Reef Fish Spawning Aggregation Monitoring Protocol for the Meso-American Reef and the Wider Caribbean

Version 2.0

DRAFT DATE: 4 July 2004

Acronyms and Abbreviations

ASK	Amigos de Sian Ka'an
BAS	Belize Audubon Society
BFD	Belize Fisheries Department
BICA	Bay Island Conservation Association
CONANP	Comisión Nacionál de Areas Naturales Protegidas, México
CZMA&I	Coastal Zone Management Authority and Institute, Belize
DIGEPESCA	Dirección General de Pesca y Acuicultura
FUNDAECO	Fundación para el Desarollo y la Conservación, Guatemala
FON	Friends of Nature
FSA	Fish Spawning Aggregation
MBRS	Mesoamerican Barrier Reef Systems Project
MPA	Marine Protected Areas
PROLANSATE	Fundación para la Protección de Lancetilla, Punta Sal y Texiguat
SCRFA	Society for the Conservation of Reef Fish Aggregations
SPAGs	Spawning Aggregations
TIDE	Toledo Institute for Development and Environment
TNC	The Nature Conservancy
UB	University of Belize
UNIPESCA	Unidad de Manejo de la Pesca y Acuicultura
USC	University of South Carolina
WCS	Wildlife Conservation Society
WWF	World Wildlife Fund

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Acknowledgements

This updated (version 2.0) monitoring protocol has been amended as a result of two Spawning Aggregation Monitoring Training Courses, and two years of SPAGs monitoring using the guidelines and methods outlined in the protocol. Fishermen, dive guides, students, research scientists, marine biologists, fisheries managers, marine reserve rangers, government and non-governmental agencies who used the earlier draft provided recommendations and comments that were incorporated into this final document.

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At the 55th Annual Meeting of the Gulf and Caribbean Fisheries Institute Meeting held in November 2002, The Nature Conservancy sponsored a Spawning Aggregation Symposium. Follow up meetings during the week of the meeting lead to an agreement that a standardized protocol would be useful for monitoring of spawning aggregations throughout the Meso-American Reef and Caribbean region. Brian Luckhurst, of the GCFI board generously offered to compile the existing protocols into one single document that could be used as a standard. This document is based in part on a similar protocol developed by The Nature Conservancy's Asia Pacific Marine Program, The Nature Conservancy's Belize Program Protocol and a spawning aggregation monitoring protocol used by Green Reef in Belize in January 2001.

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This document was compiled and edited by Will Heyman (The Nature Conservancy - Belize), James Azueta (Belize Fisheries Department), Oscar Lara (MBRS), Isaias Majil (Fisheries Department), Dwight Neal (OAK Foundation and formerly Belize Fisheries Department), Brian Luckhurst (Department of Environmental Protection – Bermuda), Mito Paz (Green Reef - Belize), Imani Morrison (Coastal Zone Management Authority and Institute), Kevin Rhodes (The University of Hong Kong), Björn Kjerve (University of South Carolina), Beverly Wade (Belize Fisheries Department) and Nicanor Requena (TNC) and should be cited as:

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Evolution and use of this document

This document was developed in consultation with the Belize Spawning Aggregations Working Group, to whom we are greatly indebted. The working group consists of all of the major government and non-government organizations in Belize that are working together to monitor and conserve spawning aggregations. The group has been instrumental in designing mutually agreed upon data sharing agreements, protocols, databases, and support for marine protected areas legislation.

This document is designed for managers and field practitioners and so is intentionally streamlined and practical. We have purposely left out comprehensive references and background material as those are covered more fully elsewhere. Moreover, the document is designed as a flexible guideline that should be used to assist practitioners in designing a monitoring protocol recognizing the inherent physical, biological and economic conditions among various sites. Additional documents that can also be used as companion guides include:

- R2-Reef Resilience Toolkit. 2003. The Nature Conservancy
- Introduction to Monitoring and Management of Spawning Aggregations and Aggregation Sites for Three Indo-Pacific Pacific Grouper Species: A Manual for Field Practitioners. 2003. The Nature Conservancy.
- Manual for Study and Conservation of Reef Fish Spawning Aggregations (http://www.scrfa.org)

This manual is a work in progress and is considered a "living document". After three years of use by multiple organizations and people, this draft has been updated to the present version 2.0. This version includes practical pointers, from field practitioners who have used the protocol. The authors recognize that the manual can be improved and we urge you to contact us with suggestions. The document will be updated periodically as needed, with suggestions to be reviewed by an editorial committee. For now, the committee will be chaired by The Nature Conservancy and consists of Belize Fisheries Department, MBRS Project, and Green Reef Environmental Institute, and Belize Audubon Society - the chair of the Belize Spawning Aggregations Working Group.

List of Tables, Figures and Data Sheets

1. Introduction

- Fisheries form an important sector of the economy of the countries along the Mesoamerican Reef and the entire Caribbean Basin.

- Most commercially important reef fish (e.g. groupers and snappers) migrate to specific places at specific times to reproduce in what are called spawning aggregations or "SPAGs", also referred to as fish spawning aggregations, or "FSAs".

- SPAGs, once discovered by fishers, are often heavily exploited. In some cases, SPAGs may become so depleted that they no longer form. For example, Nassau grouper SPAGs have disappeared from approximately one-third of all known SPAG sites in the wider Caribbean region. These include sites in Belize, Mexico, Honduras, Puerto Rico, the U.S. Virgin Islands, Florida, the Dominican Republic and Bermuda.

- If management/conservation intervention occurs before complete collapse, spawning aggregations may have the potential to recover.

- SPAGs are critically important in the life cycle of many reef fishes and reproduction at these sites may represent the total annual reproductive output for that species population.

- SPAGs provide substantial economic benefits to subsistence and commercial fisheries and may play a significant role in the marine tourism industry, e.g. dive tourism.

Protecting spawning aggregation sites within marine reserves can provide regional benefits to fisheries and the tourism industry and assist in the conservation of marine biodiversity

General Goal of this Document

- The purpose of this document is to provide a standardized methodology for the evaluation and routine monitoring and conservation of transient multi-species spawning aggregations along the Meso-American Reef and the wider Caribbean. This document is intended for use by resource managers, conservationists, biologists, fishers, students and trained recreational divers.

Specific Objectives of the Monitoring Protocol

The monitoring protocol is designed to provide methods to:

1. Describe and monitor the ecological context of each spawning aggregation site by providing a map and description of the spawning location that includes a general description of the physical environment (geomorphology, coral cover) and the oceanographic parameters (currents, temperature, salinity).

2. Quantitatively evaluate and monitor each spawning aggregation by species, documenting the time and location of each aggregation with estimates of the numbers of fish and their sizes using underwater visual census techniques.

3. Evaluate and monitor the status of the fishery and the effects of fishing effort on the SPAG site using measures of catch per unit effort, sex ratio and length frequency analysis.

4. Evaluate the site fidelity of fish at each SPAG site and the extent and distance of migrations to and from SPAG sites using tag-recapture techniques.

5. Share data and information from all sites to promote appropriate management measures both locally and regionally.

6. Involve displaced fishers in the monitoring program (e.g. by including them as assistants in the data collection program) as one of several possible economic alternatives to commercial fishing at spawning aggregations.

7. Collect appropriate biological samples from specimens for analysis of life history information for target species e.g. gonads for evaluation of reproductive status, otoliths for age and growth analysis.

2. Background

Coral reefs are among the most diverse environments on the planet and are second only to rainforests in biodiversity. Caribbean nations have increasingly recognized the natural wealth of coral reefs and are creating and implementing conservation measures that include the use of marine protected areas (MPAs) to conserve reefs and to combat overfishing. MPAs are touted as effective means of increasing fisheries productivity within and adjacent to MPAs and for the protection of the spawning stocks of commercially important species. However, many reef fish species (including commercially valuable snappers and groupers) may migrate out of existing MPAs for spawning, whereupon they are often heavily exploited, particularly at SPAG sites.

Among commercially important species that form spawning aggregations, grouper populations have drastically declined in the Caribbean. Nassau grouper, *E. striatus*, are listed as "endangered" by the IUCN, in association with an estimated decline in population abundance of 40% within its distributional range. Goliath grouper, *Epinephelus itajara* (previously called jewfish), are listed as critically endangered and face an extremely high risk of extinction in the wild. Other grouper species appear to be showing similar declines. These trends are common throughout the Caribbean, where many known grouper spawning aggregations have been fished to near extinction. Although the effects of aggregation fishing are well-documented, few SPAG sites have been included within MPAs. Data collected in Belize over the past few years indicates that Nassau grouper SPAG sites also serve as spawning sites for most of the Caribbean's other large and commercially important food fish. On the basis of these findings, The Nature Conservancy has proposed that the conservation of multi-species SPAG sites throughout the Mesoamerican Reef, and by extension, the wider Caribbean, will help to conserve marine biodiversity and provide benefits to local and regional economies through tourism and fisheries.

The purpose of this document is to provide a standardized methodology for the evaluation and routine monitoring of spawning aggregations along the Mesoamerican Reef and the wider Caribbean. It is anticipated that the information collected using this methodology will be shared regionally via the Gulf and Caribbean Fisheries Institute, the Society for the Conservation of Reef Fish Aggregations (SCRFA) and other regional initiatives such as the MBRS project. Data and experiences shared through these organizations will form the basis for strengthening in-country capacity, as well as national and regional policies on spawning aggregation conservation. National and regional marine reserve network designs should incorporate known SPAG sites.

Further, fishers who are presently fishing spawning aggregations should be encouraged to participate in the research, monitoring, tour guiding, and management of these sites. In this way, displaced fishers will gain economic alternatives to SPAG fishing, and become more active stewards of these resources. The collaboration of scientists, managers, fishers, government and non-government organizations (NGOs), agencies, and the private sector is viewed as the best hope for the conservation and sustainable use of Caribbean marine resources.

3. Criteria to define a spawning aggregation

Reef fish aggregations are groups of fish gathered for either spawning, feeding or shelter. By definition, a spawning aggregation is a group of con-specific individuals grouped together in densities three times higher than those found in non reproductive periods (Domeier and Colin 1997). Spawning aggregations are best documented with direct evidence - observations of actual spawning or documenting the presence of hydrated oocytes within gonads of females on the site (Colin et al. 2003; www.scrfa.org). If direct evidence is not available, indirect evidence include density increases, spawning-specific color changes and behaviors, swollen abdomens and increases in the gonadosomatic index. A combination of indirect observations increases the likelihood that the aggregation is actually a spawning aggregation. Reef fish spawning aggregations are predictable in space and time.

Two different types of spawning aggregations have been defined ("resident" and "transient") using the following three criteria; the frequency of aggregations, the longevity of aggregations, and the distance traveled by fish to the aggregation. Spawning in resident aggregations is common to most rabbitfish (siganids), wrasses (labrids) and angelfish (acanthurids). In this type of aggregation, spawning is brief (often 1-2 hours), occurs frequently (often daily) and involves migration over short distances to the spawning site. This manual does not focus on these aggregation types or species. Spawning in transient aggregations, by contrast, is the strategy used by most grouper (serranids), snapper (lutjanids), and jacks (carangids), along with several other families. Transient aggregations generally exhibit the following characteristics:

a. Fish frequently migrate long distances to the aggregation site, sometimes using specific routes
b. Aggregations typically form during only a few limited months of the year, possibly in relation to day length or seawater temperature.
c. Within each month of occurrence, aggregations typically remain from a few days to a few weeks, entrained to the lunar cycle.

For the species that use this strategy, it appears that all reproductive activity for the year takes place within these aggregations, as there is no evidence of spawning in these species outside the aggregation. Transient, multi-species spawning aggregations are the focus of this manual.

4. Locating Reef Fish Spawning Aggregations

Locating spawning aggregations generally requires the use of a variety of different tools and approaches including secondary sources, fishery-dependent sources, and fishery-independent sources.

Searches should be initiated using secondary sources and available published materials. Secondary sources include published scientific studies (basic life history studies, including seasonal gonosomatic indices and studies of age and growth) and other published materials such as popular articles and trade magazines.

In locations where there is commercial, subsistence or recreational fishing, managers can collect fishery-dependent data. The best means of obtaining information is to compile traditional knowledge from resource users. Patriarch fishers can be a particularly valuable source of information as they can provide a temporal perspective on given SPAG sites. This information is generally obtained by in-person interviews with fishers as well as other members of the fisheries sector e.g. fish marketing agents. Historical records from government landings statistics, from fish marketing facilities and export records can also be helpful in identifying the periodicity and abundance of aggregating species. The above sources can all be classified as fishery-dependent data. Any anecdotal information about SPAGs should be verified through at least one of the above information sources, if possible. Fishing vessels concentrated in a small area for a limited time period can often be an indication of a SPAG site. Such observations should be verified by checking at landings sites to determine the quantities and reproductive status of the fishes being harvested.

A fishery-independent method for attempting to locate SPAG sites is the use of satellite imagery, aerial photographs and bathymetric charts for potential site location, since many documented SPAG sites globally in the tropics occur at reef promontories, and/or seaward extensions of reefs near deep water. In locations where SPAG fishing has never been documented, this methodology can be used to predict SPAG site locations. Predictions must be subsequently verified by divers during the appropriate lunar phase of the reproductive season.

In summary, a combination of tools for locating spawning aggregations is usually necessary. Researchers will generally need to include fishers' knowledge of traditional spawning sites, published and "grey literature" studies, nautical charts, aerial photos and surveys, and satellite imagery. After spawning aggregations have been located, they can be monitored using techniques that are detailed in subsequent sections of this manual - underwater visual assessments, fishery-dependent monitoring (catch per unit effort), fish tagging, biological sampling and surface oceanography studies.

5. Target Species and Seasonality

Detailed studies of spawning aggregations at Gladden Spit and Lighthouse Reef Atoll in Belize have revealed a seasonal pattern of spawning site utilization by a variety of reef fish species. These same patterns are consistent with the Bahamas, Cuba, the Cayman Islands, Mexico, and Florida. Further research may show this to be a more general pattern at SPAG sites throughout the region. This monitoring protocol is designed to identify and survey *all* species forming transient spawning aggregations at a given site throughout the year. It is focused primarily on the most commercially important grouper and snapper species, and secondarily on other species.

For the Mesoamerican Reef groupers and snappers the pattern is as follows: Dec – March: Nassau grouper, black grouper, yellowfin grouper and red hind; and March – June: yellowtail snapper, mutton snapper, cubera snapper and dog snappers (Table 1). Aggregations typically form and persist for a period of around two weeks within a spawning month but this varies by species. All of these species seem to aggregate for about 5 – 10 days within their respective spawning seasons after each full moon , although the precise timing depends on the species (Table 2). These spawning patterns can serve as a guide and appear to be similar throughout the Caribbean region, but timing each location must be verified.

Table 1: Common spawning months for some groupers and snappers. *Numbers of x's indicates relative abundance for the particular species so that xxx indicates peak abundance*.

Species name	Common name	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Epinephelus striatus	Nassau grouper	XXX	XX	X								X	XX
Mycteroperca bonaci	Black grouper		XX	XXX	XXX	X							
Mycteropera venenosa	Yellowfin grouper		X	XXX	XXX	XX	X						
Lutjanus jocu	Dog snapper	X	X	XX	XXX	XXX	XXX	XX	X	X	X	X	X
Lutjanus cyanopterus	Cubera snapper			XX	XXX	XXX	XX	X	X	XX	XX		
Lutjanus analis	Mutton snapper			XX	XXX	XXX	XX	X					

Table 2: The common spawning period during the lunar month for various species. *Numbers indicate the numbers of days after full moon. Full moon (fm) is assumed as day 0, and the numbers of days after full moon are indicated to the right. The figure indicates, for example, that the Mycteroperca species spawn later in the lunar month than other species.*

Species name	**Common name**	**0** fm	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14** nm	**15**
Epinephelus striatus	Nassau grouper				X	X	X	X	X	X	X						
Mycteroperca bonaci	Black grouper									X	X	X	X	X	X	X	
Mycteroperca venenosa	Yellowfin grouper										X	X	X	X	X	X	
Lutjanus jocu	Dog snapper			X	X	X	X										
Lutjanus cyanopterus	Cubera snapper					X	X	X	X	X	X						
Lutjanus analis	Mutton snapper					X	X	X	X	X	X	X	X	X	X		

In Nassau grouper, the best-studied in the region, spawning generally occurs over only one to three days during two to four consecutive months, such that the total annual reproductive output for the population occurs in a tight window of time and space. Several species have been documented aggregate for spawning at the same times and locations as Nassau grouper, which have lead us to describe these as "multi-species" SPAG sites. Data on all transient spawning aggregations should be recorded while monitoring.

During each month, each species tends to build up in numbers to a peak, until spawning occurs often over several consecutive evenings. The fish usually disperse immediately after the spawning event for the lunar month has concluded.

Commonly, fish exhibit geographic, inter-annual, and lunar variations in the timing of spawning aggregations. It is important to consider these variations in designing the monitoring program. Aggregations form in consecutive months for at least two months and fish abundance peaks during one month of the spawning season. Although some consistent patterns emerge, inter-annual variations within a site are common, shifting peak spawning times by a month or so. Geographic shifts in peak spawning times for single species may also occur in relation to environmental conditions, such as water temperature. Careful documentation will be needed at many sites in order to discern these regional differences.

An optimal monitoring and management program would include initial observations during every month of the year, with specific focus between December and June annually. In cases with limited resources, however, it is suggested that the peak grouper moon (January) and the peak snapper moon (April-May) be selected as the two times when sites are fully monitored, if possible, for 30 consecutive days to accurately document the build-up and subsequent dispersion of the aggregations of each species. Data collected at other times can also be useful in defining these patterns. Monitoring frequency and duration, as well as the species to be monitored, depends on the objectives and resources of the management authority.

Table 3: Fish species identification chart

	Belize/English	Guatemala	Honduras	Garifuna	Creole
	***Lutjanus buccanella***				
	Blackfin snapper	Calau/Colorado	Cubera aleta negra	Jiyaba fanatii	Black fin snapper
	***Lutjanus cyanopterus***				
	Cubera snapper	Cubera	Cubera	Jiyau auiriti	Black snapper
	***Lutjanus jocu***				
	Dog snapper	Pargo colorado	Pargo colorado	Galalp	Dog teeth
	***Lutjanus griseus***				
	Gray snapper	Cubera sacatal	Cubera de mangle		Black snapper
	***Lutjanus synagris***				
	Lane snapper	Calau	Galale	Galali	Silk snapper
	***Ocyurus chrysurus***				
	Yellowtail snapper	Xalatil	Yalatel	Galali	Yelatil
	***Lutjanus analis***				
	Mutton snapper	Pargo criollo	Botisnapa		Mutton snapper
	***Lutjanus campechanus***				
	Red snapper	Cubera	Corruncha ojo rojo	Gagubanagai	Deep water silk
	***Mycteroperca bonaci***				
	Black grouper	Mero	Abadejo	Waga'nut	Rockfish
	***Epinephelus itajara***				
	Jewfish	Yerno/Wasa	Yerno/Wasa	Inegii	Jewfish
	***Epinephelus striatus***				
	Nassau grouper	Wasa	Grupamanchada		Groupa
	***Epinephelus morio***				
	Red grouper	Mero	Grupa roja		
	***Mycteroperca venenosa***				
	Yellowfin grouper	Mero	Payaso		Yellow wing
	***Mycteroperca tigris***				
	Tiger grouper	Mero	Mero tigre		Fringy tail

	Belize/English	Guatemala	Honduras	Garifuna	Creole
	***Epinephelus fulvus***				
	Coney	Mero de arrecife de pantiel	Mero mantequilla		Butterfish
	***Epinephelus guttatus***				
	Red hind	Mero	Quimijay		Jimmy hind
	***Caranx hippos***				
	Crevalle jack	Jurel	Jurel	Yawariga	Crebally
	***Caranx ruber***				
	Bar jack	Jurel bajo el arrecife			
	***Caranx crysos***				
	Blue runner	Quinoa	Cabo de año	Gülilagaii	
	***Seriola fasciata***				
	Amberjack	Jurel ojudo	Cabo de año grande	Gülilayüai	
	***Trachinotus falcatus***				
	Permit	Pampano	Palometa	Jauarawiia	Pompus jack
	***Haemulon album***				
	White Margate				Margaret fish
	*Anisotremus surinamensis*				
	Black Margate				Margaret fish
	***Lachnolaimus maximus***				
	Hogfish	Boquinete			Hog snapper

6. Spawning Indicators

The documentation of a spawning aggregation requires that the observer first be able to distinguish it from a feeding or schooling aggregation. For example, fish aggregating in unusually large numbers relative to non-spawning “normal” periods may serve as indirect evidence of spawning aggregation formation. The recognition of this increase requires the observer to have an idea of what "normal" abundance is at the site to make this evaluation. (If baseline data are unavailable, then monitoring the site for at least one 30 day period is desirable to conserve and document potential changes in abundance.) In addition to increased abundance, spawning aggregations are often associated with behavior patterns and species-specific color changes indicative of reproductive periods (Figure 1, top panels). These may include sometimes-erratic schooling behaviors, biting, chasing, and species- (and sometimes sex-) specific color changes. For example, Nassau grouper frequently exhibit four different color patterns during spawning (Colin 1992). Male hogfish guard territories on hard-bottom substrates to attract harems of females. Other fishes, such as ocean triggerfish create and vigorously guard clean sandy nest patches on the bottom. A wide variety of spawning behaviors and reproductive adaptations may be recorded by the keen observer.

For most transient spawners, courtship and spawning activities commence in the late afternoon (about 45 - 30 minutes before sunset) and often peak close to sunset. For others, spawning may be midday, overnight, or close to sunrise. Though many species spawning behaviors are documented, close observations and photographic and video documentation of spawning behavior can provide additional details of these rarely witnessed events. Any instances of spawning should be carefully described on the data collection sheets to verify the function of the aggregation, i.e. spawning, as opposed to feeding. Particularly, gamete release (Figure 1, bottom panels) provides the most incontrovertible evidence that spawning has occurred.

Figure 1: Observed spawning colorations and spawning events

7. Monitoring Teams and Equipment

When in the field, monitoring teams can collect a variety of useful data, but to do so requires thoughtful and efficient use of the time available. Monitoring efficiency is particularly important, since the cost and logistics of conducting monitoring surveys is considerable.

In all likelihood, monitoring teams will vary for a variety of reasons. For each monitoring survey, and for each specific dataset, a lead person should be chosen to ensure complete and efficient data collection. A recommended outline of team and equipment components needed for the most effective monitoring is provided below. To increase the probability of success in the field, it is highly advised that fishermen, particularly those experienced in fishing aggregations, be involved in every aspect of data collection.

Suggested Aggregation Monitoring Team:

- Team Leader (biologist)
- Boat Captain/ fish tagger/CPUE
- Boat assistant/ fish tagger/CPUE
- Camera operator
- 3 Divers: that can also film or count, and do tagging and CPUE at the surface

General Equipment List:

- Boat and engine (or preferably two) with fuel, depth sounder, GPS, VHF radio, flares, reef anchor and long rope
- Full gear for 4 divers: Mask fins, snorkel, BC, regulator, weights and belts, watch, depth and pressure gauges and dive compass
- Dive computers are highly recommended for every diver
- Dive safety equipment, including dive flag, safety sausages, whistles and dive flashlight or strobe
- DAN Oxygen Kit
- DAN Insurance for all divers
- At least one person on the trip has taken CPR training
- Food and Camping Equipment (or a place to stay)
- Rain Gear for all participants
- CPUE measuring equipment (scales/board) and data sheets
- Site maps with GPS coordinates and aerial photos, all laminated if possible
- Underwater measuring tape or marked rope (50-100 m)
- Tagging equipment and tags, data sheets
- Current drogues
- Digital underwater video camera, tapes and batteries
- Still camera for both surface and/or underwater use
- Cannula, microscope slide or piece of glass
- Magnifying glass or 10X eyepiece

8. Mapping the Site

A good base map of a spawning aggregation site is an invaluable tool for monitoring, outreach, and management. There are several techniques available for making such maps, as described below. A map should be developed for each site where detailed monitoring will occur. Good maps will be geographically referenced, include accurate scales, provide an accurate indication of the geomorphology, and show the locations of the aggregations of various species. Each map should be appended with a site description that provides geographic context and detailed descriptions of biological cover and physical attributes of the site. No matter the method, aggregation boundaries should be clearly defined and areas measured as accurately as possible, since these measures will be used in calculating fish densities and assessing future changes to the aggregation.

Example of Mapping Techniques:

Weighted Line - Underwater Mapping

Perhaps the simplest and most reliable method entails using a weighted, measured line to measure (and map) the aggregation boundaries underwater. Once the boundaries of the aggregation are located, the weighted, measured line can be laid along each of the aggregation boundaries, with the measurement recorded on underwater paper. A compass reading can be taken along the weighted line (in degrees) to assist in defining aggregation shape and subsequent area calculation. For convenience in measuring borders and ease in calculating aggregation areas, it is best to use straight line boundaries whenever possible and have the entire aggregation areas fit a simple geometric pattern, e.g. square, triangle, or combination of patterns. Maps can be created on graph paper and areas calculated.

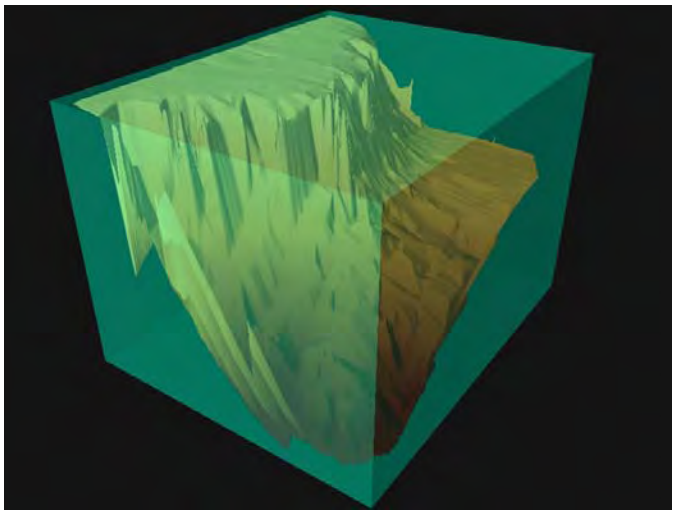
Mapping with Floats and a GPS

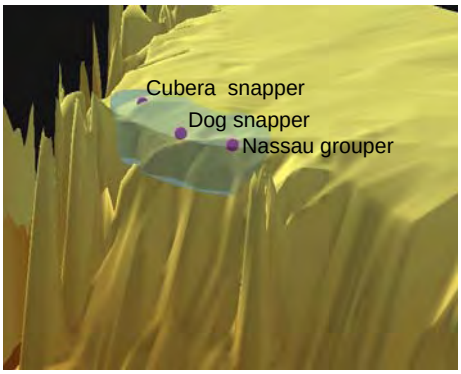
Aggregations can also be mapped from a boat with a handheld GPS. The location of the aggregation can be communicated to the surface by divers from below. As above, divers swim around the perimeter of the aggregation site, sending a float line to the surface from major points along the aggregation boundary. GPS coordinates and directions are then taken from the boat and the distances between points derived by using the "Go To" function (e.g. from Point 1 to Point 2, Point 2 to Point 3, etc.) on the GPS receiver. Compass bearings can be taken between points to get the angles between borders to determine the shape of the aggregation. Once the boundaries are established and distances and angles derived, the area can be calculated from basic geometric equations. Maps and area calculation can easily be created using GIS software such as ARCGIS.

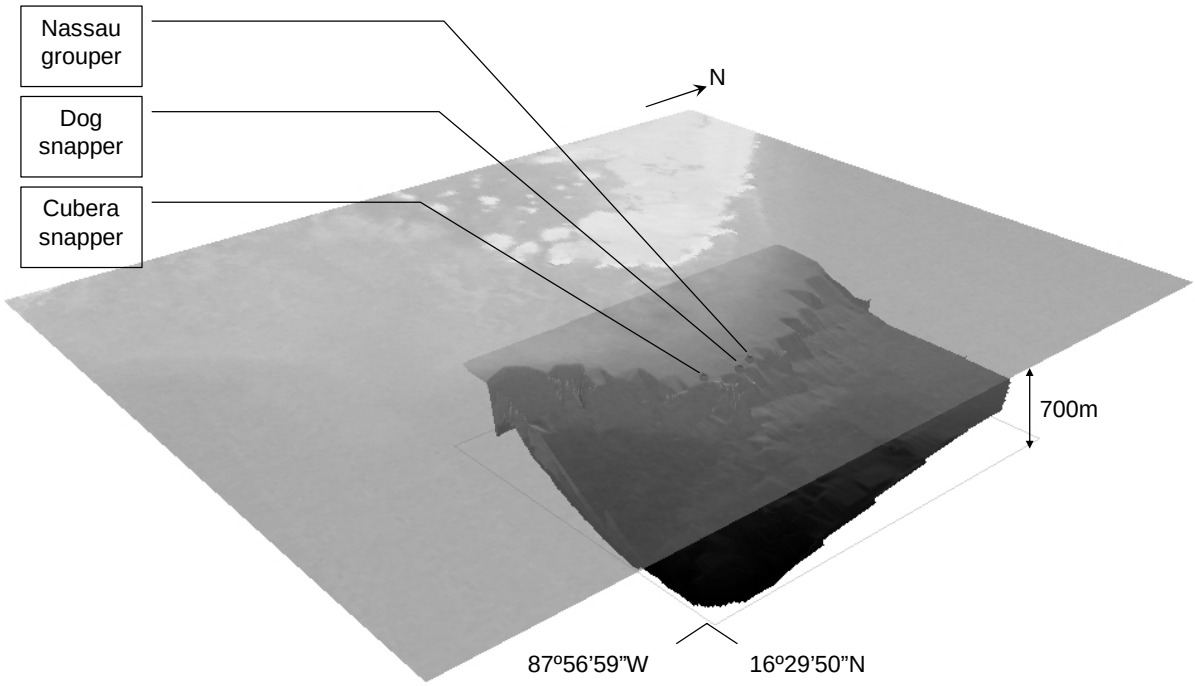
Adaptive Bathymetric Surveys (ABS)

Adaptive bathymetric survey (ABS) technique was recently developed to provide resource managers with a relatively quick and inexpensive way to develop detailed, three-dimensional, georeferenced maps of spawning aggregation sites. A full description of the techniques and its application are available elsewhere (www.nature.org). Examples of maps created with this technique are provided below (Figure 2).

Figure 2: Adaptive Bathymetric Surveys (ABS) map views. *Gladden Spit spawning aggregation site is shown from various angles, indicating the locations of three of the various species that aggregate there.*







Reef area: 768 Hectares

Example of Site Descriptions:

Example: Geographic Context Description

"Glovers Reef is an elongate rectangular atoll about 32 km long and 12 km wide with an area of 260 km^2. It is surrounded by reef that is about 400-700 m wide with the windward side better developed than the leeward side. There are three main channels in the atoll: one in the northeast corner of the atoll, another in the south and another between Northeast Caye and Long Caye. On the seaward side of the peripheral reef, the fore-reef slopes gradually to the drop-off. The fore reef is about 400 m to 1.5 km wide and the edge of the drop-off lies at variable depth between 15-45 m, the further north the deeper the edge. There is spur and groove system that runs perpendicularly to the fore-reef on the windward north and east sides of the atoll. The drop-off falls steeply, or vertically, to a depth of about 1000 m on the windward and 500 m on the leeward side of the atoll. In some areas there are a series of ledges or terraces that occur at various depths below the drop-off" (from Sala and Ballesteros, 2001).

Example: Detailed Spawning Site Description

"The spawning site, which is located approximately 300 m off the reef crest and outside a big channel, was located in a spur and groove system at 25-30 m depth, at the northeastern part of Glover's Reef, off one of the main channels of the atoll. The coral ridges are higher and more rugged than any other at Glover's Reef, with more crevices, overhangs, and shelters for the groupers. There are eight main coral ridges and nine sand channels within the spawning site. Below the coral ridges there is a flat bottom (shelf edge) covered by corals with a few sandy patches, down to 45 m depth. The slope of this area is quite gentle, but after 45 m it becomes steeper, going down to 70 m, where the reef wall becomes completely vertical going down to 1000 meters. The spawning site is limited to a surface of about one hectare" (from Sala and Ballesteros, 2001).

9. Underwater Visual Survey Protocol

Accurately estimating the number and size of fish within an aggregation is considered the most important and often most difficult monitoring technique within the manual. These skills are typically acquired through training by divers experienced in underwater visual census. Aggregations vary naturally by species in abundance, density, area, individual size and behavior. For example, species, such as hogfish or trunkfish may form aggregations of tens of fish over small area close to the substrate that are easy to approach, identify and describe. Others, such as jacks, may form large (up to several hundred individuals) roving aggregations in the water column. These schools are often mixed with other species and constantly moving. Groupers, on the other hand, are often wary of divers, with aggregations spread over substantial areas along the bottom in relatively deep water. As such, the manner of measuring abundance, individual size and behavior may also vary. For these reasons, training in underwater visual census is highly recommended prior to any attempt at monitoring aggregations. A brief training section is provided as Appendix 2 (p. 53) of this document.

Many aggregations have strong site fidelity, occurring in the same location each year. The site maps described above will therefore serve as a basis to locate and re-locate aggregations. However, anecdotal accounts from fishers suggest some aggregations may shift from their traditional sites after heavy fishing pressure or disruption from divers. Divers must keep a keen eye and be ready to recognize and document any new phenomena, including shifting times and locations of aggregations daily, monthly and annually.

Objectives of Visual Survey

- Quantify the numbers and length of fish (by species), timing, and locations of multi-species reef fish spawning aggregations
- Describe courtship and spawning behaviors
- Accurately describe, monitor and map biological and physical characteristics of the spawning site including geomorphology, benthic cover and structure, winds, current direction and speed, wave height and direction, air and water temperature, salinity, other physical measurements
- Assess changing patterns of site usage, such as changes in the horizontal or vertical area and distribution of the aggregation(s), aggregation density or sex-specific changes in spatial usage

General Equipment List:

- Data Collection Sheets
- Plastic Slates
- Underwater pencils
- SCUBA gear
- Video Camera and Underwater Housings
- Still Camera and Underwater Housing
- GPS
- Current drogues

Methodology

1. Physical measurements at the spawning site

- Record the location of the spawning site. Estimated positions of most aggregation sites are acquired during the information gathering phase of the work that may include anecdotal information from fishers, preliminary surveys, geo-referenced materials, etc. When the aggregations have been located underwater, new GPS points should be taken in the field using UTM coordinates.

- Evaluate the surface conditions at the site including the direction and speed of the wind and the height of the waves. The speed and direction of the prevailing currents both at the surface and at depth, as well as the methods used to determine these values should be documented.

Survey Date:	**Time In:**	**Time Out:**
Team Leader:	**Team Members:**	
Location[1]:	**GPS coordinates[2]:**	
Surface Conditions		
Air Temperature		
Water Temperature		
Surface Current Speed and Direction[3]		
Sea State		
Wind Speed and Direction[4]		
Number of fishing boats nearby		
Underwater Conditions		
Depth		
Temperature		
Visibility		
Estimated Survey Area		
Estimate of Current at Spawning Depth		

Species	**<10**	**10-20**	**21-30**	**31-40**	**41-50**	**51-60**	**61-70**	**71-80**	**81-90**	**91-100**	**101-110**	**111-120**	**121-130**	**Total**	**Spawning Behaviors[5]**

Date entered in SPAGS Database: _____

User Name: _____

Survey ID: _____

[1] Write a detailed site description including sketches of the spawning bank, on the back of this sheet or additional sheets. In addition, please write any anecdotal information gathered on the spot, and note any further or abnormal observations.

[2] For all sites, GPS coordinates should be taken in UTM with datum, WGS 84.

[3] Sea current direction is the direction in which the current is moving, e.g. current is moving, e.g. current is moving to the south at .5 knots so write, "south current, 0.5 knots". Please also note if current speed is estimated or calculated with a current drogue.

[4] Wind direction is the direction from which the wind is coming, e.g. wind is from the northeast at 5 – 10 knots then write, "Northeast wind, 5 – 10 knots".

[5] Please note any of the observed spawning behaviors using the following letters: a) grouping, b) fighting, c) color changes, d) bite wounds, e) gravid, f) courtship, g) spawning.

2. Diver Surveys:

a. When to count: Teams should, ideally, conduct two dives daily at the spawning sites and utilize the visual estimation techniques described below to estimate the numbers and sizes of all aggregating finfish. It is important that all team members synchronize their watches so that all observations relate to the same time datum.

At least one of these dives should be conducted between 1500 – 1600 hrs. to quantify the spawning aggregations. These late afternoon dives will be used for abundance comparison with subsequent surveys. If a team will quantitatively survey more than one site, two mid-day dives can be taken.

If possible, another dive should be made 60-30 minutes before sunset, to observe courtship, spawning behavior and possible spawning. Since many fish rise up in the water column in preparation for spawning, it is probably best to quantify aggregations in the late afternoon. The timing and number of dives is ultimately left to the discretion of the team leader and boat captain. However, once dive times and locales are established, all efforts should be made to dive consistently at the same times and locales within and among survey periods, since activity levels and, therefore, abundance may vary throughout the day. Alternatives are: one dive per day (if too rough) to collect all data, or two dives that include one sunset dive specifically to observe fish behavior. Optimally, specific tasks should be assigned to each team member to ensure that all parameters are recorded (e.g. one person does size ranges and numbers, one does physical observations, such as depth, temperature current direction and speed, one person does video and observes courtship and coloration changes.) Alternately, one person can focus on a single species, while others look at a different species. At some fish aggregations it is necessary to have an additional diver observe and record semi-pelagic (mid-water) fish aggregations, such as jacks and permit.

b. SPAG Site Area Estimation and Density Calculations: Estimate the size of the aggregation area in square meters (m^2). Several methods can be used for area estimated as described in Chapter 9 – Mapping the Site. Using the aggregation area estimated and abundance data, calculate the fish density.

For repeated measures and census in a given area, it is advised that small floats or colored rocks be anchored and left on the bottom to indicate aggregation boundaries and the start and stop points of previous and future dives. For groupers, this technique works well, since many species maintain fidelity to bottom areas during aggregation periods. For snappers and jacks, which tend to roam in their aggregations, several dives will be needed to verify the most common area of the aggregation.

It is recommended that members of the dive team prepare underwater slates before the dive, making columns for fish size ranges and fish species that the team

anticipates will be observed. Using size ranges enables the researcher to compare the underwater size estimations to actual sizes of fish collected from fish landings data.

Due to differences in the sizes of the aggregations and their shapes, each site team should design a monitoring protocol so that the entire area is surveyed systematically, and without double counting or missing fish. On a straight section along a reef dropoff, a set of divers swimming parallel to the wall edge, each counting and examining a swath (belt transect) may work well. When an aggregation is nestled in a spur and groove system, divers can be deployed to survey different spurs simultaneously. If divers are working as teams to count the fish, the boundaries or widths of the area to be covered by each diver should be established beforehand to prevent double counts, leading to overestimations of aggregation abundance.

c. Video recording: On the evening dive before sunset, video cameras in underwater housings should be used to record courtship and spawning behavior. Video records can also be used to calibrate abundance counts in highly abundant or dense aggregations. If two divers with video cameras are available, they should film the aggregation from different perspectives (e.g. opposite sides) to capture variability in the form of the aggregation which could influence abundance estimates. Observations and video tapes will be used to describe the succession of events that lead to spawning and note reproductive coloration, color changes, interactions between individuals, and schooling patterns. These data are also important to verify that fish are spawning, not merely aggregating for other purposes. If dive teams are used (e.g. paired divers), one diver should video while the other makes raw visual observations.

d. Data recording: As soon as the dive is complete, all divers should work together as a team to record all of the information from the dive on the data sheet. Any unusual events or observations should be discussed and recorded in detail. Use as much paper as is necessary to record observations in writing.

e. Dive safety: Always make dive safety a top priority. To ensure the safety of divers, use the provided Dive Safety Log Sheet (Data Sheet 2), to record the times in and out, and the pounds of air used on each dive. These data can be very helpful to avoid decompression sickness and diving accidents. In the unlikely event that these accidents do occur, these data can be useful for treatment. Each team should have a pre-arranged evacuation plan, in the case of emergencies (e.g. location of nearest recompression chamber, available modes of transport to chamber, etc). Most importantly, all teams should be equipped with a DAN oxygen kit. Delays in treating decompression sickness can result in permanent paralysis or death. In the event of accident, oxygen treatment should begin immediately. In addition, all members should carry DAN dive insurance in case of the need for evacuation. At least one member of the team should be trained in cardio-pulmonary resuscitation (CPR).

Dive Safety Log

Location:		Date:		Dive #:	
Boat Captain:					
Mate:					
DIVE TEAM		Time In:	Pounds In:	Time Out:	Pounds Out:
Dive Master:					
Data Prime:					
Videographer:					
Still Photographer:					
Diver 1:					
Diver 2:					
Diver 3:					
Diver 4:					

Location:		Date:		Dive #:	
Boat Captain:					
Mate:					
DIVE TEAM		Time In:	Pounds In:	Time Out:	Pounds Out:
Dive Master:					
Data Prime:					
Videographer:					
Still Photographer:					
Diver 1:					
Diver 2:					
Diver 3:					
Diver 4:					

Location:		Date:		Dive #:	
Boat Captain:					
Mate:					
DIVE TEAM		Time In:	Pounds In:	Time Out:	Pounds Out:
Dive Master:					
Data Prime:					
Videographer:					
Still Photographer:					
Diver 1:					
Diver 2:					
Diver 3:					
Diver 4:					

10. Physical Measurements at Spawning Sites

Since spawning aggregations for various species occur seasonally, and are timed with various moon cycles, it is valuable to establish a baseline of physical monitoring at spawning aggregation sites. While it may not be feasible at the beginning, eventually spawning aggregation site monitoring should include physical data collection using *in situ* data loggers that can be moored on site and downloaded at appropriate intervals.

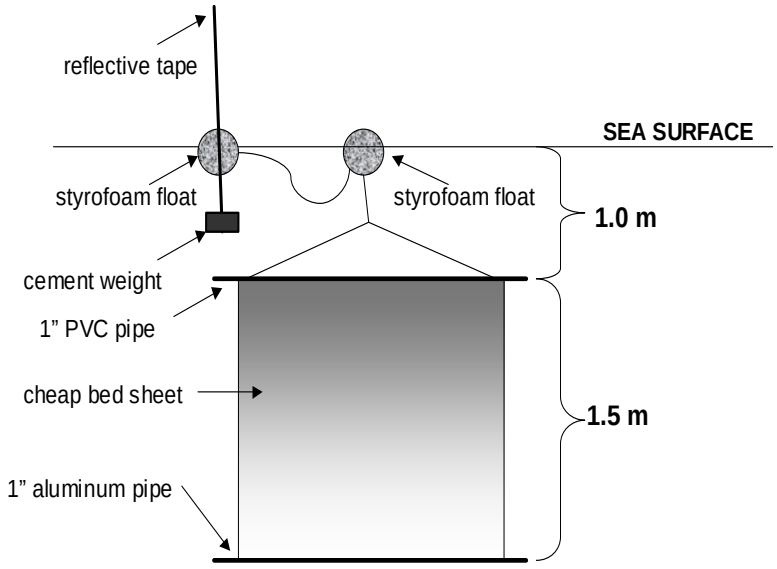
Temperature

Temperature measurements at the site should include air and surface water temperature, and, in particular, water temperature at the spawning (aggregation) depth. These measures are important, since spawning in certain species (e.g. Nassau grouper) has been shown to be correlated with specific water temperatures. Therefore, it may be possible for certain species to predict potential aggregation formation and spawning by water temperature profiles. Temperature should be taken with a water-proof, non-mercury filled (preferably LED) thermometer that can record to 0.1 degrees Celsius (^{o}C). Alternately and preferably, underwater temperature can be monitored using an in-situ temperature logger such as a Hobo Temp, or other recording thermisitor.

Surface currents

Once fertilized, fish eggs from demersal (water column) spawners float to the surface prior to hatching, which typically occurs within the first 24 hours post-spawning. Therefore, information on surface currents may be used to determine the initial direction of dispersal of eggs and potentially larvae. Common surface current directions during spawning periods can provide insight into the ecology of aggregation spawning species. The surface currents can be measured quantitatively with a GPS and a current drogue (Figure 3).

Figure 3: Current drogue design



The protocol is as follows: Place the drogue in the water at the spawning site. Let the drogue drift for 5 minutes to overcome inertia. Record the initial location, as accurately as possible, using the average function on the GPS. At regular intervals of around 30 minutes, record the new location of the drogue and the time. The GPS distance and bearings functions and the data sheet is provided below can be used to plot the speed and direction of the surface currents. The most useful data can be provided by surface currents at the time of spawning to evaluate egg and larval dispersal, particularly during the first 24 hours after spawning.

Tides, winds

Information on tides and wind are also important factors that affect the fate of eggs and their dispersal. Again, common trends among spawning periods may provide important clues in understanding spawning aggregation dynamics and fish ecology, as well as potential settlement areas for larvae, once hatched Tides can generally be assessed from local tide charts. Wind direction can be assessed by a handheld compass.

Current drogue Data Sheet

Location:
Team members:
Data recorder

Date	Drogue Depth (m)	From Northings	Eastings	Local Time	To Northings	Eastings	Local Time	Bearing (degrees)	Distance (km)	Elapsed Time (min)	Rate (km/hr)	Rate (cm/s)	Wind From	Wind Speed	Notes

11. Fishery Dependent Data Collection: Catch per Unit Effort and Size: Frequency Analysis

Background and Objectives

The purpose of this section is to gather quantitative information about the fishery and the landings. We offer two techniques in this section, both which can be done at the time and location where fishers are cleaning and processing fish. We use catch per unit effort (CPUE) as one relative index of the status of the fishery. We use life history data size frequency distribution to analyze the size (and potential age) structure of the population, and to gather information on the size (and potential age) of reproductive maturity for the species. These are standard fishery-dependent methodologies used to monitor the status and trends in a population or fishery to assist managers faced with adaptive management decisions.

Methodology

The best time and place to collect CPUE data is at a spawning aggregation fishery landing site, while fishermen are capturing, cleaning and processing freshly caught fish. Catch per unit effort and size frequency data can also be collected while tagging and releasing fish, if no commercial fishery is exploiting the SPAG site.

For size frequency analysis, record the following information on the appropriate data sheet:

- Fork Length (measure length from tip of snout to middle rays of caudal fin to the nearest cm).
- Weight (record whether gutted or whole and record weight to the nearest gram)
- Sex: examine gonads macroscopically, from gutted fish or sub-samples of the gonad (ovary or testes) following cannulation (see Chapter 11). Record the sex of the fish and the maturity stage, i.e. immature, early development, late development, ripe and running, or spent (Data Sheet 4).
- If collaborating with fish geneticists or physiologist, collect any needed biological tissues following their instructions carefully (otoliths, or tissue samples for genetics). Record the sample numbers and carefully label the samples with the numbering system required by the researcher.

For CPUE analysis, for each boat present, record the following information on the appropriate data sheet:

- Hours and or number of days (with dates) spent fishing. This can also defined as “soak time” or the number of actual number of hours fishing, which for traps is every hour that the traps are baited and underwater, while for handlines would include only the hours that fishing is done at the site.
- Number of fishing units, e.g. number of traps, lines, etc.

- Gear type used for fishing (nets, handlines, traps, etc)
- Estimated expenses of fishing per person/day
- Number of fish captured per fisher per unit time

Equipment

- Data Sheets
- Fish Measuring Board
- Scale

Spawning Aggregation Survey:
Catch/Unit Effort Data Sheet

Location:	
Date:	Time:
Survey Team Members: Name of data recorder:	
Boat Name or License Number:	
Names of Fishers:	
Hours spent fishing (soak time):	Method (gear type) employed for fishing:
Estimated cost of fishing per person/day:	
Number of fish of each species caught:	
Mean weight of each species caught:	

Catch Data

Species	Weight (g)	Length (cm)[6]	Sex	Gonad state[7]

Date entered in SPAGS Database: _____

User Name: _____

Survey ID: _____

[1] Length should be measured to the fork of the tail.
[2] Record if gonads are: **I**mmature, **E**arly **D**evelopment, **L**ate **D**evelopment, **R**ipe and **R**unning, or **S**pent (see Table on Tissue Collection and Processing).

Fish Landings Data Form

TL: Total Length
FL: Fork Length

I: immature
ED: early development
LD: late development
RR: ripe and running
S: spawned

DNA (H/F/G) H: heart
F: fin
G: gill

Species	Location	TL (cm)	FL (cm)	Wt (lbs)	SEX	Gonad Weight (g)	Gonad state	Date	Fishermen	Otolith #	DNA (H/F/G)	DNA tube #	Data Recorder
Mutton Snapper	Gladden Spit	56	52.2	8.8	F	150	RR	18/05/03	Lennox Leslie	code	H	code	Athen Marin

12. Fish Tag and Recapture

Background

Fish tagging has become a standard research tool in fish biology for estimating fish population sizes, migration routes and distances, and to assist in estimating growth rates and potential sex change in many species. The tag-recapture component of the SPAG monitoring program primarily addresses the issue of site fidelity of SPAG species and the distance traveled by individuals to reach aggregation sites. Each aggregation monitoring team should be equipped with appropriate tags and trained taggers. Several important research questions can be addressed using this methodology:

- How far do fish migrate for spawning?
- Do individual fish return to the same site month after month, and year after year?
- At what time of day or night do the fish spawn? (by determination of gonad condition at time of capture)
- When, according to the lunar calendar, do fish aggregate to spawn, and subsequently disperse?

The protocol is relatively simple - fish are captured, measured, tagged with a dart tag with an identifying number, and the location and date of tagging are recorded. When a fisher catches the tagged fish, the tag (and fish, if possible) should be returned to the organization/tagger/researcher (listed on the tag) with the catch location, date, and size of the fish. From the tag and subsequent capture locations, the researcher can determine the distance, time at liberty and, if accurate lengths are recorded, the growth rate of the fish during the tag-recapture period. It may even be possible to document sex change in certain sex-changing species, e.g. groupers.

Figure 4: Conventional ID tag

Methodology

The capture of fish will be conducted from moored boats at the aggregation site using standard hand line gear. If desired, circle hooks can be used instead of regular J-hooks to increase "lip hooking" and decrease hooking mortality. Fish should be handled with wet towels, with eyes covered to reduce stress, measured and weighed, tagged with conventional or acoustic tag, and released. Data on the tag number, time and location of release will be recorded. Conventional tags generally used in this study are Floy® brand "spaghetti" tags with nylon dart tips. These are most suitable for the larger species of groupers and snappers. For the smaller species, Floy® anchor T-bar tags work well. Other tag manufacturers produce similar tag designs. In addition, if funding is available, acoustic tagging may be considered. VEMCO® Brand Acoustic Tags have been found to provide reliable results in monitoring aggregation sites in Belize. These tags give off a specific frequency at a pre-designated, individual ping rate for each tag. The acoustic tags are recognizable by hydrophone receivers – both fixed and mobile. The VEMCO® mobile receiver is called a VR60, while the fixed receivers are VR1s and VR2s. The fixed receivers are moored along the reef (generally at spawning aggregation sites), in approximately 80 feet of water, near the shelf break. The can receive pings from a tagged fish as much as 500 m away. Battery and data storage capacity allow the instruments to remain on location for up to 6 months at a time. As each acoustically tagged fish swims near the receiver, the date and time of that visit are recorded within the instrument. Data are downloaded to a PC periodically, to indicate the timing and presence of the fish at the site. This allows researchers to test various hypotheses about site fidelity and visitation patterns of reef fish at aggregation sites.

Figure 5: Sonic Tags and Receivers

During capture, fish are often brought rapidly from depths of 30 – 40m to produce barotraumas, where the swim bladder becomes distended owing to the rapid change in pressure between the capture depth and the surface.

Therefore, prior to releasing the fish, the air bladders will need to be deflated. To do this, monitors will need to puncture the air bladder by passing a hypodermic needle (e.g. 10 gauge) through the body wall near the posterior end of the laid-flat pectoral fin. To help avoid infection, the needle should be dipped in alcohol each time that a fish is winded. Many fishers may already have experience with winding but the use of hypodermic needles is strongly recommended to reduce the trauma of the winding process. Fish, particularly most grouper species, tolerate the procedure well, as evidenced by the recapture of many winded specimens (Luckhurst, 1998; pers. obs.). Snappers appear to be more susceptible to the effects of handling trauma than groupers. Therefore, the entire procedure, including data collection, should be conducted as quickly as possible. Involving experienced local fishers is highly recommended.

Educational/awareness pamphlets should be distributed to fishers in the vicinity of the aggregation site. Information about the tagging program should be distributed throughout the region in the event of a long distance migration and recapture. For example, Nassau grouper have been shown to migrate up to 250 km to reach spawning sites. The pamphlets should briefly describe the tagging program and what to do if a tagged fish is recovered. A small reward should be offered to participating fishers to encourage reporting of recaptured fish.

Expected Results

It is probable that some fish will be caught at the aggregation site during the tagging period. Fish caught 2-5 days after tagging, for example, provide an indication of residence time at the site, whereas fish caught at the aggregation site in subsequent months or years help to confirm site fidelity. If fish are recovered from large distances away, these data will help in the estimation of the "catchment" area and can be used to promote the regional importance of the spawning aggregation site.

Distribution of Results

Data collected from these studies should be published in peer reviewed scientific journals whenever possible, along with other data on the aggregations to make them widely available to researchers. In addition, it is important to provide feedback to all participants about the findings. Fishers should be specifically recognized for their contribution to such programs.

Equipment

- Fishing lines, weights, circle hooks
- Bait net and bucket, with ice
- Reef Anchor and sufficient rope
- Boat and Gas
- GPS
- Taggers

- Tags
- Data Sheets
- Marking Pen
- Alcohol bottle
- Towel
- Fish measuring board
- Hanging scale
- Long nose pliers
- Knife
- Biological sampling kits (histology and/or genetic samples)

Simplified Tagging Protocol

1. Catch fish using circle hooks. Gently remove fish from the water and immediately cover the head and eyes with a wet towel and remove the hook from the fish's mouth.
2. Measure the fork length (to the nearest cm) and record on the data sheet.
3. Record the tag number.
4. Wash the tagging insertion device and tag tip in alcohol.
5. Insert the tag into the fish in the upper flank above the lateral line with barb side down such that the barb hooks behind a lateral rib bone. Remove the tagging device and pull gently on the tag to ensure that it is properly embedded.
6. Wind the fish, if necessary.
7. Complete data sheet with species, date of tag and release, name of tagger, fish condition, release type, location and any other relevant comments.

Fish Tagging Data Sheet

Tag No.	Species	Date	Location	Fork Length (cm)	Weight (g)	Fish condition	Sex (m/f)	Tagger Names

Date entered in SPAGS Database: _____

User Name: _____

Survey ID: _____

13. Tissue Collection and Evaluation

Monitoring assessments of reef fish spawning aggregations provide the maximum benefits when protocols involve the collection and evaluation of tissues, such as ovaries and testes for assessments of reproductive maturity and spawning, skeletal or other tissues for genetic analyses and otoliths for assessments of age and growth. In general, tissues require some care in handling, and extraction and preservation techniques may require some training beforehand. Following the collection of most tissues (e.g. those for genetic study), detailed analyses are conducted by trained researchers at academic or government institutes. However, for determinations of sex or reproductive readiness, monitors can easily record and collect useful data using simple extraction techniques and tissue examination in the field.

Tissue collection for macroscopic analysis:
The various stages of sexual development are indicated and defined in Table 4. The collection of tissues for macroscopic analysis is relatively simple and requires little or no specialized equipment. Most tissues can be extracted with a cannula or slight abdominal pressure, particularly when individuals are being released, for example, during a tag-recapture protocol. For cannulation, a flexible vinyl tube (30-50 cm long) with a small bore (2 mm inside diameter) or an empty ballpoint pen ink tube is inserted into the fish's gonopore to approximately 4-6 cm. Following insertion, a slight vacuum is applied to the tube to extract eggs or milt into the tube by sucking on the end of the tube. The tube is then removed from the fish and the contents expelled onto a glass microscope slide for examination using a 10X eyepiece or even a magnifying glass. Alternatively, when spawning is approaching, females often expel eggs and males expel milt by slight pressure applied to the abdomen. The sex and stage of development can be recorded in a column on the datasheet alongside weight/length measurements, such as on the tagging datasheet, or the CPUE data sheet. From this simple assessment, aggregation parameters can be determined, such as sex-specific length-weight relationships, temporal changes in aggregation sex ratio and possibly fishery selectivity. Using the stages of oocyte development, it is also possible to determine spawning times. It is important to remember that hydrated eggs or spent gonads are indirect methods of determination of spawning.

Finally, for tag-recapture protocols involving potentially sex-changing species, these quick and easy methods–using no more than a cannula and magnifying instrument–can provide insight into the pattern of sexual development for mature fish. For example, a grouper is captured and tagged as a female in the first year and later recaptured as a male, providing strong evidence that sex change has occurred since the fish was tagged i.e. protogyny or female-to-male sex change.

Figure 6: Photographs of snapper gonads in early and late development phases

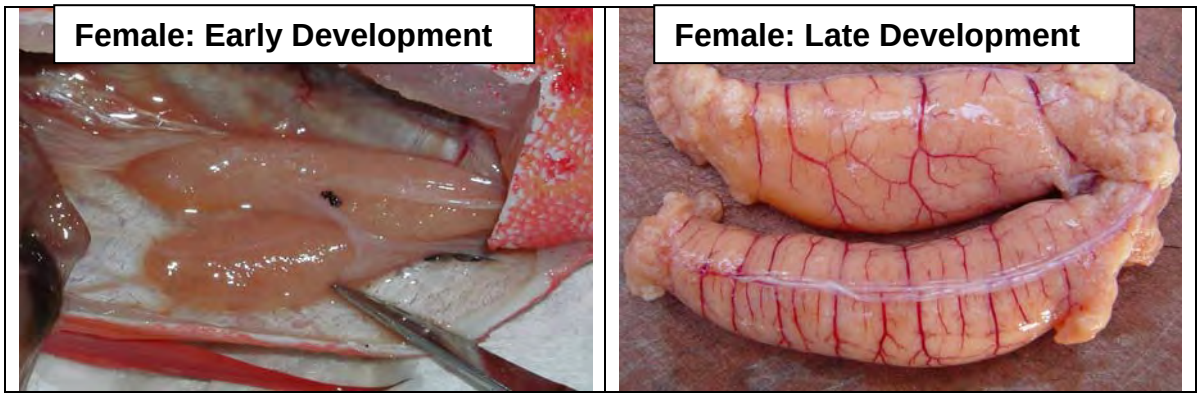


Table 4: Macroscopic evaluation of stages of maturity used for analysis of sex.

Maturity stage	Visual characteristics
Females	
immature	Gonad small and strand-like, compact, pink or cream; oocytes (eggs) indiscernible; indistinguishable from immature males. Individuals should be recorded as immature or inactive
mature (early development)	Gonads relatively small but rounded, grey or orange with thickened gonad wall; Eggs difficult to visualize and small (<0.4 mm); Indistinguishable from immature or developing males. Yolk development has not begun.
mature, active (late development)	Gonad large and grey or orange with transparent gonad wall; large yolky eggs becoming clearly visible and tightly packed
mature, ripe (ripe and running)	Gonad large, clear, hydrated eggs visible through wall; typical of individuals just prior to spawning; egg release possible with application of light abdominal pressure
post-spawn (spent)	Gonad flaccid with obvious blood capillaries; few eggs visible
Males	
immature/inactive (early development)	Indistinguishable from immature females (see the description). Individuals should be recorded as immature or inactive
mature (late development)	Gonad expanding and becoming rounded and large; greyish in appearance; early maturing individuals are indistinguishable from developing females until milt becomes evident in the sperm sinus, or gonad wall
mature active (ripe and running)	Gonads large and white with sperm visible in sinuses or wall; milt release with light abdominal pressure
post-spawn (spent)	Gonads flaccid and bloody (not round); sperm release still possible on application of abdominal pressure

14. Data Processing and Analysis

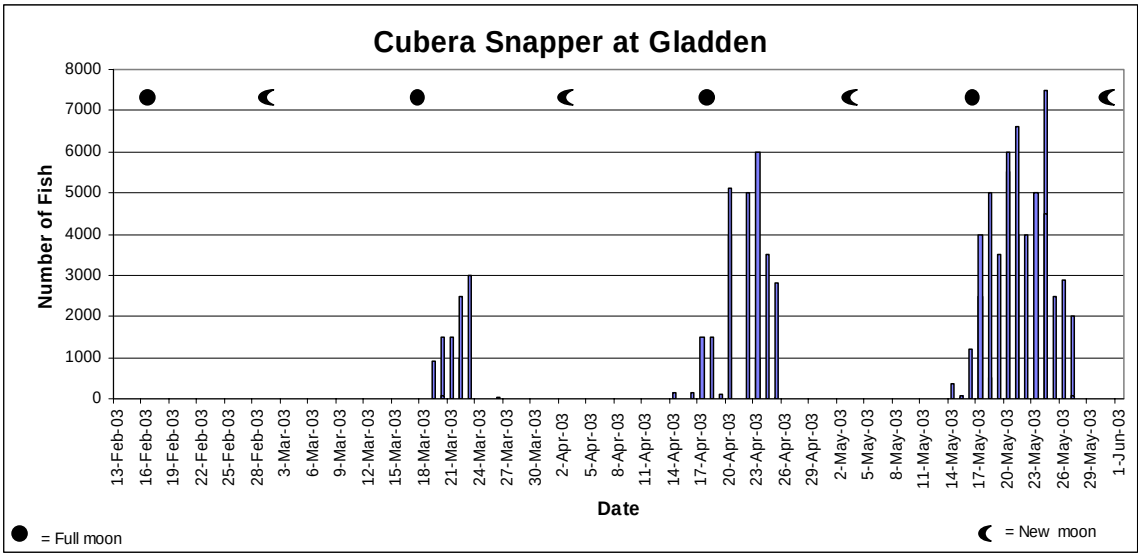
Fisheries dependent and fisheries independent data gathered from the use of this protocol can be analyzed and plotted for use by fisheries managers. Importantly, the survey teams should carefully check all raw data sheets, and copies of the original sheets should be made and stored in a backup location. When one considers the actual cost of obtaining this information, the need for backup is clear. User-friendly spreadsheets and databases are under development by several organizations and should be available in early 2003 in order to help analyze the data gathered using this protocol. This section is offered as a brief set of examples of the kinds of data analysis that can be done but should be considered only an introduction for more comprehensive data analysis as needed by managers.

Underwater Visual Survey Data:

These data are used to determine the sizes, seasonality, and number of various species that utilize spawning aggregation sites. They can be plotted by species as a function of time/lunar day, and or by numbers of various species as a function of site. Examples are provided below. These data can be entered into an Excel spreadsheet or an Access database. Comparisons between sites of the numbers of various species, changing abundance by lunar day and month, etc. permit an evaluation over spatial and temporal scales.

The graph below indicates the numbers of cubera snapper, aggregated at Gladden Spit in Belize by date, and in relation to the moon phase. These real data were collected by the Friends of Nature during 2003.

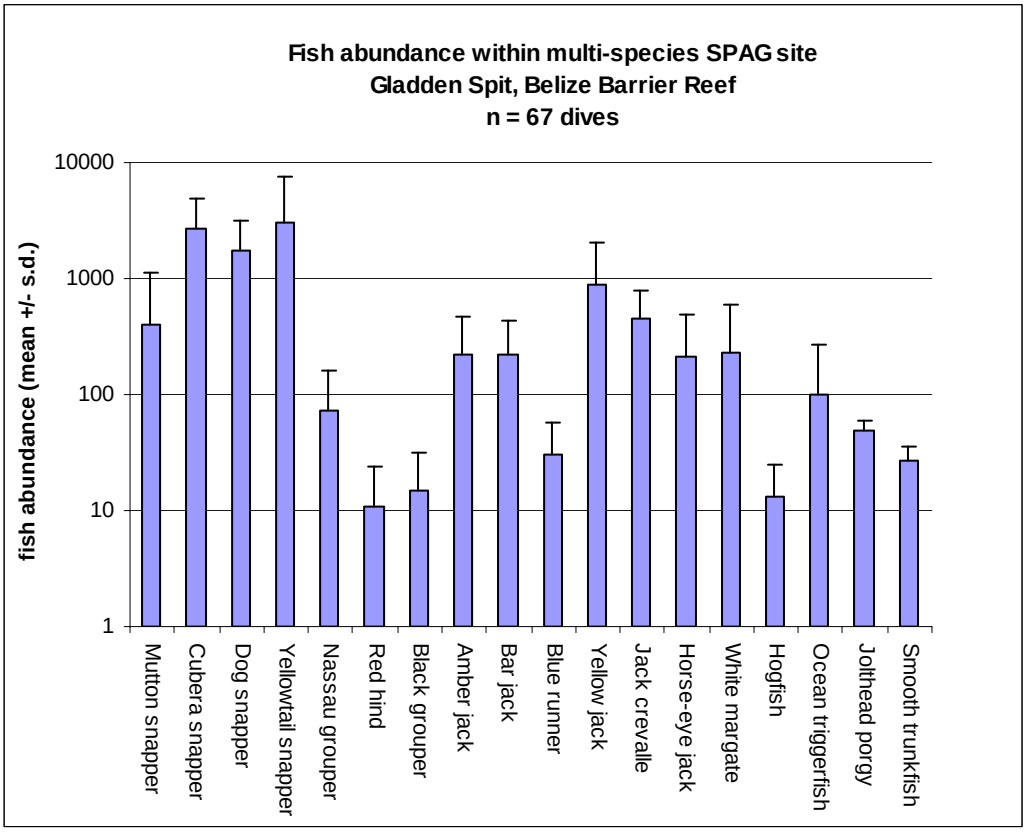
Figure 7: Cubera Snapper at Gladden, Belize



In order to illustrate the multi-species phenomenon at a site, the following graph might be more useful. The graph indicates the mean (and standard deviation) of the number of fish from various aggregating species, as averaged from 67 dives at Gladden Spit prior to 2003.

The graph shows that the site harbors spawning aggregations of at least 18 species, but does not indicate the timing or distribution of these aggregations.

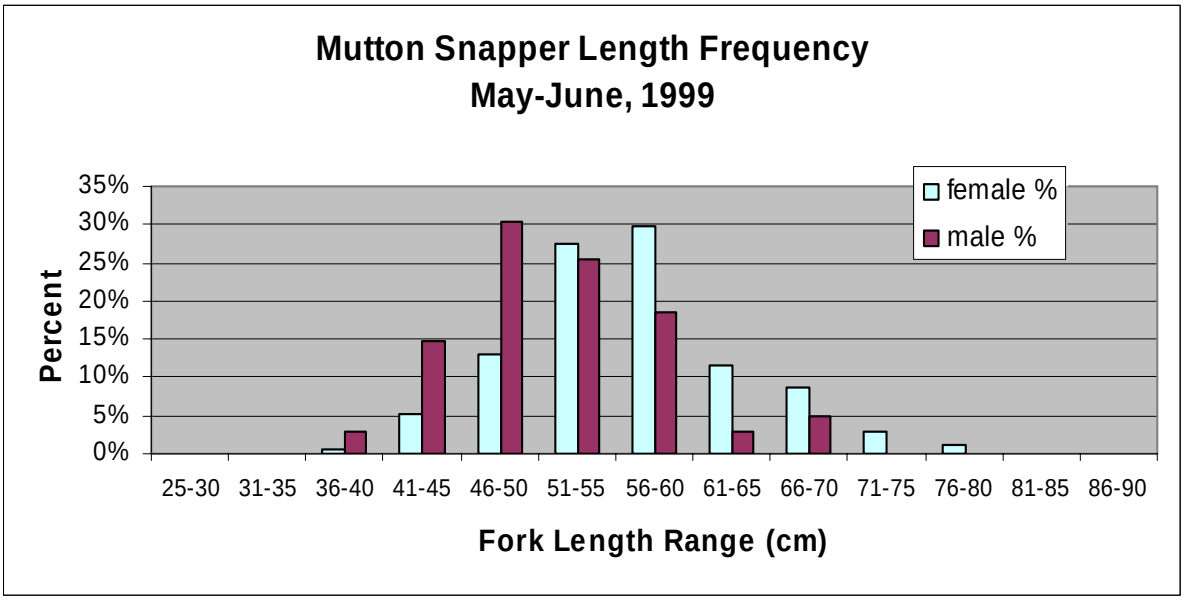
Figure 8: Fish abundance within multi-species SPAG site, Gladden Spit, Belize



Catch per Unit Effort Monitoring and Length: Frequency Analysis:

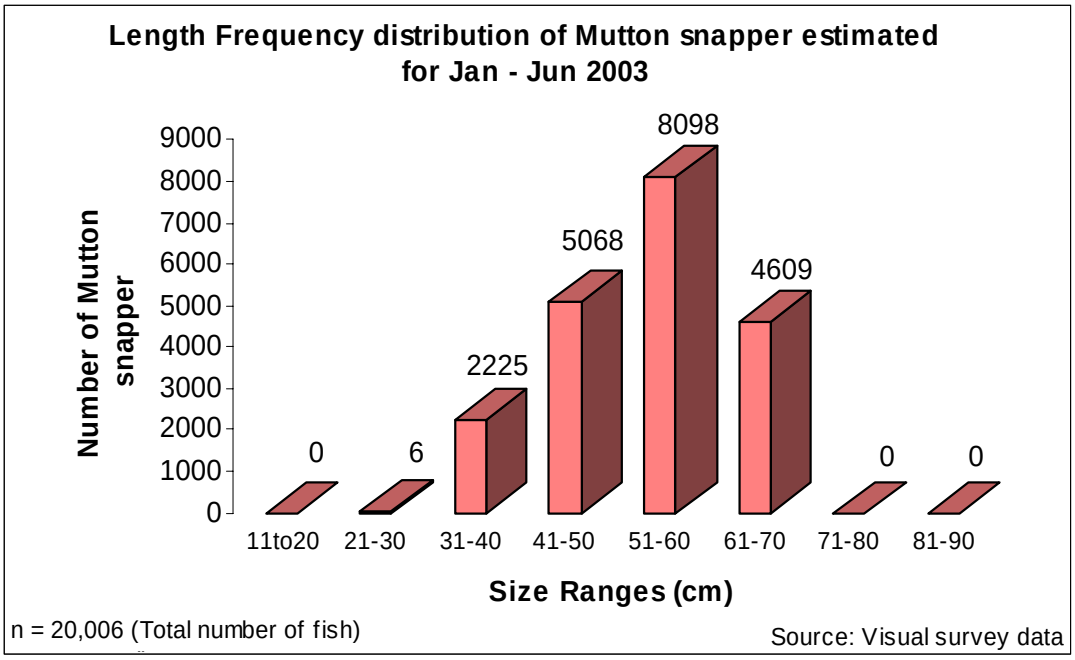
When aggregations are being fished, catch per unit effort data can be collected and entered into an Excel spreadsheet or Access database. Size-frequency and size-weight relationships can be plotted by species as well sex ratios as indicated below.

Figure 9: Mutton snapper length frequency calculated from data collected at Gladden Spit



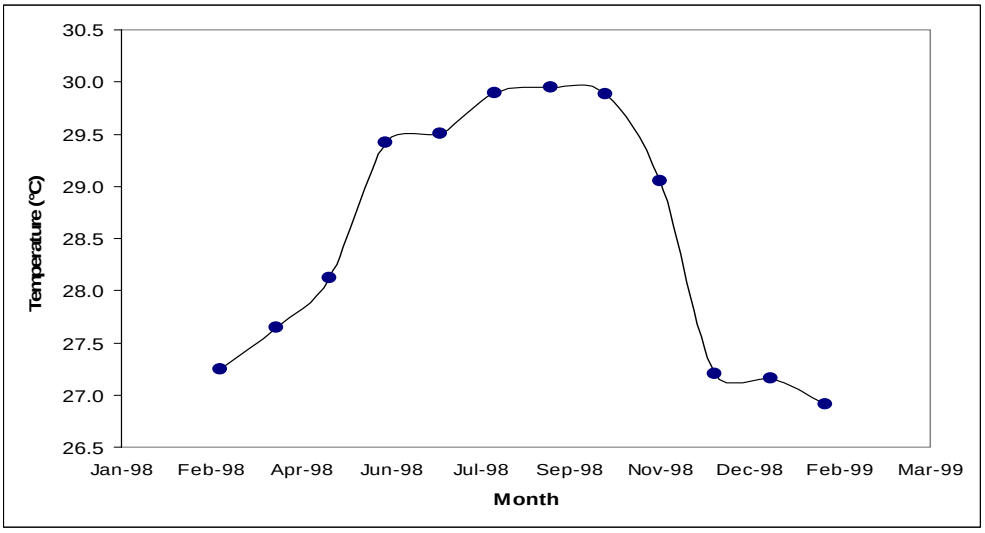
Using the underwater visual survey technique, size:frequency distribution data can be gathered and compared to those data gathered using fishery-dependent methods. Comparing fishery-dependent and fishery independent data gathered at the same time on the same aggregation can help to validate the data, and/or to illustrate differences in what is being landed, from the size frequency distribution within the aggregation underwater.

Figure 10: Length Frequency distribution of Mutton snapper estimated



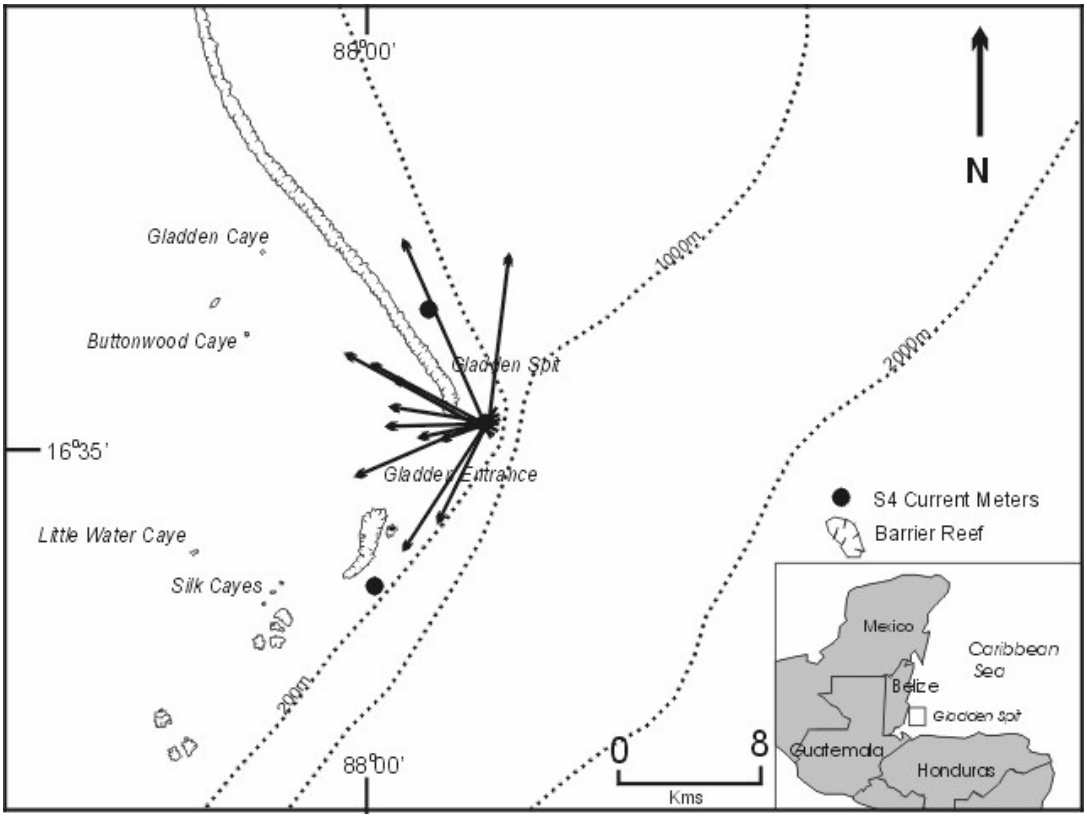
Temperature data was recorded at Gladden Spit Belize using an in-situ S4 electromagnetic current meter. Mean monthly data are plotted below for the period between March 1998 and February 1999.

Figure 11: Temperature data at Gladden Spit, Belize



The speed and direction of currents at aggregation sites can be evaluated with current drogues, as described in Chapter 9. The vectors superimposed on the map below (Figure 12) represent real data from several drogue releases at the time and location of spawning at Gladden Spit. These vectors represent 18 hours of drift, extrapolated from the direction and speed of drogues, as calculated using the drift data sheet.

Figure 12: Surface current drift patterns as calculated using a current drogue in various repetitions at Gladden Spit spawning site. *Track vectors show distance traveled in 18 hours, based on the rate of speed calculated at the time the drogue was deployed.*



15. Regional Collaboration

Ocean currents link the islands and coasts of the Caribbean Basin. These currents serve to transport and disperse larvae produced at spawning aggregations to downstream locations, dependent on the current patterns in the area. Recent genetic studies of Caribbean reef fishes, such as the endangered Nassau grouper, show that populations are quite similar throughout the basin, indicating a high level of connectivity. In order to preserve these species, regional collaboration will be needed. It is hoped that this monitoring protocol manual will serve to help countries coordinate monitoring and protection efforts at SPAG sites. It is suggested that regional monitoring be coordinated such that countries can share data and learn from each other's management successes.

16. Management Measures

Traditional users of spawning aggregations should be intimately involved with the research, monitoring, legislation development, alternative use, and enforcement of spawning aggregations. In Belize, a strategy of comprehensive involvement of fishers has lead to the successful closure of most of the Nassau grouper SPAG sites in Belize as well as a closed season for the species (G.O.B. 2003a; 2003b). By working with fishers, scientists and managers have been able to efficiently collect data and have garnered critical support from the fishing community for widespread management. Fishers should continue to be involved in monitoring and enforcement after management / conservation action. They should be included in a variety of alternative economic training activities for research, as scuba dive guides and for sport fishing (Luckhurst, 2001). It is crucial that links to traditional users are maintained as management / conservation measures are contemplated.

There is a great need to conserve and manage spawning aggregation sites and the most effective way to do this is to close these sites to fishing, year round. As these sites represent most, if not all of the reproductive output for the species that spawn there, and many of the site serve as multi-species spawning locations, then closing these sites should contribute to local and regional fisheries management efforts.

If it is not possible to close the sites year-round, it is suggested that seasonal closures be enacted during the time of year that the most vulnerable fishes are spawning (Luckhurst, 2001). For Belize, and perhaps other Caribbean areas, closure between January and June would provide protection for many of the most important grouper and snapper species. Specific times, however, would need to be evaluated after detailed survey work at the sites in question. Alternate year closures might also be considered, but when tried, have lead to last minute changes from complications among user groups. Alternative year closures are, therefore, considered difficult to enact and manage.

Seasonal closures of various species, both for possession and for sale, can also be an effective, particularly, in tandem with area closures, but can only be effective when strong monitoring and enforcement is implemented. Catch quotas can also be used but are in many cases

difficult to enforce, and may cause incidental death of other individuals that are aggregated to spawn. Size restrictions and bag limits are often difficult to enforce, particularly in multi-species fisheries of the tropics, where fish is often sold as filet.

Gear bans can be helpful but generally are not sufficient. The use of any traps, nets, and spears (especially with SCUBA), and poisons are certainly not appropriate for the management of aggregations. If fishing is to be permitted at any level, then the **only** technique which should be considered is hand line fishing.

Traditional use of SPAG sites might be permitted at some limited level, depending on circumstances, in order to gradually decrease fishing mortality on aggregations while still maintaining traditional user rights in these areas. This step should only be taken after careful evaluation of the particular circumstances pertaining to the site.

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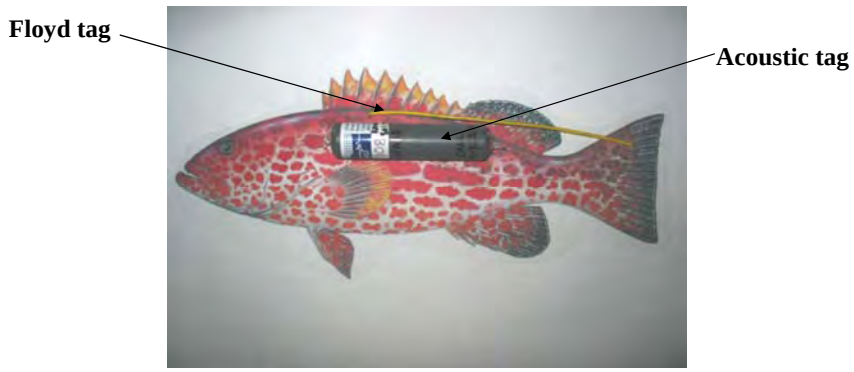
18. Appendix 1: Tagging Program Educational Flyer

ATTENTION FISHERMEN

If you catch a tagged Fish



Please carefully follow the instructions written on the tag

Record tag number, date and location of capture and return tag for a reward

By doing so you are contributing to an on going research project.

Please contact: The Nature Conservancy, Punta Gorda, Belize

Phone: 501-722-2503 or Fax: 501-722-0096 email: wheyman@tnc.org or nrequena@tnc.org

Teams doing tagging are asked to include their contacts here, print and post the pamphlet in fishing communities in the area.

19. Appendix 2: Underwater Visual Survey Training

This section provides recommendations and techniques for training of new and returning observers. Instructors should first explain the objectives of monitoring as well as the SVC technique to all students so that they understand the context for this training. It is crucially important that any observers that collect data, using this protocol, have recently passed both species identification test (100% correct is passing) and a size estimation test (75% within 3 cm is passing).

Data collected by observers that can not pass these tests are not of sufficient quality to be useful for analysis purposes and should be discarded or used with extreme caution.

Species Identification

The instructors and observers should first discuss the fish species that will possibly be encountered in the monitoring area. Key "field marks" or identifying characteristics that distinguish each species should be pointed out and discussed. Include behaviors in the consideration of characteristics. A Microsoft PowerPoint presentation has been created for this purpose, with slides of each of approximately 37 species and should be available along with this protocol. Each slide has arrows indicating the field marks on each fish, and accompanying notes on its identification.

Observers should practice identification skills in pairs, small groups, or as individuals. A set of waterproof flashcards can be created or will be provided for this purpose. Fish identification books are also helpful tools.

Observers should be tested on their identification skills, and should achieve 100% accuracy. A template form for fish ID worksheets has been provided.

Length Estimation

Fish lengths (total length) will be recorded from visual estimates using metric units (cm). Some observers may not be familiar with the metric system. Begin by giving observers a reference to their normal length measurements using visual aids (e.g., meter/yard stick) to demonstrate how many inches/feet are in 10, 20, 50, and 100 cm, and vice versa. Have observers measure the width of their hand, the length of their arm from elbow to fingertips, their arm span, and their height in metric units to gain a better feel for the measures.

Practice fish size estimation above water, using the prepared paper fish cutouts, PVC pipe lengths, or, if needed, draw fish in the sand. Together as a group, with the instructor leading go one-by-one through a series of 10 fish, everyone writes down their estimate of total length in centimeters. Have everyone say their estimates, and give the actual fish size. Repeat through the set of fish models several times.

When comfortable doing so, trainees should be quizzed by holding up a series of the fish models (or walking along a line of them) while everyone records size estimates. Trainees can

use the visual size estimation training sheet provided below to record their answers. After completing all 20 estimates, participants should trade sheets for corrections as the actual sizes for each fish are presented. Observers should note how much and in what direction (+/-) their estimates differ from actual values. This allows for correction of consistent over- or under-estimates. A value within 3cm of the true length should be recorded as correct. (For fish over 80 cm, a difference of 5cm is acceptable as correct. A score of 75% or greater is considered passing.

When observers are successfully passing the size estimation test on land, they should be tested underwater. The wooden fish should be strung on lines and laid out along the sea bottom. Observers should swim along the line (on SCUBA) recording their length estimates on a data sheet as they swim by. As was done on land, tests should be scored such that 75% correct should be considered passing – and making an observer qualified for underwater visual estimation data collection using this protocol.

Practice Practice Practice

Skills in fish identification and size estimation will degrade without practice. Experience has shown that size estimation training particularly should be repeated at the beginning of each data collection cycle. Monitoring teams should brush up on these skills as often as possible, but definitely before each actual survey period in order to ensure quality data are collected.

Materials needed

Wooden fish for size estimation (also possible to use drawing in sand or PVC pipes)
Visual Assessment Protocol
Visual size estimation testing data sheet

Visual Size Estimation Training Sheet

Date:_____

Observer Name:_____

Fish No.	Length (TL) estimate (cm)	Actual (cm)	Difference (cm)	+	-	Correct*
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
11						
12						
13						
14						
15						
16						
17						
18						
19						
20						
Total						

* Estimates are correct if they are within 3 cm of the actual length. In cases of very large fish, e.g. greater than 80 cm, estimates are correct if they are within 5 cm of the actual length.