Revised Sections of the Spawning Aggregation Monitoring Protocol (2011)

(To be used as a Supplement to the 2004 Spawning Aggregation Monitoring Protocol)

4. Locating Spawning Aggregation Sites

Whereas some schools of fish show high site fidelity to a given spawning aggregation (SPAG) site, others are more mobile and the exact location may shift from year to year or even within the same spawning season. These SPAG sites have proven problematic to monitor resulting in fish counts that may misrepresent the actual status of the spawning aggregation population. A lot of time and resources are spent locating these SPAG sites and on many occasions, they are not found. The techniques described below should be used, weather permitting, when having difficulty locating SPAG sites.

Methodology:

Once the general site has been located using a GPS or marine chart, a manta tow should be done to determine the exact location of the SPAG. Essentially manta towing involves being pulled behind a boat with snorkeling gear on. Each team should consist of two snorkelers, one boat captain and one spotter. Two snorkelers should be pulled slowly behind the boat, one on either side of the stern and each should be approximately 20 feet from the stern of the boat (Figure 1). The distance from the stern is important for both safety, as well as to improve visibility. The spotter should keep a watchful eye on the snorkelers to report any messages back to the boat captain. Hand signals should be agreed upon prior to entering the water. The depth of water chosen for the surveys should not exceed the visibility depth. Each snorkeler should scan the water column below them looking for schools of fish. The tow should continue until the extent of the school has been mapped using a GPS that is located on the boat.

Depending on the weather conditions, current, water visibility and depth of the water, the boat should try to maintain a constant speed on a set compass bearing which would ensure that the team stays in relatively the same depth of water. In other words, the boat should travel slowly parallel to the reef/drop off; alternatively one of the snorkelers can direct the captain using hand signals. Manta tows are most easily conducted using a manta board to hold onto – this is less tiring for the snorkeler. However, for the purpose of locating the SPAG, a simple loop at the end of the rope, that allows the snorkeler to slip their hand through, will suffice.

This method is most successful in good visibility and when bottom depths are less than about 20-25 m. The best time of day to do these surveys is between 10:00 a.m. – 2:00 p.m. If visibility or weather conditions do not allow manta tows to be used, a fish finder may be useful to locate the site especially if fish aggregate well above the substrate. The boat should travel parallel to the reef while a member of the team observes the fish finder. Once a school is located, the extent of the school should be mapped either with a manta tow, fish finder or a combination of both. Ideally divers can go down and survey the area (see Section 8).

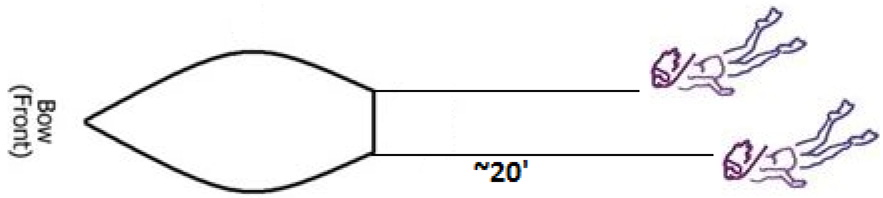


Figure 1: Manta tow layout

8. Mapping a Spawning Aggregation Site

A good base map of a spawning aggregation site is an invaluable tool for monitoring, outreach, and management. A map of the site is essential for ensuring consistency in survey areas by different workers and over the long term. Standardization of methodology, including the area surveyed over time is essential for ensuring that results can be compared between studies and over time. Given that a major value of surveys is when they are conducted over the long-term it is highly likely that multiple workers are involved and all should be using the same methods and approaches, including surveying the same area or aggregation. **An agreed-upon protocol for studying the aggregation and site is essential for standardization methods and study details.**

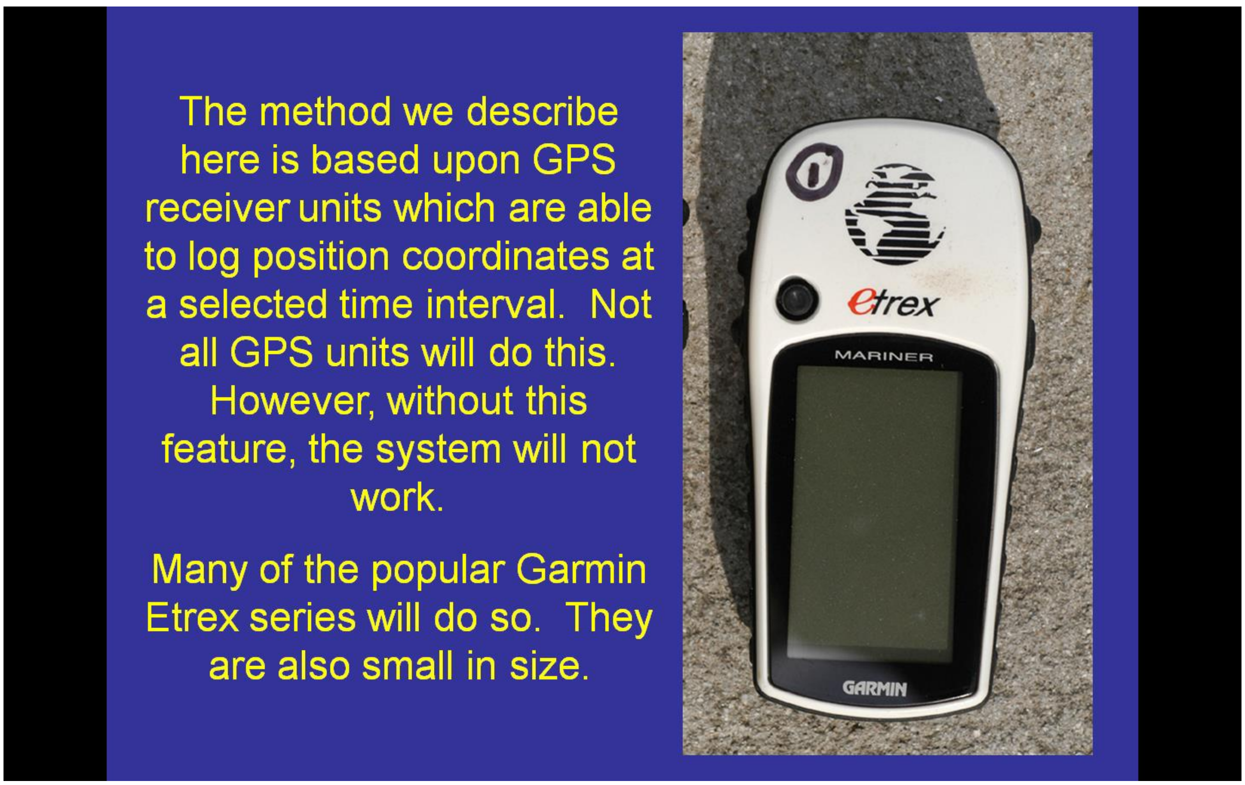
There are several techniques available for making such maps, as described below. A map should be developed for each site where detailed monitoring will occur. Good maps will be geographically referenced, include accurate scales, provide an accurate indication of the geomorphology, and show the locations 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, as needed, and areas measured as accurately as possible, since these measures will be used in calculating fish densities and assessing future changes to the aggregation.

<u>Weighted Line - Underwater Mapping</u>
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 i.e. by locating, during the aggregation period, the points beyond which no aggregating fish are found, 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.

<u>Mapping with Floats and a GPS</u>
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.

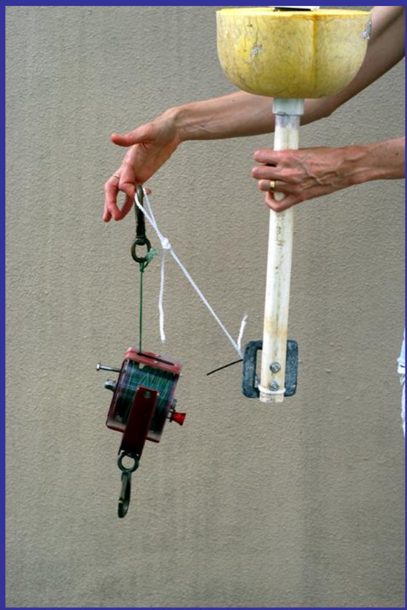
If the weather is favorable the technique below from Pat Colin's Marine Resource Assessment: Use of general and GPS based Techniques to Document and Quantify Fish Abundance, Fish Distribution and Fish Spawning Aggregations (contact Pat or Yvonne Sadovy for more details: crrf@palaunet.com, yjsadovy@hku.hk) could also be used to delineate the outer distribution of an aggregation, to determine area-it is easier and far more efficient than the above Floats/GPS method but requires a specially (easy and cheap) constructed housing for the GPS and exact synchronization between the time on the GPS and the diver's watch. The time-referenced GPS reading will give the location of the diver as she/he moves across the substrate once data are downloaded from the GPS.



The GPS receiver is housed in a PVC plastic housing. The GPS units we have tested work well inside a PVC housing.

A diving watch is synchronized to the second with the GPS time and used for all timing of counts and events

The GPS unit is started, making sure that the logging is functioning, and is sealed into the housing.



The surface float has a weight at its bottom, to keep it upright. For scuba diving use a reel is used to provide enough line to keep it on the surface while it is being towed.

Equipment necessary for mapping:

- GPS
- Compass
- Base maps
- Dive watch
- Weights
- Fishing line
- Floats (Styrofoam)
- Pvc pipes
- O-rings
- Dive computer for safely

Describe courtship and spawning behaviors

• Accurately describe 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. Counts could be made of fish in different colour phases – there is a bicolour phase spike (percentage of bicolor to other phases) about 24-28 hours before spawning so it is a really good indicator of imminent spawning – should be a dedicated task (Whalen et al., 2007)

9. Underwater Visual Census Protocol

Based on a review of the 2004 SPAG monitoring protocol and SCRFA manual (Colin *et al*., 2003), revisions have been made to the Underwater Visual Survey Protocol with a new section added: "b. How to Count: Estimating the Number of Fish by Underwater Visual Census (UVC)"

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. Necessary 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 a 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 and 3-dimensional (3D) 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.

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. Below are the revised objectives for the visual surveys.

Objectives of Visual Survey

- Quantify the numbers and/or length of fish by target species (depending on pre-agreed study priorities), specifically Nassau grouper (see list of species), timing, and locations of multi-species reef fish spawning aggregations. Sizes should only be estimated if divers have been trained sufficiently in size estimation and if there is enough time. If the priority is to register abundance of a major species then this task must be completed fully and satisfactorily before any other task (i.e. estimating size, counting other species, etc.).

- 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
- Dive watch
- Dive computer for diver safety.

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. Ideally the full spatial extent of the site should be mapped (Section 4) but if surveys are only conducted along a linear extent then, at the minimum, two points must be mapped. This ensures that the same linear area is surveyed each time.

Diver Surveys:

It is extremely useful for diver orientation and for discussion after each dive to have several physical markers in the water. These can either be permanent or temporary buoys (polystyrene) to help people learn the site layout as well as compare spatial observations.

It is also important that data be recorded in a standardized format so a data-recording form should be decided upon to include data and any necessary notes (see end of file for suggestion).

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, if time, sizes of all aggregating finfish, or focal fish species. It is important that all team members synchronize their watches so that all observations relate to the same time.

For species that spawn at dusk at least one diver survey should be conducted between 1500 – 1600 hrs to quantify the spawning aggregation(s). 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, and shortly before, 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 within the dive time available (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. Note that for challenging dive sites or limited bottom times, priorities for information collected must be determined ahead of time to ensure that the most essential information is collected first.

For long-term surveys, divers will change over time so it is essential to be disciplined in recording data and collecting information in a standardized way on each dive.

b. How to Count: Estimating the Number of Fish by Underwater Visual Census (UVC)

It is challenging to meaningfully monitor aggregating fishes for several reasons. Diving conditions are often difficult; deep water and limited bottom time, at dusk or even at night time if spawning is to be observed, as well as strong currents and often the presence of hooks or other gears in the water. But these may be the easier problems to deal with. The single most difficult task is accurate assessment of the number of fish at an aggregation site. As we have come to learn more about aggregations of different fish species, or the same species over time, we have also come to know how variable aggregations can be in time and space. For example, the area of greatest fish density can vary within a given aggregation site from year to year as can its timing in a given month or in relation to moon phase. There may be diurnal patterns in density or total numbers at specific aggregation sites. Only long-term study with good record-keeping can develop our understanding of such variability.

Numbers, density and sex ratios can change substantially during the days leading up to spawning or at any one moment at different places within a given aggregation, depending on the species. The timing of aggregation formation of the same species can vary even within different aggregations located within 20 km of each other. In other words, it is not possible to simply go out to an aggregation site, do a couple swims and expect the counts to be meaningful. Careful planning is essential, it must take into account the various factors that could influence the quality of your results, and ensure that your data are representative of the natural situation. Multiple dives at a properly mapped site will be necessary.

This section covers different approaches available for measuring fish numbers and assessing density (for species that spread out over the substrate), the biases involved and problems associated with the various approaches. It covers the considerations essential for developing a **robust, consistent, standardized and repeatable sampling protocol** and briefly touches on other aspects of underwater surveys such as the assessment of size and sex of fish underwater. It also touches on questions of

accuracy (= the closeness of a measurement, or estimate, to the true value of the variable being measured, or parameter being estimated) and **precision** (= a measure of the degree of concordance among a number of measurements or estimates for the same population, reflected by the variability of the estimate).

Methods of assessing the numbers of fish in an aggregation by UVC should be **repeatable** and consistent over space and time. Basic standards for underwater sampling (e.g. English *et al*., 1994) and diving safety apply, and survey team members should familiarize themselves with these

If actual numbers are considered to be impossible to count or if transects/counts cannot, for some reason, be completed, then it is valid to make an estimate of fish numbers present by using some form of **index of abundance**. For the SCRFA global database, an index to describe the peak (maximum) number of fish observed for a given species in an aggregation at one time was created. While only approximate, these categories nonetheless provide an indication of aggregation numbers that can be compared over time: 1-10 fish; 11-50 fish; 51- 100 fish; 101-500 fish; 501-1,000 fish; 1001- 5,000 fish; 5,001-10,000 fish; > 10,000 fish.

Estimating the Number of Fish in Spawning/Aggregating Column/Ball

If aggregations are small or fish are few, such as less than 100 fish, it might be possible to count all the fish with a high degree of accuracy. However, most cases are different. When there are too many fish to count, at least two different approaches to estimating fish numbers should be applied and total counts made by at least 4 divers. The problem of how to sample fish numbers in an aggregation is tricky and each species and site presents its own set of challenges. The worst case is where fish are dense, distributed from the bottom up into the water column some distance, moving constantly, are disturbed by human presence and are often hiding in the reef. In this sort of a case, we would be fortunate to obtain a value that is within two or three times the true number. Under such circumstances it is not possible to know whether any one estimate is correct. Therefore, it is essential to have multiple estimates (at least 4) to determine an mean and standard deviation and hence to be able to gauge the degree of precision of the count. This is essential for long-term studies to be able to meaningfully compare estimates of fish numbers across aggregations and at the same aggregation over time. This approach also provides valuable information that can be used to refine counting methods. For example if 4 divers come up with a mean of 1,000 fish plus or minus 700, then there is far less confidence in the estimate of 1,000 compared to a situation in which mean counts were 1,000 plus or minus 150 fish. In the final counts recorded for a particular day or year, the estimated fish number should always be provided with the standard deviation and (N) number of people counting. (If there are more than 4 people counting, even better).

There are several alternative means of estimating fish numbers when it is not possible to accurately count all fish present and the fish are up in the water column in a group or 3-dimensional ball.

1. Estimate the total number by assessing the number of fish in a small and consistent-sized sub-area of an aggregation and factor up these numbers by an estimate of the total aggregation (i.e. total number of sub-areas). . In estimating numbers in large (over a few hundred fish) 3-D aggregations, however, is difficult and in most cases, we must accept that such estimates are likely to have a high, and unknown, error value. This situation can be addressed by ensuring that multiple counts are made and using a mean and standard deviation for the final estimate. It should also be cross-checked using an alternative method, such as counts from videos or still photos (see below).

Essentially, if the abundance of fish is too large for each individual to be counted, a best estimate possible is needed. For the best estimate of total number of fish observed, a team of at least 4 divers should be used and each diver should record his/her observations, which will be compared after the dive. It may be appropriate to disperse the team at different depths throughout the water column, as this will allow a more accurate representation of all fish dispersed throughout the water column to be monitored, especially if some fish remain at deep depths before rising to spawn. (This may not always be appropriate, but will be site-specific). In order to count a large school of fish, it will be necessary to observe and count every individual within a small section (volume) of the school and then replicate this in order to cover the entire volume. For example, in a school estimated to be in the hundreds of individuals, a group of 50 individuals could be counted. Once the volume covered by 50 individuals is known, this volume size can be replicated until the entire school is accounted for. For example, if the volume covered by 50 individuals must be replicated 10 times in order to cover the entire school, the total number of fish would be 50 x 10 = 500. For larger schools, the number of individuals accurately counted within a specific volume may be 100 fish and this would subsequently be scaled upwards. For schools less than 100 fish in total, 10 individuals may be counted and this area replicated to cover the entire school area.

This method is one that involves individual divers making for themselves 'mental grids' to count fish in sub-areas and factor up to total area (or volume). This approach has been used by other workers including Sala and co-workers in Belize and Whaylen and co-workers in the Cayman Islands (Sala *et al.* 2001; Whaylen *et al.* 2004; Whaylen *et al.* 2007). Their counts have been cross-referenced to video counts. An alternative possible approach is to use real grids instead of mental grids. Several small grids were trialed at Glover's Reef Nassau site in 2012 (January) and small (BEING TESTED) thin-walled squares were found to be useful for some divers. In either case, care must be taken to consider the 3D (rather than 2D) form of the aggregation and to factor this into counts of fish.

Obviously the conditions encountered during the dive will determine the ease of counting, the level of accuracy in the numbers recorded and how the numbers are treated post-survey. For this reason it is always important for divers to discuss their counts and experiences as soon as possible after the dive. A monitoring dive undertaken at a deep site, which is darker and has reduced visibility, may require a slightly modified methodology compared to monitoring a shallow, brightly lit site. This situation will also occur if the visibility is reduced due to the time of day – i.e. close to sunset. For a **shallow water scenario**, it may be that the number of fish observed will be more similar between members but this should be determined after each dive. In this case, it would be applicable to take the average of all numbers of fish observed. However, for a **deep water scenario**, the team should be spread vertically through the water column during the survey to maximize the accuracy of the observations at staggered depths. In this scenario the most accurate numbers will be gauged based on the depth at which each team member was swimming during the survey. If certain species remain higher in the water column and certain species remain deeper (especially if the survey takes place before rising up in the water column/spawning actually occurs), then the most accurate numbers of abundance per species will be based on which team member was closest to (and therefore had the best view of) those fish. (This is particularly relevant when monitoring a multi-species spawning site). It may be necessary in such cases for counts to be summed rather than averaged. Whatever the final figure there should be consensus reached within the dive team.

2. Photographing or video recording the entire extent of an aggregation and subsequently counting all the fish present is a different method of attempting visual counts. If the observer is at all serious about accuracy of counts, and sufficient funding is available, it is recommended to video record transects for later analysis while visual counts are being made. Potentially a divers' buddy could do the video recording at the same time that counts are being made. Concurrent manual counts and videotaping will be useful in eventually addressing the questions of the differences in data obtained from the same aggregation by both methods.

   When videotaping or photographing an aggregation, notes should be taken regarding the extent to which the diver has recorded all of the fish. It is best to take video using a slow pan across the aggregation and to document, if possible, the depth of the aggregation if it is 3D. Ideally, a series of overlapping stills can be taken across the width and depth of the aggregation for subsequent counts (see additional notes on video recording below).

3. Fish numbers can be estimated by using a tagging method (Sala *et al*. 2001)). Fish are tagged in the water on site and then the ratio of tagged to untagged fish used to estimate numbers. See reference for details.

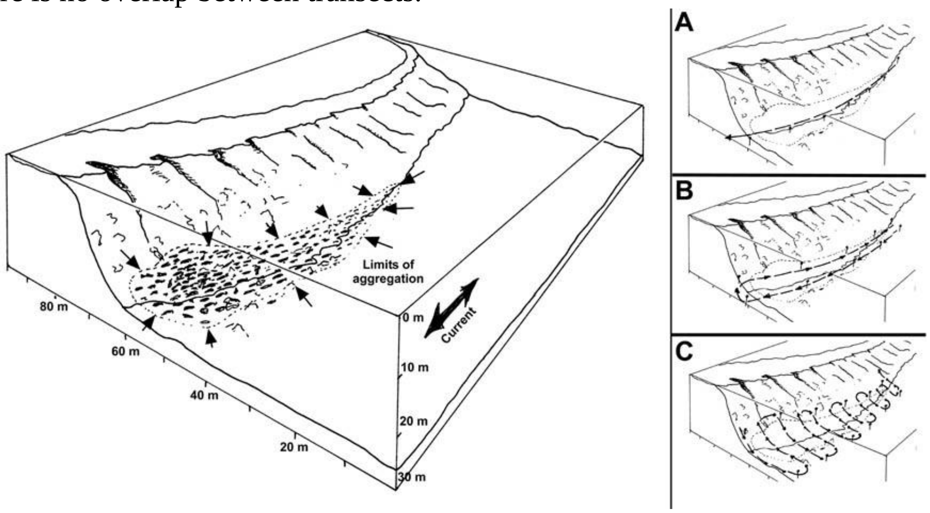
Estimating the number of fish over the reef - Visual census by moving divers counting 2-dimensional aggregations

When assessing the abundance of fish using a strip transect for fish spread 2-dimensionally over the substrate, a SCUBA diver normally swims along a transect of predetermined length and counts all the fish encountered within a set distance either side of the centre of the transect over a predetermined distance (English *et al*., 1994). A timed swim method may also be used, where a team swims for a certain time period (e.g. 30 minutes), recording all fish and/or fish species observed during this time. It is assumed that if the team keeps a constant swim speed, the same distance will be covered during each monitoring dive.

Visual estimates use quantitative measures that either includes the whole aggregation or a subset of the aggregation that is then extrapolated to include the entire aggregation. Where the aggregation is contained spread 2-dimensionally over a specific area belt-transect methods are generally used. Unless part of the aggregation site is too deep or the aggregation site is too large, the whole of the aggregation area should be surveyed. If a sub-area is surveyed instead, then it must be a known proportion of the total aggregation area and involve multiple transects in stratified samples that account for possible differences across the site in density and species.

Using the aggregation area estimated and abundance data from fish counts, the fish density (and total numbers of fish if only a sub-area is surveyed) can be calculated. For repeated measures and census in a given area, small floats or colored rocks can be anchored and left on the bottom to indicate aggregation boundaries and define the area for surveys. 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. Underwater slates should be prepared before the dive, making columns for fish species, fish numbers and size ranges.

For belt transects the area within which the aggregation is occurring must be identified and the total area defined. This can be obtained from the aggregation mapping exercise. Based on the area mapped, fish should then be surveyed within 5 m (or other width depending on conditions) wide belts along the length of the entire aggregation (or aggregation sub-area). Transect length will vary based on the distance along which fish are aggregating. Depending on the total area of the aggregation area several transects may be required. Several transects will be required to ensure that areas of different density are sampled. Surveys can be conducted moving up and down the length of the area counting within 5 m (or other) belts until the entire area is covered. In large or wide areas, several transects will be required and at least two divers will need to do counts parallel to each other ensuring that there is no overlap between transects.



Source SCRFA manual by Colin, Sadovy and Domeier (2003)

Ideally it is best to completely sample the area within which fish are aggregating. When several divers are involved in counting fish, the widths of the area to be covered by each diver should be clearly established to prevent double counting, which can result in overestimation of aggregation abundance. Careful planning is needed if sub-areas only are to be sampled (because part of the aggregation is too deep or the aggregation area is too big).

Fish size estimation: For size estimation, errors are inherent in underwater visual surveys and despite training with wooden models or experience conducting underwater surveys, there is still considerable error involved in size measurements underwater. Given that estimating sizes is time-consuming and given that deep dive sites allow for only short dive periods, the value of taking size information should be carefully considered. The most accurate size information comes from catch landings data. Therefore size estimation of aggregating fish can at best only be semi-quantitative. Do not attempt to estimate size for individual fishes as this is impossible with more than a few fish. The use of calibration rulers helps to improve the accuracy of visual size estimates and should be used where possible. Size is best estimated using ranges and even so the level of accuracy of this must be taken into consideration into data analysis and reporting. For size estimations the following size ranges are recommended: <10 cm, 11-20 cm, 21-30 cm, 31-40 cm and > 40 cm. There is a method that uses parallel laser pointers for more accurate size determination, if this is considered to be important.

Video recording: The visual surveys should be complimented with video to verify or calibrate abundance counts. Video can also be used on the evening dives to record courtship and spawning behavior. 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 focuses on the visual surveys.

Data recording: As soon as the dive is complete, all divers should work together as a team to record all of the different information (physical measurements, visual surveys) from the dive on the data sheet. Any unusual events or observations should be discussed and recorded in detail. See suggested standardized table below. The counts from each diver should be shown separately because it is not possible to determine which count is the most accurate. Moreover, the variability among the different counts gives an indication of the confidence in the precision.

Date	**UVC Counts (recorded by diver name)**	**Average count and S.D. (also provide N for total number of divers)**	**Depth of aggregation and location in dive site**	**NOTES on UVC counts**	**Video record Notes**	**Dive details**
	(one count per diver – would be good to have at least 4 counts per dive- this column should therefore have multiple columns, one for each diver			For example was entire aggregation surveyed? Any conditions that might affect count?	For example note if the video recording captured the whole aggregation or not	Time and name of dive site and other dive and environmental details such as current, wind, etc. Also notes on whether fishing gear was found on site.

References:

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