the area; b) contain high biological diversity, both in terms of species and ecosystems; c) receive critical inputs of pollutants from urban and/or industrial areas; and d) are shared by two o more countries, therefore, their sustainable management requires true international cooperation.

Within these TS, representative Sites have been selected (see Table 2.3). For Bahía de Chetumal in particular, the following four areas need to be monitored:

- The northern portion of Bahía de Chetumal (Mexico)
- The area around the mouth of the Rio Hondo and the City of Chetumal (Mexico)
- The south of Corozal Bay (Belize)
- The area around the mouth toward the sea (Belize and Mexico)

In the case of the Golfo de Honduras, the following Sites need to be monitored:

- The outlets of the rivers Dulce and Sarstún
- The area around Puerto Barrios
- The mouths of the rivers Motagua, Chamalecón and Ulúa. Additionally
- Southern Cayes: Glover's Reef, Gladden Spit and Sapodilla, to determine if there is a south to north gradient from Bahía de Amatique
- Turtle Harbor, in the Bay Islands, due to its proximity to the area of influence of the rivers

The selection and incorporation of control Sites will be critical. Such Sites may be within protected areas, far from industrial and urban areas, tourist developments and river outlets.

Number of Replicates for Pollution Monitoring
In each Site at least five replicate stations selected at random should be sampled. In the case of organisms, at least 10 individuals of the same sex and size per site should be collected.

Site Description
A Site description should be prepared for each and all Sites surveyed during the first visit to such Site. Site descriptions will only be done once, unless the Site has suffered significantly from a major event, e.g. storm or flooding. It is critical to explain how the site was selected and to provide an explanation of any possible deviations from the recommended selection protocols. The site description should also include information on:

- Location (GPS coordinates)

- For reef areas: approximate relief, slope, size and shape and relief features (e.g., spur and groove)
- For seagrass areas: proximity to reefs, size and shape of salient features; extent of the habitat
- For mangroves: characterization / zonation, details of habitat types; percentage cover of a) channels; b) open water bodies; c) areas of degraded mangroves; summary of past human impact if known; and location and magnitude of freshwater input
- Orientation (windward, leeward, or both, if wind direction changes seasonally)
- Depth range corrected for tidal variations

For those monitoring sites that are already mapped, it will be useful to record changes in coverage by particular habitats, accompanied by site descriptions. An additional useful activity would be to conduct ground-truthing of habitat maps whenever possible to build up a more accurate picture of the sites. If the acquisition of maps is being considered, useful scales are: 1:50,000 to 1:100,000 for landscape views and 1:5,000 for more detailed work. Aerial photographs 1:5,000 will also be suitable and can provide a baseline to record future changes in habitats.

2.3 DATA COLLECTION, DATA PROCESSING AND DATA ENTRY TO THE REIS

Data should be entered in the SMP data sheets, examples of which are provided at the end of each Chapter. They can also be downloaded from the MBRS web site at: http://ww.mbrs.org.bz. Please hand the completed data sheets to your Monitoring Coordinator (MC), who will be responsible for validating the data, for carrying out the initial data processing and entering the data in to the MBRS REIS. Examples of data validation tasks include ensuring that there are no illegible or missing entries, checking for potential errors and correcting these while the team is still on the Site. If necessary, re-run the transect to correct such mistakes. Common errors include mis-identification of organisms, mislabeling entries, and failure to make particular measurements.

The first level of data checking is done at the Site immediately following the dive or before leaving the field. The second level of data evaluation occurs when the original data are compared against the data entered into the spreadsheet. This should be done by the MC and a second person. A final level of checking will occur at the time data arrive at the MBRS PCU. **It is critical that these checks are carried out every time as stated above.**

Spreadsheets for submission of data are available to the monitoring teams. Such spreadsheets have macros to facilitate simple data entry and calculations such as means and standard deviations. Standard statistical analyses e.g. totals for parameters of interest, comparisons of Sites, will be carried out directly from the REIS. Sample data sheets will be available at the MBRS web site (see above), from where they can be easily downloaded.

At the time of writing this manual, the REIS was nearing completion. The detailed procedure for data entry to the REIS will be made available as soon as possible to the Monitoring Teams and Monitoring Coordinators. Please also visit the MBRS web site for announcements in this regard.

2.4 CORAL REEFS AND ASSOCIATED ECOSYSTEMS IN THE MBRS

There are numerous coastal environments in the Mesoamerican Region including coral reefs, mangroves, seagrass beds, coastal lagoons, estuaries, deltas and rivers among others. Attempting to measure changes in the long-term health of all these environments and/or their faunal and floral components would be a massive task, well outside the scope of the current level of support that the SMP has available to it through its Support Agencies.

Therefore, the ecosystems that have been selected in the First Phase of the Project include:

- **Coral reefs** [shallow, back-reef (leeward); 1-5 m depth; shallow fore-reef (windward) in 1-5 m depth; and deep fore-reef habitats in 8-15 m depth];
- **Mangroves** coastal and fringing
- **Seagrasses** coastal and fringing

The taxonomic groups included at this stage are as follows:

- **Coral communities:** Stony corals, gorgonians, algae, reef fish, sponges, *Diadema* urchins.
- **Mangrove communities:** Red (*Rhizophora mangle*), black (*Avicennia germinans*) and white (*Laguncularia racemosa*) mangroves, and several mangrove-associated species such as *Conocarpus erectus*.
- **Seagrass communities:** Turtle grass (*Thalassia testudinum*), Manatee grass (*Syringodium filiforme*), *Halophila decipiens*, *Halophila engelmannii* and *Halodule beaudettei*.

Parameters to be Measured

The parameters to be measured have been divided into **Core** and **Specific Parameters**. Core parameters are those that will be taken at all sites and at every visit and will be the same for all ecosystems. Specific parameters for corals, mangroves, and so on, are defined under each corresponding section and should be determined in addition to the core parameters. Certain physical measurements are also made at each Site. The user will find full details under each section below.

3. METHODOLOGY FOR CORAL REEF COMMUNITIES

In common with other protocols, the SMP will include measurements on corals, fish, and other selected biota, most of which can be made using transect and/or manta tow procedures (see Appendix 1). In addition, we consider it important to attempt to obtain a general picture of coral community dynamics, so far neglected by most monitoring programs, as well as stress to the ecosystem so that we may build a picture of the health of the coral ecosystem.

Useful attributes to monitor include percentage cover, community composition, size distribution, extent of bleaching, frequency of coral diseases and mortality index. In addition, the relatively low diversity of the Caribbean certainly permits recording coral abundance at the genus level, and we certainly encourage efforts to ensure that all members of the SMP Monitoring Teams are able to record accurately at the species level. We also believe that monitoring coral and fish recruits in selected Sites will give us a useful indication of the health of the ecosystem.

Table 3.1 Table of Standardized MBRS-SMP Procedures for Coral Communities

Category	Sites	Parameters	Frequency	Time Window
C_1	All Sites	**Core parameters:** Date, time of visit. Location name, Site ID, GPS coordinates. Collectors' names. Weather conditions, water and air temperature, rainfall, wind, sea state, salinity, light, turbidity, pH, DO, sedimentation, nutrients (to be analyzed in the laboratory), chlorophyll **a**. General site description (new Sites only), including depth range, relief, slope size, shape, features and orientation. Video panoramic view or manta tow. **Specific parameters for coral communities:** Percent cover of algae (turf, coralline, macro), sponges, gorgonians, coral genera (advance teams = species level). For ~50 coral colonies: genus, species, colony height, average diameter, mortality index (recently and old dead), extent of diseases, bleaching, storm damage. Abundances of selected species of fish. Abundances of newly settled juveniles of selected species of fish. Abundance of *Diadema* sea urchins.	Minimum of once a year at all Sites A subset of Sites to receive more intensive monitoring	June 1-July 31 for annual surveys Otherwise throughout the year
C_2	Specific Category 2 Sites	As for Category 1, plus coral recruitment on settlement tiles, algal growth (production) as a proxy for nutrient availability, detailed water chemistry, sedimentation using settlement traps	Once every 3 months **Sedimentation:** monthly or bimonthly	March September December (middle 2 weeks of each listed month)
C_3	Specific Category 3 Sites	As Category 2, plus increase in spatial coverage	Once a year; same time each successive year	June 1–July 31

The following methodology adopts and modifies some attributes from the Atlantic and Gulf Rapid Reef Assessment (AGRRA, 2000) Protocol, for benthic organisms to take into account the needs identified for the MBRS. However, the user should note that there are a number of important differences between the SMP methodology presented here and the original AGRAA. One such difference is the adoption of a point intercept approach instead of the original line intercept method. Segal and Castro (2001) showed that both point- and line-intercept approaches can yield comparable community composition data, provided that the former use a sufficient number of points. Both methods can detect benthos with less than 2% cover. Further details on the level of replication and other supporting materials for the SMP can be found in Sale *et al.* (2002). The original AGRRA text can be found at: http://www.coral.noaa.gov/agra/method/methodhome.htm

CORALS AND OTHER SESSILE ORGANISMS

EQUIPMENT

The following equipment is required for each diver in addition to basic snorkeling gear and SCUBA gear (including depth gauge):

- Underwater data templates
- Underwater cards to aid species identification (see Plate 3.1)
- A 30 m long transect line
- A 1m long measuring device
- Plastic slates or writing cylinder
- A small plastic ruler tied to the slate or writing cylinder

Underwater data templates for sessile organisms

Attach the data template for sessile organisms onto a clipboard, underwater slate or writing cylinder (see below). An example of the datasheet template designed for the benthic surveys can be found at the end of this chapter. You can photocopy the data template onto white underwater paper to provide a **permanent** paper record of the data collected. Templates can also be copied onto 3 mil double matte (=frosted on both sides) 'Mylar' by hand feeding the sheets through a photocopier, provided that the records can be kept permanently. Use rubber bands, clips or cable ties to attach the template to the slate. Use each template only once and keep in a safe place as a future record of the survey.

A 30m PVC measuring tape

This can be the same tape used for visual transects of fish. It will help to use a brightly colored PVC electrical tape, or an indelible felt-tip pen to **boldly** mark this tape every 25cm. These marks will be used when measuring percentage cover of sessile organisms.

A 1 m measuring device

A 1m long PVC stick (~1/2" diameter) marked in 10-cm intervals or metric measuring tape can be used.

Alternatively, a polypropylene line marked at 10-cm intervals (as above), plus a loop at one end to go around the wrist of your non-writing hand.

A small plastic ruler tied to the slate or writing cylinder

Trim the ruler to have a narrow, tapered point, but still be legible, at the basal 5 cm.

Writing cylinders – an alternative to a slate

A writing cylinder is a "thick walled" (1/4" thick) PVC pipe that is 4" internal diameter x ~18 cm long, with 3 holes drilled near one end through which surgical tubing is strung to fit over your wrist. The advantage is that it keeps hands free to hold other surveying equipment and to hold on in strong

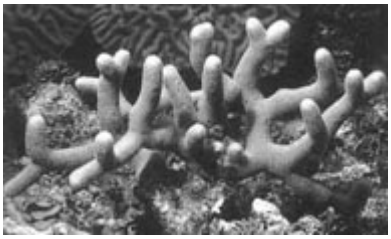
Plate 3.1 Example of Plate to be Used in the Identification of Benthic Organisms (from Human, 1998)

Staghorn Coral
Acropora cervicornis

Elkhorn Coral
Acropora palmata

Pillar Coral
Dendrogyra cylindrus

Yellow Pencil Coral
Madracis mirabilis



Thin Finger Coral
Porites divaricata

Branching Fire Coral
Millepora alcicornis

Encrusting Fan Leaf Alga
Lobophora variegata

surge or waves. A data entry form template is attached to the cylinder with tape. Writing cylinders can also be used to collect data on fish or in other habitats.

FISH AND URCHINS

EQUIPMENT

- Underwater data templates for fish and urchins
- Underwater cards to aid species identification (see Plates 3.2 and 3.3)
- Plastic underwater slates
- 2 x 30 m fiberglass surveying tapes OR 30 m nylon cord attached to a reel
- 2 x 3 lb weights
- Graduated T-bar

Underwater data templates for fish and urchins.
Please see above for details of templates. These datasheets are designed for the fish transect surveys and the roving diver census.

An underwater slate
Since the diver will need a new page for each transect, we suggest the following design for an underwater slate: 5 mm plastic slate, 21.6 cm x 28 cm (8.5 x 11”) with two “picture frames” cut from ~3 mm (1/8”) plastic, also 21.6 cm x 28 cm in outer dimensions with the frame ~12 mm (1/2”) wide. In the simplest case, frames are held tight to each side of the slate using bulldog clips top and bottom (4 in all), and frames hold the datasheets in place. To facilitate working underwater, the slate can be attached to the T-bar (see below).

> ***Note:*** The slate would be set up with one side containing the juvenile recruitment form and the other the adult fish data entry form. For those members conducting the benthic surveys, one side could have benthic component datasheet with the point intercept datasheet on the other side. In addition, the diver will have a large ziplock bag with a fishing weight sewed to it in which to keep completed datasheets.
>
> Once a datasheet is completed, remove from the slate and store in the plastic bag. The form can be folded and carried in a BC pocket, or left beside the tape reel. The diver will swim the remaining transects, picks up the reel (and data bag) and rewinds the tape ready for the next transect. These steps are continued for the requisite number of transects. Divers are cautioned to avoid rubbing their fingers against the data sheet underneath the slate, especially towards the end of the dive, when the paper starts to get saturated.

Two PVC or fiberglass tapes
At least two 30m fiberglass transect lines with a 3 lb weight attached at one end of each line. Commercially available PVC surveying tapes are suitable for the transect line, or a 30 m nylon cord attached to a home-made reel will work. A clip can be attached to the reel and suspended from the diver's belt, which allows for the tape to deploy freely as the diver swims.

A graduated T-bar or other measuring device.
Construct a T-bar using 1/2" diameter PVC pipe and a T connector available at hardware stores. It has a 60 cm long handle and two equal length arms providing a total width across the top of 1 m. Use PVC electrical tape or paint to create a scale along both arms showing divisions at each 5 cm.

Plate 3.2 Example of Plate to be Used in the Identification of Adult Fish in the Fish Belt Transects (from Humann, 1994)

Banded Butterflyfish
Chaetodon striatus

Foureye Butterflyfish
Chaetodon capistratus

Blue Tang
Acanthurus coeruleus

Hogfish
Lachnolaimus maximus

Spanish Hogfish
Bodianus rufus

Redband Parrotfish
Sparisoma aurofrenatum

Gray Snapper
Lutjanus griseus

Cubera Snapper
Lutjanus cyanopterus

Gray Triggerfish
Balistes capriscus

Queen Triggerfish
Balistes vetula

Plate 3.3 Example of Plate to be Used in the Identification of Juvenile Fish (from Humann, 1994)

Rock Beauty
Holacanthus tricolor

Banded Butterflyfish
Chaetodon striatus

Redband Parrotfish
Sparisoma aurofrenatum

CATEGORY 1 MONITORING

3.1 SURVEYS OF CORALS, ALGAE AND OTHER SESSILE ORGANISMS

At each SITE, replicate 30m line transects will be surveyed for sessile organisms. Transects will be deployed haphazardly – that is, you should deploy them in a quasi-random manner, seeking to avoid choosing places to include or avoid. To do so, lay the 30-m transect line just above the reef surface in a direction that is perpendicular to the reef slope (parallel to the reef crest). Make sure the line is taut. The objective is to sample 5 replicate transects per Site (this level of replication will be reviewed once the first sampling period's data are available for analysis. ***Remember to complete the Site Description as described in Section 2.2*.**

> ***Note:*** Be sure to avoid other transects that are being set by your companions, and stay away from the edges of the reef. Also try to avoid areas with abrupt changes in slope, deep grooves, and large patches of sand or unconsolidated coral rubble. Place the transect in areas where corals are likely to grow. Unusual reef features should only be included to the extent appropriate to their relative abundance at the site. In deploying successive transects, stay within ~100m of the anchored boat (the GPS location for that Site).

Point Intercept Method for Percentage Cover
Approximate the percentage cover of sessile organisms by swimming the transect, recording the nature of the organism directly below every 25cm point along the transect. Do your best to avoid being selective (especially in places where the tape is held above the substratum providing ample opportunity for parallax error to play a role). One helpful approach is to swim directly above the tape, and close one eye when lining up the point on the tape with the substratum. Classify organisms as:

1. **Coralline algae:** crusts or finely branched algae that are hard (calcareous) and extend no more than 2cm above the substratum
2. **Turf algae:** may look fleshy and/or filamentous but do not rise more than 1 cm above the substrate.
3. **Macro algae:** include fleshy algae whose fronds are projected more than 1 cm above the substrate.
4. **Sponges**
5. **Gorgonians**
6. **Specific genera of stony corals**

If the point is over bare rock or sand, or dead coral, record that fact also. Do not record mobile organisms such as urchins or conchs. Encourage these to move out of the way if necessary in order to record the substratum underneath them.

Particularly with gorgonians and macro algae, avoid recording presence under a point when the organism has been held down horizontally by the tape. This will exaggerate the actual abundance of these species. Instead, focus on points that overlie points of attachment of the organism to the substratum.

Recording every 25cm will yield 120 records per transect from which it is possible to compute percentage cover of each substratum type (as (# records/120) * 100%).

Characterization of the Coral Community under the Transect
After completing the point-intercept swim, swim back along the transect and stop at the first coral head, cluster, or thicket (or a portion) that is located directly beneath the transect line, is **at least 10 cm average diameter**, and which is in original growth position. For a colony that has fallen or been knocked over, only assess it if it has become reattached to the substratum or is too large to move. If the colony is loose and rolling around, then skip it. For each coral surveyed, record each of the following:

a) Name (genera). ***Note:*** For advanced members of the monitoring team, identification will be done to species level.

b) Record the water depth at the top of the corals at the beginning and end of each transect. In cases where bottom topography is very irregular, or the size of the individual corals is very variable, record the water depth at the top of each coral beneath the transect line at any major change in depth (>1m).

c) Identify the colony's boundaries based on connective or common skeleton, connective living tissue, polyp size, and polyp color. Using a measuring device, measure to the **nearest cm**, its maximum projected diameter (live + dead areas) in plan view and maximum height (live + dead areas) from the base of the colony's substratum (not from the base of the reef). The diameter should be measured perpendicular to the axis of growth. The height should be measured parallel to the axis of growth. Plan view is assessed from an angle that is parallel to the axis of growth (see Figures in Appendix 2).

d) Estimate the percent (%) of the coral that is "recently dead" and the % of the coral that is "long dead" as viewed from above in "plan" view (See *Estimating Coral Mortality* in Appendix 2). Plan view is assessed from an angle that is parallel to the axis of growth (be prepared to tilt your head to find the axis of growth and establish the proper plan view).

- **"Recently dead"** is defined as any non-living parts of the coral in which the corallite structures are white and either still intact or covered over by a layer of algae or fine mud. For recent mortality, there are several "stages" of recent:

  Very recent = white intact skeleton is still visible (dead w/in 1 month or less)

  Recent = corallite may be covered by thin turf algae or sediment (up to 6 months)

  Older recent = corallite structure may be slightly eroded or covered but can still be identified to genus (< 1-2 yrs), unless covered by clionid sponge (see Note below).

  Combine all of these recent mortality categories into **RECENT**. In some cases circular or oblong lesions or excavations caused by fish biting may result in destruction of the corallite. If fish bites are identifiable and constitute part of the mortality, consider it as recent mortality.

- **"Long dead"** is defined as any non-living parts of the coral in which the corallite structures are either gone or covered over by organisms that are not easily removed (certain algae and invertebrates). If it is entirely "long dead", indicate this on your data sheet as 100% "long dead", as long as you can identify it to generic level based on morphology (e.g., *Acropora palmata*) or skeleton (e.g., *Diploria sp.*).

Note: In some cases, a coral may be partially or completely overgrown by the brown encrusting sponge, *Cliona* sp. At a quick glance, it may look like live coral tissue, but if you look closely you may observe the in/ex- current holes of the sponge and sponge tissue and will not observe live coral polyps. If you can see the coral skeletal structure underneath and are able to identify it to genus (e.g., *Diploria* and *Montastraea*), this should be considered old mortality and you should note *Cliona* overgrowth in comments.

Note: If a coral like columnar *M. annularis* or *Dendrogyra* has been knocked over, has either reattached to the substrate or is too large to move, has started to regrow towards the water's surface, it now has a different diameter and height because of its new growth direction. You should measure diameter, height, and mortality in the correct plan view along the new axis of growth. Only measure

the recently "live" part of the *M. annularis* when estimating mortality and not the old "base" because this will skew old mortality.

e) Scan over the surviving portions of the ENTIRE coral colony and note if there are any DISEASES and/or BLEACHED tissues present. Characterize any **DISEASES** using the following nine categories taken from CARICOMP, 2001 (Table 3.2):

Underline any of these sources of disease (not bleaching) that are visible in plan view and which contributed to your estimate of "% recently dead". For more information about coral diseases see the disease cards (Bruckner & Bruckner, 1998) or visit the following web site:

http://www.uwimona.edu.jm/centres/cms/caricomp/methods_manual.html

Characterize **BLEACHED** tissue as approximate severity of discoloration:

P = Pale (discoloration of coral tissues)
PB = Partly Bleached (patches of fully bleached or white tissue)
BL = Bleached (tissue is totally white, no zooxanthallae visible)

Table 3.2 Coral Disease Categories to be Used in the MBRS-SMP

1- Black-Band Disease (BBD)	Multispecific
2- White-Band Disease (WBD)	Types I and II (only in acroporids so far as known)
3- White Plague-II (WP-II)	Multispecific
4- Yellow-Blotch Disease (YBD)	Only described in species **of** *Montastraea* but also reported in many other species.
5- Dark Spots Disease I (DS-I)	Small, dark areas with no apparent tissue mortality. This disease is common in *Siderastrea spp*.
6- Dark Spots Disease II (DS-II)	Large dark areas, larger than DS-I, common in *M. annularis* and *S. intersepta*.
7- Red Band Disease (RBD)	**Careful** here since BBD can be seen as red bands too. RBD has been reported for *Gorgonia* spp and Agaricids in the Caribbean.
8- Aspergillosis (ASP)	In *Gorgonia ventalina, G. flabellum, P. americana* and other octocoral species as well (*Plexaura flexuosa*).
9- Other -	This category includes all other "unconfirmed-pathogen-produced" diseases (tumors, hyperplasia, white pox, and all the "blotches").

Severely bleached coral tissues in many species are translucent, but you can still see the polyp tissue above the skeleton. Bleached tissues should not be included with the "recently dead" estimates. We are only interested in large scale bleaching events due to elevated sea surface temperatures, not bleaching due to algal overgrowth etc. Several months after a mass bleaching event, you may notice some corals (especially massive mound corals) still have pale or partly bleached tissues due to a previous bleaching event. Often these corals are still recovering.

Note: It is important to differentiate between tissue/skeleton with fish bites (=recently dead), recovering tissue from fish bites (=live tissue), bleaching and bleached tissue (=live tissue), and recent or old dead skeleton.

f) Whenever possible, record any other sources of recent mortality that can still be unambiguously identified: possibilities include sediments, storm damage, parrotfish bites, damselfish bites and/or algal gardens, predation on the soft tissues by snails like *Corallophilia abbreviata* or the bristle worm *Hermodice carunculata*, various effects of adjacent benthic algae, and any other spatial competitors (e.g., *Erythropodium caribaeorum*, other stony corals). Underline any of these sources that contributed to your estimate of "% recently dead".

4. Go to the next appropriate coral and repeat the measurements above. Continue evaluating each coral head (>10 cm) until you reach the other end of the transect.

The SMP requires a sample of at least 50 coral colonies from each Site. At the completion of your first transect tally the number of colonies evaluated. Repeat this process for each successive transect. In most Sites you will reach 50 colonies after 2-3 transects. Always sample all appropriate colonies on a transect, but once you have exceeded 50 colonies, subsequent transects can be run for point intercept estimates of percent cover only.

In cases where more than one person is running transects, you should compare notes after your first 1-2 transects. The total of at least 50 colonies is for the Site, not for the individual observer.

3.2 CORAL REEF FISH AND *DIADEMA* URCHINS

OVERVIEW

The AGRRA Protocol for Coral Reef Fishes (AGRRA, 2000) has been adopted by the MBRS Synoptic Monitoring Program as the standard methodology for assessing the effects of over-harvesting and/or changes in community dynamics due to natural or anthropogenic causes. We also note that CARICOMP (2001) have opted to include the AGRRA Protocol for their fish assessments. It is hoped that by adopting this protocol, data and information on fish species and populations will be collected that would allow comparisons over a much larger geographical area, and would be mutually beneficial for scientists and managers already using AGRRA and CARICOMP methods in the MBRS Region.

In the following pages we present the AGRRA fish protocol, with minor changes to adapt it for the MBRS Region. The original text can be found in the web page of the AGRRA program at: http://coral.aoml.noaa.gov/agra/. The AGRRA approach includes two distinct survey methods that provide different types of data and both should be applied at each site (AGRRA, 2000).

Method I uses belt transects. It is designed to measure the density and sizes (used for biomass estimations) of selected Caribbean key fish species, such as predators, herbivores, and "indicator" species, many of which are commercially exploited**.**

Method II does not use transects. It is designed to give information on species composition and diversity with crude information on abundance.

Transects for fish will tend to be further apart and may have to range deeper and shallower than transects for benthic organisms, however the goal should be to keep them within 100m of the GPS-defined Site. The integration of fish and benthic sampling, while beneficial, will require close coordination between team members for the two parts. However, expertise in visually identifying numerous fish species is required and it may be appropriate for the monitoring team to specialize as 'fish experts' and 'coral experts'. It is also recommended that the fish observations be conducted between 10:00 and 14:00 hours if at all possible, when underwater visibility is at a maximum due to overhead sunlight. Many fishes are wary of humans; hence it is necessary to keep away from other people while making these observations.

Method I: Belt Transect Counts for Defined Species List

1. For each transect, record the following information: recorder's name, date, time of start of transect, Site name and GPS location, transect number (See also Table 3.1).

2. Lay a 30 m transect line by first placing the weighted end of the line on the bottom (Figure 3.1), at a point selected haphazardly within the general confines of the Site. Then swim in a straight line while releasing the tape from the reel as you count the fish. Try to swim along depth contours so as to minimize changes in depth. Try also to avoid swimming across spurs and grooves. Instead, swim along spurs. By counting as you deploy the tape, you minimize the disturbance to the fishes prior to their being counted. Periodically fixing on an object in the distance as you swim will help you swim in a straight line. (You can clip the transect tape to your weight belt to allow for easy release of the tape).

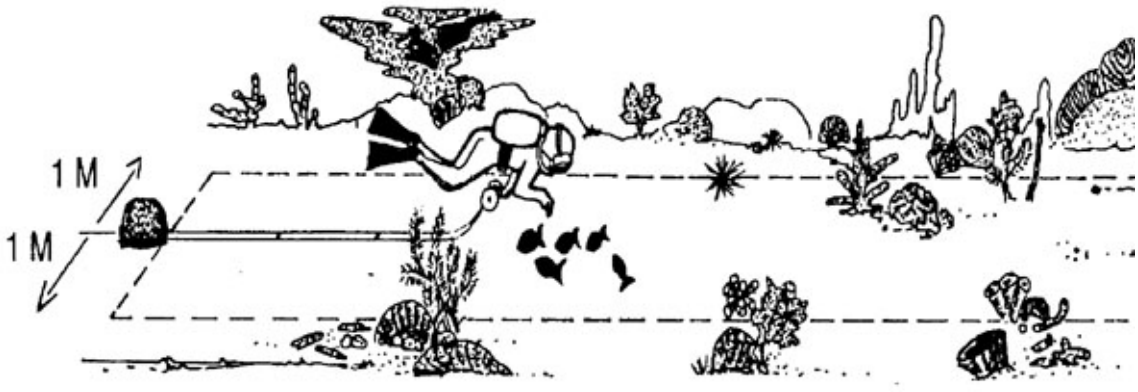


Figure 3.1 Diagrammatic representation of a diver conducting a belt transect (from Rogers *et al.* 2001). These method will be utilized to set up the transects for benthic organisms. A variation of this method will be used for the belt transects for fish censuses, in which the diver will have a T-bar.

3. As you swim out the full 30 m transect line, count and record fish found within a 2 m wide visually estimated belt transect. Carry a data sheet in standard format, and a 1-m wide T-bar to ensure accurate monitoring of the 2 m wide belt. Hold the T-bar ahead of you angled downward at about 45 degrees, and try to focus your gaze on the several meters of the transect ahead of the T-bar. Count only those SPECIES listed in Table 3.3, and do not count juvenile parrotfishes or grunts less than 5 cm in total length. This list of species has been chosen to provide coverage of a number of the species most likely to be affected by human impacts, while preserving a relatively consistent search image. This should enhance the precision of transect data.

4. Estimate the size of each fish and assign them to the following size categories (<5 cm, 5-10, 10-20, 20-30, 30-40, >40cm) using the T-bar to assist in estimating sizes. Large groups of individuals of a species will be classified by attempting to put them into one or more size categories as necessary. By remembering to keep effort equivalent on all segments of the transect, you can limit the tendency to count all members of a school crossing the transect, instead of just those members which happen to be within the transect as counting of that segment takes place.

   Note: Sample the transect belt giving uniform attention to each successive 2-m segment. This requires swimming at a more or less constant rate, while looking consistently about 2 m ahead of your current position. You may pause while recording data, and then start swimming again. It is important to swim

in a uniform manner. A speed that covers each 30-m transect in 6-8 minutes should be attempted. High densities of counted species will slow this rate in some cases. You will be tempted to count all members of a school as they swim across your transect unless you concentrate on giving equal effort to sampling each successive portion of the transect. Only those school members that are actually within the 2 m wide strip of that segment of the transect at a given time are included in the census. Fish observers should be trained to estimate fish lengths by using consistency training methods both on land and underwater.

Table 3.3 Fish Species to be Included in Counts along the 2m Wide Belt Transects

Include EVERY SPECIES within the following families.		
1.	Acanthuridae (Surgeonfishes)	*Acanthurus bahianus, A. chirurgus, A. coeruleus*
2.	Chaetodontidae (Butterflyfishes)	*Chaetodon capistratus* etc.
3.	Haemulidae (Grunts) Count **NONE** less than 5 cm long	*Haemulon flavolineatum, H. chrysargyreum, H. plumieri* etc.
4.	Lutjanidae (Snappers)	*Lutjanus apodus, L. mahogoni, Ocyurus chrysurus* etc.
5.	Pomacanthidae (Angelfishes)	*Pomacanthus paru, P. arcuatus, Holacanthus tricolor* etc.
6.	Scaridae (Parrotfishes) Count **NONE** less than 5 cm long	*Sparisoma viride, Scarus taeniopterus*, etc.
Count EVERY SPECIES in the following genera of Serranidae		
7.	*Mycteroperca*	*Mycteroperca bonaci*
8.	*Epinephelus*	*E. guttatus, E. fulvus, E. striatus* etc.
Count ONLY the following SPECIES of Balistidae		
9.	*Balistes vetula*	Queen triggerfish
10.	*Balistes capriscus*	Gray triggerfish
11.	*Melichthys niger*	Black durgon
12.	*Aluterus scriptus*	Scrawled filefish
13.	*Cantherhines pulles*	Orangespotted filefish
14.	*Cantherhines macrocerus*	Whitespotted filefish
Count the following five miscellaneous SPECIES		
15.	*Bodianus rufus*	Spanish hogfish
16.	*Caranx ruber*	Bar jack
17.	*Lachnolaimus maximus*	Hogfish
18.	*Microspathodon chrysurus*	Yellowtail damselfish
19.	*Sphyraena barracuda*	Barracuda

5. When you reach the end of the transect line, during annual summer surveys only, position the tape reel on the substratum, wait 2 min, and commence the survey for recruits. This will be a 1m wide belt transect back down the tape, recording presence of newly settled fish of target species. It will be important to count only individuals of each species that are smaller than the target size, to ensure that only recruitment from the current season is being counted.

   Note: The decision on the species to target depends on the time of year for the annual monitoring survey. A number of distinctive, moderately conspicuous species that can be reliably surveyed are available. The proposed set of suitable, summer-recruiting species and their size limits are listed in Table 3.4.

6. On completion of the recruitment census (or immediately following the census of adult fish in non-summer seasons), swim back along the tape, using the T-bar to delimit a 1m belt. Carefully inspect the substratum, including under overhangs, within this belt, and record the number of *Diadema* urchins seen. Since these can be cryptic, especially when young, swim slowly and explore crevices carefully.

Table 3.4 Shows the Proposed List of Fish Species to be Censused to Monitor Recruitment Maximum sizes are shown as TL = total length. Fish that are larger than these sizes will have settled too far in the past to be included, and should NOT be counted

Family	Species	Common name	Max. TL (cm)
ACANTHURIDAE	*Acanthurus bahianus*	Ocean surgeon	5.0
	Acanthurus coeruleus	Blue Tang	5.0
CHAETODONTIDAE	*Cheatodon striatus*	Banded butterflyfish	2.0
	Chaetodon capistratus	Four-eye butterflyfish	2.0
GRAMMATIDAE	*Gramma loreto*	Fairy basslet	3.0
LABRIDAE	*Bodianus rufus*	Spanish hogfish	3.5
	Halichoeres bivittatus	Slippery dick	3.0
	Halichoeres garnoti	Yellowhead wrasse	3.0
	Halichoeres maculipina	Clown wrasse	3.0
	Halichoeres pictus	Rainbow wrasse	3.0
	Thalassoma bifasciatum	Bluehead wrasse	3.0
POMACENTRIDAE	*Chromis cyanea*	Blue chromis	3.5
	Stegastes diencaeus	Longfin damselfish	2.5
	Stegastes dorsopunicans	Dusky damselfish	2.5
	Stegastes leucostictus	Beaugregory	2.5
	Stegastes partitus	Bicolor damselfish	2.5
	Stegastes planifrons	Threespot damselfish	2.5
	Stegastes variabilis	Cocoa damselfish	2.5
SCARIDAE	*Scarus iserti*	Striped parrotfish	3.5
	Scarus taeniopterus	Princess parrotfish	3.5
	Sparisoma atomarium	Greenblotch parrotfish	3.5
	Sparisoma aurofrenatum	Redband parrotfish	3.5
	Sparisoma viride	Stoplight parrotfish	3.5

7. On completion of the *Diadema* transect, retrieve the reel, and rewind the tape.

8. Continue conducting haphazardly-positioned 30 m transects at least 5 m laterally away from the previous position. Repeat the above steps for each transect.

9. Conduct a minimum of eight (8) transects at each Site. Following the first year's monitoring, data should be evaluated to ensure this level of replication is sufficient for the desired precision in estimates.

Modifications: Some workers may want to census other species of fish. This is encouraged, provided that these other species are counted on a **SEPARATE** pass over the transect, after the AGRRA run. Otherwise the census method is substantially changed, and your data may not be directly cross-comparable with other MBRS or AGRRA assessments.

Method II: Rover Diver Technique

After finishing the belt transects (or concurrently depending on the number of surveyors), conduct a roving diver census of ALL SPECIES of fishes following the methodology of Reef Environmental Education Foundation (REEF) (http://www.reef.org/) as outlined below. See Schmitt et al (1998) for more details.

1. The Rover Diver census is conducted in the same general area as the belt transects.
2. Swim around the reef SITE for 30 minutes and record **ALL** fish species observed. Use all knowledge you have of fish habits, and search under overhangs, in caves, and so on. The objective is to find the maximum number of species that you can during your search time at the Site. Do not extend your search even if you anticipate there may be additional species to see, and remain within the approximately 200m diameter Site throughout your survey.
3. Approximate the density of each species by using logarithmic categories: Single (1fish), Few (2-10 fishes), Many (11-100 fishes), or Abundant (>100 fishes).
4. Record your observations on the standardized data entry sheet, verify the data with your Monitoring Coordinator for entry into the MBRS REIS.
5. Each diver fills out one form per dive per Site, **copies** of which are submitted to the SMP-REIS database.

CATEGORY 2 MONITORING

3.3 RECRUITMENT OF BENTHIC ORGANISMS

Coral and fish recruitment are important components in the SMP to build up our understanding of processes and reef condition in the region; they will also play a role in identifying areas likely to function as sources or sinks for recruitment.

We are confident that the method outlined (Section 3.2) to quantify fish recruitment is appropriate and effective. Procedures for assessment of coral recruitment are less well-established, and while photographic methods can identify young corals once they are 5mm across, a method that will quantify recruitment earlier in the life of the coral is desired for the SMP.

The general procedure will require establishment of settlement plates at Sites, and for this reason, coral recruitment will only be measured at Category 2 Sites. The following methodology is a modified version of Mundy's (2000) method for coral recruitment (Steneck, 2003). This methodology was adopted to make results comparable with other studies in Belize and the Caribbean region. It is hoped that results will also be comparable to other regions of the world already utilizing this method.

It should be noted that monitoring of coral recruitment will not commence until there has been an opportunity for training under the guidance of an individual experienced in monitoring coral recruitment on Caribbean reefs.

EQUIPMENT

1. 50 (10 x 10 cm, and 1 cm thick) **unglazed (rough) terracotta** tiles at each depth (2 and 10 m) per Site
2. Pneumatic drill running off a SCUBA tank, with a masonry bit 5/16 inch or approx. 4 cm
3. Plastic wall anchors. A "wall anchor" is ~4 cm x 2 cm PVC plate that will hold ~5 mm (¼ inch) screws. Into the wall anchor, screw a ~5 cm (2.5 inch) stainless steel hex-head bolt (use a bolt driver). The bolt with a flat and lock washer goes through a ~5 mm (¼ inch) hole drilled

into the plate. Below the plate, there should be a 1 cm long piece of ~12 mm (½ inch) PVC pipe (a spacer).

4. Plastic trays
5. Dissecting microscope

METHOD

Mount 50 unglazed terracotta tiles onto wall anchors (Figure 3.2) and deploy at 2 m and 10 m (total 100 tiles) in the fore-reef at the chosen Category 2 Sites. Tiles need to be in place at all Category 2 Sites six months before the main spawning event in late August or early September. Therefore, tiles will be deployed in **late February or March** and should be retrieved in **late September**. Collected tiles will be kept in trays of seawater until they can be examined under a dissecting microscope to locate, identify (to the lowest possible taxon), and enumerate all recruits. From this data, it will be possible to determine the abundance and density of recruits per taxonomic group, per settlement tile, or square meter. A minimum of **50 tiles** will be retrieved annually per Site.

The plates will have a hole drilled in the center and each will have a unique number etched into its surface on the bottom side near the hole. At each Site, a small drill-hole will be made with a pneumatic drill attached to a SCUBA tank so that a stainless steel bolt can be fixed upright in the hole. The bolts will protrude above the substrate by at least 3 cm so that a 1 cm PVC spacer can be added onto which the coral plate will be placed before being bolted down. This leaves the plates affixed horizontally with a distinct upper surface that mimics the upper surface of the reef and an undersurface that mimics cryptic space.

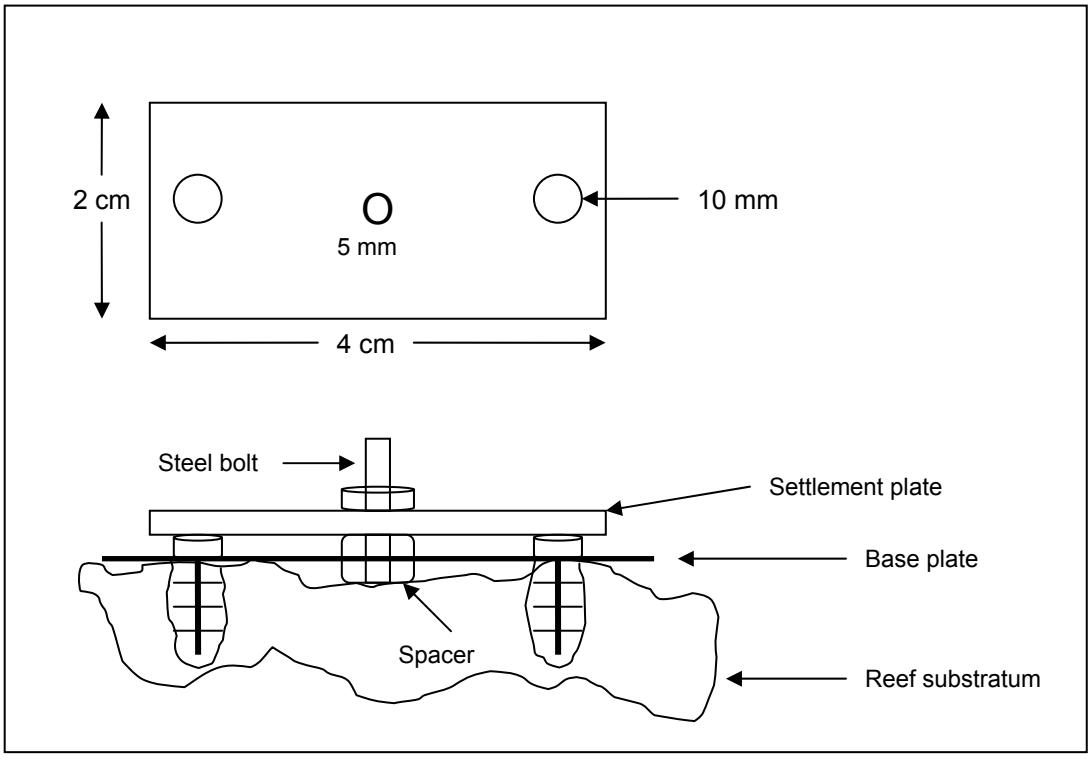


Figure 3.2 Diagrammatic representation of a wall anchor into which, the coral settlement plates (terracotta tiles) will be fixed to a portion of dead coral for the SMP studies of coral recruitment (Mundy, 2002). Drawing not to scale

3.4 METHODOLOGY TO ASSESS SEDIMENT DEPOSITION

Sedimentation, nutrification and contamination by other anthropogenic pollutants are major forms of negative pressure on coral reef ecosystems. Indirect measurements with the horizontal secchi disk visibility are simple and can be obtained in a matter of minutes at all Sites. However, we consider it important to supplement those recordings with measurements of the rate of sedimentation over time.

Yund *et al*. (1991) described a sampling method that modifies tube traps used to monitor sedimentation in order to also monitor recruitment of fish and invertebrate larvae. Tube traps create a volume of still and protected water into which sediments fall and remain, thus providing an undisturbed record of sedimentation. By filling the base of the trap with formalin, larvae settling into that volume will be killed, retained and preserved. Sedimentation and recruitment have both been largely overlooked in existing monitoring protocols and the tube traps described here can be a simple and inexpensive method to address such gap. This approach is analogous to monitoring using *bioindicators* as the traps can be deployed and left to passively collect and store information over defined periods of time, after which they can be retrieved and their contents analysed.

Sediment tube traps thus will allow the collection of sediment and can also aid in the monitoring of recruitment of fish and invertebrate larvae at selected Sites so that the nature of such sediments may be determined (terrigenous or reef-derived). **Monthly** or **bi-monthly** records of rate of arrival of zooplankton and phytoplankton, will provide direct indices of rates of sedimentation and rates of supply of planktonic food and propagules. Sediment tube traps are easily built (Figure 3.3), and the sorting of contents requires only a microscope. The traps will have to be maintained and protected from vandalism. Ideally, sediment traps should be sampled **monthly**; therefore, those Sites with permanent staff make suitable choices.

In their study, Yund *et* al. (1991) comprehensively discussed the use of cylindrical tubes for the collection of larval and sediments. They stated that for a given velocity, re-suspension can be controlled or eliminated if the sediment trap has a sufficiently high aspect ratio (Lau, 1979; Hawley, 1988). In their study, they utilised an **aspect ratio of 12** and further suggested that this trap design can reliably remain in place for periods of at least 2 months (Yund *et al.*, 1991). We therefore propose the following method for the MBRS Region:

EQUIPMENT

- Sediment trap - PVC tube, approx. 5 cm in diameter and 60 cm long, with a plastic cap glued and taped on the bottom. Such tubes give an aspect ratio of 12 (60 cm / 5 cm).
- Spare plastic caps to fit the tubes
- Stakes at least 1.5 m long. The stakes can also be attached to a cement block in groups of four, so that there is a stake or rod in each corner of the block
- Duct tape to secure the plastic tubes to the stakes
- Whatman filters
- Buchner funnel
- Drying oven
- Plastic tweezers

METHOD

1. Secure the tube traps to reference stakes at 50 cm and 10 cm above the substrate. Take at least three samples at each height at each Site.
2. Ensure that the tube traps are deployed at least a couple of meters below the surface of the water in calm areas and even deeper at more exposed Sites.

3. After a selected number of days (generally no more than 14), cap the jars underwater and bring them to the laboratory. Remove any small organisms in the jar with the tweezers.
4. Weigh #2 Whatman filters and filter the samples by pouring the jar contents through the filter, using a Buchner funnel.
5. Rinse each filter several times by running distilled water gently through the filter in the funnel to remove slats from the sediment.
6. Dry the sediment filters in a drying oven at 70^0C until a constant weigh is obtained.
7. Calculate the sedimentation rate as mg of sediment per cm^2 per day. The sediment weight is the total weight minus the filter weight, and the area of the jar opening is πr^2 (r = radius in cm).

$$\textbf{Sedimentation Rate} = \frac{\textbf{Sediment Weight}}{\textbf{Number of days at site x } \pi\textbf{r}^2}$$

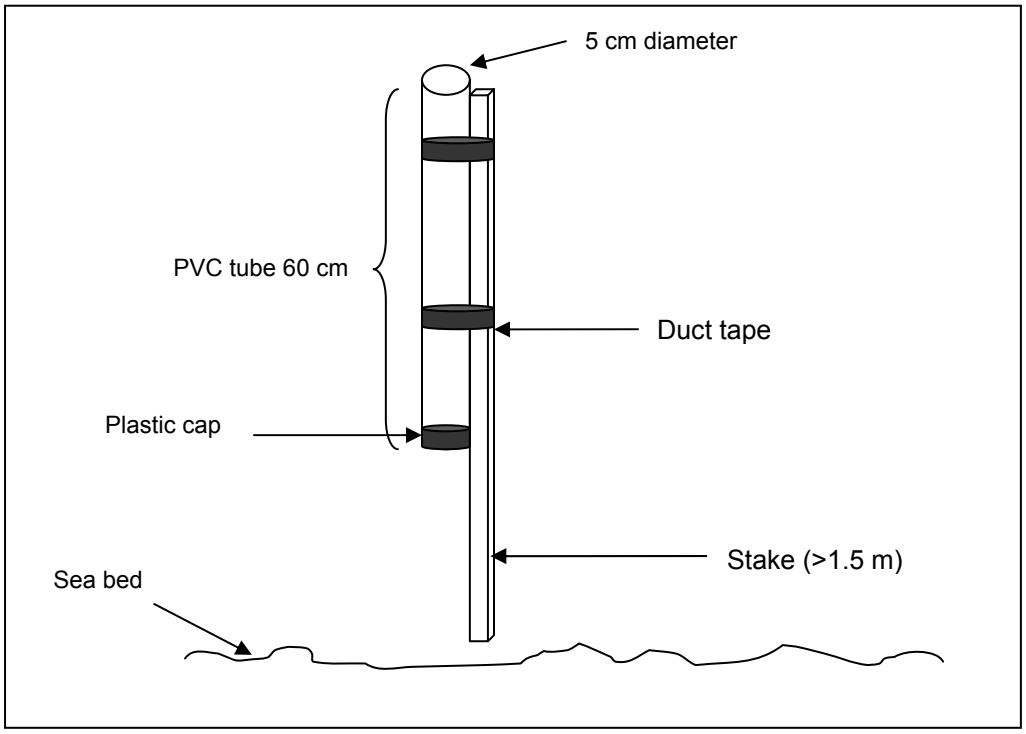


Figure 3.3 Diagram showing the recommended style and measurements for the tube trap to be used in the SMP studies on rates of sedimentation in the MBRS Region. Drawing not to scale

CATEGORY 3 MONITORING

In addition to Category 2 parameters, these surveys will expand the spatial cover of Sites surveyed.

3.5 DATA PROCESSING AND REPORTING

There are many ways to process and analyze data collected from surveys. We request that all data be initially entered into the preformatted spreadsheets that we provide. This will facilitate data collection and will ensure consistency in the way data are reported. Furthermore, the data structure will be compatible with the MBRS REIS database.

From these data entry forms, or datasheets, it will be possible to obtain means and standard deviations for the parameters measured. As agreed at the Technical Working Group in Flores, Guatemala (June 2002), the raw data will be fed in to the MBRS REIS by the Monitoring Coordinators, or an agreed focal person, who will be responsible for data validation. Statistical analyses will be carried out directly from the REIS. It is expected that some of these analyses will include size frequency distributions and mortality (at various spatial scales), and more detailed statistical comparisons between the data, such as differences between Sites, Locations, depths, various temporal and spatial scales. Below is the basic approach to data management and analyses.

There are three pre-formatted Excel spreadsheet files to make data processing and analysis easier and more standardized. The first Excel file is the Benthic spreadsheet which is for processing and analyzing coral information (abundance, size, condition, etc.) and abundance of other sessile biota. The second and third files are the Fish spreadsheets (adults and fish recruits) to process and analyze data collected from the fish belt transects.

Benthic Data Entry Form: This datasheet is designed to facilitate all data collection for two 30m transects for sessile organisms, i.e. corals, algae and other sessile biota. Once 50 or more coral colonies have been assessed at the Site, those segments of the datasheet may be left blank for subsequent transects.

Adult Fish Data Entry Form: This datasheet facilitates collection of adult fish data on two transects. Please note that the example only shows one transect. Four sheets (8 transects) are required per Site. The datasheet includes names of species likely to be common, plus blank spaces for additional species in each family that will be seen.

Fish Recruitment Data Entry Form: This datasheet facilitates collection of data on young fish on two transects (only one transect is shown). Four sheets (8 transects) are required per Site. The datasheet includes names of species of interest to the SMP at present. The datasheet provides a space for ***Diadema*** counts.

Rover Diver Data Entry Form: This entry form is based on the field form kindly provided by the Reef Environmental Education Foundation (REEF).

For all surveys, after each dive, verify data for accuracy and completion. Then, transfer all transect data from the survey datasheets directly into the pre-formatted spreadsheets for each Site surveyed (See below). The spreadsheets are similar to the datasheets so that it is easy to enter data. In the spreadsheets, some data cells are calculated and not entered and relevant headings have been marked in *italics*. Each Site will have its own spreadsheet file. Enter all the data for an entire transect at a time, then enter the data for the next transect directly next to it, without skipping a row. After all data are entered, recheck for any errors or mis-entries. IMPORTANT: For mortality data, DO NOT enter zeros if one category is 100%.

MBRS BENTHIC DATA ENTRY FORM

MSMP_1A

Loc:	Recorder:	Water depth (start):		Time:		Latitude:	Longitude:
Site ID:	Date:	Water depth (end):		% Dead		Condition	
Tran #	Coral Species	Max Diameter (cm)	Max Height (cm)	% Old	% Recent	Disease: BB, WB, WP-II, YB, DS-I, DS-II, RB, ASP	Bleached P, PB, BL
Comments:							

MBRS POINT INTERCEPT TRANSECT DATA ENTRY FORM

MSMP_1B

Location:		**Recorder:**		**Time:**	**Latitude:**	
Site ID:		**Date:**			**Longitude:**	
	Points per Transect					
Benthic Components	**Trans 1**	**Trans 2**	**Trans 3**	**Trans 4**	**Trans 5**	**Comments**
Bare rock						
Sand						
Coralline Algae						
Turf Algae						
Macroalgae						
Dictyota (Brown algae)						
Lobophora (Brown algae) *Halimeda* (Green algae)						
Blue-green Algae						
Sponges						
Gorgonians						
Coral Genera						
Montastrea spp.						
Diploria spp.						
Porites spp.						
Agaricia spp.						
Acropora palmata						
Acropora cervicornis						
Madracis spp.						
Myctephyllia						
Siderastrea						
Colpophyllia						
Leptoseris						
Millepora						
Other sessile fauna						

MBRS ADULT FISH DATA ENTRY FORM

MSMP_2A

Location:		Latitude:			Date:		
Site ID:		Longitude:			Time:		
Recorder:		Transect # _____ of _____					
Families	**Scientific Name**	0-5 cm	6-10 cm	11-20 cm	21-30 cm	31-40 cm	>40 cm
ALL SPP in the following families							
Surgeonfishes	*Acanthurus bahianus*						
Acanthuridae	*A. chirurgus*						
	A. coeruleus						
Butterflyfishes	*Chaet. capistratus*						
Chaetodontidae							
Grunts	*Haemulon plumieri*						
Haemulidae	*H. chrysargyreum*						
	H. flavolineatum						
Snappers	*Lutjanus apodus*						
Lutjanidae	*Lutjanus mahogoni*						
	Ocyurus chrysurus						
Angelfishes	*Pomacant. paru*						
Pomacanthidae	*P. arcuatus*						
Parrotfish	*Sparisoma viride*						
Scaridae	*S. taeniopterus*						
ALL SPP in these genera of Serranidae							
Mycteroperca	*M. bonaci*						
Epinephelus	*E. guttatus*						
	E. fulvus						
	E. striatus						
Balistidae	*Balistes vetula*						
only these SPP	*B. capriscus*						
	Melichthys niger						
	Aluterus scriptus						
	Canther. pulles						
	C. macrocerus						
Five misc. SPP	*Bodianus rufus*						
	Caranx ruber						
	Lachnol. maximus						
	Microsp. chrysurus						
	Sphyr. barracuda						

MBRS FISH RECRUITMENT DATA ENTRY FORM

MSMP_2B

Location:	Site ID:	Recorder:		Date:	Time:	Latitude:		Longitude:	
Species	**Common name**	**Max. TL cm**	**Trans 1**	**Trans 2**	**Trans 3**	**Trans 4**	**Trans 5**	**Trans 6**	**Trans 7**
Acanthurus bahianus	Ocean surgeon	5							
A. coeruleus	Blue Tang	5							
Chaetodon striatus	Banded butterfly.	2							
C. capistratus	Four-eye butterfly.	2							
Gramma loreto	Fairy basslet	3							
Bodianus rufus	Spanish hogfish	3.5							
Halichoeres bivittatus	Slippery dick	3							
Halic. garnoti	Yellowhead wras.	3							
Halic. maculipina	Clown wrasse	3							
Halic. pictus	Rainbow wrasse	3							
Thalassoma bifasciatus	Bluehead wrasse	3							
Chromis cyanea	Blue chromis	3.5							
Stegastes diencaeus	Longfin damsel.	2.5							
Steg. dorsopun.	Dusky damselfish	2.5							
Steg. leucost.	Beaugregory	2.5							
Steg. partitus	Bicolor damselfish	2.5							
Steg. planifrons	Threespot damsel.	2.5							
Steg. variabilis	Cocoa damselfish	2.5							
Scarus iserti	Striped parrotfish	3.5							
Sc. taeniopterus	Princess parrot.	3.5							
Sparisoma atomarium	Greenblotch parrot.	3.5							
Spar. aurofren.	Redband parrotfish	3.5							
Spar. viride	Stoplight parrotfish	3.5							
Diadema antillarum	Long-spined urchin								

MBRS ROVER DIVER ENTRY FORM (PART 1)

IMPORTANT: Only record species if you are certain. Use a tick √ on the left side of the column (√ S F M A) *AND* circle the relevant code. Abundance Codes: S = Single; F = Few, 2-10; M = Many, 11-100; A = Abundant, >100

MSMP_2C

Angelfish	**Eels**
_ S F M A French *Pomacanthus paru*	_ S F M A Brown Garden *Heteroconger halis*
_ S F M A Grey *P. arcuatus*	_ S F M A Goldentail Moray *Gymothorax miliaris*
_ S F M A Queen *Holacanthus ciliaris*	_ S F M A Green Moray *G. funebris*
_ S F M A Rock Beauty *H. tricolor*	_ S F M A Spotted Moray *G. moringa*
Basslets	**Filefish**
_ S F M A Blackcap *Gramma melacara*	_ S F M A Orangespotted *Cantherhines pullus*
_ S F M A Fairy *G. loreto*	_ S F M A Scrawled *Aluterus scriptus*
Blennies	_ S F M A Whitespotted *C. macrocerus*
_ S F M A Redlip *Ophioblennius atlanticus*	**Goatfish**
_ S F M A Saddled *Malacoctenus triangulatus*	_ S F M A Spotted *Pseudopeneus maculates*
Boxfish	_ S F M A Yellow *Mulloidichthys martinicus*
_ S F M A Honeycomb Cowfish *Lactoprys polygonia*	**Gobies**
_ S F M A Scrawled Cowfish *L. quadricornis*	_ S F M A Bridled *Coryphopterus glaucofraenum*
_ S F M A Smooth Trunkfish *L. trinqueter*	_ S F M A Colon *C. dicrus*
_ S F M A Spotted Trunkfish *L. bicaudalis*	_ S F M A Goldspot *Ginatholepis thompsoni*
Butterflyfish	_ S F M A Masked *C. personatus*
_ S F M A Banded *Chaetodon striatus*	_ S F M A Neon *Gobiosoma oceanops*
_ S F M A Foureye *C. capistratus*	_ S F M A Pallid *C. eidolon*
_ S F M A Longsnout C. aculeatus	_ S F M A Peppermint *C. lipernes*
_ S F M A Reef *C. sedentarius*	**Groupers/Seabasses**
_ S F M A Spotfin *C. ocellatus*	_ S F M A Black *Mycteroperca bonaci*
Chromis/Damselfish	_ S F M A Coney *Epinephelus fulvus*
_ S F M A Blue *Chromis cyanea*	_ S F M A Graysby *E. cruentatus*
_ S F M A Brown *C. multilineata*	_ S F M A Nassau *E. striatus*
_ S F M A Sunshinefish *C. insolata*	_ S F M A Red Hind *E. guttatus*
Damselfish	_ S F M A Rock Hind *E. adscension.*
_ S F M A Beaugregory *Stegastes leucostictus*	_ S F M A Tiger *M. tigris*
_ S F M A Bicolor *S. partitus*	**Grunts**
_ S F M A Cocoa *S. variabilis*	_ S F M A Bluestrip. *Haemulon sciurus*
_ S F M A Dusky *S. fuscus*	_ S F M A Caesar *H. carbonarium*
_ S F M A Longfin *S. diencaeus*	_ S F M A French *H. flavolineatum*
_ S F M A Sergeant Major *Abudefduf saxatilis*	_ S F M A Black Margate *Anisotremus surinamensis*
_ S F M A Threespot *S. planifrons*	_ S F M A White Margate *H. album*
_ S F M A Yellowtail *Microspathodon chrysurus*	_ S F M A Porkfis *A. virginicus*
Drums	_ S F M A Sailors Choice *H. parra*
_ S F M A Highhat *Equetus acuminatus*	_ S F M A Smallmouth *H. chrysargyreum*
_ S F M A Jackknife *E. lanceolatus*	_ S F M A Spanish *H. macrostomum*
_ S F M A Spotted *E. punctatus*	_ S F M A Tomtate *H. aurolineatum*
	_ S F M A White *H. plumieri*

MBRS ROVER DIVER ENTRY FORM (PART 2)

IMPORTANT: Only record species if you are certain. Use a tick √ on the left side of the column (√ S F M A) *AND* circle the relevant code. Abundance Codes: S = Single; F = Few, 2-10; M = Many, 11-100; A = Abundant, >100

MSMP_2C

Hamlet/Seabass	**Squirrelfish**
_ S F M A Barred *Hypoplectrus puella*	_ S F M A Blackbar Soldier *Myripristis jacobus.*
_ S F M A Butter *H. unicolor*	_ S F M A Dusky *Holocentrus vexillarius*
_ S F M A Black *H. migricans*	_ S F M A Longjaw *H. marianus*
_ S F M A Blue *H. gemma*	_ S F M A Longspine *H. rufus*
_ S F M A Indigo *H. indigo*	_ S F M A Reef *H. coruscum*
Hogfish/Wrasse	_ S F M A Squirrel *H. adscensionis*
_ S F M A Hogfish *Lachnolaimus maximums*	**Surgeonfish**
_ S F M A Spanish *Bodianus rufus*	_ S F M A Blue Tang *Acanthurus coeruleus*
Jacks	_ S F M A Doctorfish *A. chirurgus*
_ S F M A Bar *Caranx rubber*	_ S F M A Ocean A. *bahianus*
_ S F M A Horse-eye *C. latus*	**Triggerfish**
Parrotfish	_ S F M A Black Durgon *Melichthys niger*
_ S F M A Blue *Scarus coeruleus*	_ S F M A Ocean *Canthidermis sufflamen*
_ S F M A Greenblotch *Sparisoma atomarium*	_ S F M A Queen *Balistes vetula*
_ S F M A Midnight *Scarus coelestinus*	**Wrasses**
_ S F M A Princess *Sc. taeniopterus*	_ S F M A Bluehead *Thalassoma bifasciatum*
_ S F M A Queen *Sc. vetula*	_ S F M A Clown *Halichoeres maculpinna*
_ S F M A Rainbow *Sc. guacamaia*	_ S F M A Creole *Clepticus parrae*
_ S F M A Redband *Sp. aurofrenatum*	_ S F M A Puddingwife *H. radiatus*
_ S F M A Redfin *Sp. rubripinne*	_ S F M A Slippery Dick *H. bivittatus*
_ S F M A Redtail *Sp. chrysopterum*	_ S F M A Yellowhead *H. garnoti*
_ S F M A Stoplight *Sp. viride*	**Others**
_ S F M A Striped *Sc. croicensis*	_ S F M A Great Barracuda *Sphyraena barracuda*
Puffers	_ S F M A Chub, Ber/Yel *Kyphosus sectatrix./incisor*
_ S F M A Ballonfish *Diodon holocan.*	_ S F M A Glasseye Snap. *Heteropriacanthus cruentatus*
_ S F M A Porcupinefish *D. hystrix*	_ S F M A Redspotted Hawkfish *Amblycirrhitus pinos*
_ S F M A Sharpnose *Canthigaster rostrata*	_ S F M A Yellowhead Jawfish *Opistognathus aurifrons*
Rays	_ S F M A Saucereye Porgy *Calamus calamus*
_ S F M A Southern Sting *Dasyatis Americana*	_ S F M A Cero *Scomberomorus regalis*
_ S F M A Spotted Eagle *Aetobatus narinari*	_ S F M A Yellowfin Mojarra *Gerres cinereus*
_ S F M A Yellow Sting *Urolophus jamaicensis*	_ S F M A Sand Diver *Synodus intermedius*
Seabass	_ S F M A Sharksucker *Echeneis naucrates*
_ S F M A Creole Fish *Paranthias furcifer*	_ S F M A Silversides
_ S F M A Harlequin Bass *Serranus tigrinus*	_ S F M A Greater Soapfish *Rypticus saponaceus*
_ S F M A Tobaccofish *Serranus tabacarius*	_ S F M A Glassy Sweeper *Pempheris schomburgki*
Snappers	_ S F M A Tarpon *Megalops atlanticus*
_ S F M A Dog *Lutjanus jocu*	_ S F M A Sand Tilefish *Malacanthus plumieri*
_ S F M A Gray *L. griseus*	_ S F M A Trumpetfish *Aulostomus maculatus*
_ S F M A Lane *L. synagris*	
_ S F M A Mahogany *L. mahogoni*	
_ S F M A Mutton *L. analis*	
_ S F M A Schoolmaster *L. apodus*	
_ S F M A Yellowtail *Ocyurus chrysurus*	

4. METHODOLOGY FOR SEAGRASS COMMUNITIES

In contrast to coral reef monitoring studies, there have been fewer protocols to monitor changes in the health of seagrasses and mangroves. We have thus, adopted the CARICOMP Protocol since it is a recognized regional methodology currently in use in the MBRS Region (CARICOMP, 2001). We have introduced minor variations from the methodology to take into account the needs and resources available to the MBRS Monitoring Teams. One such variation is that productivity, leaf area index and C:N:P content will only be estimated in Category 3 Sites.

Table 4.1 Table of Standardized MBRS-SMP Procedures for Seagrass Communities

Category	Site	Parameters	Frequency	Time Window
C_1	All Sites	**Core parameters:** Date, time of visit, Location name, Site name or number, GPS coordinates, Collector's name, weather conditions, water & air temperature, rainfall, wind, sea state, salinity, light, turbidity, pH, DO, sedimentation, nutrients, chlorophyll **a.** General Site description (new Sites only), proximity to reef, shape, features, orientation, depth range. Video panoramic view or manta tow. **Specific parameters for seagrasses:** Percentage seagrass cover, abundance, species composition, standing crop, biomass	Twice per year	June and December
C_2	Specific Category 2 Sites	As for Category 1, plus increased seasonality (frequency), spatial coverage, temporal and spatial variation (additional sites and biomass stations), medium and low density sites, growth (new leaf biomass)	Y1: Every 3 months Y2: Every 6 months	March September December
C_3	Selected Category 3 Sites	As for Category 2, plus productivity, leaf area index, leaf area & width, carbon, nitrogen, phosphorous content (C:N:P)	Once per year	June1-July 31

The methods and measurements described below have three general purposes:

a) to determine the abundance and growth rates of seagrasses at specific Sites;
b) to allow determination of seasonal trends in these measures at the site; and
c) to allow comparison of the static, dynamic, and seasonal measurements across the region, including the CARICOMP network of Sites.

All measures will be expressed on a basis of **grams dry weight per square meter** (g dw/m^2).

CATEGORY 1 MONITORING

Sampling Strategy

Probably the most difficult task facing the investigator initially is the selection of appropriate sites for study. Ideally the site should be one that is the most representative of an area. On a practical basis, this can be difficult for even a trained investigator to judge. For Category 1 Sites, there will be ideally at least two Sites studied. The primary site will be the portion of a seagrass bed that visually has the most luxuriant or well-developed *Thalassia* community with clean, green leaves. This site is the

simplest to select and will be indicative of the maximum that the area is capable of producing. If possible, a second site will be selected which appears to the investigator to be average and representative of the area in general.

At each site, two stations will be picked for statistical replication. These should be at least ten meters apart, but can be greater distances. They should be visually equivalent.

Timing and Frequency
These Category 1 seagrass samples should be collected twice a year. Because the times of maximum and minimum productivity are not known throughout the region, samples will be taken at times of maximum and minimum day length, which occur in late December and June. As the maximum and minimum day lengths occur late in the months, moving sampling to early January or July is satisfactory. While more frequent sampling is desirable, the processing of these samples requires a great deal of effort. If additional resources are available, it would be best to first increase the frequency of the productivity samples. Additional times of sampling for biomass could then be added later. See Section 5 for a full description of enhanced sampling programs.

4.1 BIOMASS AND COMMUNITY COMPOSITION OF SEAGRASS BEDS FROM CORE SAMPLES

Introduction
The primary measurements to be made here are the standing crop biomass and total biomass of the plant. For our purposes, the standing crop, or aboveground biomass is composed of the entire short shoot (i.e. the green and non-green leaves and bundle sheath; see Figure 4.1). Leaves will be mainly green, but may be fairly heavily epiphytized. For our purposes here total biomass comprises the standing crop and the belowground, non-photosynthetic portions of the plants. This method for determining seagrass biomass will also yield the seagrass community composition.

The accuracy of the biomass estimate will depend largely on the maximum number of samples that reasonably can be taken and the structural complexity of the seagrass bed. Obviously a better estimate will be obtainable with the same effort for a small monospecific seagrass bed with fairly homogenous shoot density and coverage than for a large mixed-species bed with highly variable shoot densities and coverage.

EQUIPMENT

1 ea: Corer; PVC pipe (beveled and notched); 80 cm long and 15-20 cm diameter
A plug, approx. 5-7.5 cm diameter
45 cm long handle

EITHER
4 ea: Plastic buckets
1 ea: Sieve box; 2 mm mesh

OR
4 ea: Fine mesh (2-4mm) bags (i.e. diving bag)
1 ea: Deep tray
2 ea: Plastic kitchen strainers; 6-8" (15-20 cm) diameter
10 ea: Plastic basins (for sorting different biomass fractions into)

Miscellaneous:
Aluminum foil
Pink flagging tape

Ziploc plastic freezer bags; quart and gallon size
Hydrochloric or phosphoric acid (10% v/v; 10% concentrated acid + 90% water)
Drying oven (45, 60, or 90 ^{o}C; see text)
Analytical balance

The best way to obtain biomass samples in Caribbean seagrass beds is by the use of corers. Corers must be sturdy enough to slice through *Thalassia* rhizomes and dense calcareous sediments. Corers may be made from a variety of sources, but those made from polyvinylchloride (PVC) pipe are economical and durable. The diameter should be about 15-20 cm and length about 60-80 cm. The cutting end of the corer should be beveled and notched to provide a better slicing edge. In very compacted sediments, or those with coral rubble, the conventional corer will not penetrate far enough to be useful. In these areas a metal cutting edge needs to be affixed. The simplest material to use is the blade of a band-saw or hacksaw with coarse teeth. This can be affixed on the inner edge of the corer with pop-rivets, and replaced periodically. A continuous handle should go transversely through the corer and be sealed where it passes through the corer barrel. Handles should be at least 15-20 cm on each side for leverage, and be of sufficiently large diameter to be strong and comfortable. The corer must have a removable plug so that a vacuum can be obtained upon extraction, or else much of the material will be lost.

METHOD

Sample Collection
Force the corer into the sediment to at least 45-50 cm to obtain over 90% of *Thalassia* rhizomes and roots. If possible, the corer should be sunk up to the handles so that the sample does not slip down in the corer when removed from the sediments. Important: do not try to just push the corer into the sediments. It must be rotated rapidly back and forth to cut its way into the sediments as it is pushed. **Cut - do not just push!**

Core samples can be placed in individual buckets. Topside transference of sample from corer to bucket prevents loss of biomass underwater. Alternately, as the cores are taken underwater, they can be immediately extruded into fine (2-4mm), pre-labeled mesh bags (e.g. diving bags, laundry bags), while still underwater. With this method, each core can be taken, extruded into its individual bag, and all taken to the surface at the same time, thus eliminating many trips to the surface.

The first times that cores are taken, they should be taken back to the boat and carefully extruded. Then they should be cut in half lengthwise, and carefully inspected to determine that most of the roots are being collected, and the length of the core from the surface to the bottom of the root zone recorded. There should be a zone at the bottom of the core of 5-10 cm with no roots. This will serve as a guide in the future to just how deep the later cores must be taken.

Treatment of Samples
Clean the samples completely of sediment, and separate them first into species of seagrasses, fleshy macroalgae and green macroalgae of the order *Caulerpales* that grow from the sediment. Separation of macroalgae into species is at the discretion of the Site Team. It is desirable but not required.

If the samples are in mesh bags, they can be shaken and massaged while still underwater to remove most of the sediment. If these bags are not available, coarse sorting can be done on a sieve box with a mesh of about 2 mm and washed. Size is not critical but a box about 60 by 40 cm, with sides about 8-10 cm (made of standard 1x3 or 1x4 lumber) is quite satisfactory. The screen must retain small pieces of plant matter and all coarse shell material and fragments must be removed by hand. After coarse sorting, fine sorting can be done on the screen, but is often more conveniently done in a tray of water about 10 cm deep. This greatly aids in sorting the fine fragments. While not a perfect guide, live roots and rhizomes *tend to floa*t, while dead ones *tend to sink*. Live roots are white or very

light grey and crisp when squeezed or broken, while dead roots are dark and more flaccid. Short shoots and rhizomes can have both live and dead roots intermixed. Likewise live rhizomes have a whiter, crisp interior, while dead rhizomes are darker, both inside and out, and are less crisp when broken.

The resulting sample must be all organic matter with no contaminating carbonate fragments. Uncleaned samples can be held without disintegration for a day in shade submerged or several days if running seawater is provided over the sample. Cleaned samples can be held likewise, or, are best held chilled.

Divide all *Thalassia* plants into the following 5 separate fractions for biomass measurements 1) green leaves, 2) non-green leaves and short shoots, 3) live rhizomes, 4) live roots, and 5) dead below ground material (see Figure 4.1). Note that the green leaves should simply be torn off at the green/white interface (this is usually at the point where the leaves emerge from the bundle sheath, but may be higher up the leaf if the sheath is buried below the sediment). Note also that the "non-green leaves and short shoots" fraction comprises the non-green portions of the leaves and the sheath bundle, and is simply referred to as "short shoots" in the data sheets.

For all other grass species, it is generally possible (and often necessary) to simply sort them into 2 separate categories 1) green and 2) non-green tissue.

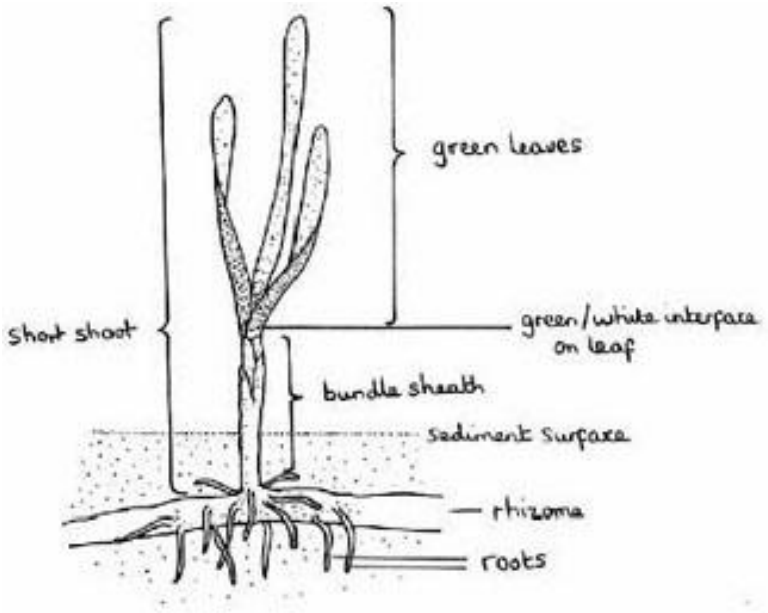


Figure 4.1 Seagrass plant showing short shoot and components along with rhizome and roots (original drawing from CARICOMP, 2001)

LABORATORY PROCESSING

After separating the plants into their various biomass fractions, make sure that any remaining sediment is removed from the seagrass rhizome and root biomass fractions by scrubbing with a soft toothbrush. Also, epiphytes on the green leaves must be removed in 10% phosphoric (gentler but expensive) or hydrochloric acid (more commonly used and cheap) unless detailed chemical analysis of the components is contemplated. The leaves should be placed in the kitchen strainer and lowered into the acid until bubbling (evolution of CO_2) stops. Note that acid emersion should be as briefly as possible and certainly should not to exceed 5 minutes. The acid bath must be changed periodically as it becomes less effective.

Rinse or soak all biomass fractions thoroughly in freshwater (using the second kitchen strainer) to remove salt and acid. This is greatly helped if the fine sorting is done in a bath of *fresh wate*r. Then place each fraction on pre-weighed and marked, heavy-duty aluminum foil tares and dry them at 60-90 ^{o}C to constant weight (no more than 60 ^{o}C if chemical analysis is planned). Some below ground fractions may take several days to dry completely. Until one is familiar with the drying time necessary, it is best to periodically weigh several of the heaviest fractions until they show a constant weight for 12 hours. At this point, all the smaller samples should be thoroughly dry. At this time the samples should be placed in a desiccator to cool before weighing. If no desiccator is available, allow the oven and samples to cool to about 45^{o}C before weighing. When through weighing, store the dried samples in a plastic bag for at least 6 months in case any errors have been made and reweighing is necessary.

Divide calcareous macroalgae into above and belowground tissue if desired. Remove all sediment and then decalcify in 20% glacial acetic acid. This may take several days. Fleshy macroalgae need to be rinsed in freshwater, dried, and weighed. Separation into species is generally not required, unless there is a clear dominant macroalgal species in the community.

DATA MANAGEMENT

Enter the data on the Seagrass Biomass Data Forms provided. To convert the weights per core sample to weights per square meter, multiply by a factor (f) based on the area of the cores. Example: If the area of the core is 200 cm 2, multiply by 50 to obtain data on a square meter (10,000 cm 2) basis.

CATEGORY 2 MONITORING

The work plan given above is for the basic Category 1 seagrass monitoring. The next logical step for those interested in additional seagrass studies would be to intensify samples both temporally and spatially utilizing the methodologies described above. This list provides guidelines for additional studies in a coordinated fashion.

Seasonality The next step is to increase seasonal coverage at the initial site. Instead of collecting samples twice per year, they would be collected 4 times per year, at 3-month intervals.

Spatial Coverage This will involve making a more detailed Site map. The area of interest, a single bed, a lagoon, or a bay, should be gridded off on a map and samples taken throughout the area to determine the **Spatial** variation of seagrasses in the area. For this purpose, the 10x20 quadrats may be used initially instead of the very laborious corers. With a sufficient number of samples, it is possible to obtain a mean density for the area, and to be able to map the areas of high, low, and mean density.

Temporal and Spatial Variation With the information obtained in (2), it is then possible to add additional sites that will enable the researchers to capture much more of the local seagrass

dynamics. Biomass stations should be added and may still be done twice per year. Stations can then be located in areas of medium and low density and the more complete variation of the system captured.

4.2 MEASUREMENT OF GROWTH OF *Thalassia testudinum*

Introduction

While the standing crop and biomass measurements will be made on the entire representative seagrass community, initial growth and productivity measurements will only be made on *Thalassia. Thalassia testudinum* is the dominant Caribbean seagrass species as it typically contributes more biomass, and thus areal productivity, to total seagrass bed production than *Syringodium filiforme, Halodule wrightii*, and *Halophila* spp., and also because *Thalassia* is the competitively dominant and "climax" species in Caribbean seagrass succession.

While standing crop and biomass measurements provide a *static* measure of the condition of the plants at a point in time, the productivity measurements give a *dynamic* measure of the health and growth rate of the plants. In addition to areal productivity (the growth per square meter of sea bottom per unit time), the method allows the determination of the turnover rate of the plants, a measure of the growth per unit weight of the plant.

Growth is measured as the production of new leaf biomass. Production of roots and rhizomes is extremely difficult to measure in tropical seagrass beds and for this reason has rarely been attempted. Measurement of leaf growth alone will serve the initial purposes of the SMP as well as CARICOMP, although it will not provide an estimate of total seagrass growth.

EQUIPMENT

Quadrats: 6 per station marked. Typically 10x20 cm; see text also.
Identification labels for quadrats (see text). Typically engineering flagging tape marked with magic markers.
Hole puncher: today the most commonly used device is a hypodermic needle.

Depending on the size of the leaves, sizes 16, 18, and 21 are most useful.
Scissors (for harvesting shoots after growth)
Plastic Ziploc type bags (the best are Ziploc freezer bags)
Plastic pans to hold acid wash and fresh water wash; typically 3-4.
Plastic kitchen strainer type sieves; 3.
Forceps (to pick seagrass from acid wash)
Pre-weighed receptacles in which to dry seagrass (e.g. aluminium foil, weighing boats, dishes)
Hydrochloric or phosphoric acid (10% v/v; 10% concentrated acid + 90% water)
Drying oven (45, 60, or 90 ^{o}C; see text).
Analytical balance.

METHOD

Sample Collection

At this point, measurements will be made for both productivity and standing crop. The prospective worker is also referred to Zieman (1974) which is both detailed and illustrated, although modifications have been made from the original description (**mainly** the substitution of a needle or hole punch for a stapler).

Although a measure of standing crop was made in the total biomass cores, it is necessary here to measure it *simultaneously* with productivity. Leaf growth should be measured by marking all leaves of a leafy shoot (= leaf bundle = turion = short shoot) a short distance (e.g. 2 mm) above the green-white interface, or at the sediment surface. Commonly the short shoots extend out of the sediments before the leaves divide and become green. The leaves are held tightly together by the bundle sheath and above this point the leaves separate individually, develop chlorophyll, and are green, while below it they are white. It is highly important with this method that all of the leaves on the short shoot be marked simultaneously with a single punch of the needle through all of the leaves.

In many areas, particularly behind reefs or in other high energy areas, the leaf bundle is buried and green leaves emerge from the sediments. In this case the leaves will be both **marked initially** and **collected at a later date** at the sediment surface. When placing the quadrat for marking, sink it to the sediment surface. That way it will serve as a reference point if the sediments are disturbed.

Marked leaves should be left for about 8 to 12 days. The best way to collect the sample is to harvest the entire short shoot from the sediment and return it to the lab intact in a marked Ziploc bag. In the lab, all leaves on a short shoot are clipped at the point on the short shoot at which they were originally marked. This is typically the green-white border that extends closest to the sediment, and is commonly the location of the needle punch mark on the oldest leaf. If the oldest leaf is senescent, indicated by beginning to turn brown or becoming heavily coated with epiphytes, it probably has not grown during the marking period.

In rare circumstances, if the leaves were originally punched individually at the sediment surface, because the sheaths were buried, then they are harvested individually at the sediment surface at this time.

Extinction coefficients (horizontal Secchi disc; also please refer to the Physical Measurements Section in CARICOMP, 2001) for light in the water column above the seagrasses should be measured at least several times (if not daily) during the week when growth is being measured. Two good times are just prior to marking and just prior to collecting, before the divers have stirred up the sediments. Light available for *Thalassia* growth can be calculated using the extinction coefficient and incoming irradiance recorded daily at the site's weather station.

LABORATORY PROCESSING

Leaves and leaf sections are separated into three groups:

Group 1) New Leaves: These are leaves that have emerged since the time of marking. They will be very green and fresh, and are distinguished by having no needle marks.

Group 2) Old Growth (= New growth of old leaves): This group is composed of the length of the leaf from the point of marking down to the base where the leaf was harvested at the original level of marking. It represents the growth of the marked leaves.

Group 3) Old Standing Crop: This is the section of the old, marked leaves *above* the mark. It is a portion of the material that was present when the original material was marked.

Each of these three groups is decalcified in weak acid, thoroughly washed, and dried on an aluminum foil tare. The pieces of foil should have been previously marked for identification and weighed. After drying the total dry weight is measured, the tare weight subtracted, and the actual weight of the plant fraction calculated.

Areal Productivity is the amount of new material produced per unit area per day. Here it is obtained by summing up the total plant growth (groups #1 + #2) and dividing by the number of days. This

figure is the production per quadrat. As a quadrat is 1/50 m 2, this number is multiplied by 50. Thus daily production is defined as:

$$\textbf{Daily Production} = \frac{\textbf{(Weight Group 1 + Weight Group 2)}}{\textbf{\# Days Marked}} \times 50$$

Turnover Rate can be considered in two ways. While areal productivity is the amount of plant produced per unit area, turnover rate is the amount of plant produced per unit plant. Expressed as a percent, it is the percent of the plant present that is replaced each day. Thus:

$$\textbf{Turnover Rate (\%/day)} = \frac{\textbf{Daily Production}}{\textbf{Standing Crop}} \times 100$$

Where Standing Crop is (Gp 1 + Gp 2 + Gp 3) x 50.

DATA MANAGEMENT

As before, data (entered on *Thalassia* Growth Forms) should be converted to weights per square meter. Productivity is normally measured in a 200 cm 2 quadrat (1/50 of a m 2), so it is only necessary to multiply all of the values by 50 to obtain the proper value. Thus 50 is the *factor* (f) for these quadrats.

CATEGORY 3 MONITORING

This work plan will include the parameters for Categories 1 and 2, plus it will include estimations of productivity, leaf area index & width, and carbon, nitrogen and phosphorous content (C:N:P). Productivity can be done twice per year. Locate monitoring Sites in areas of medium and low densities to capture better variation in the Sites.

4.3 LEAF AREA INDEX AND LEAF CHEMICAL COMPOSITION

Introduction

A set of measurements will be made to determine leaf area index and several highly useful plant parameters. Leaf area and leaf width are proven indicators of stress in seagrass communities. Both decrease when plants are stressed by such things as excess temperature or salinity. In addition, these measurements will allow better comparisons of the seagrass communities among the different Sites across the MBRS and the CARICOMP networks. Plants will also be collected and preserved for analysis of the carbon, nitrogen, and phosphorus content. C:N:P ratios will be calculated which will allow determination of the nutrient status of the plants. These will indicate if nitrogen or phosphorus is limiting or in excess at the various Sites.

EQUIPMENT

Ziploc bags, Small trowel, Scissors / razor blade, Ruler, Drying oven

METHOD

Sample Collection

At the time of leaf marking, the number of short shoots will be counted and recorded for each quadrat. From an area adjacent to the marked quadrats that is visually the same density collect 5 short shoots by uprooting the shoot. Take care not to tear or lose any leaves. Sometimes you will need to dig or fan the sediment away from the base of the shoot with your hand. Place these shoots with their leaves in a separate plastic bag and take to the lab.

Laboratory Sample Processing

At the laboratory, wash the shoots and leaves in fresh water. Cut the leaves from the short shoot with a razor, knife or scissors. Lay them out in the order in which they were on the shoot. Each leaf will then be measured beginning with the youngest, which will be *Leaf 1* to the oldest. Leaf 1 will typically be short, very green with a round tip and in the middle. *Leaf 2* will be the next youngest and will be adjacent to leaf 1. It will *usually* be green with no or few epiphytes but much longer than leaf 1. As the leaves are produced alternately, *Leaf 3* will then be on the opposite side of leaf 1 from leaf 2. *Leaves 4, 5* (if present) will continue to alternate from side to side (see Figure 4.1).

Measurements

Measure the total length of the leaf from base to tip and record as xx.x cm. Measure the width of the leaf about 1-2 cm from the base and record as xx.x mm. If the leaf is less than 2 cm in length, measure it in the middle. If the leaf still has a round tip record this. Finally measure the length from the base of the leaf to the first occurrence of epiphytes on the leaf and record it in xx.x cm. If epiphytes cover the entire leaf all the way down to the base then record 0.0 for the distance.

After the measurement, use a single edged razor blade or very sharp knife to scrape off all of the epiphytes possible. You may not get all of them and you may scrape off some of the green leaf. This is normal. If the leaves are very yellow and brown and completely covered in epiphytes so that they cannot be reasonably cleaned, or if they fragment so readily that you cannot work with them, throw them away.

DATA ENTRY FORMS

There are three pre-formatted datasheet files to make data entry and analysis easier and standard. These are: **Seagrass Biomass Entry Form**; **Seagrass Growth Entry Form**; and ***Thalassia* Leaf Area Index Entry Form**. The examples shown in the following pages show the first sections only because of their size. The complete sets of datasheets are available as spreadsheet files from the PCU or the MBRS web site: http://www.mbrs.org.bz

MBRS SEAGRASS BIOMASS ENTRY FORM (Partial example shown)

MSMP_3A

Location:		**Collection Date:**			**Latitude:**			**Collector:**		
Site ID:		**Processing Date:**			**Longitude:**			**Processor:**		
Site Prod:		**Time:**			**Core Diam:**			**Support Agency:**		
Core Replicate 1:				**# Living Shoots/Core**						
FRACTIONS		**Tare #**	**Tare Wt**	**Gross Wt**	**Net Wt**					
Thalassia	Green leaves									
	Short shoots									
	Rhizomes									
	Roots									
	Dead Tissue									
	Above Ground									
	Below Ground									
	Ratio A:B							**f**	**g/sq.m**	
						Total Wt				
Other Grass	Green Tissue									
	Non-Green							**f**	**g/sq.m**	
						Total Wt				
Fleshy Algae								**f**	**g/sq.m**	
						Total Wt				
Calcareous	Above Ground									
Algae	Below Ground									
	Ratio A:B							**f**	**g/sq.m**	
						Total Wt				

MBRS SEAGRASS GROWTH ENTRY FORM (Partial example shown)

MSMP_3B

Location:	**Date of Marking:**		**Latitude:**		**Collector:**	
Site ID:	**Collection Date:**		**Longitude:**		**Processor:**	
Site Prod:	**Time:**		**Duration of Expt:**		**Support Agency:**	
Sechi at marking:			**Sechi at Collection:**			
Quadrat # 1:		**Tare**	**Tare Wt**	**Gross Wt**	**Net Wt**	
New leaves (decalcify)						
Old leaves (decalcify)						
Old Standing Crop (decalcify)						
Areal Productivity	=(1+2) x 50 / # Days	(g/sq m/day)	Turnover per (green) Biomass of plant		=(1+2) / # Days (1+2+3)	x 100 = % per day
Quadrat # 2:		**Tare**	**Tare Wt**	**Gross Wt**	**Net Wt**	
New leaves (decalcify)						
Old leaves (decalcify)						
Old Standing Crop (decalcify)						
Areal Productivity	=(1+2) x 50 / # Days	(g/sq m/day)	Turnover per (green) Biomass of plant		=(1+2) / # Days (1+2+3)	x 100 = % per day
Quadrat # 3:		**Tare**	**Tare Wt**	**Gross Wt**	**Net Wt**	
New leaves (decalcify)						
Old leaves (decalcify)						
Old Standing Crop (decalcify)						
Areal Productivity	=(1+2) x 50 / # Days	(g/sq m/day)	Turnover per (green) Biomass of plant		=(1+2) / # Days (1+2+3)	x 100 = % per day

MBRS THALASSIA LEAF AREA INDEX ENTRY FORM (Partial example shown)

MSMP_3C

Location:		**Date:**		**Latitude:**	
Site ID:		**Time:**		**Longitude:**	
Recorder:		**Mean Shoot / Quadrat:**		**Sup. Agency:**	
Shoot 1	**Round Tip? (Y/N)**	**Length to epis (cm)**	**Length (cm)**	**Width (cm)**	**Area (sq cm)**
Leaf 1					
Leaf 2					
Leaf 3					
Leaf 4					
Leaf 5					
Shoot 2	**Round Tip? (Y/N)**	**Length to epis (cm)**	**Length (cm)**	**Width (cm)**	**Area (sq cm)**
Leaf 1					
Leaf 2					
Leaf 3					
Leaf 4					
Leaf 5					
Shoot 3	**Round Tip? (Y/N)**	**Length to epis (cm)**	**Length (cm)**	**Width (cm)**	**Area (sq cm)**
Leaf 1					
Leaf 2					
Leaf 3					
Leaf 4					
Leaf 5					
Shoot 4	**Round Tip? (Y/N)**	**Length to epis (cm)**	**Length (cm)**	**Width (cm)**	**Area (sq cm)**
Leaf 1					
Leaf 2					
Leaf 3					
Leaf 4					
Leaf 5					
Shoot 5	**Round Tip? (Y/N)**	**Length to epis (cm)**	**Length (cm)**	**Width (cm)**	**Area (sq cm)**
Leaf 1					
Leaf 2					
Leaf 3					
Leaf 4					
Leaf 5					

5. METHODOLOGY FOR MANGROVE COMMUNITIES

A preliminary characterization study of the different mangrove habitats within the chosen MBRS Locations will be conducted by using Point-Centered Quarter Method (PCQM) (Pool *et al.,* 1977). After habitats are characterized with PCQM, the researchers will be able to select the optimal Sites for the long-term monitoring plots.

The sections on community composition, interstitial water, and productivity have been adopted from the CARICOMP Methods Manual (CARICOMP, 2001). General methods for measurement of mangrove ecosystem structure and function are as described by Lugo and Snedaker (1975), Pool et al. (1977), and Snedaker and Snedaker (1984).

Table 5.1 Table of Standardized MBRS-SMP Procedures for Mangrove Communities

Category	Site	Parameters	Frequency	Time Window
C_1	All Sites	**Core parameters:** Date, time of visit, Location name, Site name or number, GPS coordinates, Collector's name, weather conditions, water & air temperature, rainfall, wind, sea state, salinity, features, orientation. **Specific parameters for Mangroves:** Forest characterization/zonation (once only before monitoring begins). Recognize stress. Establishment of plots, trunk diameter at breast height (dbh), height range for trees within the plot, salinity of sub-surface (interstitial) water, biomass within the plot, standing crop, community description (within plot), tidal range, abundance, percentage cover. **Seedlings and saplings (growth):** Establishment of subplots, to tag, identify, map and measure rooted seedlings (< 2.5 cm dbh), growth (new leaf biomass)	Once per year **Interstitial water**: monthly	June 1-July 31
C_2	Specific Category 2 Sites	As for Category 1, plus increase in spatial cover, biomass within a larger spatial scale (e.g. caye or island).	2 x year (dry and wet seasons)	June and December
C_3	Specific Category 3 Sites	As for Category 2, plus leaf area index, estimates of productivity (surface litter, for *R. mangle* only). Litter fall collection with traps, nutrients, turnover rate	**Leaf litter:** Y1: monthly; Y2: every 3 months, to eventually reduce to 2 x year **Nutrients:** Every 3-6 Months	June1-July 31 March June September December

CATEGORY 1 MONITORING

5.1 MANGROVE HABITATS CHARACTERIZATION

The preliminary characterization study of mangrove areas should be carried out only at the beginning of the monitoring process for each Site. It uses the Point-Centered Quarter Method (PCQM). PCQM is a fast and easy plot-less method to survey an unknown area. It is normally carried out on homogeneous habitats, but in the SMP case, this method will allow a rapid and precise overview of the different habitats encountered in the Location. Ideally, three PCQM transects should be laid to cover as much as possible of the ecosystem.

MATERIALS REQUIRED

Clipboard, data sheets, pencils
Calculator
10 m tape measures
Sighting Compass, or 4 light, 10 m lines
Nylon twine
PVC stakes for plot corner
Paint or paint pens for permanent tree marks
Flagging tape
Vinyl-coated wire
Large number of weatherproof numbered tags (preferably aluminum)
1 m cloth tape measure, or dbh tape
6 m telescopic measuring rod
Clinometer (if possible)
Hammer

METHOD

1. Select the mangrove ecosystem to be surveyed.

2. Familiarize yourself by walking around, through and/or riding around the ecosystem. Complement it by studying aerial photographs if they are available.

3. Select a starting point on the fringing mangroves close to the shoreline. Select the bearing for the PCQM transect to cover the most important habitats in the particular Site. Ideally, transects should be perpendicular to the shoreline.

4. The PCQM measures the closest tree to the center point in each quadrant defined by the transect line and the perpendicular. To carry out the PCQM, the following procedure will be necessary:

    a. Start at point zero and lay a line on the selected bearing direction.

    b. Select the distance between points to avoid measuring the same tree twice and obtain at least 20 points per transect.

    c. At each center point, four quadrants will be defined by the transect line and a perpendicular line crossing through this point.

    d. In each of the four quadrants formed by these intersecting lines, take the following measures of the tree closest to the center point:

i. species
ii. distance from the center point to the midpoint of the nearest tree (*d*, in meters)
iii. diameter at breast height (*dbh,* in centimeters);
iv. height (h, in meters).

e. Continue to the next center point and repeat the method.

f. If at any point there are trees that are already measured in the previous point, extend the distance between these points (usually adding 2 m is enough).

DATA MANAGEMENT

a. Calculate the tree density per center point with the following formula (Cintron and Shaeffer Novelli, 1984):

$$D = \frac{1}{d_{mean}^2}$$

Where:

D = Stem density in m^{-2}
d_{mean} = mean distance for all trees on a transect

b. Calculate the mean dbh per species per center point and the total mean of all species combined per center point.

c. Calculate the mean height per species per center point and the total mean of all species combined per center point.

d. Draw graphs of these means and densities against the distance from the shoreline of the center point.

5. From these analyses, field observations and aerial photographs (if available), define the different types of mangrove habitats in each Site (e.g., tall *Avicennia germinans* dominated forest; dwarf *Rhizophora mangle* dominated forest, etc.).

5.2 RECOGNIZING STRESS IN MANGROVES (From Talbot and Wilkinson, 2001)

The main damage to mangrove forests comes from anthropogenic activities such as deforestation for timber, charcoal, firewood, scaffolding, fish traps, coastal fish or shrimp farming. While the use of remote sensing methods is an ideal way to monitor changes in mangrove cover in large areas, often their use is unfeasible because of monetary constrains.

Very often, damage to mangroves is obvious (e.g. areas cleared for development, damage by storms). However, when mangroves are stressed as a result of natural factors (changes in salinity) or other human activities such as pollution, which may not show immediately, the signs of stress are less obvious.

Use the list below to recognize a stressed mangrove forest from a healthy one. Make careful examinations over time of these characters to show whether a mangrove is recovering or being damaged further. We suggest that these steps be carried out regularly when visiting mangrove Sites to look for signs of stress.

Mangrove forests may show stress in the following ways:

- There may be large or small areas where trees have been removed
- Trees may have branches cut off
- Branches and trunks may have bark with cracks or crevices
- The uppermost branches in the sun may be dying at their tips
- Leaves may be fewer, smaller, show twisting and curling, and have dead parts or spotting; the distance between leaf parts on shoots may be very much shorter than in a healthy tree
- There maybe no flowers
- Fruits may fall off before they have matured
- The seeds may be deformed – have abnormal growth
- Established seedlings may begin to grow abnormally
- Seedlings may die
- The small upright aerial roots (pneumatophores) coming up from the mud may be branched, twisted or curled, and aerial roots may develop on the tree's trunk
- Young trees may grow at an angle

5.3 COMMUNITY COMPOSITION (CARICOMP, 2001)

METHOD

Plots
Establish three 10 x 10 m plots within each representative selected habitat, defined by the PCQM. These plots will be designated A, B, C. Leave 3 – 5 m between each plot. To begin each plot, mark a tree at the first corner (left side corner, closest to the sea; Figure 5.1) with flagging tape or paint. Measure a 10 m side parallel to the shore, marking the second corner with flagging tape. Use the compass to set the second side (from the second corner to the third) at right angles to the first, and then completing the square in a similar way. To facilitate the effectiveness of the measurements, you could subdivide the plots into smaller sections. However, you should avoid walking inside the plots as much as possible to prevent damage to the seedlings and saplings. In addition, we suggest that you use flagging tape to mark all the trees to be measured (dbh >2.5 cm) at the beginning of the survey. As each tree is measured, the flagging tape can be removed, making it easier to spot those trees still to be measured.

Sub-plots
Within each plot, establish and mark five randomly placed 1 x 1 m sub-plots. Mark the corners of each subplot with PVC stakes and delineated the plots with flagging tape. In each sub-plot, all live saplings (< 2.5 cm dbh) and rooted seedlings should be tagged, identified, mapped, and measured, as above. Label each plant with an aluminum (Al) tag attached loosely with a vinyl-coated wire. Measure the total height of each plant within each subplot.

Map these plants using X Y plotting techniques. Record coordinates. The 0 point in each subplot should be at the left side corner closest to the water. The X-axis should be parallel to the water and the Y-axis should be perpendicular to the water.

> ***Note:*** It is recommended that these sub-plots be established and marked before making the tree measurements, then carefully avoid walking in them, to prevent damaged seedlings/saplings from being included in the initial sample.

> ***Note:*** Some scrub/dwarf mangrove stands may have mature trees with dbh < 2.5 cm. These differ from saplings in fringe and estuarine mangroves by the presence of aerial roots arising from branches (in addition to normal prop-roots). For scrub mangroves take the mean dbh of all major stems and record height as above.

Label Trees
For each plot, all mangrove trees with trunk diameter greater than 2.5 cm should be (a) numbered with a permanent marker such as Al tags, plastic ring-tags, or paint, (b) their position mapped (Figure 5.2), (c) identified, and (d) measured as follows:

Diameter
Measure the circumference (c) of the tree in order to obtain the diameter of the trunk (diameter at breast height or dbh). With *R. mangle,* the circumference is measured immediately above the uppermost buttressing prop root, using a flexible tape marked in centimeters. Diameter is then calculated as:

$$\mathbf{dbh = c/\pi}$$

> ***Note:*** Specialized tapes that measure dbh directly are also available through forestry suppliers.

Paint a ring around the trunk to mark the point where the dbh measurement was made. One year later, the measuring tape can be placed on top of this marker when re-measuring the circumference or dbh. Red mangrove trees sometimes have more than one trunk arising from common buttress or "prop-roots." In these cases, each trunk is measured as a separate tree. Where prop-roots grow down from high branches, these should be ignored when deciding where to measure the circumference.

Height
Height should be measured for all marked red mangrove trees in the plot using three parameters: (a) height above sediment surface of the highest prop root, (b) length of trunk, from prop roots to main area of branching and (c) total height, from ground to highest leaves (Figure 5.2). For black and white mangroves, measure: a) total height from sediment to highest leaves; and b) the length of the trunk from sediment surface to area of main branching.

For saplings and trees up to 6 m, a graduated telescoping rod is practicable. For trees taller than the 6-m telescoping rod, use a clinometer. **Where tree density is high, measuring height may be very difficult. Estimate as closely as possible, where it may be difficult to obtain actual measurement.** The measurements to be made are defined below:

Mapping
Use X Y plotting techniques to map all the trees within each plot and record coordinates. The 0 point in each subplot should be at the left side corner closest to the water. The X-axis should be parallel to the water and the Y-axis should be perpendicular to the water.

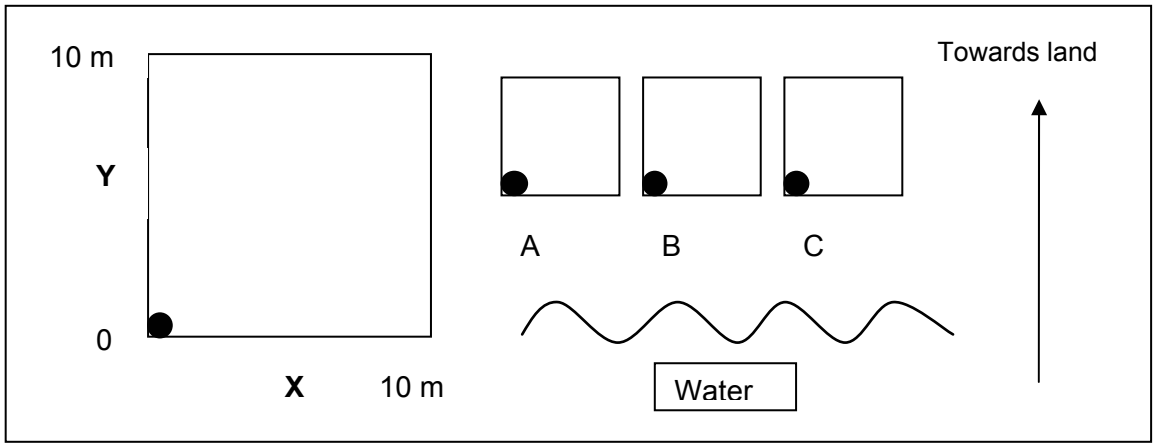


Figure 5.1 Position Mapping; use XY plotting technique within individual plots; the 0 point in each plot should be at the left side corner closest to the ocean or lagoon, as above. Drawing taken from CARICOMP (2001)

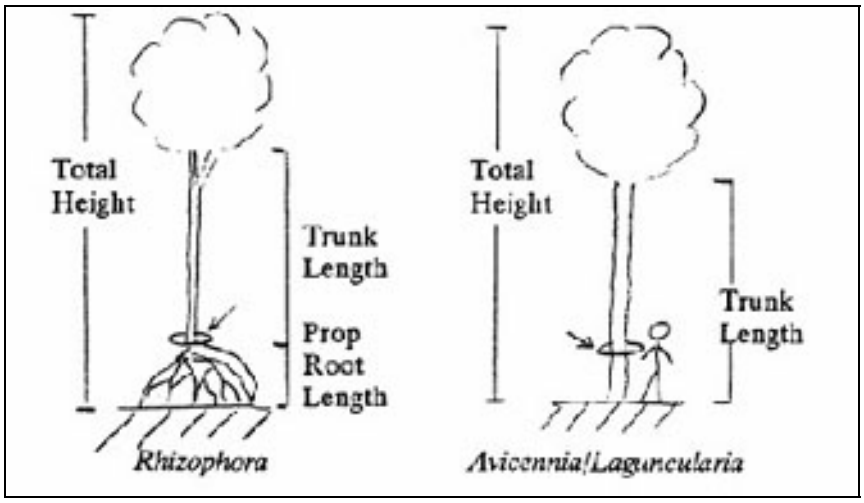


Figure 5.2 Definition of measures to be made in *Rhizophora mangle* and *Avicennia germinans/Laguncularia racemosa*. Circle on trunk marks position of circumference measurement (original figure from CARICOMP, 2001)

Timing and Frequency

Repeat the observations on all trees, saplings, and seedlings in the plots and sub-plots at one year intervals.

DATA MANAGEMENT

For comparative purposes, mean diameter will be calculated as the arithmetic mean of all dbh measurements for all plots. The mean stand diameter can be calculated for the whole site mangrove area in which the plots form a sub-sample. Basal area of the stand will also be calculated. This is a measure of the ground space covered by trees, expressed per unit area. For normal trunk forms, *A.*

germinans and *L. racemosa*, this is equivalent to the cross sectional area at the point where dbh was measured. This convention is utilized for *R. mangle* also, despite the complex prop-root development.

$$\textbf{Cross sectional area = basal area = } \pi \textbf{ x } r^2$$

This will be calculated for each tree in the plot and added together. The total basal area (cm^2) for the plot will be expressed as $m^2\ ha^{-1}$ for comparative purposes.

Mean tree height should be recorded for each plot. This and other measurements can be used for direct comparison with other plots and sites.

Community composition and diversity can be described using the 10x10 m plot data. Comparisons can be made over time (at 2 yr intervals), between plots and between sites.

5.4 INTERSTITIAL WATER

MATERIAL REQUIRED

Trowel or trenching tool
Suction pump or small container for bailing
Sample bottles

METHOD

An important parameter for mangroves is the salinity of the sub-surface water surrounding roots that penetrate the sediments. This is known as the interstitial water. Its salinity will be measured in each plot.

In areas where surface water is absent, it will be necessary to collect water to measure its salinity. To do this, dig a soil pit and remove the soil water by bailing or with the suction pump. Allow the pit to refill with water before sampling. Note the depth of the water table.

Salinity Well
An easier way to measure interstitial water for a long-term study such as the SMP is to set up a salinity well (Poche and Shaffer, 2003) in one corner of the plot. Use the same corner for each plot. If possible, correlate the salinity measurements with rainfall and tide data, to get an idea of water exchange in the Site. Salinity should be collected and measured at low tide.

The salinity wells are made of 1 m sections of 5 cm wide PVC with caps on each end. The PVC pipe segments contain slits (or small holes) every 4 cm, below the soil surface, which allow interstitial water to enter the well. Approximately 60 cm of the well is installed below the surface, with the remaining 40 cm above the soil surface. A plastic sleeve is taped around the top of the well at the sediment surface to prohibit sheetflow from affecting salinities below ground. Record the salinity in each well, then extract from the well with a hand-pump to enable new water to enter each well.

Where surface water is present, take a small core, using either a cut-off 30-50 ml plastic syringe with the cut edge beveled to aid insertion, or an equivalent technique. Mark the location (e.g. with a stake) so that the monthly samples will be taken in close proximity to the initial one. The core-hole should be refilled with a core taken from outside of the plot. Where possible, samples of interstitial water in the latter situations should be taken at low tide.

Timing and Frequency
Interstitial water should be sampled monthly at low tide.

5.5 BIOMASS

METHOD

Data from the plots and sub-plots are used to estimate biomass. In addition, it will be necessary to establish separate sub-plots from which all saplings and seedlings will be harvested to determine height-weight ratios for juvenile mangrove below 2.5 cm dbh. The sample should contain between 50-100 plants of each species sampled. When harvesting the juvenile plants, use a fork to loosen the sediment around each plant and pull up as much of the root system as possible. **This is done only once to determine the height-to-weight conversion factor, after which only heights of seedlings are measured in successive sampling**s. Data should be expressed as wet weight biomass.

DATA MANAGEMENT

Biomass of the mangrove forest trees greater than 2.5 cm dbh is estimated by using trunk diameter and tree density (number of trees per unit area). Individual tree biomass will be calculated using the dbh to weight conversion factor of (1) Golley *et al*. (1962):

$$\textbf{Biomass (g) = dbh (cm) x 3,390}$$

and (2) Cintron and Shaeffer Novelli (1984):

$$\textbf{Biomass (g) = b[(dbh)}^{\textbf{2}}\textbf{ (height)]}^{\textbf{m}}$$

where **b** and **m** are constants of 125.9571 and 0.8557, respectively (see paper for details).

Total biomass of trees will be calculated for the plots by summing individual tree measurements. Data should be expressed as wet weight for the living biomass (kg/m^2).

The annual increment in stand biomass due to tree growth is obtained from the differences between measurements of tree dbh made at two yearly intervals.

Biomass of saplings and seedlings from the experimental sub-plot data, using the conversion ratio calculated, will be added to the tree data. Record sapling and seedling growth at two yearly intervals as an indication of recruitment to stand biomass. You should expect some seedling mortality that will influence your estimation of biomass added to the stand.

CATEGORY 2 MONITORING

This level of monitoring will suit those researchers interested in covering larger geographical areas. Our recommendation for Category 2 Sites is to increase the spatial cover and biomass studies following the methodology for Category 1 Sites. For more complex studies, we propose to include estimates of productivity and turnover rates, as detailed below.

CATEGORY 3 MONITORING

5.6 PRODUCTIVITY

At present, studies on mangrove productivity are not envisaged routinely as part of the standard SMP Surveys. However, it is recognized that some researchers may need information on productivity in specific Sites. In addition, further needs for the SMP may well include productivity studies. Therefore, we have included the relevant section on productivity from the CARICOMP (2001) Methods Manual to encourage students interested in such studies to follow a standard methodology already adopted for the MBRS Region.

LITTER FALL

MATERIAL REQUIRED

Litter fall traps, 10 per plot. Construct traps in the form of a basket from a 1 m^2 of nylon netting (mesh size 1.5 mm) sewn onto a PVC, wood, or metal frame, 0.5 x 0.5 m (0.25 m^2)
Sample bags, 10 per plot (c. 30 x 20 cm)
Sandwich-size paper bags for drying litter
Drying oven
Balance
Wire or string to tie traps
Forceps and white sample trays for sorting

METHOD

Within each sample plot, deploy 10 litter fall traps at regular intervals, five parallel to the shore, and five perpendicular to it. The traps should be tied to prop roots of *R. mangle* approximately 1 m above the highest high tide.

Plot the location of each trap using the X Y plotting technique (see Figure 5.1). In addition, number each litter trap so that the code for any litter trap will include a letter (for the plot) and a number (for the trap), e.g. A1.

For the first year, litter samples should be collected every month and traps should be left in place throughout the year. Each month, collect litter from each plot (10 traps) into labeled bags and oven dry it at 70º C for 48 hours. Sort the litter into leaves, flowers, fruits, bracts, wood/twigs and frass (faeces from herbivore grazing, etc.). Each fraction should then be weighed.

Simultaneously with the first period of litter collection, loose surface litter on the forest floor should be collected from ten 0.25 m^2 quadrats per plot. Bag separately, and label as surface litter from near an identified litter trap (e.g. A1). Floor litter samples should be washed carefully to remove sediment and salt and then dried, sorted, and weighed like the trap litter samples.

Timing and Frequency

Surface litter is collected only once, at the start of the survey. For the litterfall traps, litter should be collected monthly during Year 1. For Year 2, sample four times per year at periods of maximum and minimum production (normally summer & winter or wet & dry season). That is, sample for two months at each period. Eventually trap sampling may be reduced to twice per year, again at periods of maximum and minimum production and traps should be set for 1 month.

DATA MANAGEMENT

Enter surface litter data in the Initial Surface Litter spreadsheet, and monthly litter fall data in the Monthly Litter fall spreadsheet. **Litter fall rates** and **turnover rates** will be calculated as follows by the SMP data center:

Litter fall rate will be calculated in g m^{-2} d^{-1} and presented graphically, keeping components separate. For interpretation of seasonal changes, graphs of litter fall components can be placed beside meteorological and hydrologic data. Total litter fall (g m^{-2} yr^{-1}) for each plot and the whole site area will be tabulated.

The difference between total litter fall and standing crop of litter, estimated by surface litter collection (although only at one time of year), is an indication of the fate of the litter in the particular habitat. A high standing crop indicates accumulation, while low standing crop indicates removal. Removal is difficult to interpret as it could be due to *in situ* decomposition, consumption, or export. Export could be into neighboring water bodies or into neighboring zones as discussed below. Studies on decomposition rate (below) can be used to aid interpretation, but the turnover rate of litter should be used simply as an index of the amount of organic material available to other components of the ecosystem.

Turnover rate (K) will be calculated as the ratio of total litter fall (L) over 12 months, to standing crop of litter (Xss), assuming a steady state of input = output and rate of change over time as zero, as:

$$K = L/Xss$$

MANGROVE DATA ENTRY FORMS

All the datasheets for mangroves are too large to be included in this manual. Instead, we show the first portion of the entry form for illustration purposes only. There are seven Mangrove Entry Forms: a) **Mangrove Characterization/Zonation**; b) **Mangrove Forest Structure**; c) **Mangrove Forest Structure Seedling / Sapling**; d) **Mangrove Seedling / Sapling Biomass**; e) **Interstitial Water**; f) **Mangrove Surface Litter**; and g) **Mangrove Litter Fall**.

The complete datasheets can be obtained from the PCU or in the MBRS web site: http://www.mbrs.org.bz

MBRS MANGROVE CHARACTERIZATION / ZONATION DATA ENTRY FORM

MSMP_4A

Location:		**Recorder:**	**Time:**	**Latitude:**	
Site ID:		**Date:**		**Longitude:**	
Point # distance from shore	**Transect (Quadrant)**	**Species**	**Distance from Center Point (cm)**	**DBH (cm)**	**Height (m)**

MBRS MANGROVE FOREST STRUCTURE DATA ENTRY FORM

Plot ID:_____

MSMP_4B

Location:		**Recorder:**			**Time:**		**Latitude:**		
Site ID:		**Plot ID:**			**Date:**		**Longitude:**		
Position		**Tree No.**	**Species**	**Circumference dbh (cm)**	**Diameter dbh (cm)**	**Basal Area (sq. cm)**	**Height**		
X	**Y**						**Prop roots**	**Trunk length**	**Tree height**

MBRS MANGROVE FOREST STRUCTURE SEEDLING/SAPLING SUBPLOTS DATA ENTRY FORM

MSMP_4C

Location:	**Recorder:**	**Time:**	**Latitude:**
Site ID:	**Plot ID:**	**Date:**	**Longitude:**

Plot (A, B, C)	**Subplot No.**	**Position**		**Sapling or Seedling No.**	**DBH if needed**	**Species**	**Height (cm) from the sediment surface**	**Live (Y/N)**
		X	**Y**					

MBRS MANGROVE SEEDLING / SAPLING BIOMASS ENTRY FORM

MSMP_4D

Location:	**Recorder:**	**Time:**	**Latitude:**
Site ID:	**Date:**		**Longitude:**

SPECIES	Sapling/ Seedling	Height (cm)	Tare Wt (g)	Tare Wt + Sample Wt (g)	Sample Wt (g)

MBRS INTERSTITIAL WATER ENTRY FORM

MSMP_4E

Location:		Recorder:	Latitude:	Time:
Site ID:		Date:	Longitude:	
JANUARY	**Plot (A, B, C)**	**Approx Sample Depth (cm)**	**Sediment Surface Exposed**	**Salinity %**
FEBRUARY	**Plot (A, B, C)**	**Approx Sample Depth (cm)**	**Sediment Surface Exposed**	**Salinity %**
MARCH	**Plot (A, B, C)**	**Approx Sample Depth (cm)**	**Sediment Surface Exposed**	**Salinity %**
APRIL	**Plot (A, B, C)**	**Approx Sample Depth (cm)**	**Sediment Surface Exposed**	**Salinity %**

MBRS MANGROVE SURFACE LITTER ENTRY FORM

MSMP_4F

Location:		Recorder:			Time:		Latitude:	
Site ID:		Plot ID:			Date:		Longitude:	
		LEAF+OTHER SOFT MATERIAL			WOOD			
	Near to Litter Trap No.	Tare (g)	Tare + Sample (g)	Net Wt	Tare (g)	Tare + Sample (g)	Net Wt (g)	Total Wt (g)
Plot A	A-Trap 1							
	A-Trap 2							
	A-Trap 3							
	A-Trap 4							
	A-Trap 5							
	A-Trap 6							
	A-Trap 7							
	A-Trap 8							
	A-Trap 9							
	A-Trap 10							
Plot B	B-Trap 1							
	B-Trap 2							
	B-Trap 3							
	B-Trap 4							
	B-Trap 5							
	B-Trap 6							
	B-Trap 7							
	B-Trap 8							
	B-Trap 9							
	B-Trap 10							
Plot C	C-Trap 1							
	C-Trap 2							
	C-Trap 3							
	C-Trap 4							
	C-Trap 5							
	C-Trap 6							
	C-Trap 7							
	C-Trap 8							
	C-Trap 9							
	C-Trap 10							

MBRS MANGROVE MONTHLY LITTER FALL ENTRY FORM

MSMP_4G

Location:			**Recorder:**			**Time:**			**Latitude:**		
Site ID:			**Plot ID:**			**Date:**			**Longitude:**		
Plot (A, B, C)			**LEAVES**			**BRACT**			**FLOWER**		
	Trap No.	**Species**	**Tare (g)**	**Tare + Sample (g)**	**Net Wt (g/ 0.25 sq m)**	**Tare (g)**	**Tare + Sample (g)**	**Net Wt (g/ 0.25 sq m)**	**Tare (g)**	**Tare + Sample (g)**	**Net Wt (g/ 0.25 sq m)**
JANUARY											
PLOT A											
PLOT B											

MBRS MANGROVE MONTHLY LITTER FALL ENTRY FORM (Continuation)

MSMP_4G

Starting Date:			No. days Litterfall:						
Collection Date:									
FRUIT			WOOD			MISCELLANEOUS			TOTAL LITTER FALL (g/0.25 sq m)
Tare (g)	Tare + Sample (g)	Net Wt (g/ 0.25 sq m)	Tare (g)	Tare + Sample (g)	Net Wt (g/ 0.25 sq m)	Tare (g)	Tare + Sample (g)	Net Wt (g/ 0.25 sq m)	

6. METHODOLOGY FOR POLLUTION AND WATER QUALITY

The analyses of pollutants in a large marine ecosystem such as the MBRS, is a very complex and enormous undertaking as there are many factors that influence pollutant concentration such as currents (and thus dilution factor), the size of the geographic area, wind, time, temperature, rainfall and many other environmental factors. The accurate determination of pollutants requires complex and expensive analyses, which are currently not easily available throughout the MBRS Region, despite the existence of several certified laboratories.

A sensible approach under these circumstances, therefore, is to study the accumulation of selected pollutants in both organic (fish and mollusks) as well as inorganic (sediments) samples. Samples from key Sites in the MBRS will be carefully taken and preserved and will be sent to several certified laboratories in the MBRS region for essential testing. The following section includes the recommended methodologies for those analyses to be made as part of the SMP. Table 6.1 shows the procedures for pollution monitoring for the SMP.

Table 6.1 Table of Standardized MBRS-SMP Procedures for Pollution Monitoring

Category	Site	Parameters	Frequency	Time Window
C_1	All Pollution Sites	**Core parameters:** Date, time of visit, Location name, Site name or number, GPS coordinates, collector's name, weather conditions, water & air temperature, wind, current velocity (speed and direction), sea state, salinity, light, turbidity, pH, DO, nutrients, chlorophyll **a**, bacterial concentrations. **Specific parameters for pollution:** Cholinesterase activity; PAH metabolites in bile; organochlorine pesticides (including PCBs); bioaccumulation studies to assess contaminants Additional parameters for PAH metabolites in bile and organochlorine pesticides: • **Fish:** species, total and standard lengths, weight and sex. • **Mollusks:** species, length, weight and sex. • **Sediments:** grain size and organic content.	Year 1: monthly **Sediments:** once a year Year 2 and after: Seasonal* (rainy & dry, cold fronts)	July 1 – Aug 30
C_2	Specific Category 2 Sites	As for Category 1, plus increased seasonality (frequency); spatial coverage; temporal and spatial variation	Year 1: monthly **Sediments:** once a year Year 2 and after: Seasonal* (rainy & dry, cold fronts)	July 1 – Aug 30 Apr 1 – May 31
C_3	TS plus selected Sites, e.g. MPAs	As for Category 2, plus increased seasonality (frequency); spatial coverage; temporal and spatial variation **Additional components:** PAH analysis on sediments and other biomarkers e.g. vitellogenin in plasma and histopathology (to be sent away for analysis in certified laboratories).	Years 1 to 3: monthly **Sediments:** once a year. Year 4 and after: Seasonal* (rainy and dry seasons, cold fronts)	**Wet:** Jul 1 – Aug 30 **Dry:** Apr 1 – May 31 **Cold Fronts:** Dec 1 – Jan 31 **October* =** Peak period for freshwater discharge

**** = Rainy and dry seasons may vary slightly along the latitudinal range in the MBRS***

6.1 POLLUTION MONITORING

FIELD EQUIPMENT AND MATERIALS REQUIRED

Map of the area
GPS Receiver
Glassware
Acetone
Alcohol
Teflon covered kitchen spoon
Labels
Ice box with ice
Aluminum foil
Dissecting knife
Masking tape or Cellotape
Plastic bags
Hypodermic needles
Eppendorff tubes

6.2 SAMPLING STRATEGY

The sampling strategy is important as it will determine the type of data analyses that will be possible to carry out and it will condition the selection of the sampling sites. This is a crucial aspect of the monitoring exercises as it could invalidate the conclusions arrived at from such results. A simple strategy is described below. For those who are interested in complex statistical analyses, please refer to a standard statistics text.

Site Selection and Sampling Design
Sampling will be carried out in pre-selected Sites (Map 1.2; see also Section 2.2). There will be several Sites within each study Location, and in each site ***n*** random samples will be taken. Samples will be taken randomly in each site for all consecutive monitoring visits and the previous Sites should be disregarded.

If at any time it is decided to increase the number of sampling Locations or Sites, the following aspects should be taken into account:

1. Is the Site impacted by contaminants?
2. Is it near port installations?
3. Is it near coastal urban areas?
4. Is it near river mouths?

It is also important to include Sites which are considered to be free of pollution, or that have been impacted minimally so as to use them as controls for the other sites, and to establish if there are any other unknown sources of pollutants.

Given the normal variability found in biological samples, it is important to determine the number of replicates to be analyzed. There are two main strategies:

1. Using standard statistical procedures determine the number of replicates per sample. Then, this number of individual organisms must be collected and analyzed. Usually the number of replicates is too high and time and budget constraints make impossible to reach this number.

2. Take pools of samples. In this case, instead of analyzing individual samples three pools of 15 or so individuals each is analyzed. In this way, a better approximation to the "true" value is obtained.

Sampling Frequency
For sediment samples, one sampling per year is enough, unless it is known (or one has strong evidence) that the sedimentation rate is very high (more than a centimeter a year). Monitoring of the sedimentations rates (Section 3.4) will provide useful information in this regard.

For organisms, samples should be collected every month for at least during one year. Subsequently, the sampling frequency can be decreased, collecting samples only during each climatic season (i.e. rainy and dry seasons).

Number of Replicates
In each Site at least five replicate stations selected at random should be sampled. In the case of organisms, at least 10 individuals of the same sex and size per site should be collected.

Species
Little information is currently available on suitable indicator species for particular habitats in the MBRS for monitoring of pollutants. Nevertheless, potential indicator species should have, at least, the following characteristics:

- Be easy to capture
- Easy to identify
- Sufficiently abundant to be collected throughout the year
- Bottom dweller (live and/or feed on the bottom)
- Size large enough to allow for the analyses of certain tissues
- Distributed throughout the MBRS Region

In the case of fish, catfish or flatfish are suitable species. As for mollusks, bivalves, such as the mangrove oyster *Crassostrea rizophorae* and *Isognomon alatus* are widely distributed in the MBRS.

Quality Control
One reagent blank, and one recovery blank, should be included in each lot of 6 to 10 samples. Certified reference materials, due to their cost and the difficulty in obtaining them, should be analyzed at least once for each sampling campaign. These materials should be analyzed for each sample matrix type to analyze (organisms, sediments, etc.). Also one each of 10 samples randomly selected should be analyzed in duplicate, preferably in different lots. To ensure quality control, all these analyses **must** be blind to the analyst.

Sample Collection
In the environment, toxic contaminants are usually found in traces or very small amounts. It is therefore necessary to take a great deal of care to avoid contaminating the samples at the time of collection. It will be necessary for those taking the samples to avoid sunscreens or solar filters, as these can easily contaminate the samples. The boat should be head-to-wind and water samples and sediments should be collected from the bow of the boat, to prevent contamination from the engines.

All equipment to be used for the collection of samples must be thoroughly washed and all containers labeled accordingly. The type of cleaning will depend on the type of analyses to be carried out:

- **For pesticides:** all glassware should be washed in soap and water and should be rinsed with acetone and alcohol

- **For sediments:** samples will be taken with the aid of the kitchen spoon and will be placed in previously washed glass jars. Note that plastic contaminates the samples, and should therefore not be used at all for this procedure. All glass jars to be labeled and placed in the ice box until arrival in the laboratory, where they should be frozen until needed.

- **Tissue and liver samples:** These can be placed in aluminum foil; however, care should be taken to use only the opaque side, as the shiny side has a certain amount of wax, which can contaminate the samples. With a clean knife, lift the fish skin and take a small piece (cm x cm) of muscle or liver and place it in the aluminum foil, wrap it well and seal it with cellotape or masking tape. Care should be taken to fully label the sample before it is placed and sealed in a plastic bag, which is then placed in an ice box, to be frozen until the analyses can be carried out.

- **Bile samples:** Open the fish with a sharp knife and extract the bile with a hypodermic needle. Place the bile in a vial or in an Eppendorff tube properly labeled and frozen until analyses can be conducted on the samples.

Sample Preservation
All samples are to be frozen immediately upon collection and kept frozen until relevant analyses can be carried out in the laboratory.

6.3 ANALYSES OF SAMPLES

Validation and Quality Control
It will be necessary to maintain the strictest possible controls to ensure the quality and comparability of the data. This is particularly important in the Regional SMP involving four countries and with several sampling ecosystems to be monitored. It is not only essential that data are comparable between countries but also comparable in time, so that special and temporal tendencies of the pollutants can be established.

To ensure data quality, several protocols have been recognized internationally. The three most important aspects are:

1. The use of blanks
2. The use of certified reference materials; and
3. Careful log keeping of all aspects of the work

Blanks
For each sample batch, two types of blanks should be analyzed:

1) Reagent Blanks: where the entire analytical process is carried out in exactly the same conditions, but without a sample. This provides an idea of the cleanliness of the glassware and the purity of the reagents. Limits must be established for the blanks, which if exceeded, the entire bath of samples should be discarded.

2) Enriched Blanks: these are blanks to which a known amount of an analytical standard is added. Ideally, the amount analyzed needs to be the same as that which was added, although this occurs rarely as it is usual to obtain a lower result. All values for the batch are then corrected in relation to the percentage of recovery of the blank.

Certified Reference Materials
These are samples that have been analyzed by one or several high standard laboratories and thus, the amount of one or more high quality concentrates in the samples are known. Care should be

taken to select materials similar to those that are going to be analyzed, i.e. certified materials for fish tissue, mussels, oysters, sediments, water, etc. are available as well as for a large number of analytes. These should be analyzed blind, the person carrying out the analysis must not know which ones are the true samples and which ones are the certified reference materials.

After the analysis of each batch is completed, their results are compared to those from the certified concentrations, and if they are found to be within the true interval, the results of the sample batch are accepted. Given that certified materials are very expensive, not all batch samples are analyzed in this manner. However, there should be periodical analyses by each laboratory to ensure the quality of the results.

The National Oceanographic and Atmospheric Administration (NOAA), USA has recent listings of certified marine reference materials.

Activity Log
There will be a dedicated laboratory log book for all samples taken as part of the MBRS-SMP, where all aspects of the analytical work will be recorded. This will aid in the identification of any anomalous result, so that calculations, batches of reagents and standards used may be verified.

All entries will be in ink, never with a pencil; should an error be committed, this will be crossed out with a horizontal line (i.e. ~~example~~) so that it is still legible.

The following items will be included in the log book:

Date
Name and signature of analyst/Laboratory
Sample specifics
Sample weight
Standards used
Calibration curve for the analytical instruments used
Comments

Additional Notes
There are additional procedures that must be carried out in a certified analytical laboratory in order to maintain the quality of the results. For example, all analytical instruments must be calibrated periodically; standards must also be run every day to ensure that there are no instrumental biases. Laboratories should also participate in intercalibration exercises, such as those organized by international organizations such as UNEP, through its Marine Environmental Laboratory in Monaco.

6.4 ANALYTICAL METHODS

ORGANOCHLORINE PESTICIDES AND POLYCHLORINATED BIPHENYLS (PCBs)

The methodology proponed in this Section has been adopted from the UNEP Marine Environmental Laboratory in Monaco, and co-administered by the International Atomic Energy Agency. All references are included at the end of the Manual (UNEP/IAEA, 1982a; UNEP/IAEA, 1982b; UNEP/IAEA 1984, and UNEP/IAEA 1986).

The solvents used (hexane, dichloromethane) must be of the following standards: ***pesticide analyses*** or ***residual analyses*** or similar. Water to be used must be bi-distilled and extracted with hexane, to ensure that it does not contain any impurities. Florisil must be between 60 to 100 mesh. It is baked at 650ºC for 2 hours and it is kept in an oven at 110ºC until it is used.

Silica gel (70 to 230 mesh) must be baked at 220ºC overnight (a minimum of 12 hours) and kept in an oven at 100ºC until needed.

Glassware must be washed with soap and water, after which, it must be soaked in a chromic mixture for at least 2 hours; it should then be rinsed with acetone and hexane in that order. Alternatively, after washing with soap and water, glassware can be placed in an oven at a temperature of 260ºC for at least 12 hours.

SEDIMENTS

At the laboratory the sample is dried (it is best to freeze-dry it), then it is homogenized (Figure 7.1) and sieved (0.5 mm). A 20 g sub sample is placed in an extraction thimble, and extracted with 300 ml hexane in a soxhlet apparatus for 8 hrs. The hexane is removed, and the sample extracted again with 300 ml methylene chloride. The methylene chloride extract is reduced to 20 ml in a rotary evaporator and mixed with the hexane extract. The mixture is reduced to 40 ml in a rotary evaporator, and the reduced to 6 ml in a kuderna-danish concentrator. Rinse the kuderna-danish with hexane to get a total volume of 15 ml, and reduce it to 8 ml with a stream of nitrogen. Add a few drops of mercury to the extract and shake it. Transfer the extract to a clean kuderna tube and add more mercury (a few drops). Repeat this until all sulfides are eliminated (until mercury does not turn black). Reduce the extract to 1 ml with nitrogen.

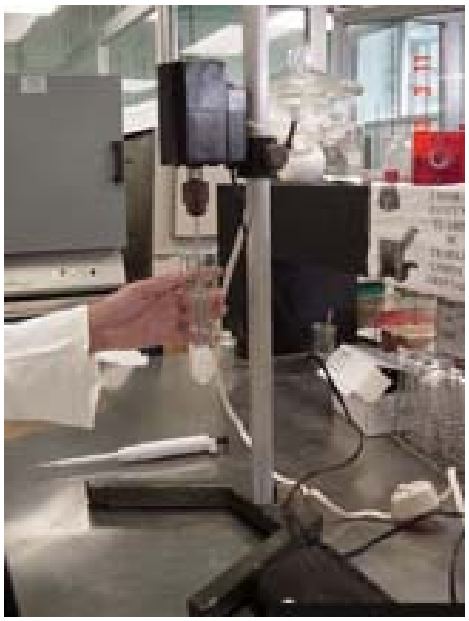


Figure 6.1 Tissue homogenizer built with a sewing machine motor (Photograph courtesy of Gabriela Rodríguez-Fuentes)

Pack a chromatography column with 16 g 5% deactivated Florisil made into slurry with hexane. Add some anhydrous sodium sulphate at the top of the column to eliminate humidity. Add the sample extract, and open the valve until the sample is drawn completely into the sodium sulphate layer. Elute with 70 ml hexane and receive fraction F1; then elute with 50 ml of a 7:3 (v/v) mixture of hexane:methylene chloride (fraction F2). Finally elute with 40 ml methylene chloride (fraction F3). Receive each fraction in a different kuderna-danish tube. Concentrate each fraction to 10 ml in the kuderna-danish and to 1 ml with a stream of nitrogen. Inject 1 to 2 µL in the gas chromatograph.

Sample extracts are analyzed in a gas chromatograph equipped with an electron capture detector, an split/splitless injector and an SE-54 (200 µm i.d.) capillary column (or equivalent). Analysis conditions are:

Carrier Gas	Nitrogen 1-2 mL min^{-1}
Make-up Gas	Nitrogen 30 mL min^{-1}
Detector Temperature	320 ^oC
Injector Temperature	280 ^oC
Temperature Program	Initial temperature 70 ^oC for two minutes, and then increase by 3 ^oC /min to 265 ^oC, hold for 25 min.

Analyzed compounds are identified and quantified using analytical standards supplied by the Marine Environmental Laboratory of the International Atomic Energy Agency (IAEA), in Monaco, or a commercial supplier.

ORGANISMS

A sample of 50 to 100 g of fresh tissue is dried, preferably by freeze-drying. Grind it in a mortar, and extract it in a soxhlet apparatus with 150 ml hexane for 8 hrs. Determine the extract volume (V_i). Take an aliquot (V_{fat}) and put it in a clean, previously weighed, beaker. Evaporate the extract with a stream of nitrogen and weigh it again. Calculate the weight of the lipids (W_{fat}) subtracting the weight of the beaker from that of the beaker plus the lipids. Calculate percent lipids from the weight difference.

Reduce the volume of the extract to 5 to 10 ml in a rotary evaporator, and then transfer it to a clean kuderna-danish tube. Add a few drops of concentrated sulfuric acid and shake it. Add more acid until lipids have been removed (until the extract is colorless and/or transparent). Transfer the extract to a new tube, and add some sodium sulfate to dry it. Pack a chromatography column to 18 cm with a silical gel and hexane slurry. Add the extract to the column, and elute with 35 ml hexane to receive fraction F1, and with 45 ml benzene or toluene to receive fraction F2. Reduce the volume of these fractions to 1 ml in a kuderna-danish apparatus, and inject in a gas chromatograph using the same conditions as described for sediments.

Analyzed compounds are identified and quantified using analytical standards supplied by the Marine Environmental Laboratory of the International Atomic Energy Agency (IAEA), in Monaco, or a commercial supplier.

6.5 BILE METABOLITES

Mix 48 mL of ethanol (spectroscopy grade) and take to 100 mL with distilled water, to obtain a 48:52 (v/v) solution. Calibration curves should be in the expected concentration range of the samples.

Take 5 µL of bile and dilute it to 2 ml with the ethanol:water mixture, shake in a vortex mixer to ensure sample homogeneity, transfer to a 1 cm fluorescence cuvette and read the fluorescence emission in a spectrofluorometer. The volume of bile can be increased if concentrations are low.

Four compounds are analyzed, representing the four most important PAH groups: naphthalene (two benzene rings); fenanthrene (three rings); pyrene (four rings), and benzo(a)pyrene (five rings). If the

concentration of low molecular weight compounds (two and three rings) is higher than the concentration of high molecular weight PAHs, then the hydrocarbons come from petroleum. If the concentration of high molecular weight compounds (4 and 5 rings) is higher then the hydrocarbons come from a pyrolitic source (burnt crankcase oil, forest fires, etc.).

The excitation and emission wavelengths for these compounds are:

Compound	Excitation	Emission
Hidroxypyrene	348	386
Hidroxynafthalene	308	470
Fenanthrene	292	363
Benzo(a)pyrene	364	404

Metabolite concentration is calculated according to the formula:

$$\text{Conc} = \frac{(INT_{muest} - b)\,(VOL_{dis}/VOL_{muest})}{a}$$

Where:

INT_{muest} = Sample fluorescence.

a = Y intercept calibration curve.

b = Slope calibration curve.

VOL_{dis} = Final volume

VOL_{muest} = Bile volume

6.6 CHOLINESTERASE DETERMINATION

Tissue preparation

1. Prepare Tris pH 8.0 buffer with a concentration 0.5 M (0.709 g in 100 ml of distilled water).
2. Weigh 200 mg of tissue.
3. Add cold Tris buffer (1 ml per 100 mg of tissue).
4. Homogenize with a piston tissue blender until the tissue is completely blended. The tube with the tissue must be kept on ice at all times.
5. Put the mixture in a centrifuge tube.
6. Centrifuge at 4000 rpm for 5 minutes.
7. Recover the supernatant, and put it in an Eppendorff tube.
8. Freeze at – 20 ^{o}C until analysis.

CHOLINESTERASE ACTIVITY (Ellman *et al.*, 1961)

REAGENTS

DNTB/Tris Buffer pH 7.4 Solution

1. Weigh 0.0758 g of Tris buffer (pH 7.4)
2. Dissolve in distilled water and take to 100 ml
3. Weigh 0.0099 g DNTB
4. Dissolve in the Tris solution, and take to 1 liter.

Acetilcholine Iodide (ASChI) Solution

1. Weigh 0.1446 g of ASChI.
2. Dissolve in distilled water, and take to 5 ml

Enzyme Activity

1. Thaw the sample.
2. Put in a test tube 960 µl of the DNTB/Tris pH 7.4 buffer solution and 20 µl sample.
3. Shake.
4. Put into an spectrophotometer cuvette.
5. Before reading absorbance, add 20 µl ASChI solution, and stir.
6. Read the absorbance in a spectrophotometer at 405 nm for 1 minute. Obtain ∆ Abs/min.

Calculation of Enzyme Activity

1. Obtain the protein content of the sample by the Lowry method (see below).
2. Use the formula:

$$\text{Enzyme activity} = \text{M/min/ gr protein} = \frac{1}{13600} * \frac{\Delta \text{ Abs/min}}{(Vh/Vt) * Cp}$$

Where:

∆ Abs/min = Kinetic parameter obtained before.
Vh/Vt = Homogenate Volumen/Total volumen in the cuvette = 20 µl/1000 µl= 0.02
Cp = Protein concentration in mg/ml

Protein Determination (Lowry Method)

1. Solution "A" for 1 liter:
   20 g sodium carbonate (Na_2CO_3)
   4 g sodium hydroxide (NaOH)
   0.2 g sodium potassium tartrate

2. Copper Sulphate Solution (Solution "B") ($CuSO_4 \cdot 5H_2O$) 0.1%
   Dissolve 0.1 $CuSO_4 \cdot 5H_2O$ in 10 ml distilled water. Prepare the same day it is going to be used.

3. Solution "C"
   Mix 50 parts of solution "A" and 1 part of solution "B". Stir.

4. Solution "D"
   Mix equal volumes of Folin Cicolteau´s reagent and water. Prepare fresh.
5. Protein standard solution
   Weigh 0.01 g of bovine serum albumin in 10 mL water.

Tube	1	2	3	4	5	6	Sample
SEP µl	0	5	10	25	50	75	0
Distilled Water	100	95	90	75	50	25	90
Homogenate	0	0	0	0	0	0	10
Solution C ml	1	1	1	1	1	1	1

Shake and leave to rest for 10 minutes

Solution D µl	100	100	100	100	100	100	100

Shake each tube when adding solution D and leave to rest for 30 minutes

6. Place the content of each tube into a cuvette, and read absorbance at 750 nm.

7. Plot absorbance vs protein concentration for each tube, filling the table:

X Protein Content in µg	**Y Abs**
0	
5	
10	
25	
50	
75	

8. Obtain the linear regression formula:

Y = mX + b

9. Isolate X: X = (Y-b)/m

10. Substitute in Y the absorbance of the simple and obtain µg of sample.

11. Since 20 µl of sample were used, protein concentration in mg/mL is:

Sample concentration mg/ml = µg protein in homogenate / 20 µl

6.7 DATA PROCESSING AND ANALYSIS OF RESULTS

For each sampling period, calculate the median concentration of each parameter. It is usually better to use medians and not means, because of non-normality of biological data. In order to determine possible spatial trends in the results, plot individual results on maps of the study area. Also, plot results versus time to look for temporal trends.

ANALYSIS OF RESULTS

Run nonparametric correlation analysis of the concentrations in organisms versus biometric measurements such as total and standard length, weight, etc. Do the same for the results in sediments, correlating the concentrations to sediment grain size and organic matter content.

When sufficient data are available, then temporal trends can be explored. Also, run an analysis of variance (ANOVA) to test for differences between sampling sites and/or times. In particular, it is interesting to see if there are differences between clean and impacted sites.

INTERPRETATION OF RESULTS

Interpretation of results is a complex issue, since the interpretation can be done from a technical point of view or from a legal point of view, in terms of whether or not the values are above or below environmental regulations. This has important management consequences.

6.8 WATER QUALITY

The selected methods for water quality analyses recommended for the MBRS-SMP are presented in this Section. There are many parameters that can be measured to determine the quality of water and while some of them are quite sophisticated, we have focused on methods that are simple and inexpensive. We have done so in an attempt to ensure that they can be conducted over a long period of time, thus, ensuring sustainability of this Section of the SMP. Such simple methods will also allow a wider participation of countries and institutions.

We have also focused on those parameters that can provide useful indications of the state of the ecosystems, as well as highlight possible problems with discharges of waste waters: nitrates, nitrites, ammonium, phosphates and coliforms (total and fecal). The methodology to analyze silicates is not included because this compound is not usually associated to discharges of sewage or industrial waters.

The selected water quality methods have been taken from classic references of this topic (Strickland and Parsons, 1972); APHA, 1989). Such procedures are carried out practically all over the world, sometimes with slight modifications. An updated summary of methods from Parsons *et al*. (1984) can also be consulted as it contains slight modifications on previous work (Strickland and Parsons, 1972). Although the analyses of nutrients are carried out routinely in many parts of the world, it is strongly recommended that the two aforementioned books be consulted carefully prior to embarking on any analyses and that such analyses be carried out by a person with due training and experience. The methods for the analysis of total and fecal coliforms were taken from APHA Standards Methods (1989).

All the methods for nutrient analyses described in this manual require the analyses of blank reagents that utilize “aged” seawater. In order to do these analyses, several liters of seawater should be collected, preferably several kilometers offshore. The seawater is to be placed in a clear plastic recipient, and left in the sunlight during several weeks.

Despite being mentioned in practically all the legislations of the world, coliform bacteria are not a good ecological indicator. Their usefulness as an indicator of sanitary conditions is unquestionable, because some species of bacteria besides being a pathogen to humans, are also indicative of the presence of fecal material, and therefore of the possible presence of other pathogen organisms as such as bacteria or parasites. However, from the point of view of environmental monitoring their usefulness is doubtful, because they are not pathogenic to aquatic organisms, and the mortality of these bacteria in warm marine waters with great penetration of solar light, such as the conditions prevailing in the Caribbean Sea, is very high in a very few hours. For these reasons, routine measurements of coliform bacteria for a monitoring program such as the SMP are not recommended. Nevertheless, in the interest of completion, we include in this manual, accepted standard methodologies for their analyses.

Water analyses have the disadvantage that the values obtained vary greatly, mainly in areas near the coasts. This variability is produced by the tides and their associated currents, changes in temperature and solar illumination, etc. The values of temperature, salinity, nutrients, and other parameters can change by a factor of two or three within a few hours. For this reason, the routine analysis of nutrients (or other dissolved components) in water is not recommended for pollution

monitoring. Should it be determined that such analyses need to be carried out, then at least four samples per sampling station in a 24 hours cycle must be taken, and the average (and standard deviation) of the measurements be reported.

6.9 AMMONIUM

The main problem in the determination of ammonium in natural waters is the need for good quality analytic blanks. Therefore, all due precautions should be taken to avoid sample contamination from glassware or reagents. Glassware should be rinsed with warm hydrochloric acid (10%), and later with distilled and deionized water immediately before use. Water must be distilled, and deionized immediately prior being used. This can be done by passing the distilled water through a column (1 to 2 x 30 cm) with a cationic exchange resin (in acid form) immediately before it is used.

Note: Never open a bottle of ammonium hydroxide while carrying out an analysis of ammonium.

REAGENTS

Alkaline Sodium Citrate Dissolve 700 g of sodium citrate and 40 g of sodium hydroxide in 2000 ml of distilled and deionized water and store in a polyethylene bottle. This solution is stable for several months.

Sodium Hypochlorite Use a commercial hypochlorite solution (bleach, *i.e*. Clorox) that should be approximately 1.5 N. This solution breaks down slowly and its concentration should be revised periodically. To do this, dissolve 12.5 g of sodium thiosulphate pentahydrated in 500 ml of distilled water. Add some crystals (around 2 g) of potassium iodide to 50 ml of water in a flask, and add 1.0 ml of the hypochlorite solution. Add 5 to 10 drops of concentrated hydrochloric acid and titrate the liberated iodine with the thiosulphate solution until the yellow color disappears. Discard the hypochlorite solution if less than 12 ml of thiosulphate are used.

Sodium Arsenite Dissolve 20 g of sodium meta-arsenite (analytic reagent quality) in 1000 ml of distilled and deionized water. Store in a sealed polyethylene flask. This solution is stable indefinitely.

Sodium Bromide Dissolve 1.5 g of sodium bromide (analytic reagent quality) in 250 ml of distilled and deionized water.

Oxidizing Reagent Add 0.5 ml of the sodium hypochlorite solution to 100 ml of the alkaline sodium citrate solution immediately before use (at most three hours before it is used). Prepare multiples of this solution, according to the number of samples to be analyzed: 10 ml of oxidizing reagent per sample.

Acidifying Solution Dilute hydrochloric acid (analytic quality reagent) with a similar volume of distilled and deionized water.

In a 125 ml Erlenmeyer flask add approximately 50 ml of distilled water, add 10.0 ml of the oxidizing reagent and 2 ml of sodium arsenite. Add one or two drops of thymol blue (0.1% in distilled water), mix and titrate carefully with the diluted hydrochloric acid solution until the color changes from blue to pink (pH around 1.7). Titrate in duplicate. The titrations should vary less than 0.1 ml of used acid, and the average of the two should be registered to the nearest 0.5 ml. If *x* ml of acid were used (approximately 5 to 6 ml) then dilute 200*x* ml of acid to exactly 2000 ml of distilled and deionized water. This solution should be prepared fresh every time that the alkaline sodium citrate solution is prepared.

Sulfanilamide Dissolve 5 g of sulfanilamide in a mixture of 50 ml concentrated hydrochloric acid and around 300 ml of distilled and deionized water. Dilute to 500 ml with distilled and deionized water. This solution is stable for several months.

N-(1-naftil)-ethylendiamine Dihydrochloride Dissolve 0.50 g of the dihydrochloride in 500 ml of distilled and deionized water. Store this solution in a dark flask. This solution should be prepared each month, or if it presents a strong brown color.

Standard Ammonium Solution Dissolve 0.100 g of analytic reagent quality ammonium sulfate in 1000 ml of distilled and deionized water. Add 1 ml of chloroform, shake and maintain protected from strong light. This solution is stable for several months if it stays tightly sealed. 1 ml = 1.5 µmol of ammonium. Place 1.00 ml of this solution in a flask and make up to 500 ml with seawater. The resulting solution is equal to 3.0 µmol ammonium/liter.

Reagent Blank Place 50 ml of freshly deionized distilled water in a flask, and add 1 ml of sodium bromide as a catalyst.

PROCEDURE

1 - Add 50 ml of the sample to an Erlenmeyer flask. Add 10 ml of the oxidizing reagent, shake and allow to rest for 10 minutes at a temperature between 20 and 25 °C.

2 - Add 2 ml of the sodium arsenite solution and shake. Add 10 ml of the acidifying solution and mix.

3 - As soon as it is possible, add 1 ml sulfanilamide and mix. After 3 to 8 minutes, add 1.0 ml nafthylethylendiamine and mix. Allow to rest for at least 10 minutes (but less than 2 hours) and measure the extinction in a spectrophotometer at 543 nm, using distilled water as reference, in a 10 cm cell.

4 - Correct the extinction, subtracting the extinction from the reagent blank from that of the sample (see below). Calculate the concentration using the formula:

$$\text{[Ammonium µmolar]} = F[E-(0.70 \times C/F')]$$

Where E is the corrected extinction, F it is a factor of proportionality (see below), C is the concentration of nitrite in the sample, and F ' is equal to 2.1 if a spectrophotometer is used.

5 - To determine the factor F, 50 ml of the standard ammonium solution is placed in a 125 ml Erlenmeyer flask in quadruplicate, 50 ml of the same water used to prepare the ammonium standard solution is placed in two additional flasks. Carry out steps 1 to 3 described before. Calculate F as:

$$F = 3.0/(Es-Eb)$$

Where Es is the average extinction of the four standards, and Eb is the average extinction of the two blanks. **The value of F should be close to 4.5**.

The reagent blank should be analyzed in triplicate (steps 1 at 3 above) for each lot of samples to be analyzed. **If the average extinction is higher than 1.5 the blanks should be discarded**.

6.10 PHOSPHATES

REAGENTS

Ammonium Molybdate Dissolve 15 g of ammonium paramolybdate (analytic reagent quality) in 500 ml of distilled water. Store in a plastic bottle away from sunlight. This solution is stable indefinitely.

Sulfuric Acid Add (carefully!) concentrated sulfuric acid to 900 ml of distilled water. Allow to cool and store in a glass flask.

Ascorbic Acid Dissolve 27 g of ascorbic acid in 500 ml of distilled water. Store in a plastic bottle and maintain frozen. Defrost before using, and freeze again. The frozen solution is stable for several months, but it should not be conserved more than one week at room temperature.

Potassium Antimonyl-tartrate Dissolve 0.34 g of potassium antimonyl-tartrate in 250 ml of water, heating if necessary. Store in a glass or plastic bottle. This solution is stable for several months.

Mixed Reagent Mix 100 ml of ammonium molybdate, 250 ml sulfuric acid, 100 ml ascorbic acid and 50 ml of ammonium antimonyl-tartrate. Prepare immediately before using (not more than 6 hours), and discard any excess. Do not store. This quantity is suitable for approximately 50 samples.

Standard Phosphate Solution Dissolve 0.816 g of anhydrous potassium dihydrogen phosphate in 1000 ml of distilled water. Add 1 ml of chloroform and store in a dark bottle. This solution is stable for several months. 1 ml = 6.0 µmol P.

Dilute 10.0 ml of this solution to 1000 ml with distilled water, and add 1 ml of chloroform. Store the solution in a dark bottle for a period no longer than 10 days. 1 ml = 6.0 x 10^{-2} µmol P.

Reagent Blank Follow the procedure described below (steps 1 to 3) substituting distilled water instead of the sample. Determine the extinction of the blank (Eb), that should be smaller than 0.02. If it exceeds this value then use double distilled water; if it continues to exceed it, prepare the solution of ammonium molybdate again. This value should be revised every week.

PROCEDURE

1. Defrost the samples and take them to ambient temperature. Determine the extinction at 885 nm against distilled water, to determine the correction for turbidity, that should be smaller than 0.1.

2. To a 100 ml sample, add 10 ml of the mixed reagent and stir immediately.

3. After 5 minutes, and before 2 hours, measure the extinction from the solution at 885 nm in a 10 cm cell against distilled water.

4. Correct the extinction subtracting the extinction for turbidity and that of the reagent blank (see below). Calculate the concentration of reactive phosphate using:

$$\mu Mol\ P/l = Ec \times F$$

Where Ec is the corrected extinction, and F a proportionality factor.

5. Prepare four standards that consist of 5.0 ml of the dilute phosphate standard solution and make up to 100 ml with distilled water. Carry out the analytic procedure (steps 1 to 3) and calculate F using:

F = 3.00/(Es-Eb)

Where Es is the average extinction of the four standards, and Eb is the average extinction of two reagent blanks. F should have a value close to 5.0, and it should be revised at not very frequent intervals.

6.11 NITRITES

REAGENTS

Sulfanilamide Dissolve 5 g of sulfanilamide in a mixture of 50 ml concentrated hydrochloric acid and around 300 ml of distilled and deionized water. Dilute to 500 ml with distilled and deionized water. This solution is stable for several months.

N-(1-nafthyl)-ethylenediamine Dihydrochloride Dissolve 0.50 g of the dihydrochloride in 500 ml of distilled and deionized water. Store this solution in a dark flask. This solution should be prepared each month, or if it presents a strong brown color.

Nitrite Standard Solution Dry some anhydrous sodium nitrite in a laboratory stove at 110 °C for one hour. Dissolve 0.345 g of dry sodium nitrite in 1000 ml of distilled water. Add 1 ml of chloroform as a conservative. Store the solution in a dark bottle. This solution should be stable at least for one month. 1 ml = µ5 mol nitrite. Take 10.0 ml from this solution and make up to 1000 ml with distilled water and use the same day. 1 ml = 1.0 µmol/l if it is diluted in 50 ml.

PROCEDURE

1 - Determine the turbidity of the sample by placing 30 ml of the sample in a flask and adding 1 ml sulfanilamide and mix. Place a sub-sample in a 10 cm spectrophotometer cell and read the extinction at 540 nm.

2 - Place 50 ml sample in a flask and add 1.0 ml sulfanilamide. Mix and allow it to rest for 2 to 8 minutes.

3 - Add 1.0 ml nafthyl-ethylenediamine and mix. After 10 minutes, but before 2 hours, read the extinction in a 10 cm cell at 540 nm.

4 - Correct the extinction subtracting the extinction for turbidity and that of the reagent blank.
5 - Calculate the nitrate concentration using the formula:

Nitrites (µMolar) = Ec x F

Where Ec is the corrected sample extinction, and F it is a proportionality factor.

6 - The reagent blanks are made following steps 2 to 4, but using distilled water instead of the sample.

7 - Prepare four standard solutions taking 2.00 ml of the dilute standard solution to 50 ml with distilled water. Transfer the solutions to dry 125 ml Erlenmeyer flasks. In two additional flasks place 50 ml of distilled water as blank. Make the determination of nitrites as described in points 2 and 3.

8 - Calculate **F** from the following formula:

$$F = 2.00/(Es-Eb)$$

Where Es is it is the average extinction of the four standards, and Eb is the average extinction of the two blanks. **F** should have a value close to 2.1 if a spectrophotometer is used. This value should vary little.

6.12 NITRATES

REAGENTS

Concentrated Ammonium Chloride Dissolve 125 g of analytic reagent quality ammonium chloride in 500 ml of distilled water. Store the solution in a plastic or glass bottle.

Dilute Ammonium Chloride Dilute 50 ml from the concentrated ammonium chloride solution to 2000 ml with distilled water. Mix well, and store in a glass or plastic bottle.

Sulfanilamide Dissolve 5 g of sulfanilamide in a mixture of 50 ml concentrated hydrochloric acid and around 300 ml of distilled and deionized water. Dilute to 500 ml with distilled and deionized water. This solution is stable for several months.

N-(1-naftil)-ethylenediamine Dihydrochloride Dissolve 0.5 g of the Dihydrochloride in 500 ml of distilled and deionized water. Store this solution in a dark bottle. This solution should be prepared each month, or if it presents a strong brown color.

Cadmium-Copper Reducer Stir 100 g of cadmium filings in 500 ml of a 2 % (w/v) copper sulfate pentahydrate solution until the blue color has disappeared and semi-colloidal copper particles begin to appear in the solution. Wind some fine copper wool with the fingers to make a small plug. Place the plug in the reducing column and fill the column with dilute ammonium chloride solution, and add enough mixture of cadmium and copper to fill approximately 30 cm of the column. Add the metal slowly, gently packing the column so that it settles well. Wash the column with dilute ammonium chloride. The flow should be such that 100 ml of the solution will take between 8 and 12 minutes to flow through. Add a plug of copper wool to the upper part of the column. When the column is not being used, the cadmium-copper mixture should be totally covered with ammonium chloride. If it is suspected that the reduction efficiency has diminished, empty the content of four columns in a glass beaker, and wash the metal with diluted hydrochloric acid (5% v/v) twice. Finally, rinse with distilled water until the water is not acidic. Decant the metal and treat it again with copper sulfate, as described previously, and fill the columns again. The cadmium-copper regenerated this way should be enough for three columns.

Nitrates Standard Solution Dissolve 1.02 g of analytic reagent quality potassium nitrate in 1000 ml of distilled water. This solution is stable. Dilute 4.00 ml in distilled water. This solution should be stored in a dark bottle, and prepared fresh immediately before being used. The concentration of this standard is 20 µmol nitrate/liter.

Reducing Column The column is prepared joining three glass tube sections: 10 cm of tube (5 cm internal diameter) joined to 30 cm tube (10 mm internal diameter) (this will contain the metallic reducer), which in turn is joined to 35 cm of 2 mm of diameter tube. The 2 mm tube is bent near the joint, so that it is parallel to the 10 mm tube. The free end of the 2 mm tube is bent near the tip, forming a siphon in the form of an inverted “U”. This last bent should be leveled with the upper part of the 10 mm tube (Figure 6.2).

Figure 6.2 Cadmium-Copper reducing column (Photograph courtesy of Dr. David Valdés)

PROCEDURE

1. Place a 100 ml sample in a 125 ml Erlenmeyer flask, and add 2.0 ml of concentrated ammonium chloride. Mix and put 5 ml in the upper part of the reducing column, and elute. Add the rest of the sample to the column, and place a beaker under the column to collect the effluent. Collect 40 ml, rinse the flask and discard. Collect another 50 ml of effluent and add 1.0 ml sulfanilamide.

2. Allow the mixture to react at least for 2 minutes, but not more than 8. Add 1.0 ml nafthyl-ethylendiamine, and mix. After 10 minutes, but not more than 2 hours, measure the extinction of the sample in a 1 cm cell at 543 nm. If the extinction is higher than 1.25 then use a thinner cell or dilute the sample. If the extinction is lower than 0.1 then use a 10 cm cell.

3. Correct the extinction subtracting the extinction of a reagent blank (using a cell of the same size that the one that was used for the sample).

4. Calculate the concentration using the formula:

Nitrates (µmol/l) = (Ec x F)-0.95 x C

Where Ec is the sample corrected extinction, F is a proportionality factor, and C is the concentration of nitrites in the same sample.

5. The extinction of the blanks is determined substituting distilled water instead of the sample.

6. Calculate the factor F as:

F = 2.00/(Es-Eb)

Where Es is the average extinction of the four standards, and Eb is the average extinction of the two blanks. F should have a value close to 2.1 if a spectrophotometer is used. Value can vary a little.

6.13 TOTAL COLIFORMS

PRESUMPTIVE PHASE

REAGENTS

Lauryl Tryptose Medium To a liter of distilled water add:

20 g tryptose
5.0 g lactose
2.75 g of dipotassium hydrogen phosphate
2.75 g of potassium dihydrogen phosphate
5.0 g of sodium chloride
0.1 of sodium lauryl sulphate

Mix the ingredients and heat to dissolve them. After sterilization, the pH should be 6.8 ± 0.2. Place the medium in fermentation tubes with an inverted vial; close them with a metal plug or heat-resistant plastic cap. Sterilize the tubes in an autoclave. Prepare 5 series of tubes for each dilution for each sample.

PROCEDURE

Inoculate increasing quantities of sample in a decimal scale (0.1, 1.0, 10.0 ml). To do this, dilute a 1.0 ml sample in 99 liters of distilled and sterilized water. For example: add 10 ml of the sample to a tube; add 1 ml to second tube; add 0.1 ml of the sample to a third one; add 1 ml of the diluted sample to the fourth; and finally, add 0.1 ml of the diluted sample to the last tube. In this way, the concentration of the samples will scale from 10 ml, 1 ml, 0.1 ml, 0.01 ml to 0.001 ml in each tube. Mix each tube several times. Prepare 5 tubes of each dilution for each sample. Incubate at 35 ± 0.5 °C for 24 hours. Mix each tube carefully and observe if any gas or a yellow color develops. Otherwise incubate for a further 24 hours and observe again. The appearance of gas, or a yellow color, at either 24 or 48 hours constitutes a positive presumptive test.

CONFIRMATORY PHASE

REAGENTS

Brilliant Green Medium Dissolve in one liter of distilled water:

10.0 g peptone
10.0 g lactose
20.0 g ox gall
0.0133 g brilliant green

The pH should be 7.2 ± 0.2 after sterilization. Place the medium in fermentation tubes with an inverted vial and sterilize in an autoclave.

PROCEDURE

Inoculate one tube with brilliant green medium for each positive sample in the presumptive phase. Stir the tubes of the presumptive phase that gave positive results; introduce a sterile metal loop (3 mm diameter) in the presumptive tube and quickly introduce it in the brilliant green tube. Stir the tubes and incubate at 35 ± 0.5 °C during 48 hours. The formation of gas at 48 hours constitutes a confirmatory test.

Calculate the value of the most probable number (MPN) starting from the number of positive tubes. Consult the table that is provided in the following section.

6.14 FECAL COLIFORMS

REAGENTS

EC Medium In a liter of distilled water dissolve:

20.0 g trypticase
5.0 g lactose
1.5 g bile salts
4.0 g dipotassium hydrogen phosphate
1.5 g potassium dihydrogen phosphate
5.0 g Sodium Chloride

After sterilization, the pH should be 6.9 ± 0.2. Place the medium in fermentation tubes with an inverted vial; seal them with a metal plug or heat-resistant plastic cap. Sterilize the tubes in an autoclave. Prepare 5 tubes for each dilution for each sample.

PROCEDURE

Inoculate a tube with the EC medium for each positive sample of the presumptive phase of the test for total coliforms. Stir the tubes of the presumptive phase that gave positive results, introduce a sterile metal loop (3 mm diameter) in the presumptive tube and quickly introduce it in the tube with the EC medium. Stir the tubes and incubate them in a water bath at 44.5 ± 0.2 °C during 24 hours. The formation of gas at 24 hours constitutes a positive test.

Calculate the value of the most probable number (MPN) from the number of positive tubes in the confirmatory test. Consult Table 6.2 next, where the MPN of colony-forming units is given per 100 ml sample. The columns 1 and 3 refer to the number of positive tubes of the five that were inoculated for each dilution.

Table 6.2 Table of Calculation of the Most Probable Number (MPN) for several combinations of positive results if five tubes are used by dilution (10 ml, 1 ml, 0.1 ml)

Combination	MPN/100 ml	Combination	MPN/100 ml
0-0-0	< 2	4-2-0	22
0-0-1	2	4-2-1	26
0-1-0	2	4-3-0	27
0-2-0	4	4-3-1	33
		4-4-0	34
1-0-0	2		
1-0-1	4	5-0-0	23
1-1-0	4	5-0-1	30
1-1-1	6	5-0-2	40
1-2-0	6	5-1-0	30
		5-1-1	50
2-0-0	4	5-1-2	60
2-0-1	7		
2-1-0	7	5-2-0	50
2-1-1	9	5-2-1	70
2-2-0	9	5-2-2	90
2-3-0	12	5-3-0	80
		5-3-1	110
3-0-0	8	5-3-2	140
3-0-1	11		
3-1-0	11	5-3-3	170
3-1-1	14	5-4-0	130
3-2-0	14	5-4-1	170
3-2-1	17	5-4-2	220
		5-4-3	280
4-0-0	13	5-4-4	350
4-0-1	17		
4-1-0	17	5-5-0	240
4-1-1	21	5-5-1	300
4-1-2	26	5-5-2	500
		5-5-3	900
		5-5-4	1600
		5-5-5	> 1600

MBRS POLLUTION SAMPLES ENTRY FORM

MSMP_5A

Location:	**Data Collector:**	**Time:**	**Country:**
Site ID:	**Sample Collector:**	**Date:**	

Station	Latitude	Longitude	Time	Sediments	Organisms	Observations

Signature:

7. METHODOLOGY FOR PHYSICAL OCEANOGRAPHY / MODELS

7.1 OVERVIEW

The MBRS is a dynamic oceanographic region, bordered by some of the most energetic currents of the world's oceans: The Cayman Current and the Yucatan Current. There is a high space and time variability in the oceanographic conditions in the region (Stammer and Wunsch, 1999; Ochoa *et al.,* 2001; Sheinbaum *et al.,* 2002a) as well as the ubiquitous presence of small and large-scale eddies in the region, which may be generated locally from instabilities of the strong currents or drift into the region from the eastern Caribbean (Sheinbaum *et al*., 2002b).

The adequate development of the SMP in the long-term, therefore, requires a better understanding and predictive capability of coastal currents, oceanographic processes, and variability on different time scales. The development of a numerical circulation model for the Western Caribbean is also a useful component of the overall MBRS Project that can add to the understanding of the biological and ecological processes taking place in the region as a whole. The information gathered under this theme will be utilized to improve on the design of a comprehensive SMP. The specific objectives of this Section of the SMP Manual are to discuss measurements needed in support of the SMP, how to make such measurements, and the strategy of what to measure under specific circumstances.

7.2 SAMPLING STRATEGY

Physical oceanographic sampling in support of environmental coastal monitoring, and in support of numerical circulation modeling, can be conducted in one of several ways.

Time Series Measurements
This is usually the most effective way of collecting physical oceanography monitoring data for analysis and to validate numerical models. Time series measurements consist of the measurement of one or more parameters at a fixed sampling rate for an extended time period from one or more locations along the coast or in the open ocean. Since most parameters are measured automatically with submerged instruments or sensors, the sampling rate should be sufficiently rapid to resolve tidal variability. A sampling interval equal to Δt = 1 hour, i.e. a sampling rate of 1 cycle per hour (cph) is an optimum choice. Sampling should be continued for a sufficiently long duration to allow meaningful resolution of tidal, meteorological, and seasonal variability. Ideally, this duration should be at least one full year. A time series measurement duration of less than 29 days (696 hours) is not meaningful. A month-long series will allow harmonic tidal analysis of the measured data. Ideally, time series measurements should be made for 1 year or longer.

Measurements along a Transect
This is usually an effective means of collecting physical oceanographic data on ship cruises when biological, chemical, and geological measurements are made simultaneously from specific sensors, water samples, or tows. In this case, the physical measurements are usually made as a vertical profile between the water surface and the bottom every time a hydrographic or oceanographic station is occupied. To be useful, transect measurements from a ship should be made systematically according to a pre-determined sample design. Vertical sampling of physical oceanographic measurements is usually accomplished pseudo-continuously every meter or few meters. In the case, when data are obtained from water samples, vertical sampling should be conducted at many depths, more closely spaced near the surface and at the pycnocline where the gradients are steepest. Spatially, stations should be sufficiently closely spaced to allow the determination of coherent features, e.g. coastal waves, meso-scale eddies, upwelling fronts, and river or inlet plumes. Temporally, adjacent stations should also be made as frequently as is convenient while traveling. Most physical oceanographic sampling can be made while a research ship is drifting; however, velocity measurements may require anchor stations, although modern bottom-mounted or towed

Acoustic Doppler Current Profiling (ADCPs) current meters do allow velocity sampling while a ship is under way (because of bottom tracking).

Occasional Sampling
Physical parameters should always be measured when biological, chemical, and geological ocean parameters are measured. However, the value of occasional sampling at dispersed times and locations in a study area, is usually of little value unless sampling is conducted in the form of a time series or systematically as vertical profiles along a ship track.

Sampling Interval, Sampling Rate, and Sampling Duration
The ideal sampling rate for time series measurements in the MBRS project is one set of measurements per hour. However, each measured parameter should ideally be burst sampled frequently, may be at a rate of 1-2 Hz, for 2-5 minutes to establish a mean value to record every hour. This is a type of filtering, which avoids spurious peaks and smooths small-scale turbulence fluctuations. Since much coastal oceanographic variability is highly related to tides, even in the MBRS region which has a small tide, it is essential that field sampling be conducted sufficiently frequently to resolve the variation within a tidal cycle and avoid data aliasing.

7.3 CORE PARAMETERS

Physical oceanographic measurements can be thought of as consisting of a) generic measurements, which must always be recorded; b) oceanographic measurements, which usually are either the main focus of a study or required supplementary data; and c) meteorological data, which can be thought of as important value-added information to be added to the physical oceanographic observations and recordings. All measurements should be recorded in standard SI units of measure, in some cases in standard oceanographic units of measure (as in the case of either **‰** or practical salinity units [**psu**] for salinity), but not in English units of measure.

The generic measurements include:

Position of Measurement
Such measurements should always be made using a hand-held Global Positioning System (GPS) recorder. The latitude and longitude of a location or the UTM coordinates should be determined to the nearest few meters, which usually translates to better than the closest 1second of latitude/longitude or to within ~0.01 minute of latitude or longitude.

Date and Time
For all measurements, date and time must be recorded as carefully and detailed as possible, including year, month, day, hour, and minute. For any year, it is often convenient for plotting purposes to express the month-day-hour information in a modified Julian Date (JD) format, where JD 0.0000 is the very beginning of a new year, 1.0001 is just after midnight on 2 January, and the year ends on 31 December at midnight with JD equaling 365.0000, or JD 366.0000 on leap years. When the JD format is used, 4 decimal places are required. Alternately, year, month, day, hour, and minute can be expressed in a spreadsheet but with each time unit in a separate column to allow conversion to JD or other time format for analysis and plotting purposes. It is essential to record time of measurement in local standard time, local summer time, or Greenwich Mean Time (GMT). The actual time reference used must be noted. For long-term time series measurements, in particular, it is advantageous to express time referenced to GMT. Alternately, time can be recorded in standard local time (without ever) converting to local summer time.

Measurement of Water Depth or Elevation above Sea Surface
For any physical oceanographic measurement, it is essential to note the depth/elevation of any measurement relative to the local Mean Sea Level (MSL). For example, in making time series

measurements of currents, the depth of the measurement should be noted; in the case of winds, the elevation of the wind sensor above both local ground and MSL should be recorded; and in the case of water level (tide) measurements, a stable zero reference datum, which in turn ideally should be referenced to a local vertical geodetic datum, must be established for each tidal measurement site and regularly on approximately an annual basis verified.

Total Water Depth
For any physical measurement, it is essential to record the absolute (total) water depth at the location where measurements are made. This measurement should be made to either the nearest meter or preferably the nearest decimeter. This information has many uses, and play an important role in e.g. the analysis of shoaling and refraction of wind waves, propagation of coastal tides, frictional effects on currents.

The oceanographic measurements include:

Water Elevation (Tide)
Time series measurements of water elevation (tide) needs to be made at least hourly at fixed locations for long time periods, typically years, making sure that elevations are resolved to the nearest ~10^{-3} m, that the sensor is serviced and exactly repositioned every few weeks, and that a local vertical datum is established and revalidated from time to time.

Current Speed and Direction
Current speed and direction need to be measured as a time series at a fixed location and as a vertical profile of speeds and directions from a research vessel during transect sampling. Current measurements, together with water elevation measurements, are essential for any physical oceanography study including SMP in the MBRS region.

Water Temperature
Water temperature measurements are essential measurements to calculate density of sea water, to relate to biological metabolic processes, to map upwelling, to recognize internal waves, to determine the depth of the thermocline, and to calibrate satellite thermal images. It should be measured both as a time series at tide gauge locations and as vertical profiles on cruise transects.

Salinity (Conductivity)
Salinity measurements are essential measurements to calculate density of sea water, to relate to biological processes, to recognize terrestrial runoff, to determine the depth of the halocline and pycnocline. It should be measured both as a time series at tide gauge locations and as vertical profiles on cruise transects. It should be noted that salinity is never measured directly, but rather calculated from simultaneous conductivity, temperature, and pressure measurements (Foffnoff and Millard, 1983).

Density
Density is an essential component of the coastal and oceanic circulation, yields a measure of the pycnocline, is an indicator of mixing, and also an indicator of vertical stability. Density is calculated from salinity, temperature, and pressure values. The computational algorithm is given by Foffnoff and Millard (1983).

Water Transparency
Water transparency is an important parameter to explain high biological productivity and is also used as a water mass tracer in interpreting satellite images (in the visual part of the electromagnetic spectrum).

Sea state
The sea state is optimally expressed as a time series of measurements of significant wave height, significant wave period, and wave direction. Such data are important to relate to and interpret coastal erosion, storm effects on beaches and natural (e.g. reefs) and manmade structures (e.g. harbors, groins, breakwaters etc.) in the coastal ocean. These data are also important in the calculation of shoaling, refraction, and littoral sediment transport. It is essential that both measurement depth and total water depth be recorded in wave studies. When a recording wave instrument does not exist, it is useful to note manually the prevailing sea sate, according to the Beaufort scale, during both transect and time series measurement periods. For a modified Beaufort scale please refer to Appendix 1.

The meteorological measurements include:

Wind Speed and Direction
Wind speed and wind direction are measured as time series at fixed location along the coast, 10 m above the ground, ideally above open water. Good wind data are essential for physical oceanographic studies. Unfortunately, most available meteorological stations are located at inland airfields, whereas the ideal location for meteorological stations for oceanographic use would be on platforms or buoys in the coastal ocean.

Atmospheric Pressure
Atmospheric pressure is measured with a recording pressure gauge (barometer) at fixed locations associated with both meteorological stations and tide gauges. The data are important for correction and correlation of sea level measurements to separate the effects of atmospheric pressure fluctuations from astronomical tidal forcing.

Air Temperature
Air temperature should be measured as time series as a standard part of a meteorological station. Air temperature data are useful to evaluate changing meteorological states, calculate air-sea heat fluxes, and make water balance calculations to assess watershed runoff.

Rainfall
Rainfall measurements are usually integrated over time to assess either daily or monthly rainfall rates as a permanent component of a meteorological station.

River Discharge
The discharge of the main rivers entering the MBRS region need to be gauged upstream of where tidal variations are noted.

7.4 INSTRUMENTS

It is not practical to discuss specific instruments in any detail, as physical oceanographic sampling in the MBRS region will depend on actual instruments purchased or otherwise made available. For any type of measurement, there are many choices of instrument companies and also types of instruments, which range from high-tech sensors with automatic sampling and data-logging, usually expensive, to low-tech or simple appropriate technology instruments, which in comparison are inexpensive to purchase or construct but usually must be operated manually with each parameter-value written down in a notebook. These manual instruments require substantial personnel time in data collections and are not appropriate for a multi-national monitoring project in the 21st century. Specific capabilities and short-coming of commercial oceanographic instruments are detailed on the Internet pages of instrument manufacturers. However, it is safe to state that the types of physical oceanographic measurements to be measured as a part of MBRS physical oceanographic sampling

in support of the SMP, will require a mixture of time series, transect, and occasional measurements. This will require the acquisition and regular use of the following types of instruments.

GPS Unit
A handheld Global Positioning System (GPS) receiver is inexpensive, operates on batteries, and does a great job of measuring position as latitude, longitude, or UTM coordinates to within 30 m or better, and also measure time as both local time and GMT to within a fraction of a second.

Fathometer
A recording fathometer or echo sounder is the preferred tool for the recording of total station water depth. A fathometer can vary by three or more orders of magnitude in price, depending on recording memory and other features. The more high-tech and expensive units integrate GPS positioning and time with depth readings.

CTDs for Transect Profiling and Time Series Recording
A profiling Conductivity-Temperature-Depth (CTD) probe with internal recording and external display is required for transect sampling and should be equipped with a cable which measures at least 100 m. In addition, a recording conductivity-temperature (CT) device should be installed at each water level (tide) gauge and be capable of recording data internally for each at least several months at an hourly rate. If the CT device is exchanged for a recording CTD device, this then becomes a capable tide gauge as well. This is recommended. It should be noted that salinity is never measured directly, but calculated (either internally within an instrument or subsequent to sampling during the analysis phase) based on conductivity, temperature, and pressure (Foffnoff and Millard, 1983). A handheld refractometer is not an acceptable instrument for long-term monitoring of salinity in coastal or open ocean areas of the MBRS because of low precision.

Current Meter
An Acoustic Doppler Current Profiling (ADCP) meter is ideal for time series measurements, either bottom mounted or mounted on a hydrographic cable, to measure horizontal vector currents, usually at anywhere from 16-25 vertical depths or bins. Such an ADCP would be oriented to look upward. An ADCP can also effectively be used for transect measurements from the surface looking down. In that case, the ADCP is either mounted to the side or keel of a research vessel, or mounted onboard a small dedicated catamaran, which is towed behind a research vessel. The ADCP unit should be able to do bottom-tracking, and this is usually a value-added feature, to resolve absolute horizontal velocities by subtracting away the ship speed. Alternately, an electromagnetic current meter is also a good choice for long-term time series measurements of horizontal current vectors (speed and direction) from a fixed buoy location. Such an instrument has no moving parts and is particularly easy to service in the field. Also, either a profiling ADCP or an electromagnetic current meter, when combined with a permanent stage gauge, can effectively be used to establish a rating curve for specific location along the lower reaches of rivers, allowing the estimation of river discharge as a function of time.

Water Level (Tide) Gauge
The ideal choice for a water level or tide gauge is a high-resolution, bottom-mounted CTD recorder. Since tidal water level variations in the MBRS region are very small and often dominated by meteorological variability, it is essential that any water level gauge is sensitive enough to measure and record water level variations with a precision of sub-centimeter precision and accuracy, which requires a digital instrument capable of 14-bit or better recording and installation of the recording CTD in water depths less than 10 m. Alternately, the installation of a stilling well attached to a dock or pier with a pressure sensor mounted interior to the stilling well will also work well, but care must be taken to install the instrument in such a way that it is protected from the direct approach of wind waves and swells. In either case, care should be taken to establish a local vertical datum, ideally a datum referenced to the national geodetic network, and relate this with sub-centimeter accuracy to the recorded values of the tide gauge. It is essential that the installation of any CTD sensor/recorder

for the purpose of serving as a tide gauge or for measurement of the relative sea level variability is installed onto a fixe stable base, which does not change elevation, tilt, or change orientation even in the case of hurricane wave assault.

Transmissometer or OBS
Either a transmissometer or an Optical Back-scatter System (OBS) can effectively be attached and electronically connected (bundled) to a profiling CTD or a bottom-mounted CTD to measure both transect and time series of water clarity. The OBS is the preferred instrument in river mouth and other areas where the total suspended material concentration in the water column exceeds ~200 ppm, whereas a transmissometer usually is the more effective instrument in locations with low total suspended material concentration. A Secchi disk is often used to integrate water calrity or turbidity in the water column during occasional sampling. A Secchi disk is a manual instrument which is not satisfactory for determining water transparency and turbidity in any situation other than occasional sampling.

Directional Wave Recorder
A directional wave recorder is a relatively expensive piece of equipment, which because of the high frequency of wind waves and swells must be able to measure and record data at a very rapid sampling rate (usually 2-10 Hz), and also store large amounts of data (Gigabytes) for later processing. A good instrument choice is an electromagnetic current meter with a high-resolution pressure sensor, allowing simultaneous measurement of pressure, u component velocity, and v component velocity, i.e. making it into a puv wave sensor. Such an instrument can be programmed to measure horizontal currents on tidal and meteorological time scales as well as on wave time scales. Alternately, a wave-riding buoy is a good choice although the coast of purchasing and maintaining such a wave buoy is in comparison extremely expensive.

Meteorological Station
A fixed location meteorological station is not only a requirement to make good sense of oceanographic data but would simultaneously be used for weather measurements and predictions and climate change data collection. A meteorological station should collect and record data automatically, including at a minimum, measurements of wind speed, wind direction, rainfall, air temperature and atmospheric pressure. The wind speed and wind direction measurements should be made 10 m above the local ground, which for oceanographic applications ideally is 10 m above MSL, with the meteorological station located on a platform or buoy in the coastal ocean. Whereas the wind vector component measurements must be measured every second or more frequently and then averaged for ~1 minute before being recorded at preset times, usually every hour. The same is often the case with the air temperature and pressure readings although they usually do not need to be sampled quite as frequently as the wind. Rainfall, on the other hand, is usually measured and recorded as an integrated value for each 24-hour period.

Handheld Anemometer
A hand-held anemometer is an instrument with the capability to measure wind speed (usually using an impeller), wind direction (with built-in compass), and air temperature sensor for manual recording of these parameters while making transect or occasional measurements in the MBRS region.

7.5 Instructions on Instrument Use

To take accurate readings for monitoring, instruments need to be used correctly and consistently. Here are some general instructions for equipment use that will improve data quality and reliability.

GPS Unit

The best way to learn how to use GPS is of course to have a GPS receiver and try it out in practice. It will always be accompanied by detailed Instructions in the form of a handbook that explains how to use it.

GPS receivers come in different sizes, from small, handheld ones to bigger, more complex systems. In most cases, they have a display panel on which the status of the receiver and the current measurement are displayed. Most of the present models will also have a computer outlet by means of which the device can be connected to a PC with suitable software.

A GPS receiver will not work well inside buildings or near many tall objects (such as building in a city) that stop or reflect the high-frequency satellite transmissions. A fairly level area with a full horizon will receive the best signal (for instance in a boat off the coast). The GPS knows its approximate geographical position (you may have to tell it in which country you are) and will establish a list of satellites that are currently above the local horizon. Next it will acquire the satellite signals and display the signal strength for each acquired satellite. When signals from at least three satellites are being received, the GPS is able to perform a calculation (using triangulation) of its geographical position, giving the longitude and latitude in either decimal degrees or meters. When more than three satellites are acquired, it will add the altitude above the sea level. This is not accurate and must be corrected using a local datum. Prolonging the session with the GPS receiver will improve position accuracy somewhat. It may take some time for the accuracy to stabilize. Once a position has been established, the GPS receiver can be moved around. The GPS can accurately indicate position and travel direction (in degrees) while moving. In this sense, it acts as a super-compass.

Many GPS devices will store a series of momentary positions which display the route of travel. This can be very useful when traversing unknown territory as it can be followed back to the original position. A series of waypoints (i.e. sampling points) can be stored in the GPS and their positions can later be downloaded to a computer. Waypoints can also be uploaded to many GPS receivers. The GPS can then be used to read the distance, direction to a specific sampling point.

General Operating Procedure to establish and relocate monitoring sites and marked locations:

1. After the GPS is on and has determined its position, press “MARK”
2. Name the landmark using the UP/DOWN arrows to change characters and the LEFT/RIGHT arrows to move the cursor. A unique four letter code is a convenient way to label each site
3. Press “ENTER” after you have input the desired landmark name
4. This should accept the current latitude and longitude, if not press “ENTER” to accept

Some GPS units will allow a user to “Go To” a waypoint (marked site). To go to a waypoint:

1. Press “GO TO”
2. Use the LEFT/RIGHT arrows until desired destination is displayed
3. Press “ENTER”
4. A compass is displayed with an arrow indicating the bearing to the next station. Directional arrow may be used in conjunction with but not in place of navigational charts when motoring between sample sites

Calibration is only necessary if it is moved more than 300 miles from the original position. Calibration is used to adjust elevation, initial earth position, date and time.

(GPS instructions for Magellan GPS 310 Handheld taken from U.S.V.I. Coastal Zone Management Program: Water Quality Monitoring Manual, 2001).

CTD Transect Profiling and Time Series Recording

Maintenance

The most important element in the effective maintenance of a CTD (or most any other oceanographic instrument) is to avoid storing the unit wet with salt water, or in a salt-spray environment. Any underwater unit should be hosed down with fresh water after use and dried with a clean rag prior to storage. Individual CTD units will have an instruction manual (either with the unit or on the web) which will give specific instructions how to conduct a preliminary checkout when the unit is first received, as well as how to calibrate, maintain and deploy the CTD.

General Instructions for Profiling

Check that the anodes have not been eroded away and that hardware and external fittings are secure. Refer to the information on programming the CTD for the desired operating routing and confirm that there is enough memory and battery power. Make sure that the self-diagnostic battery voltage is proper.

The following steps should be taken before putting the profiler in the water:

1. Turn the magnetic switch off
2. Initialize the memory
3. Check that time, date, and voltage of battery are satisfactory and that the number of casts and samples equal zero
4. Return CTD to quiescent conditions
5. When finished computer communications with the CTD put a light coating of silicon grease on the dummy plug and cover the connector I/O port

6. Remove covering used to protect the instruments during storage and prepare them for deployment (usually placing another protection cover on the CTD which allows water to flow through, sometimes with a built-in stirring mechanism)
7. Turn on the magnetic switch and CTD is ready to go in the water (CTD instructions taken from the manual for the SeaBird 19 SEACAT Profiler CTD, pictured above)

General Instructions for Long-Term Deployment

1. Install new batters or ensure existing batteries have enough capacity to cover the intended deployment
2. Program CTD for intended deployment:

    a. Date and time
    b. Upload all data and make entire memory available for recording (if not recorded data will be stored after last recorded sample)
    c. Establish setup and logging parameters

3. Install a cable or dummy plug for each connector on the main housing end cap

a) Lightly lubricate the inside of the dummy plug/cable connector with silicone grease (DC-4 or equivalent)

b) Install the plug/cable connector, aligning the raised bump on the side of the plug/cable connect with the large pin (pin 1 – ground) on the CTD. Remove any trapped air by burping or gently squeezing the plug/connector near the top and moving your fingers toward the end cap

c) Place the locking sleeve over the plug/cable connector. Tighten the locking sleeve finger tight only. Do not over- tighten or use a wrench or pliers

4. Connect the other end of the cables installed in Step 3 to the appropriate sensors.
5. Verify that the hardware and external fittings are secure.
6. If applicable remove storage tubing/casing from sensors and add any instrument safety features (such as a cage or frame)
7. Immediately prior to deployment:

Normal mode

a. With the CTD in quiescent state put the magnetic switch in the On position
b. With the CTD awake put the magnetic switch in the On position and send the GL or RL command

Standby Mode

a. With the CTD awake send the Standby command, wait and then put the magnetic switch in the On position

8. The CTD is ready to go in the water

(CTD instructions taken from the manual for the SeaBird 25 SEALOGGER CTD, pictured above).

Optimizing Data Quality for Profiling
Profiling speed of about 1meter/second is usually satisfactory, however ship motion and it's effects on data quality have to be considered as operating conditions change. Slow profiling speed can cause degraded flushing of the conductivity cell especially in CTDs without flushing pumps. Salinity spiking can be severe in areas of strong temperature gradients. In rough conditions where the ship's dynamic motion is large, profiling speed should be increased (as high as 2-3 meters per second) to reduce dynamic errors.

Downcast data is generally better quality than upcast data. Upcast data is best retrieved by inverting the CTD so that the sensors are on the top. The CTD should not be positioned so that water flowing past the sensors can be contaminated by other instruments. A CTD with a built in pump or stirring mechanism can be lowered more slowly and give greater resolution. Where water temperatures are markedly different from the temperature where the CTD has been stored, better data will be obtained if the CTD is allowed to equilibrate at the surface before profiling.

Current Meter

Transect Data Collection Using an Acoustic Doppler Current Profiler
Taken from United States Geological Survey (USGS) http://www-il.usgs.gov/adcp/reports/KJBiflow.pdf

The USGS collects flow data with an RD Instruments (RDI) 1200 Workhorse Sentinel ADCP mounted on a boom that is mounted to a 12-foot inflatable boat. The boat is crewed by a pilot and an ADCP operator. The Sentinel ADCP is relatively small (0.4 meter long by 0.2 meter wide) and lightweight (13.0 kilograms). It is designed for long-term deployment, with internal memory and battery, but can be used as a direct sensor for measurement of stream velocity and cross-sectional area.

Left: RDI's Workhorse Sentinal ADCP

The data-collection process begins by setting the measurement boundary conditions for the ADCP through modification of a configuration file. Parameters such as cell depth, blanking distance, instrument modes, and depth of transducer below the water surface must be defined before the ADCP begins collecting data. As cell depth decreases, the vertical definition of the velocity within the ensemble increases, but the errors in velocity measurement also increase. The blanking distance moves the location of the first cell away from the transducer head to allow the transmit circuits time to recover before the receive cycle begins. The instrument mode changes to a different transducer pulse for each different flow condition. The depth of the transducer below the water surface is needed to compute the total depth of the water column. Within the configuration file there is an option to set the salinity at the transducer heads, but during data acquisition, the salinity is set to zero. Adjustments for salinity are made when the data are processed.

Choosing the proper location to make a measurement transect is critical to obtaining good data. The transect location is limited to the channel of flow. It must be far enough from obstructions to avoid turbulence, which interferes with the ADCP signal processing. The location must also be chosen where the flow is not too shallow (less than 1 meter) or too slow (less than 0.05 meters per second) to be measured by the ADCP.

A measurement transect is made by navigating the boat in a straight line normal to the direction of flow, while the ADCP collects depth, distance, and velocity data. A measurement generally begins and ends at the location closest to each bank where depth is greater than 1 meter. In this case the distance from the shore to the ADCP must be measured at the beginning and end of each transect and is used later to estimate the discharge of the edges of the measurement cross section. A minimum of four transects should be made, and later averaged together during data processing.

Describing Water Conditions

1. Determine the surface conditions at the time of collection, whether it is calm or if ripples, chop, or swells are present.
2. Describe the color of the water at the collection site.
3. Sniff the air. Make note of the odour (if any) on the data sheet and describe the smell.
4. Record any general comments and signs of pollution.

Rain Gauge

Manual Rain Gauge

1. Place the rain gauge in an open area sufficiently far from buildings, trees, overhead wires and other obstructions that may cause contamination or deflect precipitation. The rain gauge may be located on the school grounds to make the weekly reading more convenient.

Note: There should be no obstruction above a 45 degree angle from the top of the rain gauge. Locate the gauge at least 20 feet from a 20 foot-tall obstruction.

2. Attach the gauge to a post mounted in a relatively secure place. Make sure the top is about 1-2 inches above the post to keep water from splashing into the gauge.

3. Read the amount of precipitation to the nearest 0.01 inch.

4. Empty the gauge after each reading.

***Note*:** On sunny days there may be some evaporation from the gauge. Use a small amount of clear mineral oil to prevent evaporation from the container. You may have to empty the gauge more frequently during stormy weather.

Digital Rain Gauge
This instrument measures and logs rainfall and must be set up within the watershed of the sites being monitored on a level location sheltered from strong wind and clear of overhangs and tree branches. It should also be easily accessible for regular cleaning.

Maintenance: A digital rain gauge should be cleaned at least twice a year.

1. Disconnect the collector cable
2. Separate the cone from the base
3. Use warm soapy water and a soft cloth to clean pollen, dirt, and other debris from the cone, cone screens and bucket
4. Use a pipe cleaner to clear the funnel hole in the cone and the drain screens in the base
5. When all parts are clean, rinse with clear water
6. Reattach the cone and replace the screen
7. Reconnect the collector cable

The logger will need the batteries replaced a few times a year. The data logger will usually have a weatherproof case (when closed and latched). The electronics can not get wet from rain or condensation. It they do get wet, remove the battery immediately and dry the board completely with a hair dryer before reinstalling the battery. There is also a moisture absorbing pack inside. This needs to be changed when the battery is changed.

A digital rain gauge will collect rain and drain automatically. To download data, the logger may be detached and taken to a computer or a portable computer may be used. A digital rain gauge should not need to be calibrated (that is done at the factory) but it is possible to adjust the calibration if necessary. Instructions for calibration can be found in the instructions manual. Digital rain gauge instructions taken from U.S.V.I. Coastal Zone Management Program: Water Quality Monitoring Manual, 2001.

Handheld Anemometer

1. Stand facing the wind
2. Grasp the gauge so that you do not obstruct wind measuring instruments
3. Hold up at approximately head height
4. Read the most consistent reading, rather than peak gusts
5. Ensure to use the appropriate scale for light winds

Transmissometer or OBS

Water Level (Tide) Gauge

Wave Buoy

Directional Wave Recorder

7.6 DATA ANALYSIS

To make any oceanographic measurement or monitoring program worthwhile, it is essential to subject the data to careful and systematic analysis to ensure high data quality and consistency and to be able to make statements about the variations in each parameter and relationships between different parameters, both locally and across the MBRS region. A series of analysis steps should be standard procedure.

Data Inspection
All data should be plotted and inspected visually as a means of quality control. It is not possible to generalize all required actions in the case of bad or suspect data. However, obvious data spikes or outlying values should be eliminated. This is often a problem with CTD data with large spikes in conductivity or salinity. In general, parameter values that lie more than three standard deviations away from the mean of any time (or space) series are suspect and should not automatically be accepted. A gradual decrease of the strength of a time series signal usually denotes biological growth (barnacles, algae, etc.) on the sensor or declining battery power. Step-like changes of a time series signal usually denotes inadvertent relocation of sensor (horizontally or vertically) or power failure. The initial processing and quality control inspection of physical oceanography data is usually performed using an electronic spreadsheet software program.

Data Table and Data Synthesis
Using an electronic spreadsheet software program, a data table should be developed on a monthly basis, including as rows in the spreadsheet all measured and some calculated parameters. All parameters should be expressed in SI units. The columns are:

a) Parameter, the name of the variable
b) Latitude, expressed as □N in decimal degrees
c) Longitude, expressed as □W in decimal degrees
d) Year (four digits)
e) Month (two digits, 1-12)
f) Day (two digits, 1-31)
g) Hour (two digits, 0-23)
h) Minute (two digits, 0-59)
i) Time Base, expressed as either “GMT” or “Local Standard Time”
j) Julian Day [JD] (0.0000-365.9999, always using for decimals - this parameter is calculated)
k) **n**, the number of acceptable high-quality hourly samples for the month
l) Mean, the arithmetic mean of the n high-quality data values
m) SD, the standard deviation of the n high-quality data values
n) Max, the maximum recorded value of the n high-quality data values
o) Min, the minimum recorded value the n high-quality data values
p) Quality, expressed as % acceptable data points for the month for each parameter. This is calculated for hourly data as (n*100/720) or (N*100/744) for a 30-day or 31-day month, respectively.

This data table and the raw data that were used to produce the table are suitable for long-term storage in a database, such as the REIS.

The above calculations are appropriate for all scalar data but are not appropriate for the vector current and wind vector data, whenever these vectors are expressed as a magnitude and a direction. The statistics calculated on the direction would yield incorrect information. Rather, both the current and wind speeds and directions must be decomposed into scalar component velocities and added to the above parameter table. This decomposition is easily done in a spreadsheet.

If the magnitude of the current speed is denoted **V**, the recorded magnetic current direction towards which the current is flowing is denoted **φ**, the local magnetic inclination is denoted **α** (positive for east declination and negative for west declination), the north-south current component velocity is denoted **v** (positive towards the north), and the east-west component velocity is dented **u** (positive towards the east), the component scalar current velocities are calculated as:

$$u = |V| \sin(\varphi - \alpha)$$
$$v = |V| \cos(\varphi - \alpha)$$

This is slightly different for wind velocity components because wind direction is recorded as direction from which the wind blows, different from currents. In the case of winds, the decomposition transformation is:

$$u = -|V| \sin(\varphi - \alpha)$$
$$v = -|V| \cos(\varphi - \alpha)$$

It should be noted that once the current and winds have been decomposed into component scalar velocities, the oceanographic convention should universally be adopted, i.e. both wind components and current components denote the direction towards which the current flows or the wind blows.

Data Plotting

All scalar time series (including the calculated component wind speeds and current speeds) and all transect data should be plotted for each month and each transect, respectively. For the time series, each parameter (vertical axis) time series should be plotted against time (horizontal axis), where the time axis should be expressed as JD. In addition, multiple bin ADCP velocity component time series should be plotted vs. time (JD), where the vertical axis is tide-varying water depth and the component velocity values are interpolated contours. The typical time series plot has a ~1:10 (vertical/horizontal) ratio. To plot transect data, the vertical axis is water depth, the horizontal axis is distance, and the parameter values are interpolated contours. In addition, to aid the interpretation of data, it is customary to plot both wind and current data as stick diagrams, progressive vector diagrams (PVDs), and a polar plot (similar to wind rose). The details of how to produce such plots can be found in Emery and Thomson (1997).

Harmonic Analysis

Standard analysis of water elevation (tide) data includes harmonic analysis of hourly (or more frequent) water level data collected for at least 29 continuous days (696 hours) but ideally for a year or longer. The details of harmonic analysis are explained in many standard physical oceanographic texts, including Pugh (1987) and Franco (1988).

Spectral Analysis

To explore the statistical variability of an oceanographic parameter measured as a time series and the degree to which the variability in the series is related (correlated) to the variability in another simultaneously measured parameter time series, it is customary to calculate variance spectra of each time series, the magnitude of the cross-spectrum, the phase of the cross-spectrum, and the coherence-squared spectrum (i.e. how well two series are correlated as a function of frequency). The details of such analyses are detailed in standard oceanographic texts such as Emery and Emery and Thomson (1997).

Oceanographic Interpretation and Modeling
The previous data analysis and visualization products are relatively standard, but far from trivial. They serve as the starting points for interpretation of the physical oceanography of a region, such as the MBRS region, and data necessary to calibrate and validate numerical hydrodynamic circulation models.

7.7 COMPUTER SOFTWARE

Physical oceanographic projects usually generate large quantities of data. For example an ADCP current meter sampling at 40 Hz at 25 vertical depths (bins) generates 31 billion sets of pairs of velocities and directions annually. These data are reduced to 200,000 sets of hourly pairs of speeds and directions after reduction to hourly values. Thus, it is completely impractical to analyze physical oceanographic data without the use of a computer. Considering the very low current cost for a powerful desktop or laptop personal computer, it is both essential and simple to make sure that a physical oceanographer has access to ample computing power. This can be provided in the form of a fast (1.2 GHz or faster) Pentium IV processor or equivalent with ample RAM (at least 512 Mb) and ample hard disk storage (20 Gb or more). In addition, a physical oceanographer needs to make effective use of several available types of software, including:

Electronic Spreadsheet Software
Both Microsoft Excel and Corel Quattro are good choices for data manipulation, quality control analysis, and basic statistical calculations.

Plotting Software
Although spreadsheet software usually come with plotting functionality, for high-quality data plots specialized programs will in general do a better job. Suggested software programs include Grapher, Surfer, Sigma Plot, and Matlab.

Data Analysis and Visualization Software
Two excellent software choices used by physical oceanographers are Matlab and MathCad with large user bases and many available program modules.

Programming Software
Matlab is an excellent software choice for programming and is commonly used by physical oceanographers around the world. However, for large complex numerical simulation models, FORTRAN is still the programming language of choice.

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9. APPENDICES

APPENDIX 1

MANTA TOW TECHNIQUE (Bass and Miller, 1998)

The Manta Tow Technique has been used as a standard long-term monitoring method for the Long-term Monitoring Program of the Australian institute of Marine Science (AIMS) (Bass and Miller, 1998). We have adopted it for the MBRS Synoptic Monitoring Program, as a tool to provide us with a general description of large or new reef and seagrass areas. In contrast to AIMS, the SMP will only utilize this technique as an annual method for Site description, or when assessing a new reef or seagrass area, and not as a main monitoring method.

Besides providing a general description of large areas of reef, it also allows to gauge broad changes in abundance and distribution of organisms on coral reefs. The advantage of manta tow is that it enables large areas of reefs to be surveyed quickly and with minimal equipment. Since the 1970's, the manta tow technique has been used extensively on the Great Barrier Reef for broadscale surveys (at the scale of entire or large part of, reef). The technique involves towing a snorkel diver (observer) at a constant speed behind a boat (Figure A1.1). The original text for this technique as well as further details of other of its uses can be found at the AIMS website:
http://www.aims.gov.au/pages/research/reef-monitoring/ltm/mon-sop1/mon-sop1-00.html

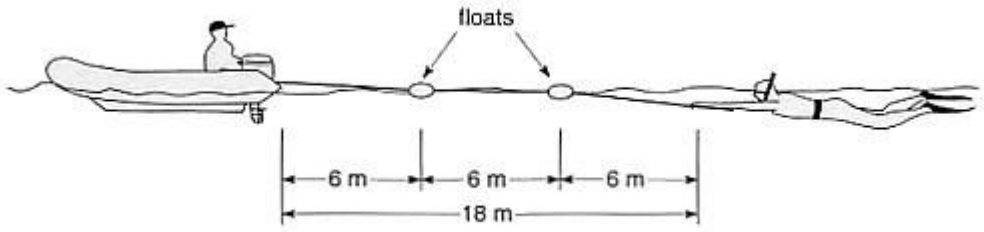

Figure A1.1 The manta tow technique (From Bass and Miller, 1998)

The observer holds on to a 'manta board' attached to a small boat by a 17 metre length of rope. This person makes a visual assessment of specific variables during each manta tow (2 minutes duration), and records these data when the boat stops, on a data sheet attached to the manta board (see Figure A1.2). The Manta Tow technique has been summarised in the next few pages.

PERSONNEL

In large areas, a minimum of four people are required to conduct the surveys efficiently. One person, nominated as the cruise leader is responsible for planning the trip and ensuring that the surveys follow a standardized and safe procedure.

The surveys are conducted using two boats. Each boat has a driver and an observer and these roles are rotated during the survey of the reef. Each person should have a good knowledge of the coral reef environment and its fauna, be an experienced snorkeller and possess a speed boat driver's license. In small areas, it should be possible to conduct the Manta tows using one boat.

EQUIPMENT

The equipment used to conduct manta tow surveys is listed below. The field gear is required in each boat while the extra gear remains on the ship.

Field gear

1. A small (4 m) boat with 15-20 HP outboard motor and necessary safety gear
2. Waterproof VHF hand-held radio
3. Rope harness which attaches to the boat's transom (Figure A1.2)
4. Manta board with fitted harness and attached pencil (Figure A1.2)
5. 17 meter, 10 mm towing rope with quick release clips on either end (Two small white floats are attached to the rope at 6 meter intervals from the manta board (Figure A1.1)
6. Data sheet printed on underwater paper (this is held in the recess on the manta board by a fixed clamp (Figure A1.2)
7. Container with spare 2B pencils, twine and rubber bands
8. **Optional:** Photocopy of an aerial photograph of the reef, attached to a slate by screw down clamps and rubber bands (The photocopy is made on waterproof drawing film)
9. Snorkeling gear (mask, fins, snorkel and wetsuit or stinger suit) for each person
10. Two large buoys, 2 ropes (10 m) and 2 dump weights
11. Waterproof, digital watch with countdown function

Extra gear on ship

1. Field logbook
2. One reef aesthetics and 2 manta tow data sheets for each reef
3. Extra data sheets (manta tow and SCUBA search)
4. Spare stationery items
5. Tide tables
6. Basic toolbox and spare snorkeling gear

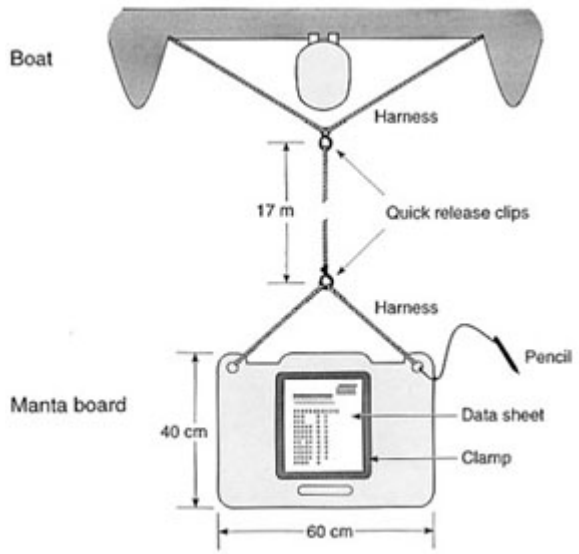

Figure A1.2 The manta board and attachments (From Bass and Miller, 1998)

PROCEDURE

On arrival at a reef, the following procedure should be adopted.

1. The two teams discuss the location of the starting point, manta tow path and weather conditions before commencing the survey. The cruise leader must ensure that everyone is aware of tides, currents, daylight hours remaining, and present weather conditions.

   Note: If the weather is expected to change for the worse, the teams should agree on a strategy before leaving the shore. Radio contact with the shore staff and between boats should always be maintained. This is particularly important on large reefs where the two boats may be out of visual contact.

2. Begin the survey at the set starting point (usually at the northern end of the reef) unless conditions are unsuitable. ***Note:*** Factors such as wind, direction of current, and angle of sun may alter the starting point. The driver should avoid towing into the sun continuously, where possible. Mark the starting point as '0' on the aerial photograph. (The two boats start together but proceed in opposite directions around the perimeter of the reef, meeting up at the other end.)
3. Clip the tow rope to the transom harnesses so that it can move freely, and attach the other end to the manta board with the quick release clip. The observer should then record the ambient variables, such as weather conditions on the top of the data sheet, don snorkelling gear, and enter the water with the manta board.
4. The observer signals the driver to commence the manta tow when he/she is ready. The observer and driver use hand signals to communicate information about the tow path and the towing speed (see English *et al*. 1994). The driver tows the observer holding the manta board, behind the boat at a constant speed of about 4 km/hr (the actual speed may vary, depending on wind and current).

   > ***Note:*** Observations are generally made from the surface, however when closer inspection is required, the observer can manoeuvre the manta board below the surface. To dive down, tilt the leading edge of the board down, and tilt upwards to ascend.

   The tow path should be parallel to the reef crest, and close enough for the observer to see as much of the reef slope as possible. The search area will vary, depending on tow path, speed of the boat, visibility, reef gradient, distance from substrate, distribution and density of organisms being counted (Moran & De'ath 1992). This variability of the reef slope and weather conditions make it difficult to define a search area however, observers should consciously try to restrict their search width to about 10 metres (see English *et al*. 1994).

5. The driver times the manta tow and stops after two minutes by idling the motor. The tow rope will become slack allowing the observer to record the data for that tow (i.e. extent of the reef, number and size, percentage cover of live and dead coral, sand/rubble and presence of feeding scars). The driver should keep a record of the number of tows and where possible, mark the tow number and position on the aerial photograph (Figure A1.3). When the observer signals that the data recording is complete, the driver recommences towing, stopping again after two minutes.
6. The observer and driver change roles after an agreed time, (usually after fifteen tows) to avoid fatigue. During the changeover time, it is important to discuss observations about the reef and sea conditions.
7. This procedure is repeated until the entire perimeter of the reef is surveyed. Thus, a completed survey consists of a series of consecutive two minute tows.

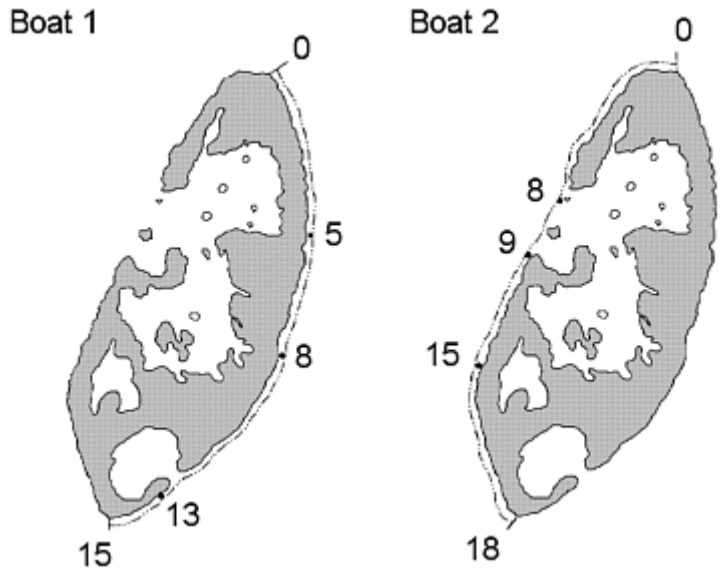

Figure A1.3 Reef aerial maps showing numbered tow paths for each boat (From Bass and Miller, 1998)

POTENTIAL PROBLEMS

At any time, if the observer or driver feels conditions are unsuitable for towing they should assess the situation giving consideration to their safety. Listed below are some of the common problems that make towing conditions difficult.

1. Rough seas: Generally, the reef front receives the roughest seas on the southeast edge. Rough sea conditions can make it difficult and dangerous to manta tow, so drivers should know their limits. As a guideline, if the waves are greater than 2 metres, or breaking erratically then it is unsafe to manta tow. ***Note:*** This is only a guide and each person should use their discretion. If at any point one team decides to stop towing, then they must notify the other team by radio, and decide upon a point to continue towing where conditions are more suitable. If a break in the tow path is made, it should be clearly marked on the aerial.
2. Currents: When there is a strong current flowing along the reef edge, towing speed and/or direction should be modified. If the current is going in the same direction as the boat, the driver should slow down to compensate for the speed of the water flow. If the current is against the boat, the driver should increase the speed of towing. However, if the observer finds towing difficult, the tow should end there and continue further around the reef perimeter where the current is less.
3. Low tide: At low tide, the water may recede from the reef periodically exposing the crest. In this situation, the driver should keep a safe distance from the reef crest, to prevent the boat or the observer being caught in the surge. On a reef front where the wind is blowing the boat onto the reef, the driver should head the boat into the wind, especially when towing on a low tide to avoid being pushed onto the reef crest.
4. Low visibility: Generally if the visibility is less than 6 metres, (ie. the nearest float on the tow rope is not visible) then surveys should not be conducted. However, if there is only a patch of low visibility water, then the observer should continue to record data if possible, and record the visibility change on the data sheet (see visibility section in data recording). The observer should beware of diving down in poor visibility.

5. Channels: Occasionally the reef perimeter is broken by a channel that may be quite deep and/or have a strong current running through it. If the channel is deep (>9 m) and wider than about 25 metres, then the tow should end at one side of the channel and begin again at the other side. This break in the tow path should be marked on the aerial photograph.
6. Sandy back areas: Some back reefs are typically shallow and lack a solid edge, making it difficult to determine the correct tow path. In these areas the driver must decide upon a straight tow path across the back reef to include as much hard substrate as possible. It is important to keep the orientation of the reef in mind when selecting the tow path and to mark any recognisable points on the aerial photograph.

DATA RECORDING

Ambient Variables

The ambient variables recorded include, information about the survey (Location name, Site ID, time, date, data collectors) and the weather conditions. The weather conditions are recorded as wind strength, cloud cover, sea state and tide, and are described as:

Wind

Wind strength is recorded as a category from 1 to 5, described in Table A1.1.

Table A1.1 Wind strength categories

Category	Wind strength
1	0-5 knots
2	6-10 knots
3	11-15 knots
4	16-20 knots
5	21-25 knots

Cloud

Cloud cover is quantified in terms of eighths of the sky area covered by cloud. The unit of measure is the okta. From a position where the entire sky can be seen, estimate the amount of cloud as a fraction of eight. Thus, a cloudless sky is recorded as 0 eighths or oktas and an overcast sky is recorded as 8 oktas.

Sea state

Sea state is described by a modified Beaufort scale (Table A1.2).

Table A1.2 Sea state description

Sea state	Description
Calm	Mirror-like to small ripples
Slight	Small waves, some whitecaps
Moderate	Moderate waves, many whitecaps
Rough	Large waves, 2-3 m, whitecaps everywhere, some spray

Tide
The tide state is defined as low, high, falling or rising and is determined from a Tide table. These states are described in Table A1.3.

Table A1.3 Tide states

State	Description
Low	One hour either side of Low water
High	One hour either side of High water
Falling	The period between High and Low water
Rising	The period between Low and High water

Live Coral Cover
Percentage cover estimates of live coral are made from the total tow path area observed during each 2 minute manta tow. Live coral refers to the living reef-building Scleractinian corals. This does not include the non-Scleractinian corals such as Millepora. Live coral is coloured by the presence of living tissue and can be easily recognised by its colour and the detailed structure of the corallites. The percentage cover estimates of live coral are recorded as one of 6 categories (Table A1.5).

Dead coral cover
Dead coral is defined as coral that is not covered by living tissue but still has distinguishable corallum structure. A 'newly' dead coral is easily recognised by its brilliant white colour. The white skeleton is then colonised by a succession of algal types, initially with turf algae giving the dead coral a dull, greenish/brown colour.

Eventually, coralline algae take over giving the coral skeleton a smooth pink appearance. By this time, the detailed structure of the coral skeleton is lost, due to the algal colonisers and weathering, and the colony is considered part of the substrate, or if fragmented, becomes rubble. The percentage cover estimate of dead coral is recorded as one of 6 categories (Table A1.4).

> ***Note:*** Once a coral skeleton has become encrusted by coralline algae it is considered to be substrate and not dead coral.

Table A1.4 Estimates for Percentage Cover. For each category (except 0) a plus (+) or minus (-) is added to denote whether the cover estimate falls into the upper or lower half of each category

Category	Cover estimate
0	0
1	0 - 10%
2	11 - 30%
3	31 - 50%
4	51 - 75%
5	76 - 100%

Soft coral cover
Soft corals, as their name implies, lack the hard limestone skeleton typical of scleractinian corals. There are many forms of soft coral, but in a manta tow survey we only look for the 'fleshy' soft corals,

such as those from the families Mussidae and Caryophylliidae. Soft coral cover is also recorded as a percent cover category (Table A1.4).

Visibility
Water visibility estimates are recorded during the first manta tow and subsequently whenever the visibility changes. An estimate of visibility is made by diving below the surface and looking at the two floats attached along the tow rope at six metre intervals.

Depending on how far the observer can see along the tow rope, the visibility is recorded as one of four categories (Table A1.5). For example, if the observer can see the boat motor and beyond, then the visibility is scored as category 4 and if the nearest float cannot be seen, then the visibility is scored as category 1 (Figure A1.4).

Table A1.5 Categories for estimating visibility

Category	Distance
1	<6 m
2	6 - 12 m
3	13 - 18 m
4	> 18 m

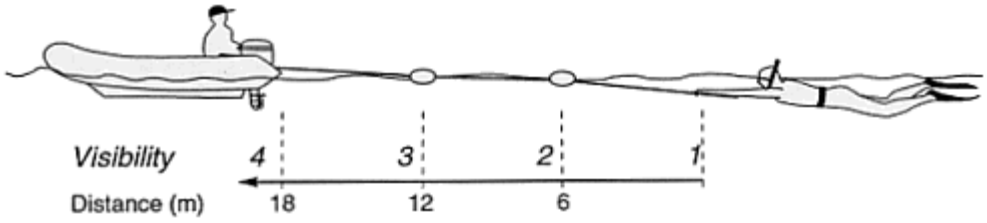

Figure A1.4 Visibility category estimates for manta tow surveys (From Bass and Miller, 1998)

Other Features
This column is for any observations such as the reef structure, slope, diversity, fish abundance, number of conch, lobsters, coral mortality or any additional information that the Recorder may find useful to obtain an overall impression of the reef (See example of Manta Tow Data Sheet Entry Form). Such descriptive information about reef aesthetics gathered over time can also be a good qualitative indicator of the overall state of a particular reef. The data are obtained from the collective manta towers' observations. The result is a qualitative impression of the reef's topography and value.

PROCEDURE
When recording the manta tow data the observer writes descriptive notes in the 'other' column on the manta tow data sheet that describes the reef slope and depict areas of change. At the end of the day's work, these notes are used in conjunction with a discussion by the observers to form an overall impression of the reef. To help form this impression, a series of attributes have been devised to describe the reef. We include a selection of the attributes below to assist with reef descriptions. For a full description of this method, please refer to the AIMS web site:
http://www.aims.gov.au/pages/research/reef-monitoring/ltm/mon-sop1/mon-sop1-00.html

Below this are four rows, each row corresponding to a reef zone. In each box a number or letter representing a category is recorded.

Zone
The zones, marked on the reef outline are labelled in a clockwise direction starting from the back reef (leeward side) (Figure A1.5).

1. Back reef
2. Flank number 1
3. Front reef
4. Flank number 2

Reef slope
The reef slope is defined as the average angle of the slope in the zone and has been categorised as follows:

1. Shallow (0 - 20°)
2. Moderate (21 - 45°)
3. Steep (46 - 75°)
4. Vertical (76 - 90°)
5. Broken - If the reef edge is not well defined, or is made up of scattered bommies.

Back reef slope - Is described as having a steep upper slope and a shallow, sandy, lower slope.

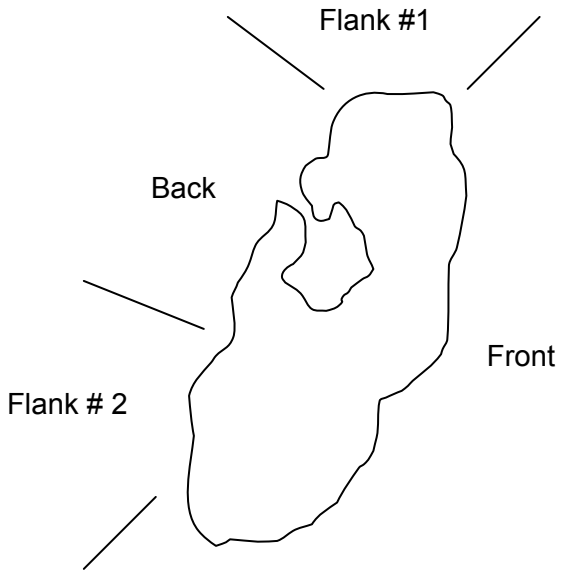

Figure A1.5 Diagrammatic representation of a reef showing the various zones

MBRS MANTA TOW DATA ENTRY FORM

MSMP_A1

Location:	Latitude:	Date:	Wind:
Site ID:	Longitude:	Time:	Cloud:
Recorder:	Sea:	Tide:	

Tow No.	Coral Cover		Cover	Algae	Other Features
1	Live	Dead	SC		
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					
16					
17					
18					
19					
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21					
22					
23					
24					
25					
26					
27					
28					
N					

APPENDIX 2

STANDARDIZATION AND CALIBRATION OF THE VISUAL ASSESSMENTS FOR CORAL COMMUNITIES (From AGRAA, 2000)

The limited ability to compare among methods is the strongest argument to adopt common sampling protocols (Sale, 1997). One of the main objectives of the AGRRA approach is to provide a standardized methodology that will enable teams working in different areas to collect and compare data on a regional scale. Visual censuses are particularly sensitive to variations in methods, yet it is important to have consistent and comparable visual estimates and to minimize individual bias among different observers. Hence, it is essential to carefully standardize methods prior to data collection. This requires "consistency training", particularly in learning to correctly identify coral and fish species, distinguish recent *versus* old coral mortality, estimate percentages of coral mortality and algal abundance, and correctly estimate fish numbers and sizes. The following highlights some of the differences found among observer and several exercises that have used to train observers on the AGRRA method, build consistency between observer estimates, and minimize differences in visual estimates. Other recommendations for maintaining data quality and consistency can be found in the literature (e.g., Bell *et al*. 1985, English *et al*. 1994).

CORALS

For assessing coral condition, differences in observer estimates can arise from differences in:

- Delineating the boundaries of an individual coral colony,
- Coral nomenclature,
- Measuring colony size,
- Estimating the percent coral mortality and distinguishing between old and recent mortality, and
- Determining potential cause(s) of recent mortality.

Colony boundaries

Before assessing individual corals for identification, size or condition, it is important to distinguish what constitutes a colony, **i.e., what are the colony's boundaries?** Colonies of mound corals (e.g., *Diploria*, *Siderastrea*) are fairly easy to distinguish, whereas recognizing distinct colonies of branching corals (e.g., *Acropora palmata* and *A*. *cervicornis*) or platy variety of corals (e.g., *M*. *franksi*) can be very difficult. When determining colony boundaries you also set the boundaries for maximum height and diameter. It is important that observers are consistent in distinguishing individual colonies prior to collecting data.

Here are some guidelines to use to establish colony boundaries:

1) A colony is defined as any autonomous, free-standing coral skeleton that is still identifiable to genus level (preferably to species level) based on the presence of living tissue or identifiable corallites.
2) Assess only colonies still attached to the substrate. Only survey a coral that has been knocked over if it has reattached to the substrate or is too large to move.
3) Include colonies that are 100% dead that you can identify to generic level based on colony morphology (e.g., *Acropora palmata*) or corallite characters (*Diploria* spp., *M*. *cavernosa*).
4) Identify the colony's boundaries based on connecting or common skeleton, connecting living tissues, polyp size, and polyp color.
5) The living tissues of some corals may be distributed among several physiologically separate units. Species like the columnar *Montastraea annularis* (= *M*. *annularis f*. *annularis*) which grow as clusters of basally-interconnected lobes having live tissues only at their summits, should be treated as one coral, and observed only at the tops of the lobes (Figure A2.1).
6) Look for one vs. two colonies. Sometimes one colony of *M. faveolata* can encircle a *M. annularis* colony in the middle and superficially look like a single colony (like an apron on a person).

7) Corals like *Acropora cervicornis* which grow as thickets should also be considered as one "supercoral", unless you are sure of the boundaries of the constituent colonies.
8) If two colonies are growing on top of one another and they both fall under the transect line, assess each colony individually. For example, this may occur when you have a mound coral growing underneath a branch of *A. palmata*.
9) If a colony has incurred partial mortality, be sure to include this when establishing the colony's boundary.
10) Include all extended plates in plating varieties.

Coral Identification

Once a colony's boundaries are established, it is easier to make a correct identification of genus and species. To avoid differences or ambiguities of common names, while still recognizing that several different systems of nomenclature are in current use by reef researchers, a species list of the major reef building corals of the Caribbean is given in Appendix 2. This list represents the Caribbean species that are the primary focus of our analysis i.e., those that contribute most to reef building. To distinguish between colonies, it is useful to use colony shape (e.g., branching, mound); corallite size (e.g., compare *Stephanocoenia intersepta*, *M. annularis*, and *Madracis decactis*), and corallite shape (i.e., *Dichocoenia stokesii* and *Favia fragum*). Particular attention should be given to correctly identifying corals with similar appearances (e.g., *Diploria strigosa*, *D. clivosa*) as well as identifying different morphologies of the same coral species (e.g., small encrusting and large mound forms of *S. intersepta*). Observers should be proficient in identifying coral genera prior to conducting the MBRS-SMP surveys. For experienced team members, the identification will be conducted to species level.

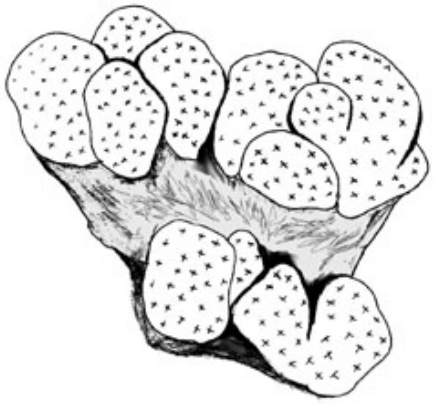
Colony size

After you identify the colony and its boundaries, measure to the nearest cm the coral's maximum projected diameter (live + dead areas) in plan view and maximum height (live + dead areas). Use these guidelines:

1) Establish the coral's axis of growth, especially for corals growing at an angle to the seafloor or on an inclined or tilted surface (see Figure A2.2).
2) The diameter should be measured in plan view perpendicular to the axis of growth. Plan view is assessed from an angle that is parallel to the axis of growth.
3) The height should be measured parallel to the axis of growth.
4) Measure height from the base of the colony, not the reef.
5) For a colony growing on top of another colony, only measure the diameter and height of the individual colony being assessed.
6) For a colony that has been knocked over, is too large to move (see Figure A2.3) or reattached, and does not yet show redirected growth, then measure size along original growth axis. But if a colony has started to grow in a new direction, be sure to measure diameter and height along the new axis of growth (not shown).

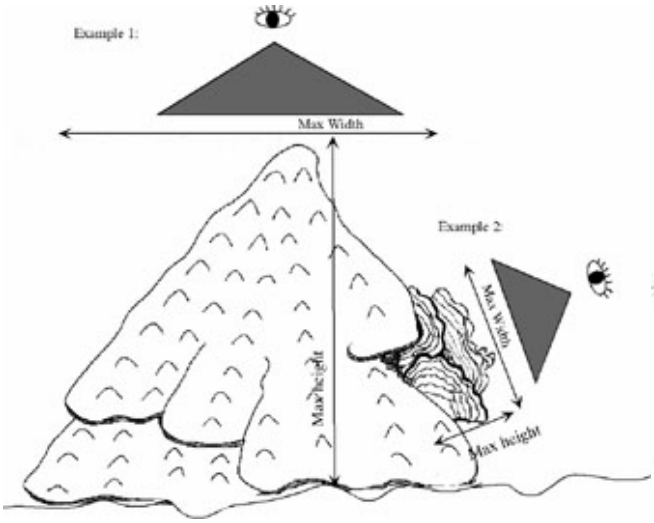
Examples of corals with complex morphologies

- Basally-connected columns of *Montastraea annularis*
- Interlocking thickets of *A. palmata*, *A. cervicornis*, or *Porites porites*
- Densely-spaced extensive colonies of *Madracis mirabilis* or *M. formosa*
- Platy or shingle varieties of *M. faveolata* or *M. franksi*, especially if they form an apron around another coral like *M. faveolata* or *M. cavernosa*
- Large mounds or heads of *Agaricia tenuifolia*, *Millepora complanata*



Drawings by E. Fisher

Figure A2.1 Above figure of basally-connected (gray area) columns of *Montastraea annularis* with only tops alive (hatched areas)



Drawings by E. Fisher

Figure A2.2 Example of size measurements for large coral (left) and small coral (right). Note plan view is tilted for small coral because its substrate is at an incline (eye and arrow)

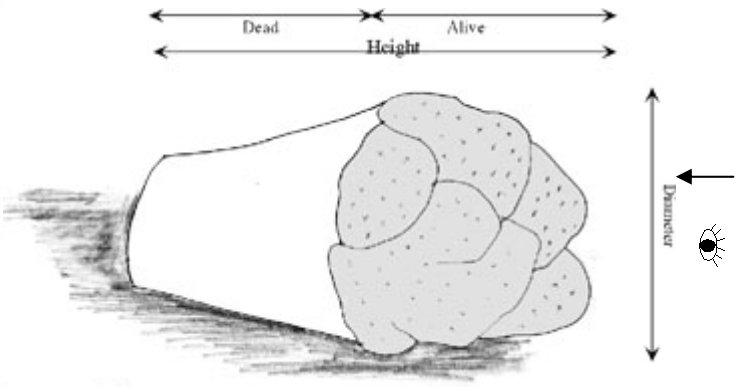
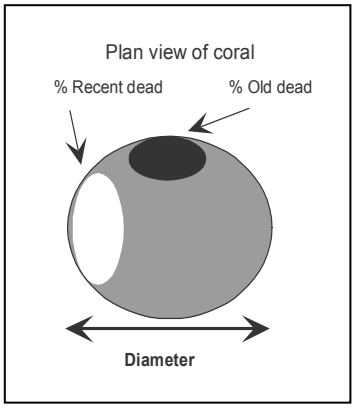


Figure A2.3 Example of size measurements for knocked over coral too large to move. Note dead area and live area. Mortality measurements would be taken from planar view from the side (eye and arrow)

Estimating coral mortality

The most common difference we found among observers is in estimating the percentage of coral mortality. Figure A2.4 to the right, shows a diagrammatic representation of coral mortality.



To minimize observer bias, follow these guidelines:

1) Quantify mortality by visually estimating the amount from above in "plan" view, and ignoring all tissues on the sides or beneath upward facing surfaces (e.g., *Acropora*).
2) Plan view is assessed from an angle that is parallel to the axis of growth.
3) Mortality estimates for *A. cervicornis*, *Madracis mirabilis*, and all other branching corals that ordinarily are dead at the branch bases, should be restricted to the peripheral parts of the colony or thicket.
4) AGRRA makes a further distinction of mortality into "recent" dead and "old" dead.
5) "Recently dead" is defined as any non-living parts of the coral in which the corallite structures are white and either still intact or covered over by a layer of fine mud or algae. For recent mortality, there are several "stages" of recent that are all considered in this category (see Section 3.2):
6) In contrast, "old dead" is defined as any non-living parts of the coral in which the corallite structures are either gone or covered over by organisms that are not easily removed (e.g., certain algae and invertebrates).
7) Corals that have been overgrown by the brown encrusting sponge, *Cliona*, but you can still identify corallite structure beneath the sponge, should be considered old dead (noticeable in *Diploria* spp., *M. annularis*, *M. cavernosa*, and *Colpophyllia natans*).

Coral Condition

Determining potential cause(s) of recent mortality is often very difficult. To standardize assessments, at least until a more common consensus on disease nomenclature is reached among reef scientists, we suggest using color categories when identifying diseased colonies: Black (BB), Red (RB), Yellow (YB), White (WB= white band, WP=white plague, WS=white spots/patches), or Unknown (UK). To become more familiar with coral diseases, we recommend using disease cards (Bruckner and Bruckner, 1998) and visiting the disease website:
http://ourworld.compuserve.com/homepages/mccarty_and_peters/coraldis.htm.

If you are unsure of disease it is better to put unknown and describe it and take a photo if possible. Remember that it is important to differentiate between large scale bleaching events due to elevated sea surface temperatures, and localized patches of bleaching due to algal overgrowth. In some cases, corals may be pale or partly bleached (or remnant bleaching) due to the previous year's bleaching event and are often still recovering. It is also important to differentiate between corals with fish bites, recovering tissue from fish bites or bleaching and bleached tissue.

***Exercise 1*:** This exercise is to help you become familiar with partial coral mortality. Figure A2.4 shows small patches of partial mortality on a "coral" and how tissue loss can expand over the entire colony. These figures also show the actual percentages corresponding to the amount of illustrated partial mortality. Review these diagrams to become familiar with patterns of mortality and visually estimating the amount of mortality. Observers should also review pictures of recent and old mortality and be able to distinguish between the two. Please refer to the Coral Disease Plates at the end of this Section.

***Exercise 2*:** An additional exercise to practice estimating mortality is to use "flash cards" of corals with different degrees of partial mortality ranging from low to high. To do this, take photographs of a coral (in plan view) then determine the amount of partial mortality through digital analysis. Make a

series of pictures with the amount of mortality written on the back of each photograph. Then look at each coral in the series of flash cards and determine the amount of partial mortality and identify the coral species. Compare your estimates with the digitally determined estimates. The resolution of the photographs usually only allows you to estimate total partial mortality and not distinguish between recent and old. Each observer should use the flash cards until his/her answer is close to the known value (+- 5%). Teams of observers should practice until differences in answers between observers is less than 10%.

***Exercise 3*:** A way to standardize visual estimates between observers is to do in the field consistency training. Snorkeling exercises are especially effective because they allow observers to share their observations and questions. For this exercise, first lay out a 10 m transect, then (with another observer) swim the transect line and estimate the amount of coral mortality for each coral under the line, writing down your estimates on a datasheet. As each coral is estimated, compare your answers with your partner and discuss any differences in estimates. After completing the transect, all observers should discuss difference among their estimates. A similar exercise is also done while diving. For this, a series of set transect lines are laid out and each observer surveys the transects for coral condition. Afterwards, the estimates among observers are compared. Variation in data estimates should be discussed and additional exercises should be done until differences are minimal. Consistency training should be conducted fairly frequently, especially if an observer has not done visual estimates in awhile.

CORAL REEF FISHES

For fish sampling, biases are often due to difficulties arising from seeing fishes, accurately counting or estimating length, and differentiating among certain similar species (Sale 1997). An effective way to census such mobile organisms as fish is through a method that most imitates an instantaneous survey of a pre-defined area (Sale 1997). The AGRRA fish method includes two different methods to characterize reef fish populations – belt transects and the Rover Diver Technique.

Estimating 2 m wide belt transect

The first challenge is to visualize the boundaries of a 2-m wide X 30 m long belt transect for fish censuses. The sample unit has been specifically delineated to a 2-m width because this is a small enough width for the observer to be able to estimate the distance easily while allowing for a large enough sample area. A 1 m t-bar (with 5 cm markings) is used to assist observers in estimating the width (below left). To estimate the 30 m length, try to visualize the transect as a 2-m wide square tunnel. Start the first box at least 1-2 m ahead of t-bar. When sampling the transect belt, the observer should give uniform attention to each successive 2-m segment (below right). This requires swimming at a more or less constant rate, and looking consistently about 2 m ahead, except when actually recording data.

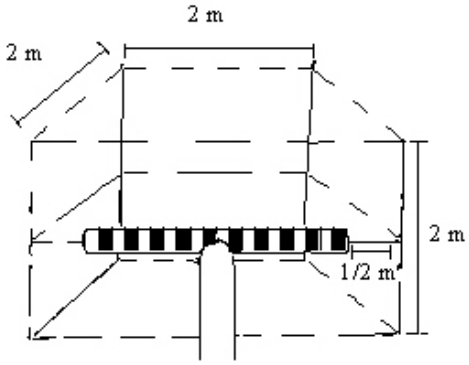


Figure A2.5 A 1 m t-bar is used to help visualize the 2 m wide belt transect

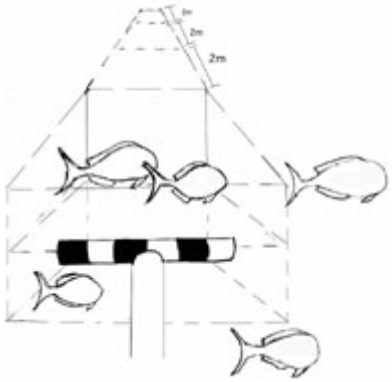


Figure A2.6 Each 2 m segment of belt transect is surveyed separately

Counting fish

Uniform attention should be given to each successive 2-m segment of the transect when surveying. It is important to swim in a consistent manner while actually sampling the fish, although it is permissible to pause while recording data, and then to start swimming again. A speed that counts each 30-m transect in 6-8 minutes should be attempted. High densities of counted species will slow this rate in some cases. By remembering to keep effort equivalent on all segments of the transect, you can focus on counting just those members which happen to be within the boundaries of the transect and limit the tendency to count all members of a large school of fish on either side of the transect. In other words, keep your eyes focused straight ahead of the transect and do no get distracted side to side as a large school swims by. Large groups of individuals of a species that occur within a 2-m segment will be classified by attempting to put them into one or more size categories as necessary.

Estimating length

Fish observers should be trained to estimate fish lengths by using consistency training methods both on land and underwater (e.g., GBRMPA 1979, Bell *et al*. 1985, English *et al*. 1994). The AGRRA method assigns fish lengths to the following size categories (0-5 cm, 6-10, 11-20, 21-30, 31-40, >40cm). A 1 m T-bar with 5 cm increments is used to assist in estimating sizes. We use a variety of

fish models cut out of closed-cell foam into the different size classes (plywood or plastic can also be used). The shapes and general colors of the fish models used represent some of the species included in the AGRRA survey.

Rover Diver Technique

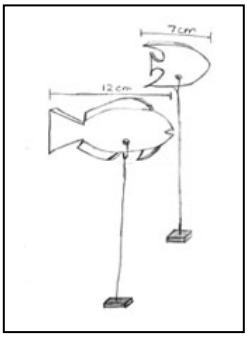
Fish surveyors should be proficient in identifying Caribbean reef fishes. For minimizing and recognizing observer bias and differences when using the REEF rover diving technique, we suggest reading the guidance provided by Schmitt *et al*. (1998). The "Reef fish identification (Florida Caribbean, Bahamas)" book and CD ROM by Paul Humann (1994) are very useful for improving and testing your fish identification.

***Exercise 1*:** While snorkelling or diving, each observer should have a T-bar marked with 5 cm increments and a datasheet (see below) and should be 2-m from the fish models. One person should have a series of different sized fish models (of known lengths). One person holds up one fish model at a time and each observer estimates the size of each fish and assigns it to one of the following size categories (0-5 cm, 6-10, 11-20, 21-30, 31-40, >40cm). The holder should write down on a datasheet the order in which the fish models that are shown and the corresponding actual lengths. After at least 10 fish models have been estimated, the observers should rotate to allow each person a chance to practice estimating length. After the exercise, the divers compare their answers to the actual answers and determine any differences from the actual known amount and the difference between observers. The exercise should be repeated until individual observer estimates are > 95% accurate and observers are consistent with each other.

Example datasheet

Size (cm)	**0-5 cm**	**6-10**	**11-20 cm**	**21-30 cm**	**31-40 cm**	**>40 list size**
Fish 1		X				
Fish 2			X			
Fish 3			X			

***Exercise 2*:** In this exercise, observers practice fish counting along a belt transect and estimating fish size. Each observer should have a T-bar, a 30-m transect tape, and a datasheet. A practice transect line is laid on the substratum and fish models (made from styrofoam and attached to a weighed line of varying lengths) are haphazardly placed along the transect (see figure to right) with some models placed just out side the 2 m wide belt transect. One observer starts at the beginning of the transect line and uses a T-bar to estimate the width of the 2-m belt transect and the length of fish. Observers should mark on their data sheet the size class for each fish (model) observed in the sample area. Each observer should run the survey. After completing the exercise, compare the answers of all the observers with the correct fish lengths. Repeat exercises until observers are consistent between each other and their answers are close to the correct answers.



***Exercise 3*:** While diving, two observers (one hovered above the other) survey a 30 m transect at the same time (with one laying out the transect). The data is then compared between the two observers. This approach is especially effective if one of the observers is already well trained and experienced.

CALIBRATION

Corals

Prior to conducting a benthic transect survey, a surveyor should be proficient in: coral identification of all major reef building coral genera and species on the list, distinguishing colony boundaries, estimating diameter and heights, and estimating partial mortality. In addition, he/she should be familiar with different types of condition (e.g., bleaching, disease, and predation) on the Disease and Predation Identification Cards (Bruckner and Bruckner, 1998). To maintain and quantify accuracy and consistency between benthic observers during the survey, the surveyors should calibrate standards every other day of the survey or as many times are necessary to attain >90% accuracy.

Standard calibration for benthic transects:

1) Using a set transect line or pre-identified coral colonies, have each observer estimate and record the following for at least 5 different coral colonies:
   Species Identification
   Maximum diameter and height
   % recent and old mortality
2) To calibrate between observers, the following standards should be maintained:
   - Species identification should be consistent and accurate between all observers
   - Maximum diameter and height measurements should be within 10 cm
   - Percentage of recent and old mortality estimates should be within 5-10% for each
3) Compare results after the calibration and discuss differences (if any) and variations between observers while still at the site
4) Assess additional coral colonies if necessary to maintain >90% accuracy

Other sessile organisms

Similar consistency training should be undertaken to ensure that all observers correctly classify other categories of sessile organisms. These categories are coralline algae, turf algae and macroalgae, gorgonians, and sponges. It is particularly important that other sessile organisms such as colonial ascidians not be confused with any of these.

Fish

Prior to conducting a fish belt transect survey, a surveyor should be proficient in: fish identification of all species on the SMP list; visualizing a 2 m belt transect, and be able to attain >95% accuracy in estimating observed fish lengths compared to actual measurements. To maintain and quantify accuracy of fish lengths during the survey, have the fish surveyor (s) conduct at least 10 standards every other day of the survey or as many times is needed to attain 95% accuracy.

Standard calibration for estimating fish lengths:

1) Find 10 immobile objects (e.g., pieces of coral, sessile invertebrates, or fish models, etc)
2) Estimate and record the length of each object using the fish size categories (0-5 cm 6-10 etc) staying at least 2 m from the object. Use the t-bar for estimating measurements
3) Then measure the actual size of the object and record length
4) Compare the differences between actual and observed.
5) Continue to collect standards until estimation accuracy is at least 95% or greater
6) Include this data as part of your final report

APPENDIX 3

RECOMMENDED PRACTICES FOR SAFE AND EFFICIENT MONITORING IN THE MBRS-SMP

The following recommendations are based on common sense and are not the only aspects that the SMP Monitoring Teams should take into account. Monitoring Teams should always be watchful of weather conditions (particularly sea state), team mates, equipment, boat, etc.

BEFORE THE DIVE

1. Divers and the SMP

a. All divers conducting surveys for the MBRS Synoptic Monitoring Program MUST be Certified Divers from a recognized international or regional organization, such as PADI, NAUI, BSAC, CMAS, etc. Basic diving level would be acceptable for ordinary members.
b. The Monitoring Coordinator, who will lead the team, must be an advanced diver, so that s/he may be able to deal with unexpected situations. S/he will also have the responsibility of double checking the technical details relating to the dive and offer support to the other divers.
c. Ensure that at least one member of the monitoring team has a Certification in Basic First Aid Administration Procedures.
d. Members of the monitoring teams must have sufficient diving experience so that they can concentrate on the monitoring tasks at hand without any external concerns. They need to also be able to deal with unexpected situations.
e. Ensure that divers are covered by appropriate insurance schemes under their own national organizations or under individual cover.
f. The MBRS Project SHALL NOT BE LIABLE for any damage, harm, loss of life or limb incurred by divers.

2. Plan the Dive and Dive the Plan

a. Always plan field trips ahead of time so that you may check the weather conditions adequately.
b. Prepare a checklist of monitoring equipment and check that all items are ready ahead of time.
c. Check all diving equipment regularly and carry out an extra check before the dive. Checks must be performed on: regulators, BCDs, O-rings in each tank to ensure that no leakage occurs, neoprene suits, fins, masks and snorkels to ensure that all equipment is in good working order.
d. Identify and name a person who will be the contact at your base. Always leave at your base: the exact information of where the team will be monitoring at all times; including departure time and expected time for returning to base; contact number (mobile phone, if available).
e. Maintain radio contact with the base at all times.
f. Always have a log book for the Monitoring Site with you and make copious notations in it, including: name of contact at base, members of the monitoring team, any departures from the original plan, and details of the agreed work plan among any other useful information.
g. Only conduct the field work that has been discussed and approved. Additional activities to be incorporated into the particular field trip **ONLY** if absolutely necessary and **ONLY** after approval from your base. Ensure that you write this information in the log book and signed by the Monitoring Coordinator.
h. Always check your buddy's equipment thoroughly before entering the water.

i. Agree beforehand when the dive should finish.
j. Carry extra sets of the datasheets, an extra slate and pencils.
k. Prepare, prepare and prepare!

3. Have an Emergency Action Plan with the Following Information

a. List of monitoring personnel included in the particular field trip, including: full names; their blood type; medications that they may be taking; known allergies and contact numbers for the next of kin.
b. Relevant dive Locations / Sites. These should be clearly marked on a map so that they are easily found.
c. Relevant telephone numbers, such as the Police Departments and Coast Guards, if any, in each country, the BATSUB in Belize (Emergency Air Lifts), and contact persons on land who can raise the alarm.
d. Ensure that the location of the nearest Decompression Chamber is know to the members of the monitoring team and that every one knows how to contact them urgently.
e. Always carry two diving tanks per diver.
f. Always carry an oxygen tank on the boat at all times as well as an oxygen kit, such as DAN.
g. Always carry extra floating devices for example, polystyrene sausages, rings, etc.
h. Carry a First Aid Kit for divers on the boat as well as a whistle to raise the alarm or to draw attention.
i. Always dive the DEEPEST dive first.
j. DO NOT dive if the sea state is questionable or the water seems otherwise inappropriate.
k. Always carry plenty of drinking water on the boat.

DURING THE DIVE

4. Dive Responsibly

a. Try to anchor the boat in sandy patches or if these are not present, try to anchor on a dead coral.
b. While on the boat, wear a hat to prevent sunstroke and try to keep cool by staying in the shade.
c. DO NOT wear sun block while diving. This may become a problem to some of the filter feeders, or other delicate biota, causing unnecessary distress. Instead DO USE biodegradable sunscreens.
d. Although the monitoring tasks may be carried out in groups, always use the buddy system as much as possible. Identify a diving partner and dive in pairs. Never become separated from your buddy except when assisting him/her to lay the transect line or other similar activity. While underwater, always remain close to other members of the team and ensure that your buddy is in view.
e. Use recognized international signals to avoid confusion, particularly during emergencies.
f. Maintain proper buoyancy at all times, to avoid crashing onto corals or other marine life.
g. Maintain control of your fins to prevent breaking of corals.
h. **DO NOT BREAK** corals or damage other flora/fauna.
i. **DO NOT HARASS** reef fish.
j. **DO NOT DISTURB** any marine life unnecessarily.
k. **DO NOT COLLECT** corals, gorgonias, sponges, algae, fish, or other marine life.

l. **DO NOT LEAVE ANY RUBBISH** behind. Always take it with you and dispose of it properly.
m. **DO NOT TOUCH, SIT, STAND OR WALK** on the coral, algae, or other flora/fauna.
n. Upon completion of your tasks, inform your buddy so that both divers should finish agreed tasks and surface together.
o. Always check your oxygen level AND depth.
p. Should one diver be running out of air, feel tired or find him/herself in a situation where the dive needs to be shortened or aborted, inform your buddy, or other team member, and surface together **IMMEDIATELY** or as soon as possible, taking into account all necessary dive safety measures for surfacing.
q. Secure regulator and other research equipment and materials closed to your body, to avoid damaging the reef and other marine biota.
r. If weather becomes questionable, **CANCEL** the dive.

AFTER THE DIVE

5. Care of the Diving and Monitoring Equipment

a. Rinse all equipment and instruments in freshwater, free of detergent. Ensure that this includes wet suits, snorkels, masks, tanks, valves, BCDs, fins, etc.
b. Carry out a check of all the equipment to ensure that it is all in good working order.
c. Store equipment in a cool, dry place.
d. Shortly afterwards, fill up some or all the dive tanks with air, to ensure readiness in case of an emergency.

LIABILITY RELEASE

I acknowledge that the Mesoamerican Barrier Reef Systems Project's Synoptic Monitoring Program is a volunteer program. I recognize that I do not have to participate. I acknowledge that I have chosen to follow the MBRS-SMP Monitoring Methodology because it provides a suitable way of collecting scientific information in the MBRS Region and not because it minimizes any of the risks of SCUBA diving. I recognize that SCUBA diving is an inherently risky activity and I expressly accept all risks associated with SCUBA diving in any way affiliated with the MBRS-SMP.

Moreover, I hereby release the MBRS Project from any and all negligent acts in any way related to MBRS-SMP activities. I have chosen to do this volunteer work of my own free will for the purpose of contributing to science and the conservation of coral reefs and associated ecosystems, and I agree that I, and only I, shall be responsible for my safety, and any injuries I may sustain.

I agree that I will not hold liable or responsible the MBRS Project or any of its staff, the Central American Commission on Environment and Development nor the World Bank, or any personnel associated with any of the above, whether employees, agents, independent contractors, team leaders or other volunteers. I absolve all of them from any responsibility for my safety or any injuries, loss of limb or loss of life, which I may suffer in the process of following the MBRS-SMP survey methods or any deviation from them.

Signature: _____ Date: _____

Full name (in block letters): _____

EMERGENCY ASSISTANCE PLAN FOR DIVEMASTERS OR SUITABLY QUALIFIED DIVERS - IMMEDIATE FIRST AID ONCE VICTIM ON SHORE (From the US Leader Incentive Program) http://www.usdivetravel.com/T-Wanna_be_a_Guide.html#H.%20EMERGENCY%20ASSISTANCE%20PLAN%20for

Treatment is the same for suspected lung-expansion injury or decompression sickness

1. **Calm the victim,** if he or she is conscious. Send somebody quickly to the national emergency number with details of the emergency.

2. Keep the victim's **airway open.** This is imperative.

3. For the first 20 minutes after the accident, place the victim **head down, left side down.** After that, shift the victim to a prone position flat on his or her back. Keep the victim still.

4. If victim is conscious and breathing, administer **100% oxygen** through demand-regulator valve system. Keep oxygen flowing smoothly until emergency medical help arrives.

5. If victim is not breathing and no pulse is detectable, **administer proper CPR,** as per rescue training (use the **"A-B-C"** memory jogger):

   - **A**rousal & **A**irway, **B**reathing, **C**heck Pulse.
   - 15 compressions of sternum then two breaths, repeat indefinitely until emergency medical help arrives. If you have 2 rescuers, split duties.
   - For adults, compress sternum 1.5 - 2" inward at a point two finger spaces above bottom nub of breastbone.
   - Do compressions at a rate of 80-100 per minute for adults. Stay steady.

6. **Protect victim** from excessive heat, cold, wetness or hazardous exhaust fumes.

7. **Give victim fluids -- but absolutely no alcohol, caffeine or stimulants of any kind!** When qualified medical technicians arrive, advise them that IV (intra venous) fluids are in order for the entire ride back to a hospital emergency room or a hyperbaric chamber.

8. Arrange for **immediate evacuation** when help arrives. Advise the emergency medical team to keep the oxygen flow on non-stop for the full ride back to medical facility. Just like the IVs, **this is vital**.

DIVERS ALERT NETWORK EMERGENCY HOTLINE: [001] (919) 684 8111

EMERGENCY TELEPHONE NUMBERS AND ADDRESSES IN THE MBRS

BELIZE

GENERAL EMERGENCY NUMBER FOR ALL SERVICES: 911

EMERGENCY AIR AMBULANCE SERVICE
BATSUB (British Army Training Support Unit Belize) Ladyville. **Tel: [00 501] 225 2024, ext 241**

SEA/RIVER EMERGENCIES (SEARCH AND RESCUE). Belize Defence Force Maritime Wing (24 hrs). **Tel: [00 501] 225 2174, ext 156**

NATIONAL SECURITY EMERGENCIES (B.D.F. Ladyville): Tel: [00 501] 225 2087

MEDICAL EMERGENCIES:

Belize City:	**Tel: [00 501] 223 1548; 2231564**
San Pedro:	**Tel: [00 501] 226 3668**
Dangriga:	**Tel: [00 501] 522 2078**
Punta Gorda:	**Tel: [00 501] 722 2026**
Corozal:	**Tel: [00 501] 422 2076**
Independence:	**Tel: [00 501] 523 2167**

HYPERBARIC CHAMBERS

SAN PEDRO AMBERGRIS CAYE
The Hyperbaric Chamber San Pedro (Decompression Chamber)
San Pedro, Ambergris Caye
Tel: [00 501] 226 2198

Sub-Aquatic Safety Services
San Pedro Airstrip, Ambergris Caye
Tel: [00 501] 226 2851; 226 2852; 226 3195

GUATEMALA

GENERAL RESCUE SERVICE: 911

PUERTO SANTO TOMÁS DE CASTILLA, IZABAL
BANATLAN (Base Naval del Atlántico): **Tel: [00 502] 948 3102; Fax: [00 502] 948 3127**

MEDICAL EMERGENCIES
Patient Transport Services: **Tel: [00 502] 332 9422; 332 9423**

RESCUE SERVICE
Patient Transport Services: **Tel: [00 502] 361 4001**

PUERTO BARRIOS, IZABAL
Voluntary Fire Department: **Tel: [00 502] 948 0122**

24 HRS EMERGENCIES
Clínica Belen. Calle Principal, Col. El Progreso. Puerto Barrios. **Tel: [00 502] 948 0613; 948 7118**
Red Cross: 14 Calle entre 15 y 16 Av. Puerto Barrios. **Tel: [00 502] 948 1351**

National Hospital: Col. San Manuel cruce a Santo Tomás de Castilla, Izabal. **Tel: [00 502] 948 3077; 948 3071**

Comandancia y Capitanía de Puerto. 9ª Calle entre 3 Av y 4 Av. Puerto Barrios. **Tel: [00 502] 948 0416**

There are no hyperbaric chambers in Guatemala. The nearest is located in Honduras (see below).

HONDURAS

HYPERBARIC CHAMBERS

ROATAN, BAY ISLANDS
Dr. Fermín López Arrazola; Rafael Díaz Zelaya
Anthony's Key Resort
Hyperbaric Chamber
Roatan
Tel/Fax: [00 504] 445 1049; or thorough the Hotel: **Tel: [00 504] 445 1003**
Email: cms@globalnet.hn
Bonne Beach Resort (previously the Hotel Fantasy Island)
Roatan
Tel: [00 504] 455 5222; 455 5191
Email: fantasyislandresort@bonnebeach.com

CAUQUIRA – MOSQUITIA
There is a hyperbaric chamber in the area although it is difficult to access the area and is only contactable by radio. Further details of this service are unknown at this stage.

TEGUCIGALPA
Clinica Hiperbárica New Life
Colonia Alameda , Edificio Correduría de Seguros, Planta Baja
Tel: [00 504] 239 2498 (Clinic)
Tel: [00 504] 224 4871
Cel: [00 504] 967 0929
Dr Mirna Lizette Martínez Iglesias

MEXICO

GENERAL EMERGENCY NUMBER FOR POLICE, AMBULANCE AND FIRE DEPARTMENT: 060

EMERGENY NUMBER FOR QUINTANA ROO: 066

MEDICAL EMERGENCIES:

CANCUN
Red Cross: Tel: [00 52] (998) 884 1616; 879 511
Air Ambulance: Tel: [00 52] (998) 886 0626; 8860627 Administración de Servicios Aeronáuticos de México (Cancun Airport)
Operator for Medical Emergency Services: **Tel: [00 52] (998) 887 9844** (Operadora de Servicios Médicos de Emergencia SA de CV)
Capitanía de Puerto: Tel: [00 52] (998) 880 1360 to 63 Boat assistance in main ports

HYPERBARIC CHAMBERS IN CANCUN

Buceo Médico Mexicano. Multiplace Chamber
Calle Claveles # 5 SM 22 Lt 11 Int. Hospital Total Assist. Cancún, Q. Roo
Tel [00 52] (998) 887 1688. Email. ssscun@produgy.net.mx
Dr. Enrique Minor

Hiperbárica de Cancún S.A de C.V.
Alcatraces #44 Mza. 10 SM 22. Cancún, Q. Roo
Tel: [00 52] (998) 892 7680; Cel: [00 52] (998) 1057791; (998) 845 4257
Beeper: [00 52] (998) 881 5252 Keys 13344 and 3230
Email. hiperbarica@prodigy.net.mx
Dr. César Soto Fernández

COZUMEL
Red Cross: Tel: [00 52] (998) 872 1058
Secretaría de Marina -Armada de México: Tel: [00 52] (987) 872 1330; 872 1241; 872 1229

HYPERBARIC CHAMBER IN COZUMEL
Servicios de Seguridad Subacuática (SSS). Multiplace Chamber
Calle 5 sur # 21B entre 5ª y Av. Rafael E. Melgar. Cozumel, Q. Roo C.P. 77600
Tel: [00 52] (987) 872 1430; 872 2387
VHF Radio Channels: 2 and 16
Email. bmmrocio@prodigy.net.mx; servicios@cozunet.finred.com.mx
Dr. Wilma Padilla

CHETUMAL
Red Cross: Tel: [00 52] (983) 832 0571
Emergencies: Tel: [00 52] (983) 837 1065
Blood Bank: Tel: [00 52] (983) 832 5644 (Giving or receiving blood)
Capitanía de Puerto: Tel [00 52] (983) 832 0422 Boat assistance in main ports
Secretaría de Marina: Tel: [00 52] (983) 832 0226; 832 0225

ISLA MUJERES
Secretaría de Marina: Tel: [00 52] (998) 877 0575 (Coast guards and beach security)

PLAYA DEL CARMEN

HYPERBARIC CHAMBERS IN PLAYA DEL CARMEN
Servicios de Seguridad Subacuática (SSS). Single Chamber
Avenida 10 Esquina 28. Playa del Carmen, Q. Roo
Tel: [00 52] (984) 873 1365
Email. sssply@prodigy.net.mx

INTERNATIONAL
Diver Alert Network (DAN) http://www.diversalertnetwork.org/

Diving Emergencies (Remember: Call local EMS first, then DAN!)
Tel: [00 1] 919 684 8111
Tel: [00 1] 919 684 4DAN (collect)
Tel [00 1] 800 446 2671 (toll-free)
Tel: [00 1] 267 520 1507 (Latin America Hotline)

International Emergency Hotlines
Travel Assistance for Non-Diving Emergencies
Tel: [00 1] 800-DAN-EVAC [00 1] 800 326 3822)

If outside the USA, Canada, Puerto Rico, Bahamas, British Virgin Islands or U.S. Virgin Islands, call
Tel: [00 1] 215 245 2461 (collect)

Send DAN a Fax: [00 1] 919 490 6630; [00 1] 919 493 3040 (Medical Department)

Send DAN a Letter:
Divers Alert Network; The Peter B. Bennett Center; 6 West Colony Place; Durham, NC 27705 USA

Visit DAN:
Directions to DAN International Headquarters

International Contact Information
DAN Europe
DAN Japan
DAN South East Asia-Pacific (SEAP)
DAN Southern Africa

APPENDIX 4

GLOSSARY OF TERMS

Algae: One celled or many-celled plants that have no root, or leaf systems. They lack reproductive structures and all cells are potentially fertile

Algal turf: Densely packed algae that project less than one centimetre above the substrate they are growing on; usually filamentous

Ambient: Surrounding

Anthropogenic: Produced or caused by humans

Aspect ratio: Height to diameter ratio

Assemblage: A collection of individuals, usually of different types

Atoll: A type of coral reef formation that grows in the shape of a circle, enclosing a lagoon partially or completely

***Avicennia germinans*:** Black mangrove

Barrier reef: A type of coral reef formation that lies parallel to the coast and protects a lagoon

Basal area: The total area of the ground covered by trees, measured at breast height

Baseline: First assessment of a situation against which subsequent changes are measured

Belt transect: A narrow band of predetermined width set across a study area, and within which, the occurrence or distribution of plants or animals is recorded

Benthic: Bottom-dwelling organism, associated with the bottom or bed of a water body; or that is living on or under sediment, pilings, etc.

Benthos: Animals and plants living on the bottom of the sea

Bioerosion: The breakdown of skeletal material when organisms bore into it

Biodiversity: Is the totality of genes, species, and ecosystems in a region. Biodiversity can be divided into three hierarchical categories, genes, species, and ecosystems, that describe quite different aspects of living systems and that scientists measure in different ways

> ***Genetic diversity*** refers to the variation of genes within species. This covers distinct populations of the same species (such as the thousands of traditional rice varieties in India) or genetic variation within populations (high among Indian rhinos, and very low among cheetahs)
>
> ***Species diversity*** refers to the variety of species within a region and can be measured in many ways. The number of species in a region -- its species "richness" -- is one often- used measure, but a more precise measurement, "taxonomic diversity", also considers the relationship of species to each other. For example, an island with two species of birds and one species of lizard has a greater taxonomic diversity than an island with three species of birds but no lizards

Ecosystem diversity is harder to measure than species or genetic diversity because the "boundaries" of communities -- associations of species -- and ecosystems are elusive. Nevertheless, as long as a consistent set of criteria is used to define communities and ecosystems, their numbers and distribution can be measured

Biomass: The total weight of organic material of a particular species or in a particular habitat per unit of area or volume

Biota: The total plant and animal life of a given area

Biotic: The living components of an organism's environment

Bleaching: Loss of color from reduction in a number of zooxanthellae and/or the amount of photosynthetic pigments

Bloom: A sudden increase in the density of phytoplankton or benthic algae in an area

Calcium carbonate: White limestone material that makes up the skeletons of coral polyps and the shells of molluscs

Chlorophyll: A group of pigments present in plant cells which are essential in the use of light energy for photosynthesis

Cnidarian: A member of the Phyllum Cnidaria (also known as Coelenterata), which includes corals, octocorals, hydroids, jellyfish and anemones

Community: Any group of organisms belonging to a number of different species that co-occur in the same area and interact through trophic and spatial relationships

***Conocarpus erectus*:** Buttonwoods, an important mangrove associate

Coral colony: Group of coral polyps that takes specific shapes

Cryptic: Concealed

Data sheet: A paper form used to record field data in a set format

Depth contour: Horizontal line joining points of the same depth

Disturbance: Changes caused by an external agent, could be natural (e.g. weather) or human induced (e.g. pollution)

Diversity: Variety, often expressed as a function of a number of species in a sample, sometimes modified by their relative abundances

Ecosystem: A dynamic complex of plant, animal, fungal and micro-organism communities and the associated physical environment interacting as an ecological unit

Epiphyte: A plant that uses another living organism as a substrate

Fire coral: A member of the Class Hydrozoa which forms a calcium carbonate skeleton. Members of genus *Millepora* are very conspicuous on Caribbean and Western Atlantic reefs

Fringing reefs: Reef surrounding emergent land

Gorgonians: A soft coral of the Order Gorgonacea. Include most octocorals, together with sea fans, whips and branching soft corals

Global Positioning System (GPS): Satellite-based navigation system

Gyre: Circulating currents that spin in circles as they move westward across the Caribbean Sea. Most gyres in the northern part of the basin are anticyclonic eddies, spinning clockwise, while those in the southern Caribbean are cyclonic eddies, spinning counter clockwise

Hardground: Cemented hard rock surface that has become lithified

In situ: Latin term meaning 'in the normal or natural position'

Interstitial water: Water in the soil

Intertidal: The littoral region between the mean high tide and mean low tide; exposed at low tide, submerged at high tide

Invertebrates: Animals that lack a backbone

Lagoon: A body of water separated from the sea by a bank or coral reef. The region between a shore and a barrier reef or inside a ring of islands making up an atoll

***Laguncularia racemosa*:** White mangrove

Larvae: An immature stage of an animal that undergoes metamorphosis; a developing embryo that is independent of the parents, but has not yet assumed their adult characteristics

Leaf area index: A measure of the area of photosynthetic surface expanded over a given area of ground (m^2 leaf area m^2 ground)

Leeward: Side protected from the wind

Macroalgae: Algae that project more than one centimetre above the substrate, such as *Dictyota* and *Halimeda*

Mangal: The mangrove environment

Mangrove: An assemblage of tropical trees and shrubs that grows in the intertidal zone; a non-taxonomic term used to describe a diverse group of plants that are all adapted to a wet, saline habitat. Mangrove may typically refer to an individual species. Terms such as mangrove community, mangrove ecosystem, mangrove forest, mangrove swamp, and mangal are used interchangeable to describe the entire mangrove community

Methodology: Collection of methods used in a particular activity

Monitoring: Repeated observation of a system, usually to detect change

Octocoral: A member of the Subclass Octocorallia, which includes gorgonians, sea fans and other organisms. The polyps bear eight tentacles which usually have small projections

Okta: The unit of measure for the amount of cloud cover

Parallax: A distortion that occurs when viewing the same object from different angles or distance so the object's position and size may appear to change

Parameter: A measure used to describe some characteristics of a population

Patchiness: Uneven or variable distribution

Pavement: Hard carbonate substrate of low relief, sometimes dominated by octocorals

Pelagic: Free swimming or floating organisms that live exclusively in the water column, as opposed to living on the bottom

Phytoplankton: Microscopic plants floating in the water column

Plankton: Microscopic organisms floating in the water column, not associated with the substrate, found at various depths in aquatic habitats

Pneumatophores: Vertical extensions from cable roots in black and white mangroves

Polyp: The basic structural unit of a cnidarian, consisting of a tubular or cylindrical body having an oral end which bears the mouth and tentacles

Population: All members of a species that live in a common area

Population structure: The composition of a population according to age and sex of the individuals

Predators: Secondary consumers; living prey is consumed in whole or in part

Productivity: 1) The potential rate of incorporation or generation of energy or organic matter by an individual or population per unit time per unit area or volume; rate of carbon fixation; 2) Often used loosely for the organic fertility or capacity of a given area or habitat

Propagule: Mangrove dispersal unit; seedling

Prop root: Aerial roots of red mangrove

Quadrat: A two-dimensional square or rectangular sampling unit which organisms are counted or measured. It can also denote the frame that marks the area in question

Qualitative: Descriptive, non-numerical, assessment

Quantitative: Numerical, based on counts, measurements or other values

Recruitment: Process where floating fish eggs divide from a single cell and differentiate into tiny swimming larvae, also know as recruits, which inhabit marine plankton. Recruits follow ocean currents and eventually, if they encounter suitable habitat, they will metamorphose into their juvenile forms. The measure of new individuals (recruits) arriving in a population

Reef crest: The highest point at the seaward edge of a coral

Reef slope: The face of a coral extending seaward from the reef crest

Refractometer: Optical instrument used to measure salinity

Replicate: A repeated sample from the same location and time

Rhizophora mangle: Red mangrove

Salinity: Measure of the total concentration of dissolved salts in water

Sample: Any subset of a population; a representative part of a larger unit used to study the properties of the whole

Sand: Cohesionless sediment particles measuring 2.0-0.0625 mm in diameter

Scleractinian: A member of the Order Scleractinia, the stony corals of the reef that produce calcium carbonate cups called corallites

SCUBA: Self-contained Underwater Breathing Apparatus

Seagrass bed: Large, dense stands of seagrass such as turtle grass

Secchi disc: Device used to estimate the transparency of water

Sedimentation: Process of deposition of particulate matter

Sessile: An organism that is attached to the bottom or to rocks, pilings, etc. and unable to move

Settlement: A transition in the development and growth of an animal in which pelagic larvae or juveniles colonise and take up residence in benthic habitats

Slate: Rigid surface for writing on when underwater

Soft coral: Animal consisting of anemone-like polyps with eight feeding tentacles surrounding the mouth

Spatial: Pertaining to space

Spur formations: Radiating ridges in the upper reef slope dissected by deep regularly-spaced grooves

Standard length: Measurement of fish length from snout to hypopural bone in the tail

Standing crop: Instantaneous measurement of the biomass of a population

Standing stock: Instantaneous measurement of the density of a population

Substrate: The surface on which an organism lives

Substratum: The base to which a stationary animal or plant is fixed, but can be any benthic surface

Succession: Progressional change in species composition of an ecosystem over time as the organisms alter the environment

Survey: Organized inspection

Taxonomic: Relative to taxonomy; the formal guidelines for classifying organisms based on evolutionary relationships

Temporal: Pertaining to time

Thermocline: A boundary region between two layers of water of different temperature

Transect: A line or narrow belt used to survey the distributions of organisms or substrate across a given area. Also, an ecological method particularly useful in examining zonation or gradients

Turbulence: Non-linear motion

Upwelling: Vertical movement of water currents that brings up nutrients from deep regions

Variable: Any measurable aspect of a sample that is not constant

Visibility: Distance at which objects may be sighted during a survey

Water column: A volume of water between the surface and the bottom

Windward: Side exposed to the wind

Zonation: A distinct arrangement of species along an environmental gradient

Zooplankton: Microscopic animals floating in the water column

Zooxanthellae: Photosynthetic, dinoflagellate algae (unicellular) that live symbiotically in the tissues of certain marine invertebrates, including reef-building corals