Reef Fish Spawning Aggregation Monitoring Protocol For the Wider Caribbean

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Acknowledgements

At the November 2002, 55th Annual Meeting of the Gulf and Caribbean Fisheries Institute Meeting, 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 Caribbean. 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. Thanks are due to a number of people who have contributed to these different protocols and their development in a variety of ways: Eloy Cuevas, Nicanor Requena, Dwight Neal, Beverly Wade, Dan Castellanos, Jr., Alfred Williams, Athen Marin, Elvis "Waga" Leslie, Carleton Young, Sr., Brian Young, Glen Eiley, Maggie Sommer, Bjorn Kjerfve, Rachel Graham, and Janet Gibson.

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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".

- 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 have the potential to recover

- SPAGs are critically important in the life cycle of many reef fishes and reproduction at these sites often represents 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 Goals of this Document

- To provide a guide in the identification, description, monitoring and conservation of spawning aggregation sites. This document is intended for use by resource managers, conservationists, biologists, fishers, students and trained recreational divers.

- To provide increased awareness of the existence, status and importance of SPAGs to a wide general audience to garner broad-based support for SPAG conservation and management in the wider Caribbean.

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 location as well as 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 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.

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 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) migrate out of existing MPAs for spawning, whereupon they may be heavily exploited, particularly at SPAG sites.

Among commercially important species that form spawning aggregations, grouper populations have drastically declined in the Caribbean and two species, jewfish (previously goliath grouper), *Epinephelus itajara*, and the Nassau grouper, *E. striatus*, are now listed as "endangered" by the IUCN. Other grouper species appear to showing similar declines although these have not been as clearly documented. These declining trends are common throughout the Caribbean, where many known grouper spawning aggregations have been fished to near extinction, and yet very few of these 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 within MPAs 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 and the Society for the Conservation of Reef Fish Aggregations (SCRFA). 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 in management design and implementation.

It is further hoped that fishers who are presently fishing spawning aggregations will be encouraged to participate in the research, monitoring and eventual management of these sites, as well as to participate in tourism-related activities. 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) and 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 may occur for several different reasons including spawning, feeding and shelter. All of these three activities may be observed in adult fish whereas only feeding and sheltering aggregations are observed in juveniles. The essential element of a spawning aggregation is that it has a relatively high level of predictability in space and time. Domeier and Colin (1997) defined two different types of spawning aggregations, "resident" and "transient" using the following three criteria: 1) the frequency of aggregations 2) the longevity of aggregations and 3) the distance traveled by fish to the aggregation. Groupers and snappers form "transient" aggregations with the following characteristics: a) fish frequently migrate long distances to the aggregation site sometimes using specific routes b) aggregations typically form for only 1-3 months of the year c) the duration of the aggregation is from a few days to a few weeks d) the formation of aggregations is entrained to the lunar cycle e) aggregations occur during a limited period of the year (environmental parameters such as water temperature or rate of change of temperature appear to be important factors in the timing of aggregations). In contrast, "resident" aggregations are characterized by their frequency of occurrence (often daily), short travel distances and limited duration (often 1-2 hours).

Given these differences in the two aggregation types, there are some generalizations, which can be made concerning "transient" aggregations. Both groupers and snappers, which form "transient" aggregations, are comprised of higher trophic level species and the larger species in each of these families tend to form aggregations. It appears that all reproductive activity takes place in these aggregations, as there is no evidence of spawning in these species outside the aggregation.

4. Locating Reef Fish Spawning Aggregations

Information about the location and seasonality of SPAGs can be obtained from several different sources. In locations where there is commercial, subsistence or recreational fishing occurring, usually the best means of obtaining information is to compile traditional knowledge from resource users. Patriarch fishers can be a particularly valuable information source 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 SPAG species. In addition, scientific reports and other published materials (popular articles, trade magazines) can also be useful sources of information. Any anecdotal information about SPAGs should be verified through at least one of the above information sources if possible.

Concentrations of fishing vessels 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 species, the quantities being landed and when fish with ripe gonads are harvested.

A fishery-independent methodology for attempting to locate SPAG sites involves the use of satellite imagery, aerial photographs and bathymetric charts to determine potential sites. This is based on the finding that many documented SPAG sites occur largely at reef promontories, and/or the seaward extension of reefs near deep water. In locations where no SPAG fishing has been documented, it is possible to use this methodology to predict the location of SPAG sites that can then be assessed by divers during the appropriate lunar phase of selected months.

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

5. Target Species and Seasonality

Studies of spawning aggregations at Gladden Spit in Belize have revealed a seasonal pattern of spawning site utilization by various reef fish species. With further research in the region, this may prove to be a more general pattern at SPAG sites. Thus, the monitoring protocol recommended here is designed to identify and survey *all* of the species that aggregate at a given site throughout the year. It is focused firstly on the most commercially important grouper and snapper species, and secondly on other species. The general seasonal pattern, which has emerged for the Mesoamerican Reef for groupers and snappers, is that groupers (Nassau grouper, black grouper, yellowfin grouper, red hind) spawn around the full moon in December – March while snappers (yellowtail, mutton, cubera, and dog snappers) have their peak spawning times during March – June. These species spawning patterns appear to be similar throughout the Caribbean region but must be verified at each location. All of these species seem to aggregate for spawning most commonly after the full moon in each spawning month for about 5 – 10 days, depending on the species. Again, there may be geographic variation in the actual spawning days so that careful documentation is necessary to discern differences within the region. Inter-annual variations in reproductive seasons are common, shifting peak spawning times by a month or so. It is extremely important to consider these variations in the monitoring program, particularly when inter-annual comparisons are a key component of the program. . Many aggregations have strong site fidelity, occurring in exactly the same location each year. However, some aggregations may shift from their traditional sites after heavy fishing pressure or disruption from divers. Such shifts are generally not well-documented but researchers should be aware of this phenomenon.

Aggregations form in consecutive months for at least two months annually and sometimes up to four months. Usually, fish abundance within aggregations peaks during one of the spawning months. Aggregations typically form and persist for a period of around two weeks within a spawning month but this varies by species.
In Nassau grouper, the best-studied grouper in the region, spawning generally occurs over only one to three days, such that the total annual reproductive output for the population occurs during as few as 2-12 days. Other species aggregate to spawn at the same times and locations as Nassau grouper (multi-species SPAG sites) and data on these other species should also be recorded.

An optimal monitoring and management program would include observations during every month of the year, with specific focus between December and June of each year. 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 SPAG. Data collected at any other time 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.

6. Spawning Indicators

When fish aggregate in unusually large numbers, this serves as indirect evidence of a spawning aggregation. However, it is necessary to determine what "normal" abundance is at the site to make this evaluation. If baseline data are not available, then monitoring the site for the entire month is desirable to document changes in abundance patterns. In addition to abundance, behavioral patterns and associated color changes are also important indicators of spawning.

When observed underwater each reef fish species has very specific courtship and spawning behaviors. These behaviors sometimes involve startling color changes and sometimes-erratic schooling behaviors, biting, pairing and chasing, and even species-specific spawning sounds. For example, Nassau grouper are known to exhibit four different color patterns during the spawning season as described by Colin (1992). Male hogfish guard territories on hard-bottom substrates and attract harems of females. In comparison, Ocean triggerfish individually create and vigorously guard clean sandy nest patches on the bottom. Many other behaviors and adaptations are commonly witnessed by the keen observer. For most species, courtship and spawning activities commence in the late afternoon, about 45 - 30 minutes before sunset and often peak close to sunset. Some species spawn during the day, others at night, and some at sunrise. Though many species spawning behaviors are documented, close observations and video documentation of spawning behavior can provide further details of these rarely witnessed events. Direct observations of spawning should be carefully described on the data collection sheets, to verify that the aggregation is for spawning, as opposed to some other reason, e.g. feeding. In particular, documentation of gamete release provides incontrovertible evidence that spawning has occurred.

Table 1: 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
	***Epinephelus fulvus***				

Belize/English	Guatemala	Honduras	Garifuna	Creole
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				Hog snapper

7. Monitoring Teams and Equipment

When monitoring teams are in the field they can collect a variety of different types of data about aggregations and thereby use their time efficiently. This is important due to the cost and logistics of putting a monitoring team on site to conduct survey. Teams will vary according to the availability of personnel. The basic outline of a complete team and the equipment needed for each team is provided. To help increase the probability of success in the field, it is highly advised that fishermen, particularly those who have experience 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
Diver 1/CPUE
Diver 2
Diver 3 (optional)

Table 2: General Equipment List:

- Boat and Engine (or 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, dive compass and dive computer, if available
- Dive safety equipment, including dive flag, safety sausages, whistles and dive flashlight or strobe
- Food and Camping Equipment (or a place to stay)
- Rain Gear for all participants
- CPUE measuring equipment (scales/board) and data sheets
- GPS coordinates, maps, aerial photos, all laminated if possible
- Underwater measuring tape or marked rope
- Tagging equipment and tags, data sheets
- Current drogues
- Digital underwater video camera, tapes and batteries
- Cannula
- Microscope slide or piece of glass
- Magnifying glass or 10X eyepiece

8. Underwater Visual Survey Protocol

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 of the aggregation(s), aggregation density or sex-specific changes in spatial usage

Equipment: Data Collection Sheets, Plastic Slates, Underwater pencils, SCUBA gear, Video Camera and Underwater Housings, Still Camera and Underwater Housing, GPS, current drogues

Methodology

1. Description of the Study Area:

    a. Record location of the spawning site. Estimated positions of most aggregation sites are acquired during the information gathering phase of the work. When the aggregations have been located underwater, new GPS points should be taken in the field using UTM coordinates and the appropriate datum (e.g. for Belize, NAD27 Central American Datum).

    b. Provide detailed geographical references and topographical description of the location:
    e.g. "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).

c. Provide detailed description of spawning location: e.g. "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).

d. 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 should be accurately documented. The surface currents can be measured quantitatively with a GPS and a current drogue (see figure below). The protocol is as follows: Place the drogue in the water at the spawning site. Let the drogue drift for 5 minutes before recording initial location using the average function on the GPS. At regular intervals of around 30 minutes, record the new location of the drogue and the time. These points 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 hrs after spawning.

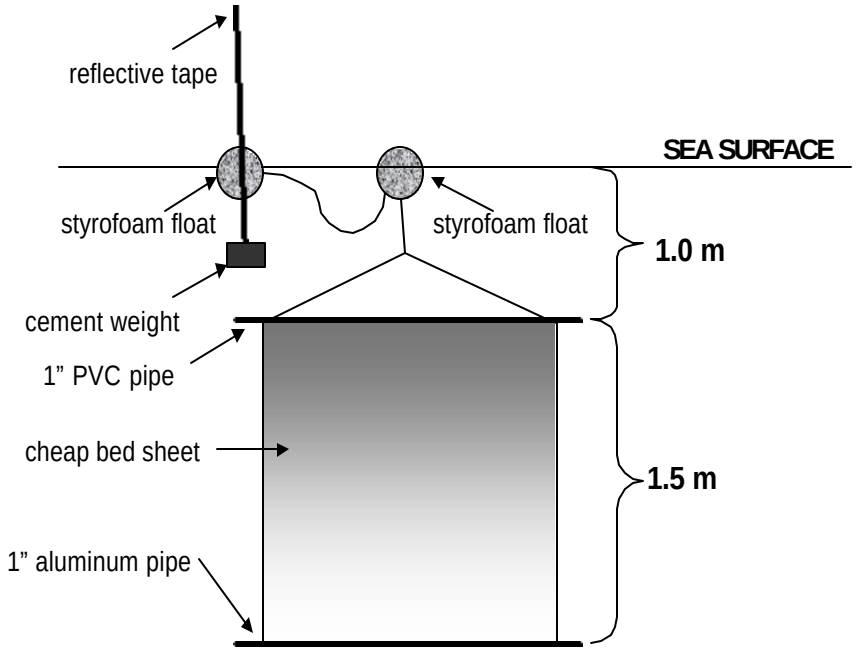

Figure 2: Current drogue design

2. Diver Surveys

a. Teams should conduct 2 dives/day at the spawning sites and utilize the visual estimation techniques described below in order 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.
One dive 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 starting about 60-30 minutes before sunset, to observe courtship and spawning behavior can also be conducted. Since many fish rise up in the water column in preparation for spawning, it is probably best to quantify aggregations in the later afternoon.

b. **SPAG Site Area Estimation**: Estimate the size of the aggregation area in square meters. Using a weighted and measured line can help to estimate the survey area. Alternately, or in tandem, GPS positions of the beginning

and end of dives can be used to estimate the length of the survey transect. This can be accomplished by sending a float line to the surface for the boat to record GPS coordinates. For repeated measures and census in a given area, it is advised that small floats be anchored to the bottom to indicate the beginning and ending of the spawning aggregation area. 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.

Using the aggregation area estimated, calculate the density of fish. Due to differences in the sizes of the aggregations and their shapes, each site team should design a stratified sampling protocol such that the entire area is surveyed systematically, 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) works well. When an aggregation is nestled in a spur and groove system, divers can be deployed to survey different spurs simultaneously.

c. 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. 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. To ensure the safety of divers, use the provided Dive Safety Log Sheet (see below), 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, and in the unlikely event that these accidents do occur, these data can be useful for treatment. Always make dive safety a top priority. Further, be sure each team has a dive evacuation plan, in the case of emergencies (e.g. location of nearest recompression chamber, available modes of transport to chamber, etc).

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:					

Visual survey data: spawning aggregations

Date:	**Time:**				
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					
Estimate of Current at Spawning Depth					
Species	**Count**	**Mean Length (cm)**[5]	**Minimum Length (cm)**	**Maximum Length (cm)**	**Spawning Behaviors (note all)**[6]

[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 Belize, GPS coordinates should be taken in UTM with datum, NAD 27 Central America – (other areas, WGS 84).

[3] Sea current direction is the direction in which the 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] Length is measured to the fork of the tail (fork length).

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

9. Catch per Unit Effort Monitoring

Background and Objectives

The purpose of this section is to gather quantitative information about the fishery and the landings in terms of catch per unit effort. This is a standard fishery-dependent methodology used to monitor changes in the fishery. Catch per unit effort data can be collected while tagging and releasing fish, if no commercial fishery is exploiting the SPAG site.

Methodology

For each target species, record the following information:

a. Fork Length (measure length from tip of snout to middle rays of caudal fin to the nearest cm)

b. Weight (record whether gutted or whole and record weight to the nearest gram)

c. Sex: examine gonads macroscopically, from extruded material or following cannulation (See Sections X and Y), state whether the gonads are early development, late development, ripe and running, or spent.

d. Collect any additional biological tissues (otoliths, tissue samples for genetics (see below).

For each boat present, record the following information:

d. Hours and days spent fishing (with calendar dates and lunar day)

e. Gear type used for fishing (nets, handlines, traps, etc)

a. Estimated expenses of fishing/day

b. Number of fish captured per fisher per unit time

Necessary Equipment for CPUE surveys:

CPUE landings
Landings protocol
Data Sheets
Fish Measuring Board

Spawning Aggregation Survey: Catch/Unit Effort Data Sheet

Location:	
Date:	Time:
Survey Team Members: Scribe:	
Boat Name or License Number:	
Names of Fishers:	
Hours spent fishing:	Method(s) employed for fishing:
Number of fish of each species caught:	
Mean weight of each species caught:	

Catch Data

Species	Weight (g)	Length (cm)[7]	Sex	Gonad state[8]

[7] Length should be measured to the fork of the tail.
[8] Record if Immature, Early development, **L**ate development, **R**ipe and running, or **S**pent (see Table on Tissue Collection and Processing).

10. 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. The tag-recapture component of the SPAG monitoring program primarily addresses the issue of site fidelity of SPAG species and the distance that they migrate to 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 the fish return to the same site for spawning 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 should be returned to the organization/tagger/researcher (listed on the tag) with the location, date, and size of the fish. From these two points, the researcher can determine the distance and time traveled and if accurate lengths are recorded, the growth rate of the fish. It may even be possible to document sex change in certain sex-changing species e.g. groupers.

Methods:

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 will 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.

Because the fish will be brought rapidly from depths of 30 – 40m and their swim bladders may be distended, two methods can be used to depressurize the fish. Optimally, when fish are ready for release, they can be hooked from the upper lip with a barbless hook, pointing ventrally. A 4–6 pound weight is attached to the eye of the hook with a 1 ft rope, while a different line is attached to the bend of the hook and is retained at the surface. The fish should be gently returned to the water and then rapidly brought to depth by the weights. When the fish is sufficiently deep, the weights can be freed from the fish via the line attached to the bend of the barbless hook (this method was developed by J. Bohnsack). Alternatively, the air bladders can be punctured by passing a 10 gauge hypodermic needle through the body wall, near the posterior end of the pectoral fin when it is laid flat against the body. 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. This procedure is tolerated very well, particularly by various grouper species 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.

Educational/awareness pamphlets should be distributed to fishers in the vicinity of the aggregation site. Information about the tagging program should be distributed nationally and internationally in the event of a long distance migration and recapture. 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. If the fish is caught 2-5 days after tagging, that will provide an indication of residence time at the site. If fish are caught at the aggregation site in subsequent months or years that will 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, 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.

Fish Tagging Protocol Equipment List:

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
Release lines with heavy weights
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 is properly embedded.
6. Hook sinker rig into fish's upper lip and return to depth as fast as possible.
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

11. Tissue Collection and Evaluation

Monitoring assessments of reef fish spawning aggregations provide the maximum benefits when protocols involve the collection and evaluation of tissues for histological examination from ovaries and testes. These samples provide information for determination of sexual pattern, maturity, spawning times and sex ratio.

- **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 (Figure A), a flexible vinyl tube (30-50 cm long) with a small bore (2 mm inside diameter) 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 morphometric measures (weight/length), 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. 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.

Table 3: 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, greyish 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 greyish 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

12. 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 another location. When one considers the actual cost of obtaining this information, it is quickly apparent it needs to be safeguarded as priority. User-friendly spreadsheets and databases are under development by several organizations and should be available in early 2003.

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.

Catch per Unit Effort Monitoring:
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.

13. 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.

14. 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 announced at the GCFI SPAG Symposium in November 2002). 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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