Manual for the Study and Conservation of Reef Fish Spawning Aggregations

by

Patrick L. Colin, Yvonne J. Sadovy and Michael L. Domeier

Society for the Conservation of Reef Fish Aggregations
Special Publication No. 1
Version 1.0

While effort has been made to verify and check the information included in this manual, no guarantees are made as to the accuracy or utility of any information included herein. It is essential that all activities undertaken on or in the water be properly planned and carried out. While the methods described in this manual have been based on the experiences of the authors and others, all users are advised to remember the conditions they encounter may not be the same and should take appropriate measures to modify the contents of this manual based on their local conditions.

This document is a technical contribution by the Society for the Conservation of Reef Fish Aggregations (SCRFA). Users may feel free to cite or use this document provided attribution is made to the authors and SCRFA (ISBN number pending).

Suggested citation: Colin, P. L., Sadovy, Y. J. and Domeier, M. L. 2003. Manual for the Study and Conservation of Reef Fish Spawning Aggregations. Society for the Conservation of Reef Fish Aggregations Special Publication No. 1 (Version 1.0), pp. 1-98+iii

Author's Affiliations: (PLC) Coral Reef Research Foundation, P.O. Box 1765, Koror, Palau 96940 email: crrf@palaunet.com, **(YS)** SCRFA, c/o 1612 Wilt Road, Fallbrook, CA 92028, USA, mailing address, Department of Ecology and Biodiversity, The University of Hong Kong, Pok Fu Lam Road, Hong Kong, China, email: yjsadovy@hkucc.hku.hk or scrfa@hkucc.hku.hk, **(MLD)** Pfleger Institute of Environmental Research, 901-B Pier View Way, Oceanside, CA 92054, email: domeierml@cs.com.

TABLE OF CONTENTS

Section I. Introduction

Spawning aggregations of reef fishes are among the most remarkable biological phenomena found in shallow marine waters. They have been receiving increasing attention, as the need to understand the biological and fisheries importance of these aggregations becomes more evident and as overfishing of many aggregations has brought questions of conservation and management to the fore. Fishermen have long targeted aggregations of some species, as they were predictable with regard to location and timing, but in past decades fishing pressure on aggregations has increased to the point that many commercially important species are threatened throughout most of their ranges (e.g., Sadovy, 1993) (Figs. 1, 2 and 3). There is a pressing need to improve our ability to study and document spawning aggregations, using sound science, the better to protect, manage and conserve them. It has become clear within the last decade that, without action, many more will decline and some may cease to form completely.

Many reef fishes aggregate in large numbers at specific times and places to reproduce. Some aggregation sites may be used by many species, either simultaneously or at different times of day, month or year, others host a single species. We do not know why fish select the places and times they do to spawn but we do know that, once discovered, the predictable nature of aggregations makes them extremely vulnerable to overexploitation. Overfishing has already depleted a substantial number of such reproductive gatherings in the Caribbean and considerable anecdotal evidence suggests that spawning aggregations, particularly of groupers (Serranidae), are systematically being depleted in the Indo-Pacific region. Many remaining aggregations throughout the tropics are seriously reduced and may disappear if not quickly protected. These aggregations are critical for the persistence of the populations that form them with many in urgent need of protection.

Until recently there has been little awareness of the problem of aggregation exploitation and little incorporation of the aggregation phenomenon into fishery management plans or marine protected areas. The Society for the Conservation of Reef Fish Aggregations (SCRFA) was formed expressly, in 2000, to promote and facilitate scientifically informed conservation and management of reef fish spawning aggregations. This manual is part of that initiative and its development was partially funded by the Packard Foundation.

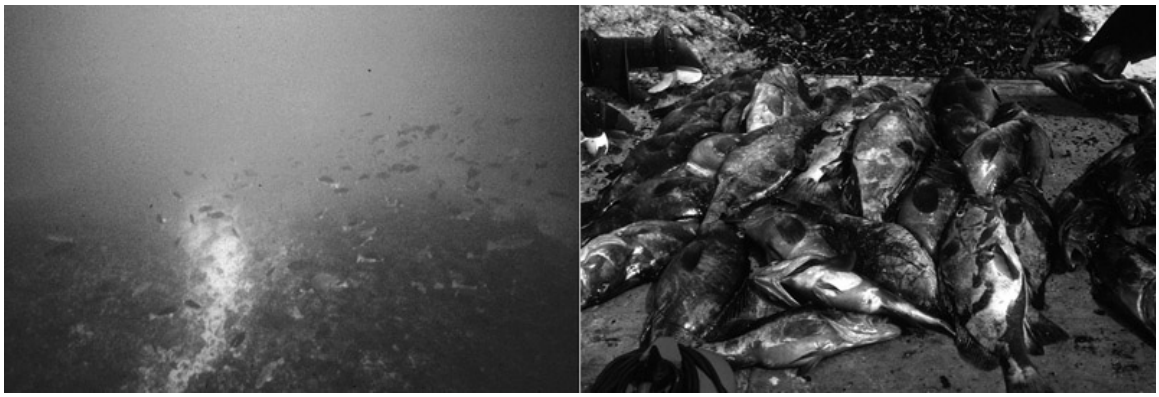


Figure 1. More and more spawning aggregations, such as these of the Nassau grouper photographed off Long Island, Bahamas are being turned into piles of fish on the shore, as shown on the right. While we are not opposed to catching fish for food, there is great need to be careful that spawning aggregations are not wiped out, ultimately affecting the entire population of the species concerned. The safest option is not to fish aggregations at all. (PLC)

Scientific study of reef fish spawning aggregations dates back to Randall and Randall's (1963) work on the redtail parrotfish, *Sparisoma rubripinne*, in St. John, US Virgin Islands in the early 1960's. In this classic work they explored many aspects of this aggregation and pioneered techniques still used today to gather biological information on aggregations. For commercially important reef fishes, grouper aggregations were probably the first known, with anecdotal records from decades to over a century ago. It was not until the report of C.L. Smith (1972) on an

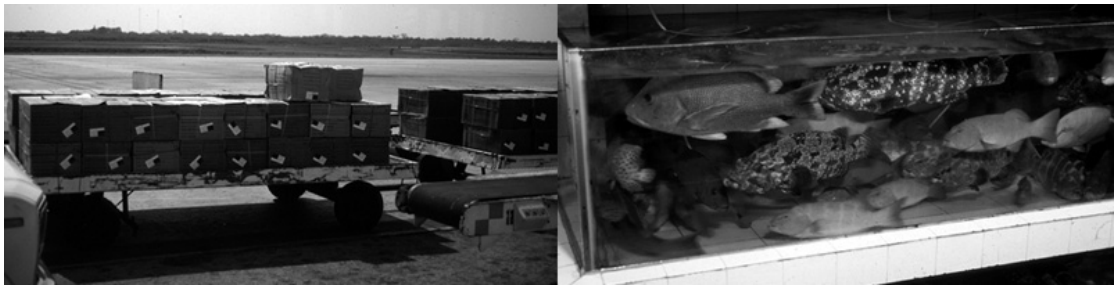


Figure 2. The targeting of reef fish aggregations for export as frozen fish fillets (left) and for the Live Reef Fish Trade (right) has put extreme pressure on many species of larger reef fishes throughout much of the world. Spawning aggregations are an easy location to capture large numbers of fishes, but their reproductive success is put at extreme risk. (PLC)

aggregation of Nassau grouper, *Epinephelus striatus*, near Bimini, Bahamas (which included some remarkable photographs) that scientists generally realized the potential biological and fisheries importance of spawning aggregations, although a report from Cuba in the 1880's recorded probable Nassau spawning aggregations (Vilaro Diaz, 1884).

Since Smith's report in the early 1970s, studies have been undertaken on reef fish spawning aggregations, last reviewed by Domeier and Colin (1997), and aggregations have been recognized as a world-wide phenomenon. Numerous papers and reports have subsequently appeared in both the scientific and conservation literature. The Society for the Conservation of Reef Fish Aggregations (SCRFA) recognizes the need for a comprehensive manual that examines past and present methods for studying spawning aggregations; a manual that makes recommendations for standardizing this field of research and that can act as a reference for the development of practical guides and protocols which address specific needs and circumstances. The objective is to have workers use methods, whenever possible, which are standardized, comparable and reproducible. Working on or in the ocean is never easy, and dealing with transient biological phenomena, often occurring in remote areas, simply adds to the difficulty. On the positive side, today we have available tools, such as sensitive underwater video cameras, reliable diving equipment, advanced telemetry, instrumentation and navigational equipment, that were unavailable when scientific work on reef fish aggregations started.

This manual is intended to serve as a resource to aid in choosing productive and scientifically sound methods for investigating reef fish aggregations and promoting their conservation and will be updated as our understanding improves, or new information becomes available. We have tried to include descriptions of methods that can be applied to aggregations of many different species, can be repeated by successive generations of workers and that provide comparable results between different aggregations. These methods vary from the simple and low tech to what we might consider "state of the art". Hopefully, there will something everyone can gain by examining what we include in this manual and the examples we cite. Many of the photos and diagrams come from our own collective work. Their inclusion is by no means intended to

imply that these are the best examples in their respective sections but these were cases most accessible to us and which we best understood, for better or for worse.

Figure 3. This mutton snapper, *Lutjanus analis****, is a species that forms spawning aggregations that have long been targeted by fishermen in the western Atlantic. Only now is it beginning to receive protection in a few areas. (copyright Doug Perrine)***

The Society for the Conservation of Reef Fish Aggregations (SCRFA) office is willing to serve as a repository for data, through our spawning aggregation database, and to archive videos, photographs, maps and other materials that need to be preserved for future use by researchers, managers and fisheries workers. These will all be made available on the SCRFA website (www.scrfa.org).

We hope that this manual will serve as a resource for those actively working with aggregations or wanting to do so. None of the recent manuals on reef fish assessment (e.g., English *et al*. 1997, Samoilys, 1997a) provide specific guidelines for dealing with aggregations. In the past decade there has developed a significant literature, both in peer-reviewed journals and as "gray literature", which has not met strict levels of documentation and reproducibility of measurements. It is hoped that this manual will help researchers adhere to reasonable standards of documentation of aggregation and spawning studies so their information can be accepted into the pool of scientific knowledge. SCRFA has developed guidelines for the level of documentation needed to include spawning aggregation information in its worldwide database and equally robust guidelines need to be applied to the planning, execution and publication of studies. Working on spawning aggregations is never easy, and we need to place all work, both the definitive and the less certain (which is valuable in its own right) in perspective to keep this field of investigation moving forward with a firm observational and scientific basis.

The manual is organized into what we hope are logical categories covering different aspects of spawning aggregations. It tries to summarize the techniques used by workers in the past and include various techniques and tips that the authors and others have used over the years. At times something as simple as diving in a certain manner, or using a piece of equipment properly makes the difference between obtaining important new information and the inability to provide any new insights. Such small things often make a project a success or doom it to failure.

Section II. What is a Spawning Aggregation? Criteria for Spawning Aggregation Identification

II. A. General

Two criteria are essential for the identification of a "spawning aggregation". They are, first, an increase in the density of fish present and second, the verification of spawning. “Transient” aggregations often involve long-distance migrations and a short reproductive season, while “resident” aggregations may form frequently, often over an extended period and occur close to, or even within, the areas of residence of participating fish (Domeier and Colin, 1997). The importance of applying specific criteria is to prevent misidentification of the nature of an "aggregation" of fish and to ensure an objective means of identifying and communicating this phenomenon. Given the limited amount of resources available for conservation and management of spawning aggregations, they need to be correctly identified to ensure that sufficient care and attention are allocated in the best possible way for aggregations to gain sufficient protection for long-term persistence. Note that there are occasions other than for spawning, such as feeding, for which non-schooling reef fishes are known to aggregate.

II. B. Increase in Density of Fish

Domeier and Colin (1997) defined a spawning aggregation as "a group of conspecific fish gathered for the purpose of spawning, with fish densities and numbers significantly higher than those found in the area of the aggregation during non-reproductive periods". This general definition, however, does not fit all situations and for some species, such as those that normally occur in dense schools, other criteria might apply (see Domeier and Colin, 1997 for more detail). The level of increase in density of spawning fish that constitutes an aggregation is, however, arbitrary. As a guideline, we suggest that a 3-fold increase in the density of spawning fish is the minimal threshold level that constitutes a spawning aggregation (Domeier and Colin, 1997). Although this value is likely too low, this inclusive first step allows us to collect information that can be reassessed at a later date when more is known about particular aggregations. There may be some examples where over exploitation has reduced an aggregation to very low levels that would not fit a more exclusive definition. Fortunately most spawning aggregations have considerably higher levels of density increase, making it easy to verify with certainty that they are actually aggregations by our definition.

Since the criteria of what is an "aggregation" are based on an increase in density, it is usually necessary to have some idea of the typical non-aggregation density of any species of interest. In many cases, we do not have quantitative density information from before, during and after an aggregation. Researchers must usually rely on general qualitative knowledge about what is a "normal", non-aggregated, density and then be able to recognize when a significant increase in numbers has occurred. When there are suddenly 500 groupers in an area that would normally have 2 or 3, there is little doubt that a 3-fold density increase in density has occurred. However, where fishes have been reduced in number, due mostly to fishing pressure, it may not be so easy to confirm the density increase. Methods are described subsequently for estimating the numbers of fish present and the area of an aggregation; in marginal cases, it may be necessary to make the same sort of determinations during non-aggregation periods.

The social systems of reef fishes cover a broad spectrum of behavior, some of which involves temporary concentrations of fish. Haremic species may have a few individuals, normally scattered, that come together daily for successive spawnings of females with a single

male. While these are irrefutably spawning groups, they do not meet the 3-fold increase criteria, hence are not considered spawning aggregations. Readers interested in the entire spectrum of reef fish spawning and/or aggregation types are referred to Thresher (1984), Sadovy (1996) Domeier and Colin (1997) and Petersen and Warner (2002) among others for more information.

II. C. Criteria to Verify Spawning within an Aggregation

The importance of documenting spawning within an aggregation of reef fishes can not be stressed enough. Reef fishes display many aggregating behaviors unrelated to spawning, allowing ample opportunity to mistakenly describe a spawning aggregation if spawning is not documented. Some reef fishes school daily in a manner that would seem to potentially be spawning aggregations. In the western Atlantic snappers and grunts typically shelter as schools on reefs during the day, milling about close to the bottom. In the western Pacific various snappers, such as *Lutjanus fulvus*, also form schools of hundreds of individuals that at times stream as a group across the reef, seemingly with a "mission" (spawning?). Actually they are really just "hanging out" during the day. Observers should always be wary of aggregations that contain more than one species since these are unlikely to be spawning aggregations (unless a planktivore is feeding on the eggs produced by spawning fish). In most cases true spawning aggregations are limited to one species. However, as in all of biology, there are exceptions to this general rule; some grouper aggregation sites can have members of multiple grouper species present. Sometimes more than one species will use a given area, and there may also be a low number of a third or fourth species present at an aggregation site.

Since it is important to confirm that the aggregation observed has formed for the purpose of spawning we have specifically identified two types of '**signs of spawning**' that include both **direct** and **indirect** indications of spawning. Direct signs provide unequivocal evidence for spawning, indirect signs are other indications of spawning that need to be accompanied by supportive information. This distinction is being applied in the SCRFA Global Database of Spawning Aggregations (www.scrfa.org).

At present SCRFA recognizes three **direct** signs to verify that a group of fish is spawning. They are 1) undisputed spawning observations, 2) females with hydrated eggs and 3) presence of post-ovulatory follicles in the ovaries of aggregating females. Each of these is discussed in detail subsequently in this manual.

If none of the direct signs of spawning have been observed, **indirect** signs can be used but should be carefully documented. Indirect signs can include behaviors or color patterns if these are demonstrably known to be associated only with spawning. GSI data, swollen abdomen and other proven indications of spawning could also be used.

Observations that do not meet the strict criteria of **direct** signs, may or may not be spawning. Such uncertain information should be suitably qualified, so that the reader has no doubt about whether the level of documentation is sufficient to confirm a spawning observation. This has not always been the case. There is considerable literature where workers have identified or reported "spawning aggregations" for many species with insufficient data to confirm that what they have seen is actually related to spawning. Some reports have included haremic spawning groups, non-spawning aggregations and misinterpretation of observed behavior as reproductive and "spawning aggregations". Often these are second hand reporting of observations of dubious veracity. The difficulty is that once in print, even in gray literature, such information tends to be considered fact, and the problem with false records is that these can lead to wasted resources and a poorer understanding of spawning aggregation related patterns in general.

II. D. The Responsibility to Verify Information before Publication or Distribution

It is incumbent upon observers to verify what they believe to be spawning aggregations before reporting their findings in the literature. This includes both the scientific and gray literature, such as newsletters and circulated reports. SCRFA can assist in this regard with any questions. Unverified reports of possible spawning aggregations are still valuable, but workers should avoid at all costs the impression that a spawning aggregation has been confirmed as such when the two criteria (spawning and aggregation) have not been met. Unverified information can be reported, but the limits of the data or observations must always be part of the reporting (e.g., Lindeman and Claro, 2003). Otherwise, others who might not be aware of the limits of the information will assume that the report is scientific "fact".

Section III. Discovering Spawning Aggregations:

There is still a very active "discovery component" to work on spawning aggregations. Quite a few species, presently not known to form spawning aggregations, may eventually be found to have them, while many aggregations remain undiscovered. Discovering the unknown is never easy, especially when you are seeking a group of fish in 30 m of water somewhere along a hundred km of shelf edge that exists only for a short period each year. Consequently, scientists and managers need to use every resource available to increase the probability of success in finding spawning aggregations. Finding a previously unknown aggregation site is exciting and may provide valuable information but carries with it the responsibility to be careful about revealing its location prematurely to avoid its possible abuse.

III. A. Talking to Fishermen

To locate a spawning aggregation unknown to researchers, fishermen will often be the best initial resource (e.g. Johannes, 1981) (see Section VI). The knowledge level of fishermen varies greatly, but a truly knowledgeable, helpful individual is invaluable. Some fishermen with considerable knowledge of the fishes may not wish to share that information with anyone. For commercial fishing, such information is often a business secret. Among subsistence fishers there is less commercial incentive, but disclosure of information may actually encourage commercial operators to move into what had previously been a subsistence fishery. Ideally the researcher can find knowledgeable fishermen with an active interest in getting their biological information recorded, either to pass on to future generations of subsistence fisheries, or to help establish workable regulations to maintain fishery yields. Always keep in mind what will happen when you make new information available to others. There may be cases where information needs to be kept confidential, such as in the case of specific locations of aggregations. You should always make certain your informants understand what you intend to do with their information.

It is important to make contact with fishermen prior to starting significant field work. This can be done in many ways, through fisheries co-ops, fish processing houses, village councils, and others. It never hurts to have some knowledge about the fish you are concerned with, but be careful about seeming to be a "know it all" when you make initial contacts. You are asking for help, and if you already know it all, why should someone help you?

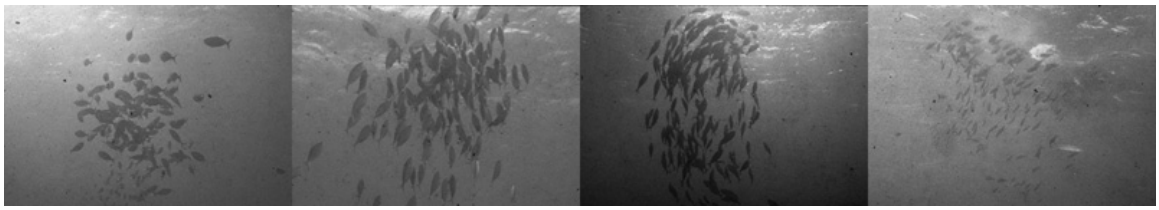


Figure 4. *Caesio teres* ***aggregation spawning, photographed at Enewetak Atoll, Marshall Islands (from Bell and Colin, 1986). In the left photograph the fish are coming together to spawn after engaging in a lengthy courtship. The remaining three photos show their ascent and swirling just beneath the surface during the actual spawning. This is an example of an aggregation discovered by "chance", in this case when behavior out of the ordinary was seen at a site that was regularly visited for other work.***

Spawning aggregations may or may not be known to local fishermen. In the cases where they are known, it is obviously advantageous to use this information to help plan field work. You should look for a fisherman, particularly someone senior (a "patriarch" fisherman), who has a longer-term perspective and is actively interested in research on an aggregation site. Try and make it clear what you are doing and why, and if appropriate consider hiring fishermen to work with you, either by taking you to the site or having them collect specimens for you. Try to involve local communities, if possible in the work, particularly in remote areas.

If you do not have a specific contact person, there may still be hope of finding aggregations. If the general area where an aggregation occurs is known, it might be possible to pinpoint the actual aggregation site looking for fishermen working the area or buoys from traps set on the aggregation site at the right time of year. For Nassau grouper aggregations, for example, aerial surveys can locate boats working at aggregations or spot the floats from traps in remote areas. While this may tell you where the fishermen think an aggregation will occur, without some information about actual catches, you will not know whether fish are actually present. For some groupers, for example, fishermen will often set traps to fish known aggregation sites, without fish being present, in the hopes they will show up and be caught.

Snooping around aggregation sites can arouse, and rightly so, sensitivities among fishermen, who may view the presence of scientists (or any non-fishermen) as a negative. Objections can range from your presence disturbing the fish (particularly if you dive on the site) or "driving them away" to "you are here to make laws to stop us fishing, so we don't want you here". Ideally, scientists can operate directly under national or local government auspices, or have the support of local fishermen who see a decrease in catches from an aggregation and want to know why, but this is by no means always the case. If researchers lack these connections, it is important to try to prepare fishermen for your presence in advance and if possible, obtain their permission to work on the aggregation site. Since fishermen are usually on the site for economic gain, it is useful if you can provide some economic benefit by your activities, such as buying specimen fish at a premium price. The time of dives or other activities might be scheduled to occur when fishermen are not actively working the aggregation. In the Bahamas, for example, most fishermen leave the Nassau grouper aggregation sites in late afternoon to return home before dark, and sites were clear of fishing activity when spawning was occurring at dusk (Colin, 1992), making diving less contentious.

Interviews of fishermen are often very useful, particularly if you know what questions to ask and how to ask them. Johannes (1981:8-9) provides some useful tips for interviews, such as asking a question you already know the answer to, so that you can assess the accuracy of other information the fisherman is giving you. Have a map of the area of interest, so you can be shown

things on the map. Be ready to draw or write all over the map (photocopies of maps are useful for this) while your source is giving you information. Before you interview, be prepared and have basic knowledge of the fishes of concern. It might be useful to have photographs of the fishes of interest and make sure you have the local names so that you are certain that everybody is talking about the same fish. Be prepared to listen to all stories, no matter how unlikely and remember that it is often the case the a particular and interesting phenomenon may be widely known but that its understanding or interpretation may not be completely correct (Johannes, 1981). When possible, at some point well after the interview has started, provide at least some information that lets those you are interviewing know you have some knowledge of the fish of concern and the ocean in general. You must demonstrate that you are not totally ignorant about what they are telling you, but that their information is important to build upon what is already known. Get the person's name, phone number (if relevant) and postal address, if possible, and give them your contact information. Don't be a ghost who fades away after you learn what you want to know. Write down notes about what this person has told you as soon as possible. If fishermen know one another, you can mention you have talked with 'so and so' and you can ask whether the person you are talking with agrees with the information someone else gave you. Careful corroboration of information obtained through interviews is a critical part of the interview approach. Think about providing a small gesture of thanks for being interviewed, such as a t-shirt or a book (fish field guide?). If possible, give talks at the local school or village council about your work and always provide reports or feedback of your results to the local community or government, wherever possible.

III. B. Finding Unknown Aggregations

Finding an aggregation unknown to anyone is likely to be a difficult proposition (see also Section VI). It is really a four dimensional problem compounded with all the factors that make working in the ocean so difficult. You need to find the right location, the right depth and the right time. Many aggregations are deep, and can not be easily seen from the surface. In the Bahamas, you could snorkel right over a large Nassau grouper aggregation and never know it was there, as the water is often not clear enough to see the fish over the dark bottom a hundred feet down. Any available information is important, as it can greatly improve your chances of success in locating an unknown aggregation. Check over records from fisheries departments, fish markets, fisheries literature and gonad data. Talk to dive operators, dive guides, tropical fish collectors, fishers and others who spend a lot of time on/in the water. Any of these may give you crucial hints as to "when" or "where" to look for aggregations.

Where you go to look for aggregations can affect your chances of success. Many species are known to spawn in shelf edge areas, reef promontories, pinnacles or passes so searching these areas can be productive. Identifying promising locations on a chart and searching from an airplane may prove effective for large aggregations in clear water. A vessel equipped with sonar and/or fathometer can also identify areas of high fish biomass that divers can then investigate. Also searching for aggregations during the late afternoon to sunset can be useful, as many species spawn during that time, but individuals might not be so obviously concentrated early in the day. If you work in an area with distinct tidal currents through passes or across reefs, many species spawn during the falling tide in the hours after high tide. Few, if any, are know to spawn on the rising tide. But don't just limit yourself to the most likely times and places. You never know what you will find if you are out there. Even aggregations already known at specific and/or specific species can vary somewhat in location and timing from year to year.

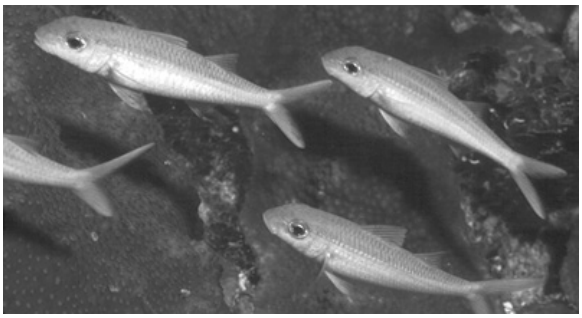


Figure 5. These goatfish, *Mulloidichthys martinicus****, are not presently known to form spawning aggregations, however, the only other shallow water western Atlantic goatfish,*** *Pseudupeneus maculatus****, does. It would not be surprising if*** *M. martinicus* ***is eventually discovered to form aggregations. (MLD)***

If conducting studies in one area over a long period of time, you can look for changes in presence and abundance of fishes that imply aggregation somewhere. It is possible to observe regular migrations of fishes through an area and then follow the migrating fish to the spawning site (e.g. Fig. 5). This worked well in the Bahamas for *Acanthurus coeruleus* (Colin, 1996) and for a variety of parrotfishes and surgeonfishes in Palau. The following of a migration route was also used to find surgeonfish aggregations in the Red Sea (Myrberg *et al*., 1988). If large fish disappear from an area one year during the spawning season (but where they went to is unknown), you might consider tagging some fish the following year with sonic tags and then trying to locate them once they have gone to a potential aggregation site.

Finally there is the "pure dumb luck factor" that increases the more time you spend on and in the water. This is how the first spawning aggregations of Atlantic ocean surgeonfishes were discovered in Puerto Rico, which led to several years of study on these sites (Colin and Clavijo, 1988), and for *Caesio teres* at Enewetak Atoll (Fig. 4) (Bell and Colin, 1986). If a general season of spawning activity is known, such as for western Atlantic groupers (December to February), this is the time to be out looking around. To maximize the area covered during a search for aggregations, you can use manta tow boards or diver propulsion vehicles (DPV's or dive scooters). Manta tows allow covering large areas at a reasonable speed, but limit travel to the route the boat takes. DPV's allow more freedom, but have less range and speed.

Section IV. Documenting Aggregations

Once an aggregation is known or located, we need to learn something about its physical and biological characteristics. The uses of this information are diverse. It may have fisheries and conservation value, to preserve aggregations for both exploitation and to serve as a source population for future generations of fish. Scientists may be interested in aggregations for the same reasons, but also have the intention of trying to understand them as a biological phenomenon. A Marine Protected Area (MPA) may be centered on a large aggregation such that its physical location is critical to locating and sizing the MPA properly.

Aggregations can be documented in many ways. These can be as simple as using our eyes to count things and make observations, which are then recorded, or as complicated as instrumentation and equipment that provide a wealth of data on an aggregation and its environment. The ultimate value of work on aggregations depends on the skillful employment of the tools and resources that are available. Like most things, it is useful to learn the skills

necessary and to practice them before needing them, so that when you are faced with a short-lived aggregation, you can gain maximum information without a major learning curve at a critical time.

At one level, we want to be able to document the biological and physical parameters of an aggregation. This includes documenting basics such as how many fish are there, their sizes, sexes, how large an area they occupy, their density throughout the aggregation, when they arrive, how long they stay aggregated, and how much they move around in that area. This may seem straightforward at first. However, once you encounter an aggregation of hundreds to thousands of fish interacting with one another, constantly moving in and out of shelter, and affected by every movement and breathing of a diver watching or counting them, these theoretically simple tasks quickly become daunting. All of this in an environment that often has limited visibility (which may mean you can't see the entire aggregation area at one time), currents, or occurs at depths where bottom time is limited. Getting an accurate census of any spawning aggregation is a difficult task and our best methods still leave much to be improved upon.

The documentation of the status of aggregations is perhaps the most important aspect of any scientific work we might do on aggregations. Aggregation demographics are essential to understanding the effects of exploitation, management, natural mortality and natural variation over time. Because this information underpins most of what we are concerned about, the methods used to document aggregations are important. It is critical to remember that the methods presently used provide us in most cases with nothing more than **estimates** of numbers of fish in an aggregation. Since they are only estimates, we must be cautious in projecting these data beyond their limits. Outside of destructive sampling, it will be some time before we have methods that provide a highly accurate census of fish. Until then, we have to use the best methods we can and remember, and record, the limits of accuracy and precision.

No single method will fit all aggregations. Keep in mind, though, that our intention is to collect qualitative and quantitative data that are comparable between aggregations and years, no matter what methods are used. When fish are too numerous to accurately count visually or by other methods, it is necessary to subsample a portion of the aggregation in some manner. Such samples need to be placed into an area or volume context. The surveys need to be repeatable, particularly by others who might not have been part of the original surveys. Ideally there should be some sort of permanent record, such as video recordings, still photographs, maps or other permanent media indicating the structure and size of the aggregation. The methods used, areas examined and other aspects of how the data were gathered need to be accurately described, with enough detail so that others can duplicate them. The bottom line is to make surveys “repeatable with precision”.

IV. A. Mapping Aggregation Sites

Preparation of a reasonably accurate site map is one of the best methods to start work on any aggregation site. Since aggregations have a physical presence, their distribution in time and space needs to be documented. This can be done by a series of observations or measurements displayed on a two or three-dimensional map or bathymetric chart of the area. The accuracy of GPS (global positioning system) plotting and the power of computer displays in two and three dimensions allow us to prepare very useful representations of aggregation structure. It is important to consider that the area outside the aggregation proper is also of interest, for example to compare geomorphology or bathymetry, and the mapping survey ideally should include a much larger area than just the aggregation site proper.

It may not be possible to prepare a map of the area in advance of the aggregation, particularly if the actual site of an aggregation is not known. The mapping process may be disruptive to the aggregation and in such a case the map survey will have to be done after the aggregation. Notes, drawings and survey markers should be used to indicate the extent of the aggregation relative to whatever features (that can be identified in the future mapping effort) may be present at the site.

Uses of Aggregation Site Maps

The benefits of a good map of the area are many:

1. It provides a reference guide for all those doing work at the site, and allows communication between participants about sites within the mapped area.

2. It allows others, who may not have participated in the original surveys, to be able to find and revisit the same sites and do repeat quantitative work on the original site(s).

3. It shows the distribution of depth and habitat relative to the aggregation, can show changes in aggregation location over time, and allows for comparison of different aggregation areas.

Maps can range from simple sketches drawn by divers, showing features of importance relative to one another with approximate distances, to detailed depictions of the bottom, with depth contours, habitat types and particular features. Maps can be prepared with basic tools no more complicated than measuring tapes and a compass, or using GPS, depth sounders and aerial photographs. Whatever the level of sophistication, a map is intended first to serve as a common reference for observers making initial observations and, just as important, for those who continue work in the future.

Some Examples of Previous Efforts to Map Aggregation Sites

Many workers have prepared maps of their study areas and these are usually included in any publications arising from the work. They are vital for subsequent work, whether for detailed scientific studies or for long-term monitoring. Randall and Randall (1963) provided a map indicating the location of their parrotfish aggregation site, plus a verbal description of the site. These materials were sufficient for Colin (1996) and Colin and Clavijo (1977) to relocate the site many years later.

As part of a study of spawning and dispersal of the eggs of *Thalassoma bifasciatum,* Hensley et al. (1994) mapped three study reefs with 48 spawning sites on the inshore shelf off La Parguera, Puerto Rico. They tracked the movement of water, represented by dye patches and current drifter, from both spawning and non-spawning sites, plotting the paths on these maps.

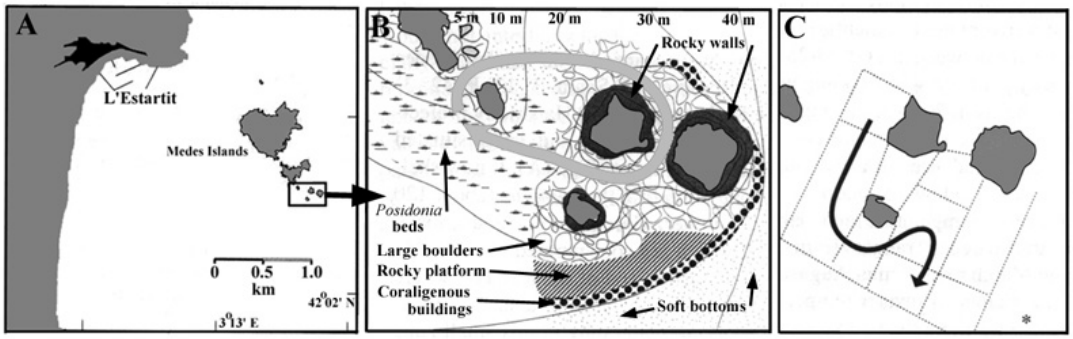


Figure 6. Maps of transect routes from Zabala et al. (1997a). A. shows the general area of the study, B. indicates the path swum in general surveys and C. details the path taken in surveys of spawning occurrence.

This study, done before GPS technology was available, used a microwave trisponder unit that allowed them to construct an x-y grid over their study reefs for plotting positions of drifters and dye patches. This equipment took a lot of effort and expense to put in to the field. We should feel lucky today that we can do the same thing with a simple hand-held GPS that costs less than $US 200!

Maps have been useful in studies of grouper aggregations. Samoilys and Squire (1994) mapped a 1700 m^2 area on the reef slope of Scott Reef, Great Barrier Reef, which had been observed to be an aggregation site for *Plectropomus leopardus*. Their map was used to standardize observations collected during censuses. Zabala *et al*. (1997a and 1997b) provided detailed maps of their study sites, including bottom communities, the route taken by divers doing underwater visual census and a rough cross sectional view of the reef communities and grouper occurrence (Fig. 6). They designated two different transects, one to estimate annual density changes (200 m long by 10 m wide swum at mid-day) and a second to survey different depths and topographies where groupers occur (100 by 10 m swum at various times of day). These provided consistent, repeatable censuses. Colin (1992) provided a map and cross section view showing the distribution of a Nassau grouper, *Epinephelus striatus*, aggregation off Long Island, Bahamas that indicated changes in the location of the aggregation between years (Fig. 7). Aguilar-Perera (1994) had a sketch map of a spawning site for another *E. striatus* aggregation off Mahahual, Yucatan, Mexico, including a vertical view of general reef and aggregation site. While he did not state how this map was prepared and it isn't particularly detailed, this map shows the value of even a simple mapping effort.

How To Do An Aggregation Site Mapping Survey

The value of a good map of the aggregation site is obvious, both as a reference during the study and for future use by others who might continue working at the site. There are a number of ways to produce a site map, and the detail and accuracy of the map can vary greatly. Since working time underwater is usually limited, anything that can be done to construct the map without working underwater at the site is probably useful. Available bathymetric or topographic maps may be a good starting point. The area of interest can be copied or scanned from available charts for use as a base map. If you are using an older map, be careful as GPS determined coordinates may not match up with the latitude and longitude indicated on earlier charts, due to errors in earlier mapping processes. If such is the case, you might be able to figure in a correction factor, based on the charted position of some known object, like a lighthouse, on the older chart.

Any aerial photographs of the area of interest are also of great use. If available, then they may show underwater features, such as large pieces of reef against a sandy bottom, which are identifiable underwater at the spawning site. If the aerial photos are properly scaled, or other known features are visible, a rough map with a scale can be prepared, and then related to bathymetric or topographic maps.

Doing Bathymetric Mapping Surveys

For areas where no bathymetric charts are available, it is possible to prepare a reasonably accurate bathymetric map using a GPS, digital depth sounder with data output, and a laptop computer. GPS coordinate data and depth data are logged through the serial port(s) by the computer using a variety of different software programs. These data are then plotted using a software program for

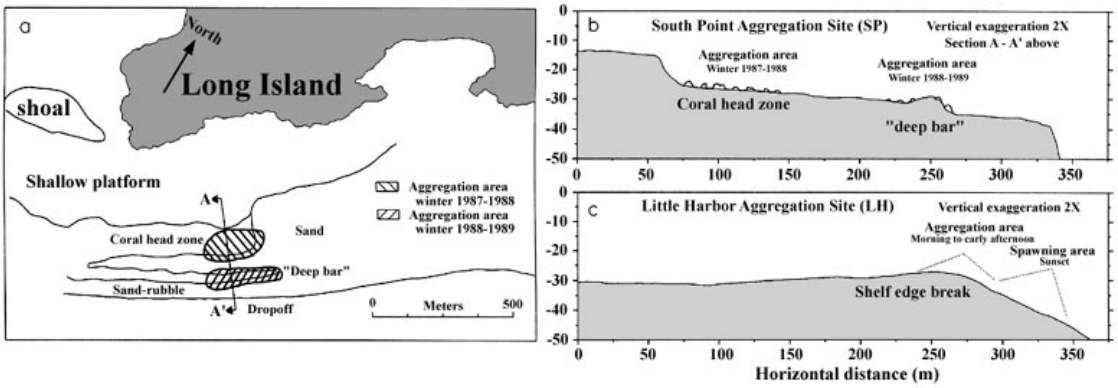


Figure 7. Example maps of Nassau grouper aggregation sites off Long Island, Bahamas (after Colin, 1992); (a) was prepared using available topographic maps and aerial photographs, (b) and (c) were based on underwater observations and other sources.

contour mapping.

Combination GPS/depth sounder units have advantages since there is only a single input cable to the computer, whereas separate GPS and depth sounder inputs would require two cables and dual com ports on the computer. Free software provided by Windmill Software, UK, which provides for system set up and logging has provided high quality data logging. This company also has commercial versions with more extensive capabilities, but for most purposes the free software has proven sufficient. Finally the coordinate and depth data are logged on a laptop computer in a watertight plastic box. The serial port cable enters the box through a watertight fitting. If the weather is good, the computer box can be left open to avoid the buildup of heat. If there is spray or rain, the box can be sealed tight after the computer has started logging. However allowance must be made for the heat produced by the computer. Be particularly conscious of not having the computer sitting on something (like a towel) that prevents heat transfer from its lower surface to the air. We would suggest running some tests in the lab to see how much heat builds up from the logging computer inside the protective box to be used.

Once the equipment is set up in a boat on the survey site, the computer can start logging and the boat is driven in a pattern over the survey area to acquire the GPS/depth data. The more sophisticated GPS units have the capability to show the tracks the boat has traversed during the survey, making it simple to efficiently cover the survey area. Care should be taken to verify that the GPS/depth sounder are acquiring good data and that the computer is properly logging it. Some electronic depth sounders use automatic depth ranging, and, if depths changes are extreme

and rapid, they sometimes lose their fix on bottom depth. In such cases the depth sounder may provide no depth data, or spit out some totally erroneous figures. Be careful when transitioning between deep and shallow water, or vice versa. When setting up the equipment, make sure the

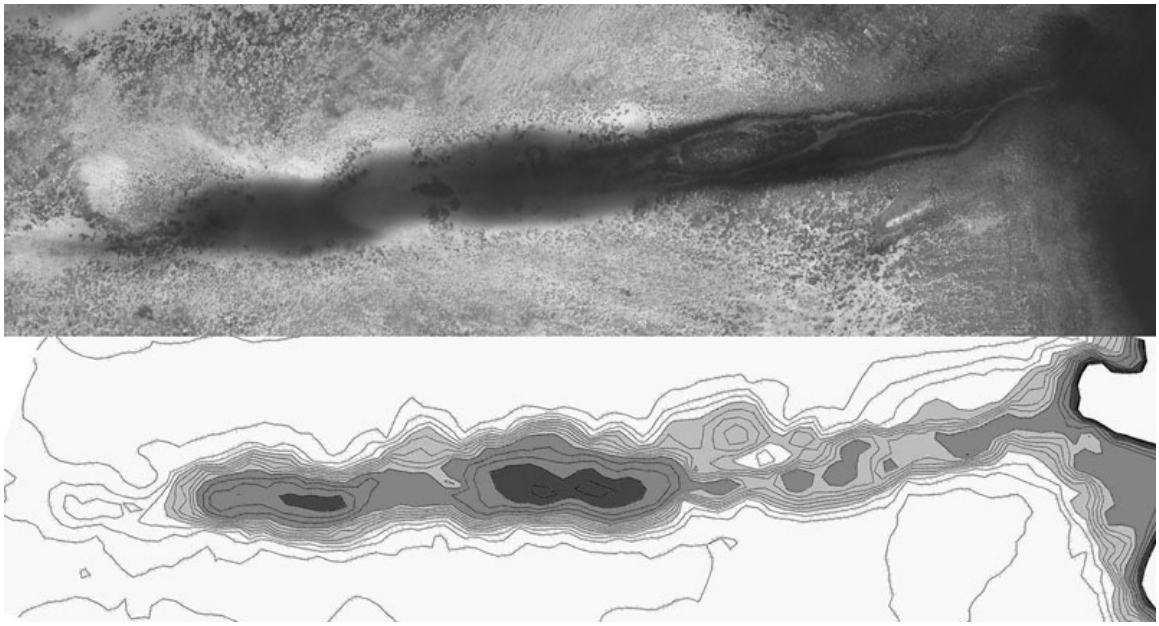


Figure 8. Aerial photograph (top) and contour bathymetric (bottom) map of Ngermekoal (Ulong Channel), Palau, a site for aggregation of groupers of the genera *Epinephelus* ***and*** *Plectropomus.* ***(PLC)***

computer clock is set on the correct time (these time data will be logged with the GPS/depth data) and set your wristwatch to the same exact time. When doing the survey have a note pad handy, so that if the depth sounder starts generating obviously erroneous data, you can quickly make note of the time. Later you can go back and delete the bad data from the file.

After returning to shore, the data are transferred to a contour mapping program for plotting. Bad data gathered can be deleted by going into the spreadsheet files based on the times such data were recorded. Once a clean data set is obtained, the data can be used to prepare a bathymetric map. We have used the program "Surfer" by Golden Software for preparing such maps, and the program can deal with 2D and 3D plots with any depth range and contouring available. Some examples of aggregation site maps are shown in Figures 8, 9 and 10. Such maps have the advantage that they are already GPS accurate and additional objects or features can be plotted accurately on the maps using GPS determined coordinates.

Additional information such as the limits of aggregation sites or migration pathways to and from a spawning site can be determined and plotted on the bathymetric map. For example, the migration pathway shown in Figure 10 was determined by having a snorkeler follow migrating fishes, while a boat closely followed him and recorded the GPS coordinates every minute. These were then transferred to the map to show the migration pathway in relation to bathymetry.

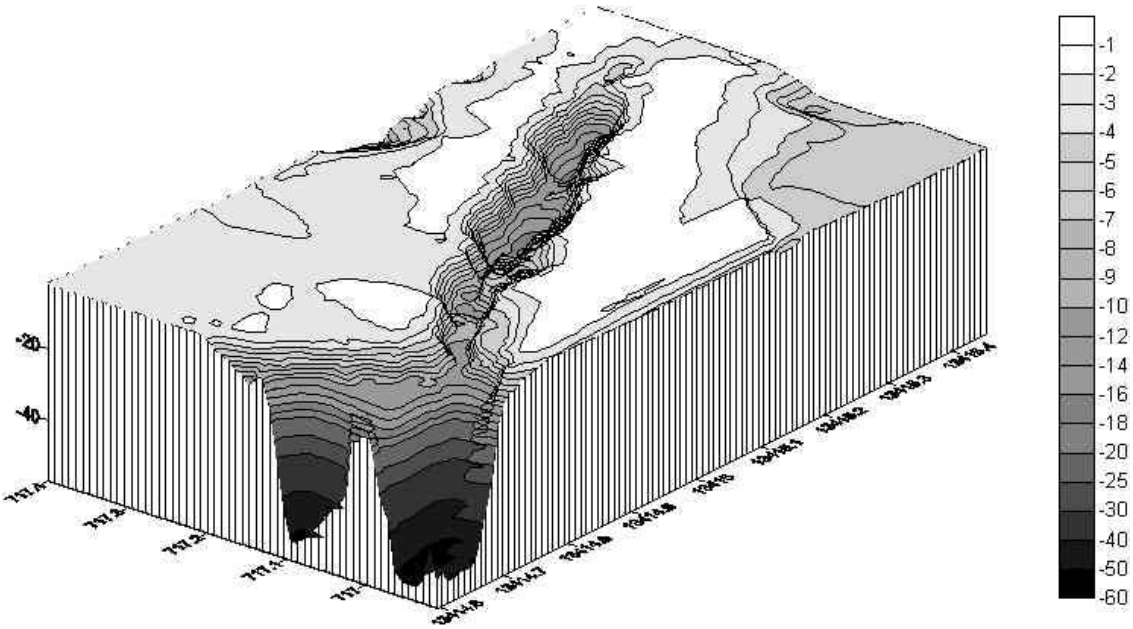


Figure 9. Contour bathymetric map in 3D of Ngermekoal (Ulong Channel), Palau, a site for aggregation of groupers of the genera *Epinephelus* ***and*** *Plectropomus.* ***Depths in meters (PLC)***

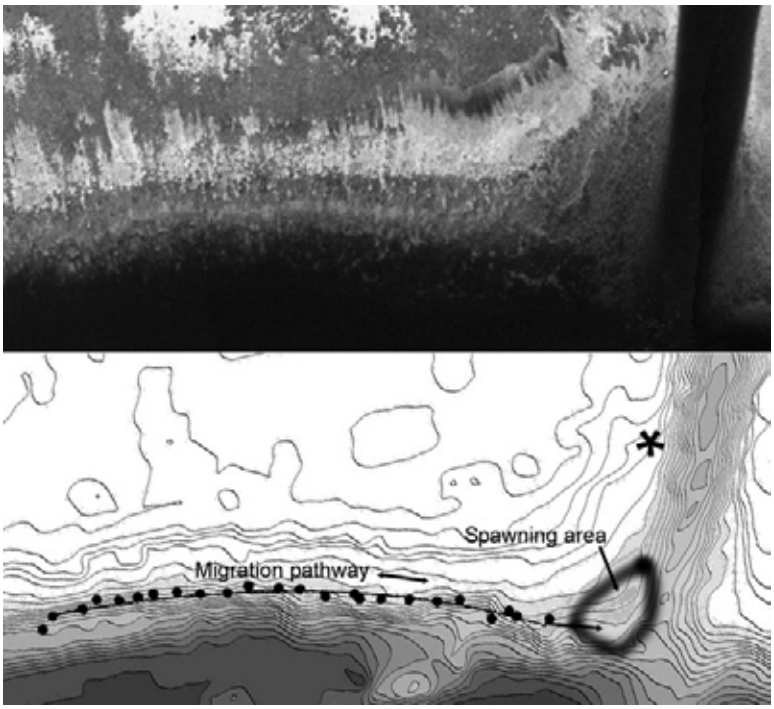


Figure 10. Aerial photograph of Lighthouse Reef, Palau with vertical aerial photograph of the reef above and the bathymetric map below with migration pathway shown (PLC).

Underwater Surveys

Surveys "in water" can be undertaken to map out the aggregation site using a measured line and underwater compass. Waterproof survey tapes, usually on a plastic reel, are well suited to underwater surveying and can be strung between identifiable points in the study area. The compass bearing and distance between each pair of points is determined. The survey might be done in a circle around the area of interest, closing the survey circle to the starting point. Or a central point might be chosen and survey lines run out from it to the surrounding areas of interest. Usually the bottom features are sketched on underwater slates relative to the survey lines. Alternately the area of the survey lines could be photographed looking vertically downward using a wide-angle lens on an underwater camera (such as a Nikonos with 15 mm lens), including the actual lines in place in the photographs (Fig. 11). The photographs are used subsequently to fill in the features of the bottom. A digital camera might be particularly useful for this type of mapping, as the images could be downloaded quickly and would probably provide sufficient resolution for mapping purposes. It is even possible to prepare mosaics of vertical underwater photos using programs such as Photoshop 4.0 and later. Again digital images would be particularly easy to use in this regard.

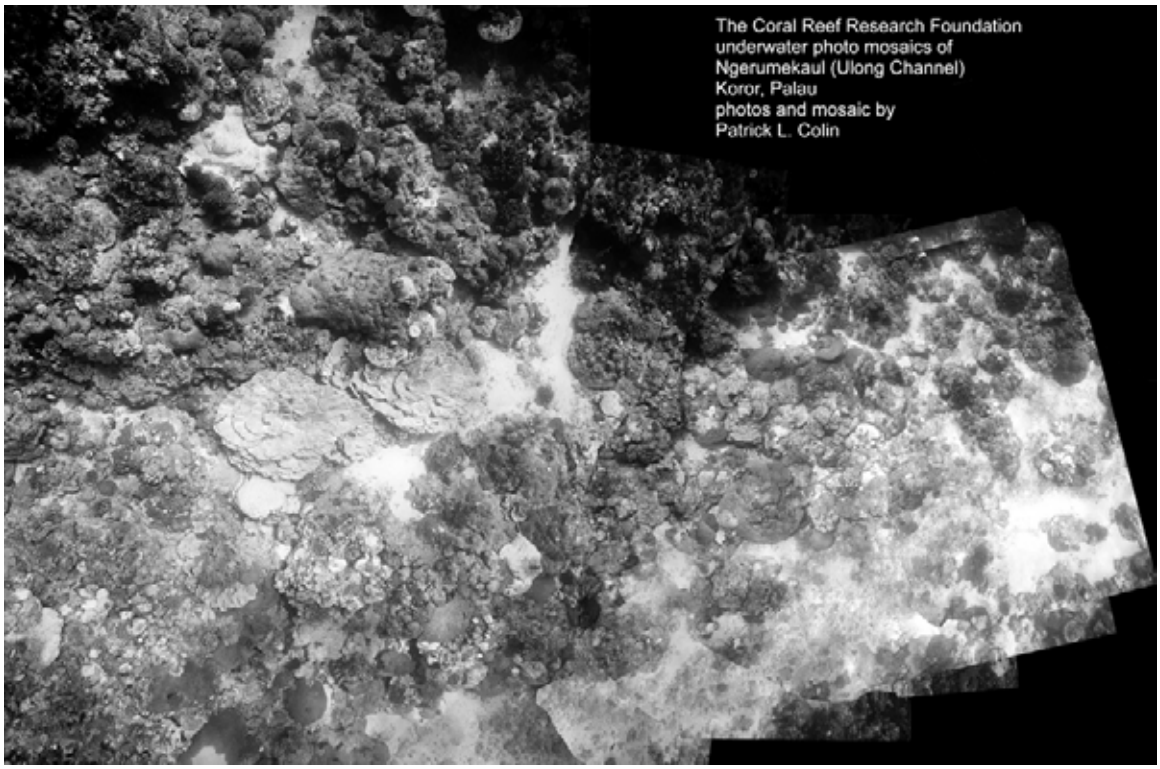


Figure 11. Vertical underwater photomosaic of area of reef in Ngermakol (Ulong Channel), Palau, a grouper aggregation area (PLC)

A readily available, inexpensive marine hand-bearing compass by Davis Instruments Inc., is excellent for taking underwater bearings. The compass has a hemispherical dome with the compass card and marks for sighting along easily. Interestingly, the liquid-filled clear plastic dome effectively disappears to sight once underwater, so it appears the compass has lost its dome (although this is hopefully not the case!) when using it underwater. Diving compasses can also be used, but be careful that the compass is sighted directly along the line for accurate bearings.

Back on shore, the survey lines are plotted out, using something as simple as a protractor and ruler, or as complicated as a computer mapping program. Then the features are sketched into place, a suitable scale added and other features included. Most underwater maps, if the maker wishes high accuracy, usually require subsequent refinement through more surveying underwater (see Fig. 12). A general, more simple, map, though, might be useable after a single survey.

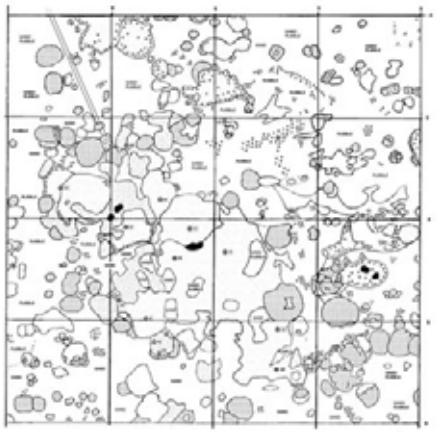


Figure 12. Detailed underwater survey map of a spawning study site of a small fish species, Koror, Palau. Each square is 1 meter square and shaded areas indicate different substrate features such as coral type. Surveys can be done with tape and compass, plotted and then refined over many hours. This process can be quite time-consuming, however. (George Mitcheson, unpublished)

In some cases, it may be of interest to map out the limits of the fish present in an aggregation area, but such a survey may not be feasible while the aggregation is present. Painted rocks, or other small heavy distinctive objects can be dropped on the bottom while swimming around the limits of the aggregation, and then surveyed, using the line and compass techniques at a later time.

It is often advisable to leave some sort of permanent reference markers on the bottom to aid in relocating a site later and quickly orienting. It is amazing how quickly the memory of a reef site can fade and make it time consuming to get reoriented. If it is aesthetically feasible (the site is not a tourist dive area) markers of some type can be left between seasons. Subsurface floats are often useful to mark an area, and various types are available. In general hard plastic floats, such as those used in the fishing industry as net floats, are best. They are pressure proof to considerable depths and have an eye for a line built in. They should be attached via a line to a weight on the bottom. The line should be heavy enough to withstand chafing, surge and motion of the float. Ideally it should be abrasion resistant and, if carefully installed and situated, it will last for years. Soft inflatable floats are convenient, but should be avoided unless the install is just temporary, a few weeks or less. Perhaps a series of substantial float anchors could be installed and the floats removed at the end of a field season, for reinstallation the next year. Where standard materials are difficult to obtain, blocks of polystyrene foam can be useful as floats for short term marking, but should be removed at the end of the study.

If visually acceptable, concrete blocks can be left in selected areas as permanent markers. They won't float away, their man-made shape is easy to recognize even years later, they are readily available in most countries, and are cheap and non-toxic. They are light enough to be moved underwater by a diver inflating their buoyancy compensator. If located in sandy patches away from reefs they can be very easy to find. Floats can be tied directly to the reef, as is often

done, but must be positioned carefully to avoid chafing of the line against the reef and possible damage to coral or other benthic life. Floats attached to concrete blocks are a good system as long as chaffing of lines is minimized. This can be done by tying lines through the holes with no slack and using abrasion resistant line.

Whatever system is used, you should try to have markers that are easily found and seen from a distance underwater. Nothing is more frustrating than spending your precious minutes on an aggregation searching for the start or end point to transects or other reference markers. In all cases, remove all temporary markers once the study has been completed.

IV. B. Censusing Fish at Aggregation Sites

While seemingly a straightforward task, counting aggregating fish and calculating the increased densities that represent an aggregation can be a challenge in both design and execution. Nonetheless, the challenge is well worth addressing. The information to be gained, especially if part of a long-term monitoring program, can be very valuable for gaining an understanding of changes in numbers over time as well as better understanding the nature and dynamics of the aggregation itself. Remember that in order to identify a site as a spawning aggregation site, surveys of the area **outside** of the spawning aggregation may also be necessary. In any event, the methodology discussed in this section can be adapted to both aggregated and non-aggregated fish.

There are three approaches to assessing numbers of fish in aggregations, underwater visual census (UVC), collection of fisheries dependent data and remote surveillance techniques. Each has its advantages and disadvantages and each must be interpreted with an understanding of associated limitations and biases. In this section we will cover UVC and remote surveillance. Monitoring via the fishery will be covered in Section VI.

Why is it such a challenge to meaningfully monitor aggregating fishes? In the first place the diving conditions are often difficult; deep water and limited bottom time, at dusk or even at night time if spawning is to be observed, 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/density 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 (e.g. *Epinephelus guttatus*). There may be diurnal patterns in density or total numbers at specific aggregation sites (e.g. *Plectropomus leopardus*). Numbers, density and sex ratios can change substantially during the days leading up to spawning (e.g. *E. polyphekadion*) or at any one moment at different places within a given aggregation. The timing of aggregation formation of the same species can vary even within different aggregations located within 20 km of each other (e.g., *P. areolatus*). 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.

This section covers different approaches available for measuring fish numbers and assessing density, the biases involved and problems associated with the various approaches. It covers the considerations essential for developing a robust, 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. We also touch 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, and is reflected by the variability of the estimate). We outline details of the methods that have been used or could be adapted to count fish in aggregations and discuss their respective merits and demerits, discuss the question of validating fish counts, address sources of error and identify some solutions to problems raised. We also provide illustrative examples (boxed) of different approaches, whenever possible, that have actually been applied and published in the scientific literature. We finish with a bulleted summary of the key points to consider when designing and implementing a monitoring program based on UVC or remote surveillance.

A Note of Caution

Given the many problems of assessing spawning aggregations, we can not, unfortunately, provide an easy step-by-step guide because one size can not possibly "fit all". Each field situation is unique and the available techniques need to be understood and adapted to each different circumstance, bearing in mind the considerable natural variability expressed even within single aggregations of single species. What we provide are **guidelines, examples and options**. To be valid, the sampling protocol must be properly designed and repeatable, not easy to achieve in the underwater environment, under any circumstances, let alone under the dynamic conditions often associated with and exhibited by spawning aggregations.

Estimating the Number of Fish by Underwater Visual Census (UVC)

If aggregations are small or fish are few, it might be possible to count all the fish. However, most cases are different. When there are too many fish to count, an estimate of the total number is achieved by assessing the number of fish in a small and known area of an aggregation and factoring up these numbers by an estimate of the total area over which the aggregation extends (see below). As a general rule we can say that most studies that have been successful in obtaining high confidence data on relative population numbers and density have either limited the area surveyed, if the overall aggregation is large, or had relatively low numbers of fish. However, in most cases, we must accept that such estimates have a high, and unknown, error value. There is no easy way to check the accuracy of such estimates, except by photographing or video recording the entire extent of an aggregation and subsequently counting all the fish present (which doesn't include those temporarily hiding or outside the area), or by having fishers capture nearly all the aggregation which can then be documented (posthumously). Neither approach is likely to be possible under most circumstances, nor, is the latter case desirable (and would certainly not be repeatable).

Methods of assessing the numbers of fish in an aggregation by UVC fall into two basic categories; those that are repeatable and those that are non-repeatable, or 'one-off', surveys. In all cases, basic standards for underwater sampling (e.g. English *et al*., 1994) and diving safety apply, and workers should familiarize themselves with these.

Underwater Visual Census (UVC) Methods

There are many important decisions that must be made when designing UVC surveys; **when, where, how** and **why** should the surveys be done are the questions to be addressed in the following section. It is important, however, that, before planning any survey of an aggregation, a preliminary roving dive at the site be conducted to provide basic information on extent of aggregation, depth range involved, water conditions, and to assess the order of magnitude of fish

present and their responses to divers. Without this important information, it will be very difficult to plan a safe and scientifically meaningful survey.

UVC - Repeatable Methods

Visual estimates are based on quantitative measures that include the whole aggregation or that can be interpolated to include the entire aggregation. Most of these approaches fall into the category of "transect methods" although fixed-point counts have also been used. Also crucial is some formal measure of the area of bottom being surveyed, which is where a mapping survey is useful (see below). At the least, some type of quantitative area measure of some portion of the substrate is important. For repeatable surveys to be possible, it is essential to have some method of surveying the same area during each survey. This could be based on distinct natural features on the bottom, used for reference, or permanent floats or markers attached to the bottom. If natural features are used for reference, it is important that these be carefully documented, principally through mapping of their positions, so that someone else can repeat a survey of the same area at a later date (see mapping Section IV.A).

The problem of how to sample fish numbers in an aggregation is tricky and each species and site presents its own set of challenges. At present the best surveys have yielded only an approximation of actual numbers for any aggregations numbering more than 30-50 fish. 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.

If actual numbers are considered to be impossible to count even while running transects, or if transects can not, 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, we created an index to describe the peak (maximum) number of fish observed for a given species in an aggregation at one time. 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; 5,000-10,000 fish; > 5,000 fish.

If the observer is at all serious about accuracy of counts, and sufficient funding is available, it is essential to video record the transects for later analysis while visual counts are being made. Modern video cameras require no more attention than to point and push the record button, so having the camera operate while swimming along counting fish is not a major problem. Potentially a divers' buddy could do the video recording at the same time that counts are being made. Concurrent manual counts and video taping will be useful in eventually addressing the questions of the differences in data obtained from the same aggregation by both methods. Such assessment will eventually allow us to refine our methods for doing surveys of aggregations.

Transects and Underwater Visual Census by Moving Divers

Here we summarize the basic techniques and principles applicable to underwater visual census surveys. These need to be carefully adapted to each situation and species but always with an eye for repeatability, scientific rigor and type of statistical analysis to be conducted (if any). Careful questions also need to be asked prior to starting such surveys regarding the specific objectives to be achieved. Most frequently, these will be questions of aggregation size (in terms of fish numbers or density) but there may also be interest in recording social structure, changes in fish numbers or diversity during the day, or over the course of the aggregation, etc.

Decisions need to be made regarding the **size** and **number** of sampling units (i.e. length and width of transect and total number of transects needed), **how long** should each be and **how wide** (the latter could be determined in part by visibility and general density of fish). The effective survey width must be estimated either visually (this needs experience) or by using markers previously placed on the substrate (see below). Length of transect will be determined by the area of the aggregation or subsection of aggregation to be sampled, as well as other factors such as depth, current, etc. and it would obviously be best to survey as large a proportion of an aggregation as possible. In terms of the length of transects; in most cases these will be used to sample larger, more mobile reef fishes in aggregations. As a rule of thumb transects should be at least 50 m long (Melita Samoilys, pers. comm.).

If there are relatively few fish and the aggregation area is small, then one transect that covers the entire aggregation area could be used. If the same transect path is followed every time and enables counts of all fish present then these counts would be comparable between different points in time. While producing very useful data, a single transect per aggregation (i.e. a sample size of N=1 transect) would not allow data to be compared statistically between aggregation periods. This may or may not be important but does need to be considered for long term monitoring programs.

If more than one replicate is needed because not all fish can easily be counted or the aggregation is large, then a decision is necessary on **how many** replicate transects must be run to provide representative counts from a given aggregation site. It is important to run sufficient replicates to account properly for variability between transects that might reflect different densities throughout the aggregation and produce a representative transect count (this applies to cases where the fish are not randomly distributed which may require a stratified sampling approach whereby sampling effort is stratified according to factors that may influence fish numbers over the aggregation such as differences in habitat or depth). To determine the necessary number of transects, a preliminary study should be carried out, which involves more transect runs than you think you will need, to produce a simple plot of cumulative mean densities (per transect) with increasing number of transects run. With the data, plot a graph (Fig. 13) of the

Transect Count	Cumulative Mean
100	100
200	150
75	125
150	131
227	150
148	150
59	135
89	131
162	134
112	132
105	130
191	135

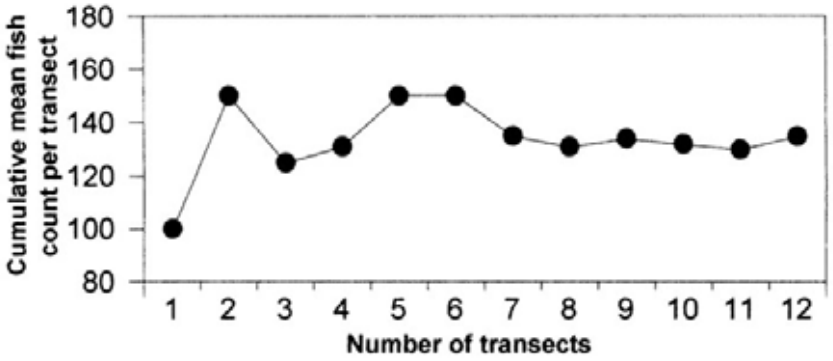


Figure 13. Example using cumulative transect counts to determine the minimum number of transects necessary to run on a given aggregation to obtain a representative fish count per transect. A similar plot of standard deviation (SD) can also be calculated from the column of transect counts to provide an indication of precision using a formula, (SD/sqrt n)/mean, where n is sample size, which could be used to compare with the precision of other similar data. (YS)

cumulative mean counts of fish per transect (this means that, say, after 4 transects, you sum all of your counts for the four transects and divide the total by four). Plotting this information will yield a graph that will indicate when the means are beginning to stabilize. In this case, and with the variability in the data provided, 7 or 8 transects are needed for the cumulative mean transect fish counts to become stable. This means that, in this case, a minimum of 7 to 8 transects were needed to assess the fish number and density at the time of the survey. The results provide a measure of average density for the aggregation at the time of sampling (remember that density can change, however, from morning to afternoon of the same day and between days – see below under **when** to survey) and some measure of variability (by calculating the overall mean and standard deviation from all transects run). In non-aggregation survey work, and in the absence of preliminary data, a 'rule of thumb' suggests that about 10 replicates are advisable (Samoilys, 1997a). Multiple transects can be run by a single diver or involve multiple divers.

Decisions also have to be made regarding **where** to place transects within an aggregation. There may be little choice because of depth constraints or because of the shape of the aggregation that may, for example, follow a shelf edge contour or run along the walls of a reef channel or slope (Fig. 14). Certainly transects should be placed in areas that appear to be representative but this may be very difficult to judge. Whatever are the practical considerations in the laying of transects, their placement should be systematic. If there are clear differences in densities around the aggregation, then the best type of design is random stratified sampling but in this case, the strata need to be identified (e.g., core area [see below], depth range, shelf edge contour, etc) as already discussed.

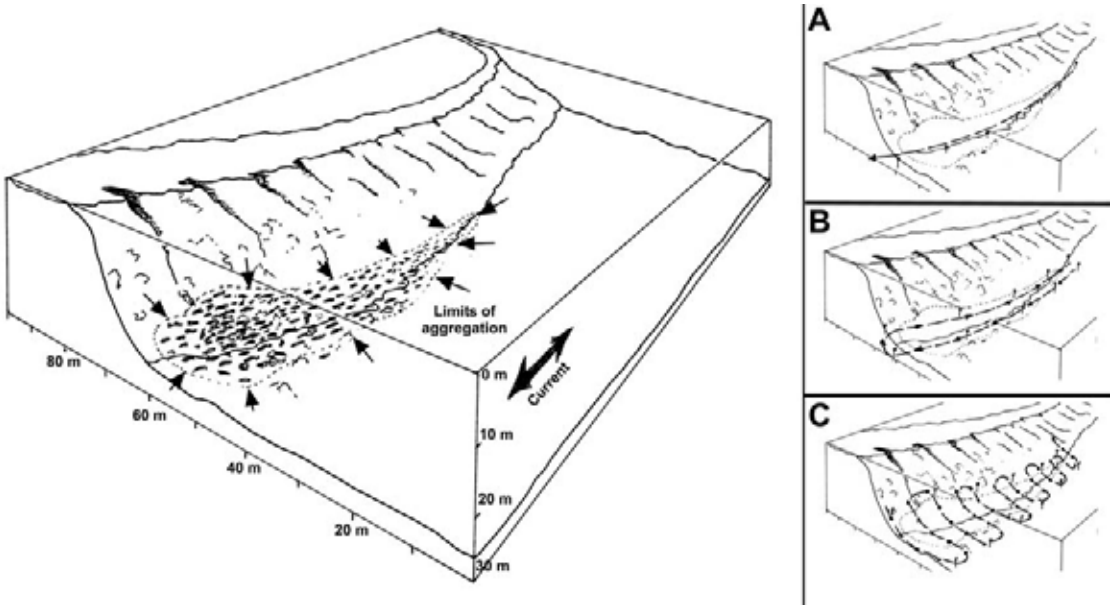


Figure 14. Typical reef slope with hypothetical aggregation showing types of transects that could be run (right panel). There could be a single transect (A) run in the direction of the current, a deep transect run first in the direction of the current and returning in shallower water upcurrent (B), or a series of transects up and down the reef slope working along the slope with the current (C). All transects are run relative to marker buoys set up on the bottom and long transects (such as A and B) would be counted as segments of equal length (PLC)

One approach that has been used in large aggregations, or where the entire aggregation is not accessible to divers, is to select a core area (i.e. a specific stratum) that appears to contain the densest concentration of fish, and follow trends in core (maximum) density within an aggregation

period as an indication of aggregation build-up (e.g. Rhodes and Sadovy, 2002). This provides a maximum density measure at the time of sampling and may include a large proportion of fish assembled in the aggregation. However, care must be taken in applying the data collected from core counts to the aggregation as a whole since core densities are less useful than average densities for estimating total aggregation numbers. This is because lower densities of fish occur in non-core areas and the core numbers can not be assumed to represent a typical, aggregation-wide, density. Importantly, it is also possible that it may not provide a good indication of changes in aggregation numbers over time. This is because of the possibility that, even if the total numbers of aggregating fish decline (say due to fishing), a core of consistent maximum density might still continue to form despite an overall decline in aggregation area (and fish numbers). At present we do not yet understand enough about aggregation dynamics to understand the significance of such 'core' measurements and so these must be carefully reported and interpreted accordingly. Further research might elucidate the significance of such cores but their use for monitoring should only be considered to address certain specific questions. This example also highlights the need to clearly establish **why** a survey is being done since this could substantially influence the survey approach taken.

In general, considerable care is needed at each decision point when designing a monitoring program for aggregations, taking into account the incredible variability of aggregation density that can occur over time and location, even within a species. Every attempt must be made to design a method that is repeatable, representative and provides some idea of precision. If not, the data obtained may be of little value, and the money and time spent getting them squandered. Attempts should also be made to evaluate accuracy by independent means although in many cases this may not yet be possible.

Underwater Visual Census and Stationary Divers

In some situations, possibly because of strong currents or because fish might be wary of divers in close proximity, it may be preferable for the diver to hold a fixed position and remain stationary. As one example, observers may remain tethered well above the substrate at a fixed location recording all fish within a prescribed area within view. Such tethering can save the diver energy and allow longer survey periods (since the diver stays in shallower water) than if swimming along the substrate. The influence of divers on fish has been demonstrated in non-aggregation studies (Watson *et al*., 1995) and needs to be considered when planning aggregation surveys.

UVC can be conducted by what are known as 'point counts' which involve a stationary diver who slowly turns through 360 degrees counting every target fish out to a pre-established, and known, radius. As for transect width, this radius will have to be adapted to local conditions of visibility and fish density and is unlikely to be more than a few meters for aggregation studies. As for transects, replicate counts should be made at the same site and also consideration given to conducting point counts at several places randomly or haphazardly selected within the general aggregation area. Replicates in the order of 10 should be considered if possible (Samoilys, 1997a), in the absence of preliminary surveys. For point counts it is also necessary to determine how long a single 360^{o} rotation should take; again, preliminary trials should ideally be conducted to ensure that rotation is slow enough to count all or most fish but not so slow that fish are double counted. An excellent reference for stationary visual census is Bohnsack and Bannerot (1986).

As for transects, the number of replicate point counts must be determined. With insufficient samples there is not enough power to distinguish between mean measures from different times and places. On the other hand, effort and funds are wasted if many more samples

are taken than are needed for scientific rigor. Methods for determining adequate sample sizes are discussed by English *et al*. (1994) and Samoilys (1997a) and references cited therein and should be consulted. The good news is that in most cases, fancy models are not needed, just good planning and design. The bad news is that there is no 'sure' formula for aggregation surveys that must be adapted to different circumstances and constraints.

How to Measure Aggregation Areas

If there is interest in assessing overall aggregation numbers, then at some point, the extent of the aggregation site must be measured. Many times it is most convenient, particularly when an aggregation is of limited duration or fish disturbed by too much diver activity within the aggregation site, to mark the edges of the site in advance, or at the time, by some type of marker that can be found later. The extent of the aggregation can be determined later based on the location of the markers, and an underwater survey done with compass and tape. If an accurate map is already available for the bottom, the edges of the aggregation could be plotted on that relative to known locations indicated on the map.

Markers could be several types. Painted rocks have been used and different colors could represent different information, such as markers placed on different days (Shapiro *et al*. 1993). Small lead fishing weights could be similarly used, and short lines with floats could be added to weights to allow the markers to be more easily found and surveyed. If lines with floats are run to the surface and the area of the aggregation relatively large, the locations of the markers could be determined by GPS receiver from a small boat, and a rough area, within the limits of GPS determination, could be made (Rhodes and Sadovy, 2002). Markers can also be used to indicate the locations of specific fish, as was done by Shapiro *et al*. (1993), which are later analyzed for spacing and social structure. Similarly, the location of a single fish, such as a large identifiable (note that, for many species, slight natural differences in body markings make it possible to distinguish between different fish) male grouper, might be marked at regular intervals during any given time period (hours, days, weeks), by placing marked weights each time.

SOME CASE STUDIES

There are many ways in which a diver or divers can survey an aggregation site for numbers, density, distribution, etc. of aggregated fish. For example, multiple divers could follow parallel transects within marked-out areas covering a significant proportion of the aggregation, and pool their data (Shapiro *et al*; 1993). Alternatively, one or several divers could randomly survey transects across an aggregation site to produce replicate transects during single aggregation periods or multiple estimates at the site throughout the year (Samoilys and Squire, 1994; Samoilys, 1997b; Zabala *et al*. 1997a, b; Rhodes and Sadovy, 2002). In one case, multiple divers sampled different areas of an aggregation site and later validated their counts using video footage (Sala *et al*., 2001). Specific cases are given of methods used and how these have been adapted to local situations, but original papers must be consulted for full methodology of survey design.

Shapiro *et al.* (1993) surveyed a large grid of roped squares each 33 m on a side that were, over a number of days, built up to seven square sections over a representative area of a red hind, *Epinephelus guttatus,* aggregation site. Squares were roped off into 8.25 m wide lanes and four divers were used to simultaneously swim across the entire grid in the four lanes. To determine the internal aggregation structure of fish, a colored rock was placed on the bottom at the location where a red hind was first seen and different colored rocks were used on different days. After the aggregation dispersed the position of each rock was determined by measuring the distance from the rock to the two nearest boundaries of the grid. For each day, the distance from

each fish to its nearest neighbor was determined and the mean observed nearest neighbor distance was compared to the mean distance expected if all fish were randomly distributed within the grid. Internal structure of the aggregation was initially estimated by counting the number of individuals in 34 clusters (a cluster was defined when individuals were within 3 m of each other). The sex ratio in 12 clusters was examined by spearing all 34 individuals within those clusters.

Samoilys (1997b) and Samoilys and Squire (1994) studied coral trout aggregations at Scott Reef (Samoilys and Squire, 1994) and Elford Reef off Cairns, northern GBR. Fish were surveyed visually over a fixed route for 25 minutes. Areas of aggregation were 1700 m^2 for Scott Reef and 3200 m^2 for Elford Reef. Counts of fish ranged up to 130 fish at Scott Reef and 60 at Elford Reef. The density of fish was also relatively low, at most 44 fish per 1,000 m^2, a level at which fish can be easily tracked individually. Counts were conducted with a minimum visibility of 7 m. Regular monitoring was conducted year round at dusk around the time of new moon monthly during the spawning season (Aug to Dec) and bimonthly at other times. During the spawning season, surveys were done weekly or biweekly at various stages of the lunar cycle. During surveys, the observer counted and estimated size of fish seen, while swimming a standard search pattern for 25 minutes. This took the observer around the aggregation site at a slow but steady pace. The observer could search a width of about 10 m, swimming about 1.5 m above the bottom. While the fish may move during the course of the census, the inaccuracy introduced was considered acceptable since the bias was present on all surveys. The location, probable sex and size of fish were noted on the map. Sex was associated with specific color phases after confirmation of sex and color by spearing selected fish for dissection (Rimmer *et al*., 1994).

Zabala *et al*. (1997a and 1997b) used two different transects, one to estimate changes in density year round (200 m long by 10 m wide swum at mid-day) and the second to survey different depths and topographies where groupers occur (100 by 10 m swum a various times of day). These provided consistent, repeatable censuses for their work on the dusky grouper, *Epinephelus marginatus* in the Mediterranean. The first transect usually took about 40 min to swim while the second took about 20 minutes. Fish numbered up to about 40 per survey on transect 1 and up to 32 on transect 2. These numbers are easily and accurately counted, but the density overall is lower than that found in many tropical species, such as the Nassau or camouflage grouper for which this method is less likely to be applicable (see Fig. 6).

Rhodes and Sadovy (2002) did morning and afternoon visual censuses of an *Epinephelus polyphekadion* aggregation in Pohnpei, FSM. The fish were concentrated along a slope-reef wall interface. Because the main part of the aggregation was at 30-35 m depth (severely limiting time for the survey), censuses were done in the direction of the current (typically along the reef) from one boundary of the aggregation to the other. The limits of the aggregation along the slope-reef interface were determined by putting surface marker buoys at those limits and later determining the positions of the buoys using GPS. An area of known size was used to count numbers of fish on each survey, providing data on trends in core (maximum) density for a given monthly aggregation

Sala *et al*. (2001) conducted daily UVC on Belize Nassau grouper aggregations starting before spawning and before fishing activity began. Their census protocol had three divers and each diver surveyed different portions of the overall aggregation area. Groupers were found on coral ridges (spurs) and each ridge 'population' was counted. Shelf edge areas were counted using continuous 75 by 20 m transects within the actual spawning site covering approximately 1.5 ha. There were 1-3 large grouper schools within the aggregation; counts of groupers in the water column were carried out for each grouper school by each diver separately and later averaged across all divers. To evaluate estimates of grouper school size, schools were videotaped using

digital video. Counts of each school were made on a monitor screen for comparison with UVC data.

Sancho et al. (2000) used a fixed area of 170 m^2 within a larger spawning area to characterize fish abundance and quantify spawning activity. Observations were always done by one person to reduce inter-observer bias with the diver's recording position kept constant, lying on the bottom and 5 m away from the edge of the sampling area. Spawning observations were noted every minute during 30 min observation periods. The total number of spawning fish was estimated for 34 species at the beginning and end of each spawning observation period. However, only data from the 11 most active spawning species were reported.

Non-repeatable Survey Methods

Non-repeatable surveys can be undertaken, but their application to long-term study of spawning aggregations is limited. This is because they are either destructive (i.e., involve removing all fish) or because the survey design does not allow repeatability. A good example of this is the "timed swim" in which a diver visually counts the fish occurring along a swath of bottom, usually only a few m either side of the diver, determined by water clarity and bottom communities (Sluka, 2001). The diver swims for a set amount of time (generally 10-20 minutes, but in some cases up to an hour), rather than covering a certain and known distance, and counts all target fish(es). If the diver swims at 1 m min^{-1} (an average sort of speed while doing another task like counting one type of fish, and assuming no current), the diver would cover about 100 m in 10 minutes, with a swath width of 10 m, equaling 1,000 square meters. The direction of timed swims is "random" or more typically “haphazard” but in either case swims can easily cross habitat boundaries, with consequent differences in fish density. In the case of aggregations, timed swims may take the observer into or out of the area of an aggregation. If the diver swims to stay within the aggregation, then the direction is no longer random. In many cases the presence of the diver will have a major effect on the fish of interest, perhaps scaring them out of sight (before the diver ever sees them) or into hiding, or driving them along the direction of diver movement. This was the case in Palau for *Cheilinus undulatus*, in which both snorkelers and SCUBA divers swimming along the reef would drive all the fish present ahead of them, making it impossible to obtain anything approaching a reliable count of numbers. In this case, it made no difference what method was used to survey the fish, which simply could not be counted accurately by a human swimming in the water. For this species a stationary video camera was placed and the assembling fish were clearly visible in the absence of humans (see remote surveillance below) (P. Colin, unpubl. data).

The true “timed swim” is one type of non-repeatable method that is not really very useful for quantifying the dynamics of aggregations. If the same route is swum each survey, by marking it by perhaps dropping painted stones, or taking exactly the same route each survey, then data between surveys would be roughly comparable. However, if the route is marked, there is no reason why it can't be measured at some point to arrive at a density of fish, as long as the same distance is swum each time in the same manner (direction, speed, water clarity and others). Given the many drawbacks associated with the timed swim, added to limitations of visual counting and others, it is dubious to base any statistical calculation regarding changes in aggregations on timed swim data. At best they are semi-quantitative and subjective. The timed swim can be improved somewhat by using a video camera to record what the swimmer is seeing, as at least the recording can provide a general impression of the abundance of fish and the conditions during the timed swim.

Of course, surveys may also be unrepeatable simply because they are poorly planned or documented. This type of survey is, of course, never recommended!

Remote Surveillance Techniques

Imaginative use of video techniques may hold promise for providing some answers to the questions of absolute abundance in the aggregation. For example, a defined area might be surveyed using normal visual census methods. Then an autonomous video camera system could be moored above the site (if the water visibility is suitable) and it could monitor the fish in the site over a given period of time. This has yet to be done, but is entirely feasible.

Some video techniques might provide useful information, particularly as the systems envisioned can function without a human present, eliminating most of the "human presence" factor. An autonomous video system mounted on a tripod that provides a view of an aggregation area could sample the aggregation at various times by recording for different periods during the day. Such a camera is limited in its field of view, but such a view would be consistent over time. Another possibility would be to "mount" a video system above an aggregation area, sort of an "eye in the sky" approach. The buoyant system would be moored on a three or four point mooring directly above the reef pointed straight down. The camera should be high enough to cover a broad area and low enough for the fish to be identifiable. It could be started to run continuously or connected to an Autonomous Underwater Video System (AUVS) timer. This and the mounted, unattended camera method have potential to address the "hidden fish" (see below) question, a big factor in visual surveys.

A useful method for perhaps covering an entire aggregation in a reproducible manner would be to mount a video system vertically on the front of a Diver Propulsion Vehicle (DPV), then from a height (depth) well above the aggregation, video record the entire area of the aggregation, making multiple passes if necessary over the site, until the entire area has been well covered. Tapes would be analyzed later and density data could be extracted also, if the area of the bottom (through a previous mapping survey?) was known. Such a method would depend on water visibility and behavior of the fish concerned.

When Should Monitoring be Done?

Having decided why, what and how to survey, decisions must also be made regarding **when** surveys should be conducted. If aggregations typically occur at the new moon, for example, then this would be an obvious period to concentrate monitoring activity but we have to first discover when an aggregation is most likely to occur. Moreover, we now know enough to say that timing of spawning can be quite variable, even for given species at particular spawning sites, both within and between years within species, such that monitoring should initially be done at different moon phases and in the typical non-spawning period to ensure that important information is not missed. We know, for example, that aggregation timing for a given species can vary within a country in a given year (e.g., Johannes and Lam, 1999; Johannes *et al*., 1999), occur in slightly different months in different years (Rhodes and Sadovy, 2002) or even at different moon phases (e.g. Sadovy *et al*, 1994). Such examples warn us to be careful in determining timing of monitoring studies. Moreover, if aggregations form in different months each year at a particular lunar phase, do we sample them in all months (the decision will reflect why the survey is being done)? We do not yet know whether the same fish spawn each time or whether different individuals might be involved in different months of the same year. Whenever the timing of aggregation is not well-known, sampling of markets and discussion with fishermen may provide additional information useful for planning monitoring activities. Decisions also

have to be made regarding what time of day to monitor an aggregation given that fish numbers can vary markedly at an aggregation site over the diel cycle. Preliminary studies may be necessary if a species is being studied for the first time.

Sources of Errors

There are many possible **sources of error** in assessing fish numbers underwater, even when a monitoring protocol has been properly and carefully designed. These sources of error include, but are not confined to, substrate complexity, fish behavior, between-diver differences, aggregation types (some types are easier to assess than others), and double-counting (i.e., counting the same fish twice).

The number of holes and other shelter available for fish can have an important effect on counts of fish found at a given site. Since most aggregations occur where there is significant coral cover, often with an abundance of hiding places, fish that remain within crevices, under ledges and otherwise hidden would produce an underestimate of the actual numbers. As an example, Sadovy *et al*. (1994) examined an aggregation area of *Mycteroperca tigris* off Vieques, Puerto Rico, in which the bottom consisted of layers of elevated coral heads that provided a huge number of hiding places and made any meaningful counts of fish abundance impossible. The number of fish hiding may also depend on the time of day, particularly for predators like groupers, and the disturbance effect of human presence. Without extensive study, it is not possible to say exactly how many fish are hiding at any given moment, although as already discussed, remote surveillance might be of value here.

A detailed discussion and attempt to deal with errors of hidden fish, or undercounting and double-counted by divers, on a species-specific basis was made by Johannes *et al*. (1999). Their solution of applying an exact percentage "correction factor" for hiding and double counts of each species was based on qualitative observations of fish behavior. To apply precise "correction factors" to counts of estimated numbers of fish (which may be inaccurate by a considerable percentage), however, confuses the issue of aggregation size. A more satisfying approach is not to attempt to apply correction factors, but simply state that a portion of the fish were missed or double-counted. The same study compared snorkel and SCUBA estimates (although not from the same time period) and tried to determine a sample design that minimized the amount of field time (and hence expense) necessary for the study objectives. The analyses were only variously successful but are interesting in their attempt to deal with some of these important issues.

Errors can also be introduced by what divers themselves are doing. For example, errors attributable to different divers being involved in monitoring (inter-diver error) are probably typically underestimated and rarely evaluated but can be important and could certainly be readily addressed by appropriate survey design. Many fish species appear to be affected by the noise/bubbles made by SCUBA gear. We may find that the introduction of rebreathing dive equipment may eliminate some of this diver error. See elsewhere (remote methods) regarding getting rid of divers altogether!

Finally, some aggregation types are simply more difficult to survey than are others. The hardest are those that involve very large numbers of fish packed densely and sometimes in 3-dimensions, coming up into the water column, or species that spread out over a large area and are very wary of divers (e.g. *Lutjanus analis*). Smith (1972) estimated at one site that the number of Nassau grouper was anywhere between 30,000 and 100,000, a massive range in estimated numbers. Species of acanthurids or lutjanids can likewise be very difficult to count while aggregated – there is a need for some bright biologist to develop survey methods applicable to

such species. One possible approach might be release a known number of tagged fish into an aggregation and film or photograph the aggregation. If random mixing of tagged animals is assumed then an estimate of total aggregation size could be made by working out the proportion of tagged to untagged fish. There are likely to be some useful applicable methods from the bird literature that might be adapted for counting aggregating fish.

Validating Counts and Data

In the end there are few ways to **validate the accuracy** of any fish counts in aggregations even for properly designed surveys and hence interpretation of data must be made accordingly and carefully. When a permanent record of fish present is made, like a video survey of an aggregation, there is some ability to refine numbers by repeated analysis of tapes compared to numbers that might have been obtained in real time by counting fish (Sala *et al*, 2001). Even the best video survey and UVC, however, have to deal with the problem of fish that hide during the survey or taping and those that move can easily be double-counted.

Despite all these efforts to reduce errors, there are some species that are simply not going to permit a human being to accurately count them. For these fish, we have to accept that counts done by divers are highly inaccurate (but may reflect relative numbers over time), or find alternate methods to census them. For difficult species, video surveying with a remote system may be a solution. In these cases fishery dependent methods may be more revealing (Section VI.A).

UVC AGGREGATION SURVEY DECISION TABLE
FOR REPEATABLE SURVEYS

Why? What is the purpose of the survey? Must it be repeatable? **When?** When in the year, month and day should the surveys be conducted? Are non-aggregation surveys also needed for reference? **Where?** Survey the aggregation site only? Survey both aggregation and non-aggregation sites? Survey the entire site or only part of the site? Which parts to survey – a core area or along randomly placed transects? **How?** First of all a preliminary survey of the site is needed to determine, depth, extent of aggregation, order of magnitude of fish numbers, effects of divers on fish and fish hiding behavior, etc. This information is critical for properly designing subsequent surveys.
(ii) Swimming
Transect; width, length, replicates (one or more), stratified sampling? (ii) Stationary Point count: speed, radius, replicates (one or more)

Other General Considerations for Survey Decisions

1.Consider potential sources of error (inter-diver variability, effects of diver on fish, double-counting, hidden fish)
2.Determine statistical approach to analyze/compare data
3.When fish numbers are high, consider using an 'abundance index'
4.Carefully document methodology so that others can repeat surveys and try to include a map of the site
5.Attempt independent assessment of fish numbers to assess accuracy, i.e., validation (e.g. underwater video)

Collecting Size and Sex Data from Aggregations

It may be possible to obtain size and sex (and thereby sex ratio) data from UVC surveys but considerable care must be taken in so doing and in clearly understanding the possible, and potentially considerable, errors that can be introduced by injudicious interpretation of results. For example, size data may be used to plot size frequency distributions of fish within an aggregation. While we recognize that it is reasonable to obtain some relatively crude information from estimates of fish size from aggregations, such as minimum and maximum sizes, and a general idea of size distributions subject to the caveats below, using such estimated information for performing comparisons between different aggregations and for the same aggregation over time requires caution. Data on sex of fish collected by UVC depends on earlier work linking particular color phases or behaviors with certain sexes, and for determining sex ratios there has to be some degree of confidence that it is equally easy to survey both males and females.

Size - care is needed in interpreting both the fish size data obtained by visual estimation underwater and the significance of the fish size data. In the first case, it is important to note that errors in fish length are involved each time a measurement is made. These can be several cm for a fish, or more, and may be different for different size classes. The use of calibration rulers can improve the accuracy of in-water length measurements and without such sticks divers may obtain a mean accuracy of 86% (St John *et al*., 1990). Nonetheless some error is likely to be involved. In the second case, we need to be cautious as to how the size data are interpreted, especially given the measurement error that might be involved (see Section IV.C for further discussion of this point).

As long as the limits to visually determined size data are properly understood, meaningful comparisons of fish sizes over time or across locations are possible under the right circumstances. For example, Samoilys (1997b) did such a comparison between two reefs for size of *P. areolatus*. However, she had long experience with the sites and fish, and was dealing with only one species. In such a case, this comparative use of data can be applied with some confidence, but this study serves to point out the need for long and careful work to be able to apply size estimate data in any but the most rudimentary way. The problem with size estimate data is that, except in the case where a significant sample of fish can be measured from captured individuals, there is no true way to verify the accuracy or precision of such data. Training of observers, through wooden models of fish or other methods, is useful. However, considerable care is needed in training and retraining workers in size estimations using fish models if they are to apply their skills under field conditions and to living fish (Samoilys and Carlos, 1997). Such data can provide an indication of sizes (Fig. 15), but must not be confused with the level of accuracy obtained from specimens captured by fishermen.

Alternative techniques hold promise for providing accurate length data from aggregations, without having to resort to estimations of length by divers. Relatively straightforward is the use of a "laser scale" in which two or more laser pointers are enclosed in underwater housings at set distance(s) apart. The laser pointer effectively penetrates clear water

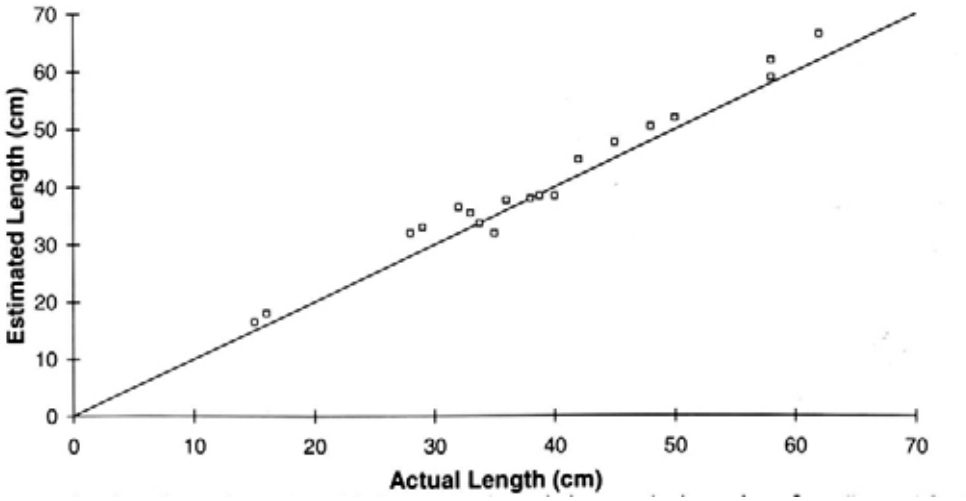
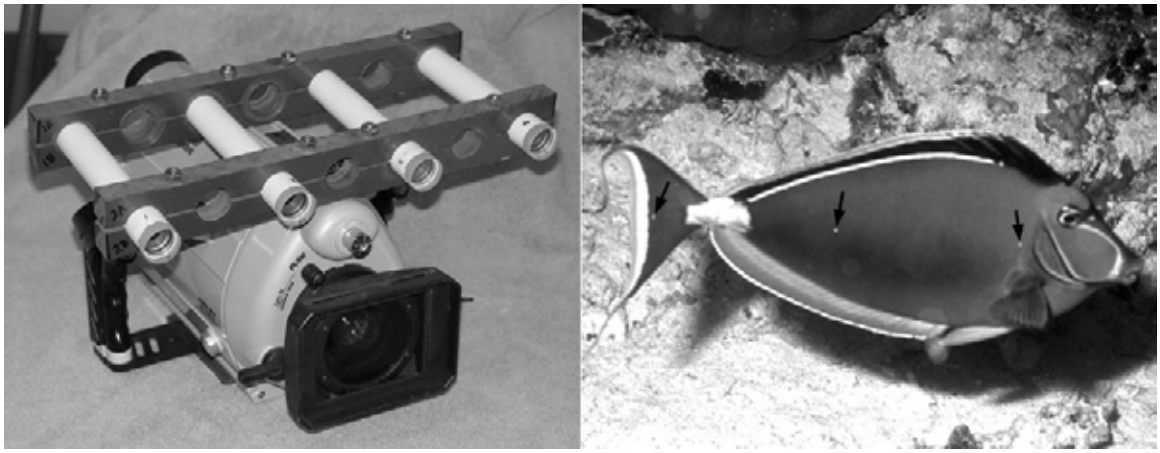


Figure 15. Comparison of estimated length by well-trained divers and actual length for models of fish assessed underwater. Redrawn from Samoilys and Carlos, 1997.

for many meters and if two or more pointers are set up parallel to each other, either on a moving bar system (calipers) or at a fixed distance separating them, they could be used to measure the length of a fish underwater without coming close to it. The fish you wish to measure is video taped with the laser scale pointed on its side and the length is later determined by using stop frame video (Fig. 16). The main drawback of this system is that the laser scale needs to be pointed essentially at a right angle to the anterior-posterior axis of the fish for an accurate length



Figures 16 and 17. Fig 16 (Left) Laser scale system on top of underwater video camera housing. The four individual laser pointers are positioned 10 cm apart and project their red laser dots outward. Fig. 17 (Right) The laser dots, 10 cm apart, on the sides of a surgeonfish, *Naso literatus.* ***The laser dots (indicated by arrows) are not particularly visible in this black and white photo. They are easily seen when in color. The total length of this fish was measured to be approximately 28 cm.(PLC)***

(Fig. 17). Any deviation beyond about 10° from perpendicular to the fish axis results in measurements that are not accurate. The laser scale system illustrated in Figure 16 can have the lasers separated by either 5 or 10 cm easily and the interval selected depends on the size of fish you wish to measure. Visual estimates can be quite accurate but accuracy depends strongly on continued training and diver experience.

Harvey and Shortis (1997) described a stereo-video system for estimating fish length and later Harvey *et al*. (2000a, 2000b and 2002) compared this system to diver visual estimates of fish length. In general, they found the stereo video system to be more accurate than diver estimates of fish length. The ability of novice and experienced divers in a swimming pool to estimate fish length, based on plastic models, was similar for models of 10-50 cm in length (Harvey *et al*. 2000a). Small models (around 10 cm) were accurately estimated for size by divers, but at lengths above about 25 cm there was considerable variation (often times 10-30% of model length) from reality in visual estimates. The means of the overall estimates of many divers did arrive at reasonably close estimates (mean error of 2.1 cm for experienced and 2.3 cm for novice divers) of model length, but individual estimates often had substantial errors. Since usually only a single observer is making size estimates in the field, the errors in such single-person estimates may often be substantial. Two or more independent estimators would be better, but may not be practical in the field. In a field comparison of the methods, Harvey et al. (2002) used three experienced divers to estimate fish lengths and found, under optimal conditions, high accuracy, but low precision to these measurements, compared to the stereo video system. They state that the estimates of experienced diver scientists have much lower statistical power than stereo-video measurements when trying to detect changes in mean length of a group of fish. If there are low numbers of fish being estimated or when the program intends to detect changes in mean lengths of 30% or less, the power is much less. These results show why all interpretations of visual length estimates must be done carefully and, despite the wishes of the observer, the human eye underwater is not a particularly accurate tool for measuring fish length from a distance.

Sex - in cases where sexes can be distinguished externally by color differences, or by distinctive behaviors, some idea of sex ratios can be obtained. This is assuming that sexes can be reliably distinguished and that both sexes are equally visible. In general for groupers, sweetlips, and some

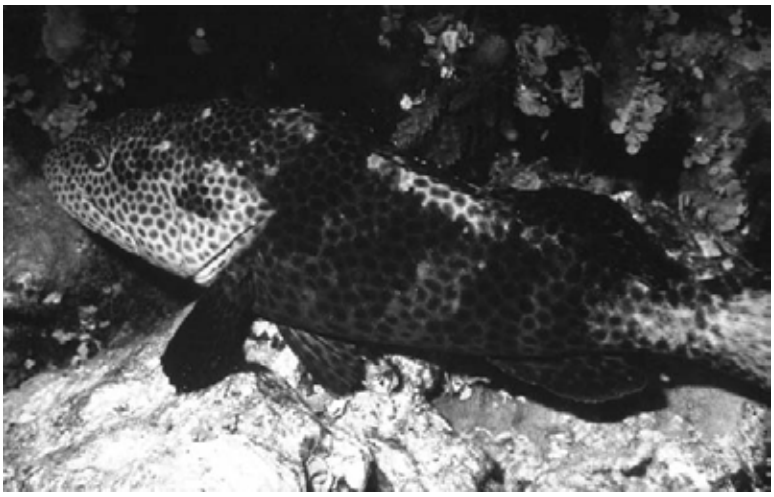


Figure 18. Grouper male, probably *Epinephelus merra****, from a probable spawning aggregation at Enewetak Atoll, date and time unknown.(PLC)***

other species, males become darker, often the black blotches, or particular color patterns are exhibited when courting and spawning (e.g. Fig. 18, Figs. 37 and 38). However, color changes can be rapid and some species may not constantly display courtship or other color patterns associated with aggregations during the time of the aggregation. While some species may not exhibit external color differences, females may become so swollen with eggs, there is little trouble identifying them (Fig. 19). Great care must be taken when associating color forms with

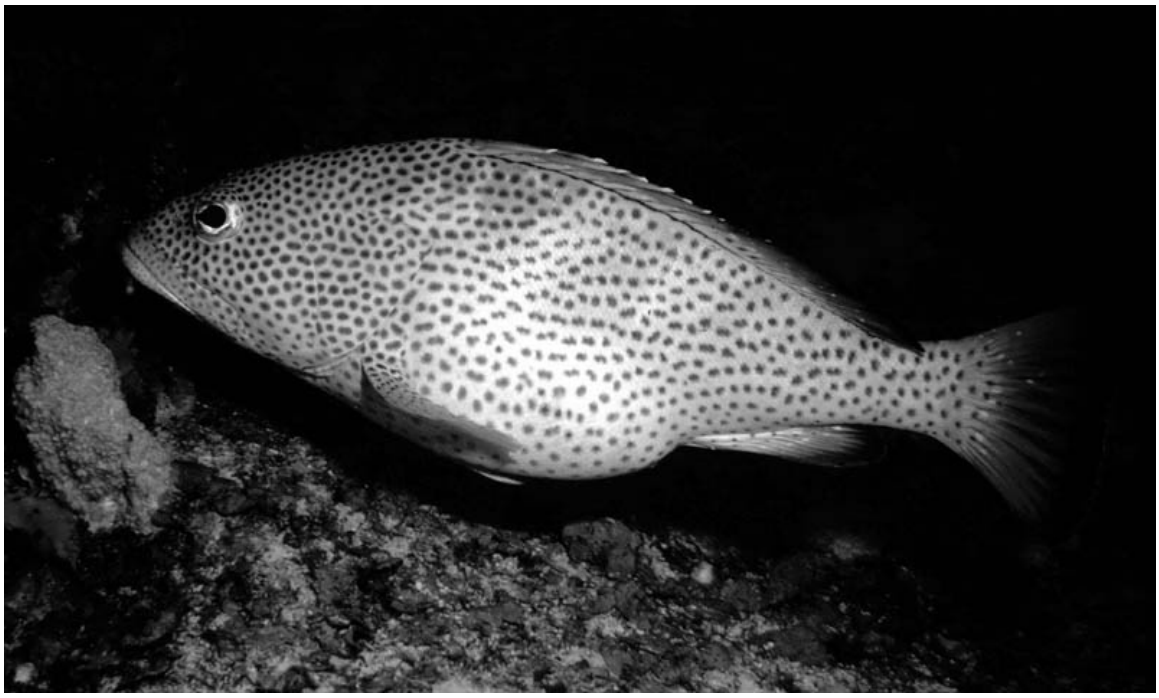


Figure 19. A female red hind, *Epinephelus guttatus****, swollen with eggs. A female like this is probably no more than a few hours from spawning, as the eggs are almost certainly hydrating and the mass of the gonad subsequently expanding. (C. Arneson)***

sex, however, and with evaluating sex ratios from visual censuses. In one study, the presence of two color forms was assumed to represent male and female when in fact both sexes displayed both colors (Colin, 1992). In another study, female behavior appeared to differ from that of males with females tending to stay more hidden and males more active and visible at the aggregation site (Sadovy *et al*., 1994). If these impressions are correct, then visual estimation of sex ratio would have been biased towards the more obvious males.

Concluding Comments

Carefully designed surveys are critical for determining numbers of aggregating fish, density, sex ratios, survey reproducibility, and to make conservation and management decisions based on that information. It is risky to draw conclusions for parameters for which you don't have data, and important to be careful how whatever data you have are interpreted. It is easy to be misled by the results emerging from a particular sampling design, as some of our examples have illustrated. Conclusions drawn from the results of surveys must recognize the limits and constraints of the survey and be made within the bounds of the sampling protocol applied. Most importantly, when designing any survey, ask yourself where, when and how the survey should be done and why it is being conducted in the first place.

Ideally, it would be nice to count all fish on an aggregation site, but more typically, subsampling is done. This can be by UVC, as we have just discussed, or by surveying the fisher catches (Section V.I). It is important to remember that any methods of subsampling will be biased and that we need to understand clearly the limits of our sample data. In monitoring aggregations of 10s to 100s of fish, total counts are very probably possible, while for 1000s of individuals, subsamples will be needed. For numbers of fish, size and sex, there are few ways of verifying data, and little check on the accuracy of data. We should, whenever possible, use data collection techniques that provide a permanent record. It is also critical to undertake surveys that are repeatable, particularly by other observers. This "repeatability by others" factor should be considered from the start in planning any aggregation survey and is the only way meaningful long-term data are going to be obtained on aggregation sizes. No matter what methods are used the data have to be comparable between years and sites at some level clearly evident to readers. The level of comparability must be understood, so that the proper interpretation of data can be made.

If we have some understanding of the limitation of the numbers we obtain, then at least we will be able to interpret and apply them in an appropriate manner. The biggest mistake would be to apply them when they will not provide a clear picture of trends in fish numbers or provide for interpretation of surveys done at different times.

Finally, two other general points need to be considered, the cost and value of monitoring. As we have come to learn more about aggregations and particularly more about how variable they can be for given species or in given locations, we have had to continually refine methodology to address potential sources of error. This means that care is sometimes needed in evaluating older (and sometimes not so old) literature that may not have had to consider a wider range of confounding factors or have involved sufficient replicates. It has also become apparent that monitoring of aggregations may not always readily provide the necessary information for management or conservation. This is the case for species that are very difficult to assess for absolute number at aggregation sites. There is certainly a need to improve our ability to count aggregated fish that occur in large numbers and to be circumspect of studies that claim to do so with any degree of accuracy (for example one attempt to use acoustic methods to measure, without ground-truthing the methodology, numbers of aggregating groupers suggested fish numbers in orders of magnitude higher than was indicated by direct observation, and in another case acoustic assessments using echo integration were found to be very sensitive to the total number of fish present with gas-filled swimbladders). While this section is about monitoring, we also need to consider why the monitoring is being done and whether there are other approaches to, say, population assessment, that can or should be done outside of aggregations. We can not answer these questions here but wish to make it clear that they need to be considered. Also to be considered is the cost of monitoring. This can be very expensive. Johannes *et al*. (1999) calculated that in Palau, the cost of monitoring aggregations probably exceeded the market value of the fish involved. This may simply not be justifiable in the long-run, an important consideration when establishing monitoring programs and determining the best way to use limited funds and human resources.

IV. C. Information from Samples Taken from Aggregations

Sampling is an important part of the study of any reef fish spawning aggregation. This can include the measuring and weighing of fish, taking whole organs or tissue samples, DNA samples, blood samples, gut contents, and otoliths. Samples provide the data to determine basic information on sex ratios, size frequency distributions, readiness for spawning, and many other

facets of the life of the fish. The methods used for sampling an aggregation often determine what information can be reliably determined about the aggregation.

Samples can be obtained by purchasing them from fishers, examining fish at landing points or just prior to sale, or by removing fish from an aggregation using various fishing methods, some of which allow fish to be returned alive. Researchers need to be flexible and opportunistic in acquiring samples, as we are seldom presented with an ideal situation for sampling. In some cases whole fish can be examined in detail if entire fish can be obtained and returned to the laboratory for work-up. Sometimes opportunities may be limited to examining and sampling from dead fish in local fish markets or at sea. Some organs may not be available even if there is a fishery. Ripe ovaries are often considered a particular delicacy, for example so may not be readily available. Fishers or traders might not want you to handle their fish at all. Also, when looking at fishes in fish markets, always remember they may have been caught in many different places and that the timing of spawning (or other biological factors) can vary somewhat among places, even within the same general region. In some circumstances, fish must be sampled live and returned to the aggregations. There are techniques and sampling considerations for each situation and type of information sought.

Assessing Aggregation Size-Frequency Distributions

Some studies have placed an emphasis on gathering data on the size of fish in an aggregation to follow changes in fish size over time, to determine the proportion mature size fish present, or for other reasons. Samples from fishermen can prove particularly useful in this regard, either measured at the aggregation as fish are caught, or obtained later from fish markets. It has been common practice to provide size-frequency curves for fish from grouper aggregations (e.g., Carter *et al*., 1994, Colin, 1992; Colin *et al*. 1987; Sadovy *et al*., 1994). In most cases the sex of the fish is also determined, although this is obviously not possible when captured fish have been gutted. Even gutted fish are of value, however, since their size data can contribute to the overall size frequency data for an aggregation.

Much care is needed in collecting and then interpreting size-frequency data taken from aggregations. First, it is important that sufficient samples have been taken to ensure a representative number of fish are available. Very often this will mean many hundreds of fish need to be measured. Second, it is most important to check whether these samples actually reflect the size distributions of the fish that are present; for example, some fishing methods are size-selective. Ideally, it is preferable to compare sizes assessed underwater (by spearing or by visual estimates) directly with fish sizes taken by fishing gears at the same time and place (e.g. Shapiro *et al.*, 1993).

Regarding **interpretation of size-frequency data** obtained from spawning aggregations. It is common to find considerable natural variation in strength among year classes in long-lived species. This means that apparent changes in size among a few years may not be entirely or largely due to fishing, or, indeed, due to fishing at all. For example, changes in mean length or in the size-frequency distributions from one year to the next could be the result of fishing but they could also arise for other, natural reasons. Many reef fishes are long-lived and can vary considerably in year-class strength. This means that, in any one year, a fishery might be dominated by just one or two age classes (e.g. Russ *et al*., 1996). Any inter-annual changes in size distributions noted from size estimates, therefore, could be due to natural causes, to fishing induced causes, or, of course, to some combination of the two. As an example, differences were noted in the size frequency distributions of aggregated fishes taken over six consecutive years from the same aggregation site of red hind grouper sampled in the same way each year, with the

same fishers, and the fish lengths determined with a high degree of accuracy in the laboratory (Fig. 20). In this particular case, how do we know whether the size differences over the six-year period are due to natural variability in recruitment and inter-annual growth, or to impacts of fishing (compare 1987 with 1992 for example and see the recruitment suggested in 1989)? Moreover, if time-series of size frequency data are too short, are too short, relative to the longevity of the study species, then it may not be possible to distinguish changes in the shape of

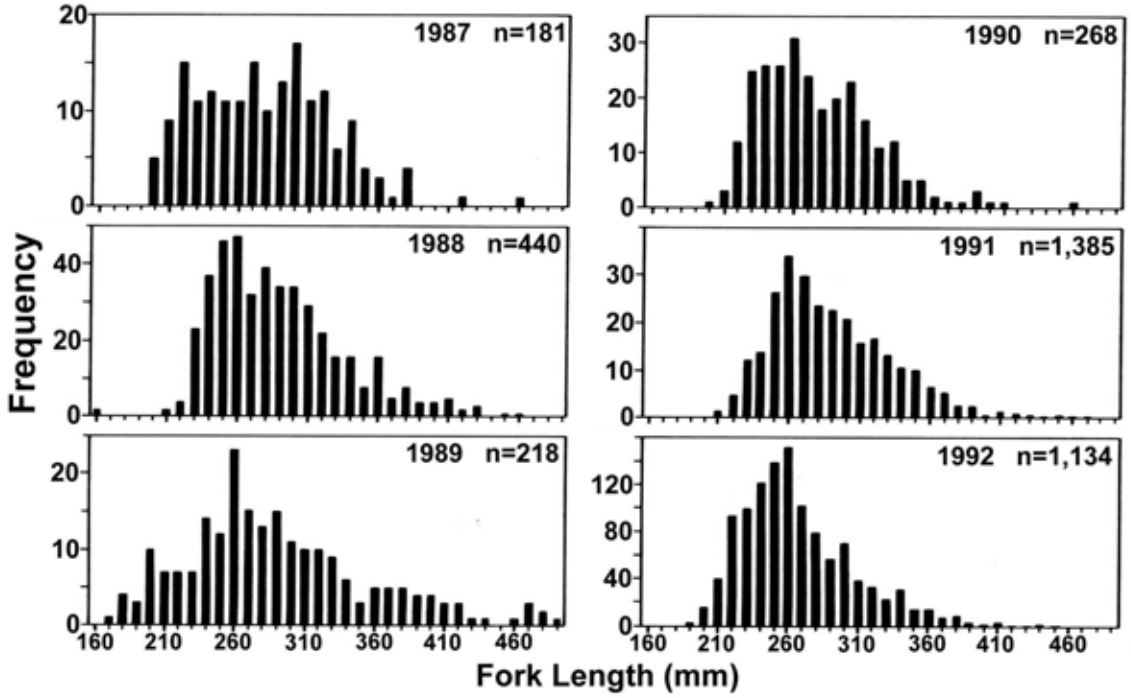


Figure 20. Size-frequency distribution for red hind, *Epinephelus guttatus****, taken by hook and line fishing, and by the same fishers, on spawning aggregations at Bajo el Cico, Puerto Rico from 1987 to 1992. n=number of fish taken each year. Figure redrawn from Sadovy et al. (1994)***

size frequency distributions over time due to fishing from changes due to major recruitment differences among years. Taking the argument one step further, if changes in size frequency distribution are noted and they can somehow be demonstrated to be due to the impacts of fishing, how do you know if they are due to aggregation or non-aggregation-based fishing? These are important questions to those of us who work on aggregations, and seek to propose or support their management. The examples serve to emphasize the need for understanding both the limits of methodology (in this case body size estimation) as well as the biology and fishery of the study species to ensure meaningful and useful interpretations of data to be made.

Methods for Length and Weight Determination

In the field it is most common to simply take the total length of the fish and its weight. When you are working on a fishing vessel or in a fish market, speed is often times very important, particularly if there are 100 or so fish waiting to be measured, and the fisher wants to get them cleaned and on ice. In such cases, it is best to have two people involved; one to handle and measure the fish, a second to record data. If additional data and samples are being taken, such as gonads, even more people would probably be beneficial.

In most cases the total length (TL) of the fish will be determined as it is simple to do with a measuring board. A measuring board has a meter stick inset into a plywood so it is flush and a board sticking up at a right angle along one side. The fish is put onto the board, the snout placed against the upright where the meter stick starts, and the length read off the meter stick at the end of the tail. It takes about 10-15 seconds to measure a fish using this board.

If necessary, a simple tape measure can be used, however remember to take into account the curvature of the tape when laid along a large fish. Weight can be taken with a hanging spring balance of the type used by most sport fishermen for weighing their catch. However, such scales are often inaccurate and need to be carefully calibrated. A more expensive hanging pan balance with a dial indicator is better, but again should be carefully calibrated both before and after. If you need to check calibration in the field, but lack standard weights to do so, take a measuring cup with which you can accurately measure out 1 liter of water (or any multiple thereof); this amount of water can be put in a plastic bag, and weighed using the scale. It should weigh approximately 1 kg per liter. Saltwater and the plastic bag add a few grams of extra weight, but are insignificant against the 1 kg of water given the precision of the equipment used.

Length and weight data should ideally be recorded on prepared data sheets, with additional spaces for sex, gonad condition and other information. If time allows, it might be useful to measure both the total length and standard length (SL) for specimens, particularly for those species where a TL-SL conversion might not be easily available. Standard length is the length from the tip of the snout to the end of the vertebral column. The end of the vertebral column can usually be determined by bending the tail of the fish so a distinct crease is seen; this being the end of the vertebral column. For those species with a forked tail, such as the jacks and trevalleys (Carangidae) a different measurement is taken, the fork length (FL). This is from the tip of the snout to the center of the tail. For many species with a deeply forked tail, the standard length is difficult to determine, as the caudal peduncle is quite hard and does not bend easily.

In nearly all cases, size frequency data obtained from collected samples should be considered superior to visual length estimates (Fig. 21). However, there is ample opportunity to bias in that part of the aggregation population that ends up captured by fishermen. At least, if samples are collected in a consistent manner over a number of years, say from fish caught by fishermen using the same methods each year, they should be comparable and provide firm data on relative changes in fish size over time. A comparison between visual estimates and caught fish would be interesting for a single aggregation. If fish must be returned alive, see the section on handling live fish below.

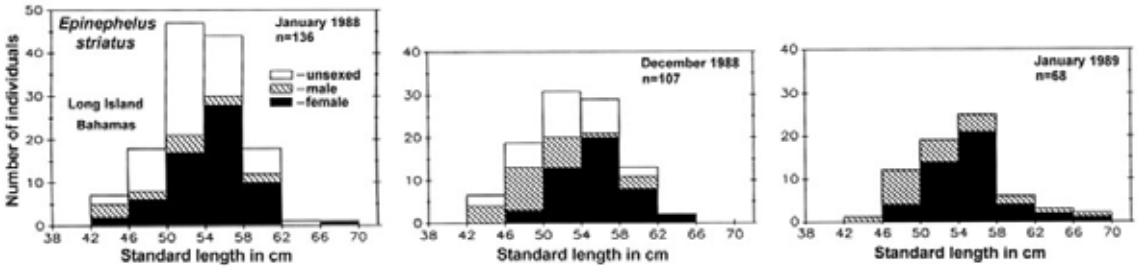


Figure 21. Size frequency of Nassau groupers, *Epinephelus striatus****, caught by hook and line fishers, Long Island, Bahamas. Unsexed fish had been gutted prior to measuring, but still provide useful information on the overall size of aggregating fish. The small size of some males implied that, perhaps, some males were not from sex-changed females; an observation which was further pursued. After Colin, 1996***

IV. D. Determination of Additional Aggregation Parameters

Methods for examining the numbers of fish in an aggregation and the area covered by an aggregation have already been discussed. There are many other parameters of aggregations that are important and can be collected or assessed using a variety of techniques. These include duration of specific aggregations, activities of individual fish within and between aggregation periods, precise timing of spawning, etc. These are covered in the following sections or elsewhere in the manual, as appropriate.

Determining the length of time an aggregation persists at a site is quite important. Fishermen are often able to provide good information relative to this question as their ability to catch fish depends on the fish being present. Fishing activity can, however, reduce the time an aggregation is present, since a large proportion of an aggregation can be fished out in a short time, hence reducing its persistence in the short term (and possibly its existence in the medium term). Making direct observation through diving or snorkeling of aggregation presence is useful, but caution must be used in doing this. It is possible the aggregation might have moved slightly since the last observation and will be missed. The surrounding area can be checked if the aggregation is not in its "normal" location, however this does not prove that it isn't present somewhere nearby that was not checked. Such problems are simply indicative of the uncertainty of dealing with many aspects of aggregation field studies.

Individual fish may not remain at the aggregation for its entire duration. There may be sex specific differences in the length of stay at the aggregation, with males generally persisting longer. Studies on individual residence at the aggregation usually require the ability to identify individual fish. Some species have individually distinctive markings, such as the Nassau grouper, which make it possible to identify individuals. The disadvantages of using such individual markings is that you generally have to approach the fish closely, something that may not always be possible, and when there are hundreds or more of a fish, finding a particular individual may be nearly impossible. Determining persistence from identifiable individuals then usually requires daily observations to be certain the fish is consistently at the aggregation site.

In assessing various aggregation parameters, it may be important to understand the manner in which transient aggregations (see Section I) build up and disperse. This can vary considerably between species, even within the same family, and might well influence the type of data taken or the way in which data are collected. Different examples are included in various sections of this manual but, in general, the three following categories of aggregation build-up are known (dispersal is usually soon after spawning and tends to include a large proportion of assembled fish). (1) Males reach the aggregation site earlier than females and establish territories. Females enter subsequently, sometimes in small groupings and stay a much shorter time at the aggregation than the females. (2) Large groups (sex composition unknown) of individuals migrate along specific routes to reach an aggregation site. (3) fish slowly build up in numbers until spawning at the aggregation site and may then quickly disappear. As we come to learn more of the details of aggregating species, additional categories may well be identified.

Assessing Aggregation Sex Ratios and Sampling Gonads

An evaluation of a spawning aggregation often involves the assessment of its sex ratio, most often the number of reproductive males and females are of interest. There are two components to such a task; identifying which individuals are reproductively active, and, of these, how many are males and how many are females. This would seem to be a relatively simple task, but, like so many other apparently easy exercises, there are a number of factors that must be

considered. The first is that not all fish at an aggregation are necessarily reproductively active. As one example, an aggregation of red hind, *Epinephelus guttatus,* in western Puerto Rico involved a significant percentage (13-36% at different times) of fish in the aggregations sampled that did not have ripe gonads; most of these fish were smaller than the 100% size of sexual maturation as determined from fished samples for which gonads had previously been examined histologically. Therefore, it can not be assumed that all fish present in an aggregation are necessarily reproductively active.

How do we sample an aggregation adequately to establish the sex ratio? Questions that need to be asked are which days to sample, how many days, what time of day to sample, how to sample (i.e. what method will be used to determine numbers of males and females) and how many fish need to be collected? It is also important to establish whether the sampling method used produces a representative sex ratio. For example, spearfishers intentionally select male tiger grouper, *M. tigris*, (to leave the females to reproduce) so their samples tend to be male-biased (Sadovy *et al*., 1994), relative to what was observed by the divers. On the other hand, the sex ratio obtained by hook and line fishing over a red hind aggregation did not differ significantly from that obtained by biologists spearing fish underwater (Shapiro *et al.,* 1993). Whatever the method of collection, every attempt must be made to determine whether the sample represents the true sex ratio and, for exploited aggregations, fishers could be interviewed to identify any possibility for bias. Ideally, sex ratios obtained from fishing methods should be validated by *in situ* observations, as long as it has already been determined that sex can accurately be assigned by divers**.** Finally, note that sex ratios must be assigned based on sexed fish. Never **assume** a size and sex relationship since size and sex frequency distributions often strongly overlap, and size in relation to sex can be markedly different in different social groups, especially in sex-changing species (e.g. Shapiro, 1981).

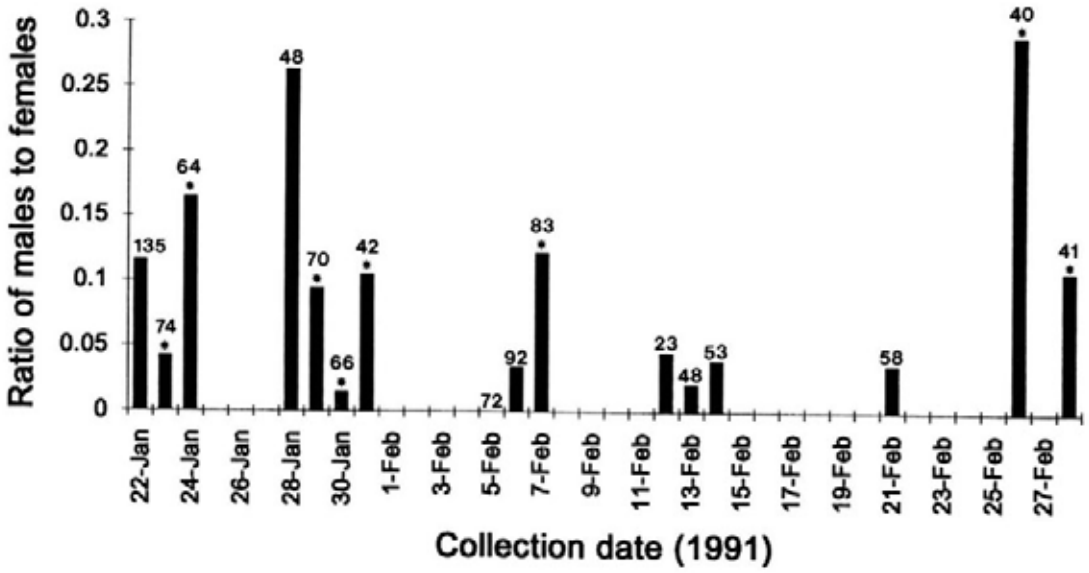


Figure 22. Ratio of active male to maturing and active female red hind, *Epinephelus guttatus* ***for 16 sampling days in 1991. Number of fish caught each day appears above each column and asterisks demote that the sex ratio differs significantly (p 0.05, chi-square) from the preceding sample date. Full moon occurred on 30 January and 28 February (after Sadovy et al,, 1994).***

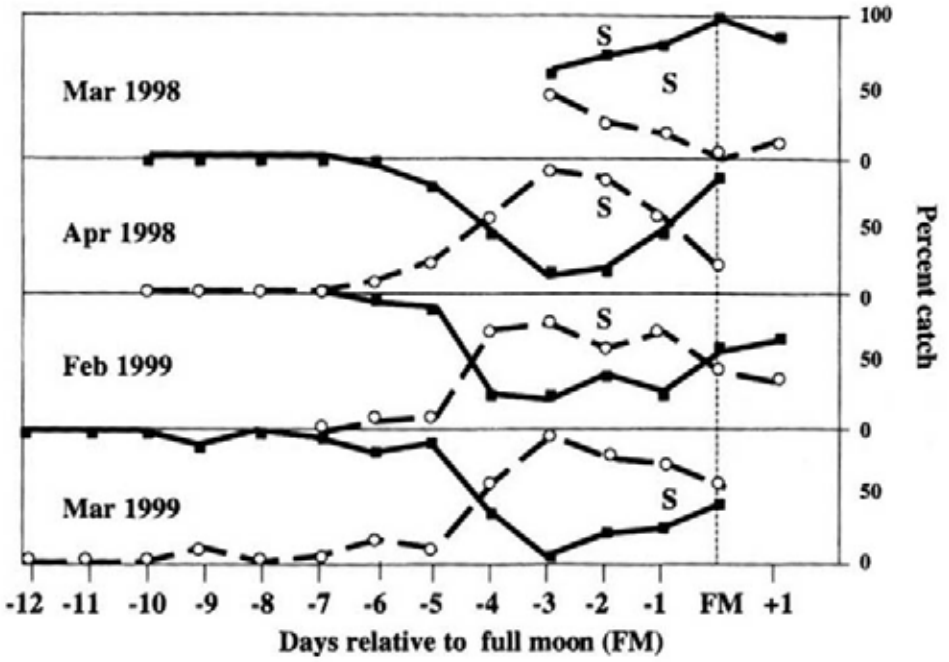


Figure 23. Catch trends for camouflage grouper, *Epinephelus polyphekedion****, taken at the Kehpara Island Marine Sanctuary February 1998-March 1999 showing the arrival of males, followed by females several days later. Spawning time(s) was determined by a combination of gonadosomatic and histological analyses (Spawning = S; solid square = males; open circles = females) (after Rhodes and Sadovy, 2002).***

Only sex ratios on the few days of spawning can be considered to be operational sex ratios. In the red hind, *E. guttatus*, modest samples of males and females collected over 16 days showed significant shifts in sex ratio from day to day both before and after spawning (Fig. 22) (Sadovy *et al*., 1994). Note that sample sizes in this case were small on any one day of sampling which could have contributed to extremes of day to day variation since inclusion of just a few individuals of the rarer sex can substantially change the sex ratio.

Establishing a meaningful and representative sex ratio can be difficult not only because of sampling problems but also because sex ratios can change from day to day and also during the course of a single day. Operational sex ratio might require assessment at the time of spawning since, in a number of species, males precede females to the spawning site. For example, in *E. polyphekadion*, which spawns around full moon, males start to enter the aggregation site 10-12 days prior to the full moon, while females start to arrive about 4 days before full moon, stay a few days until spawning and then all fish leave the site (Fig. 23).

If data on fish sex is to be taken from commercially fished samples, it is important to determine whether the gonads are valued for food and when the fish are likely to be cleaned and gutted; this will dictate how you plan your sampling. Always ask for permission to touch another person's catch. If not all fish can be sampled, then some method must be devised to obtain a representative subsample of all available fish coming off fishing boats or landing in the market place. It is important to remember that the more stages there are between the spawning site and sampling, the bigger the possibility that errors of sampling will be introduced. Check whether fishers have returned particular size classes of fish (either too big and therefore possibly ciguatoxic, or too small for a good market price or whether small fish may be retained separately in the boat for later home use) to the water. Illegal, undersized, fish may also have been returned

to the water. If the fish are weighed in markets or coming off boats, be sure to note whether or not they have been gutted.

If there is an opportunity and a need to collect gonads from fish caught at aggregations, then simple guidelines can be followed to handle and preserve them. Carefully remove the gonad trying not to rupture the ovary which, if full of hydrated eggs, will become difficult to handle. Subsequent treatment will depend on the objective(s) of gonad sampling. If fixation is necessary (i.e. the gonads will not be worked on fresh), the gonad can be placed in 10% formalsaline (10% formalin solution) in ratio of about 10 parts solution to 1 part of gonad. If the gonad is whole and very large (e.g., Fig. 24) it will be important to penetrate it or carefully slice it at intervals along its length to allow the fixative to penetrate the tissues before those towards the center of the gonad deteriorate. Fixation can take a couple of months. The gonad should then be washed and transferred to 70% ethanol for storage. If sections of the gonad are to be retained (i.e. the whole gonad is not necessary), then these should be carefully sliced (for very ripe gonads it helps to place the whole gonad in fixative for a short time [hour or so] and then to slice after the gonad has hardened a little). Slices of gonad should be as thin as possible and not too much thicker than 5 mm if this is practical – this allows for sufficient penetration of fixative. Fixation of slices is much quicker than fixation of unsliced gonads and sliced gonads can be transferred to preservative after a couple of weeks. Make sure that the jars used for fixation are large enough and seal well since formalin is unpleasant and should not be inhaled or touched if possible (use gloves while dealing with formalin solution). Place labels inside the jars with the time, date and place of capture and the size and weight of fish if possible; labels should be strong paper or card with details clearly written in pencil. If no fixative is immediately available, take the fresh gonad or gonad sections and freeze them in a bag. When fixative becomes available, place the frozen gonad or section directly in the fixative before defrosting and continue as above. Sometimes, as has happened to one of us, you may have no fixative or no freezer! There may still be hope of preserving some material if you have access to vodka or even surgical alcohol. This is not a perfect solution by any means but can make the difference between a gonad that is useable for some purposes and not useable at all. There are many good papers with instructions for histological preparation and studies - the key is to decide what you need the gonads for and to make sure that the materials are as fresh as possible when fixed.

In cases where fish are to be sampled alive, they can be measured and weighed on board the boat. In some cases the fish have been brought up from depths that require the swim bladder to be deflated with a needle. If you are working with a live fish fishery the fishermen will be very experienced in deflating these fish so you will not have to do this yourself. If the fish are to be returned to the water it is best to first see if the fish can swim back down on their own. Sometimes a little rest and a flick of the tail is all that the fish require; carefully sliding the fish into the water head first helps get them moving in the right direction. If the fish can not swim down on their own they must be deflated. This should be done with a sterile 18-gauge hypodermic needle. There is a very good spot to puncture the fish just behind and in-line with the pectoral fin. Since some species have elongated pectoral fins it is a good idea to dissect a dead specimen to identify the spot. You are looking for a location at the anterior dorsal region of the swim bladder, where the swim bladder forms a pocket in the muscle. The swim bladder is adhered to the muscle at this location so the needle will pass through muscle, directly into the swim bladder without entering the portion of the peritoneal cavity that contains viscera. Find a marker on the outside of the fish that will guide you into this part of the swim bladder. Once you've identified the spot simply remove a scale (or slip the needle under the scale) and push the needle down into the fish (at a 90 degree angle to the body) until you hear gas escaping. Many people like to do this in the water so that they see bubbles escaping from the needle; do not do this since seawater can travel into the swimbladder and cause a bacterial or fungal infection.

Live fish can be sexed by squeezing the gonads of the specimen in an attempt to express eggs or milt. Pressure should be applied (with thumb on one side and fingers on the other) to the dorsal-most region of the gut cavity. Start by gently squeezing the gut cavity about 2/3 of the way towards the head and then slide your fingers back to the vent as if you were trying to squeeze paste out of a tube. This is best done with the fish lying on its back. You will often see feces

Figure 24. (Left) Female gonads from a large Nassau grouper indicate the size that some gonads can achieve in that species. In some species ripe testes can get almost as big. (Right) Close up view of Nassau grouper ovary with hydrated eggs visible through the surface integument. The hydrated eggs are about 1 mm in diameter and appear dark (actually translucent) in this view. Scale approximate.

come out of the anus; wipe them away and keep looking for the pore just posterior to the anus. From this pore you may see milt, or, sometimes, hydrated eggs. Males are very easy to sex in this manner since they are running ripe at all times during the spawning season. Females may only exude hydrated eggs (Fig. 24) during a very short period of the day (an hour or two before spawning, often, but not always, at dusk). Hydrated eggs are usually quite runny and clear, while milt is milky white. An experienced "fish squeezer" can eventually reliably (but maybe not 100%, depending on the species) sex females simply by the appearance of the urogenital pore. However, prior to hydration, the vent may not allow eggs to pass even if ripe and sex can not readily be confirmed. In such cases, it may be possible to gently cannulate the ripe ovary, a technique that requires training and appropriate materials. Note that people assisting on projects may react in unexpected ways to handling fish or to taking samples. In one place, for example, when fishers were part of the sampling team, they did not want to touch the ripe males (the sperm) and we had to introduce gloves to continue sampling.

Seasonality of Spawning

Gonads collected at regular and reasonably short intervals over a period of time of a year or more can often provide strong evidence for seasonality of spawning in both aggregating and non-aggregating fishes using GSI or histology (Fig. 25). However, it is important to collect regularly, frequently and in sufficient number to ensure a reliable indication of spawning season. Females will tend to provide the most detailed information on the spawning season. Monthly samples sizes of 25-30 or so females are recommended as a general rule.

Determining What Time of Day Fish are Spawning

For many species (especially for those that spawn during daylight hours in shallow, accessible waters) direct observations may be made throughout the day including the period during which spawning is expected to occur (based on market samples, anecdotal evidence, etc). Where only a limited number of observation periods can be made, it is always possible that fish

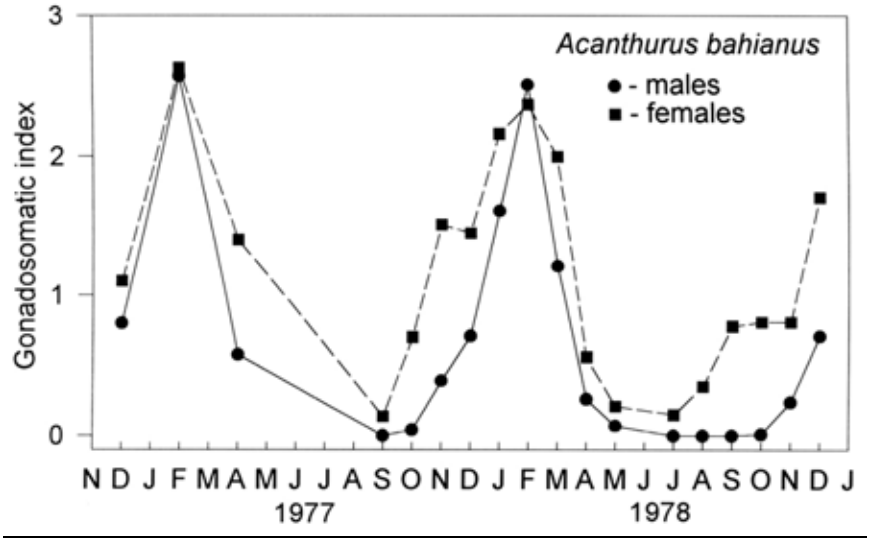


Figure 25. Spawning seasonality of ocean surgeonfish, *Acanthurus bahianus****, from southwestern Puerto Rico based on gonadosomatic index (GSI). There is a clear peak to the spawning season during the northern hemisphere winter in this species. Data from Colin and Clavijo, unpublished MS. (PLC)***

may be spawning at some time when we are not observing them, leading us to a false conclusion about the range of spawning times. We need to always keep this caveat in mind, and report our findings with suitable qualifiers.

It is more difficult to pinpoint the time of actual spawning if this occurs after dark, or in deep or inaccessible (i.e. low visibility or high current) conditions. This has been the case for many species of snappers and some groupers. For many of these, we only suspect they spawn after sunset, with definitive evidence still lacking. In such cases, indirect means may have to be used to verify spawning time. Moreover, be aware that the presence of divers could influence fish behavior. Among those species that form transient aggregations, it is sobering to keep in mind that of all the groupers that aggregate to spawn only a handful of species have been observed actually releasing gametes, as far as the published literature is concerned.

If gonad materials can be obtained then either macroscopic or microscopic techniques may be used to determine time of spawning. Once the approximate time of spawning is determined, regular (daily or even hourly) samples can be taken from the aggregation site. If gonad and body weights are available, then the GSI (the percentage that the gonad contributes to total body weight) can be calculated (Sadovy *et al.*, 1994; Rhodes and Sadovy, 2002) (Figs. 26 and 27).

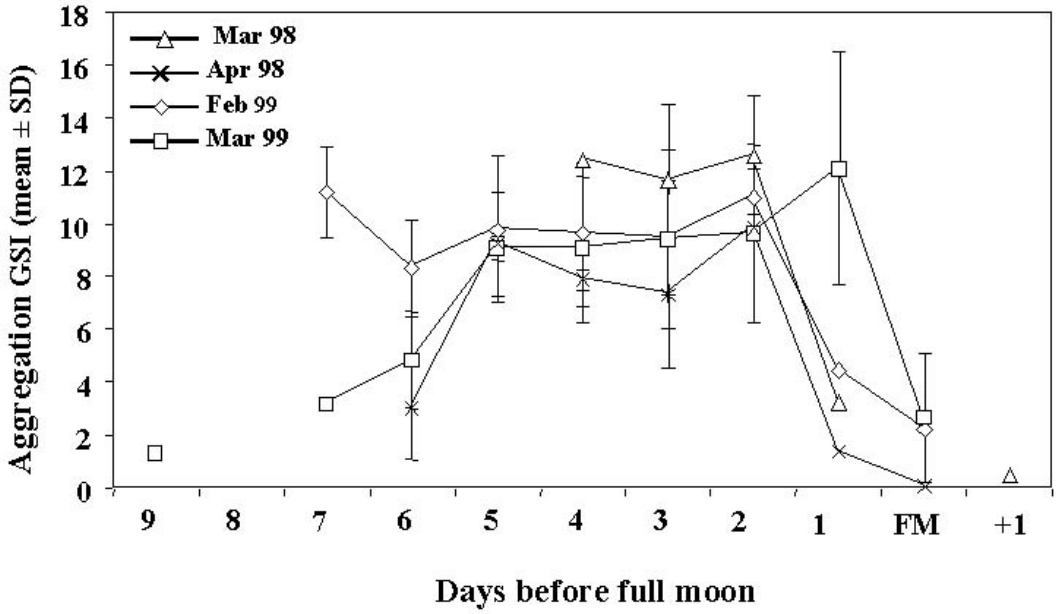


Figure 26. Mean gonadosomatic index (GSI) for ovaries taken from female *Epinephelus polyphekadion* ***in the week up to and including spawning. Sample sizes are given for each day. (redrawn from Rhodes and Sadovy, 2002).***

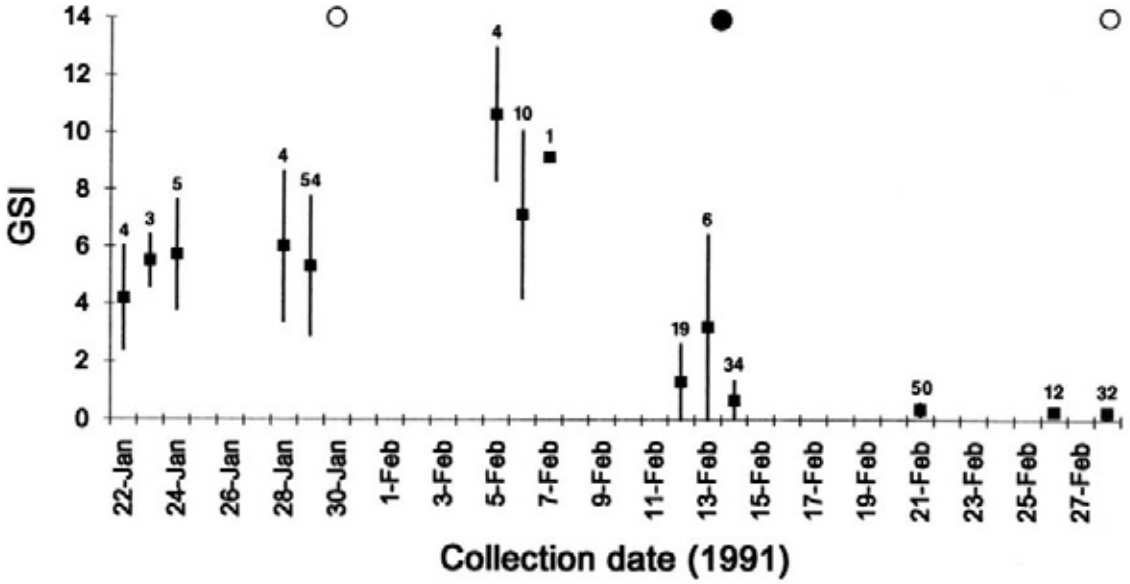


Figure 27. Mean gonadosomatic index (GSI) and standard deviation for ovaries taken from female red hind, *Epinephelus guttatus*, ***captured between 22 Jan and 28 Feb 1991 in Puerto Rico. Sample sizes are given for each day. Open circle indicates full moon and closed circle new moon (Sadovy et al., 1994).***

Figure 27 plots the GSI relative to lunar phase and shows the clear relationship in *E. guttatus* between the approaching full moon and high GSI. In the days just before the full moon, the GSI drops quickly as fish spawn and release most hydrated (heavy) eggs.

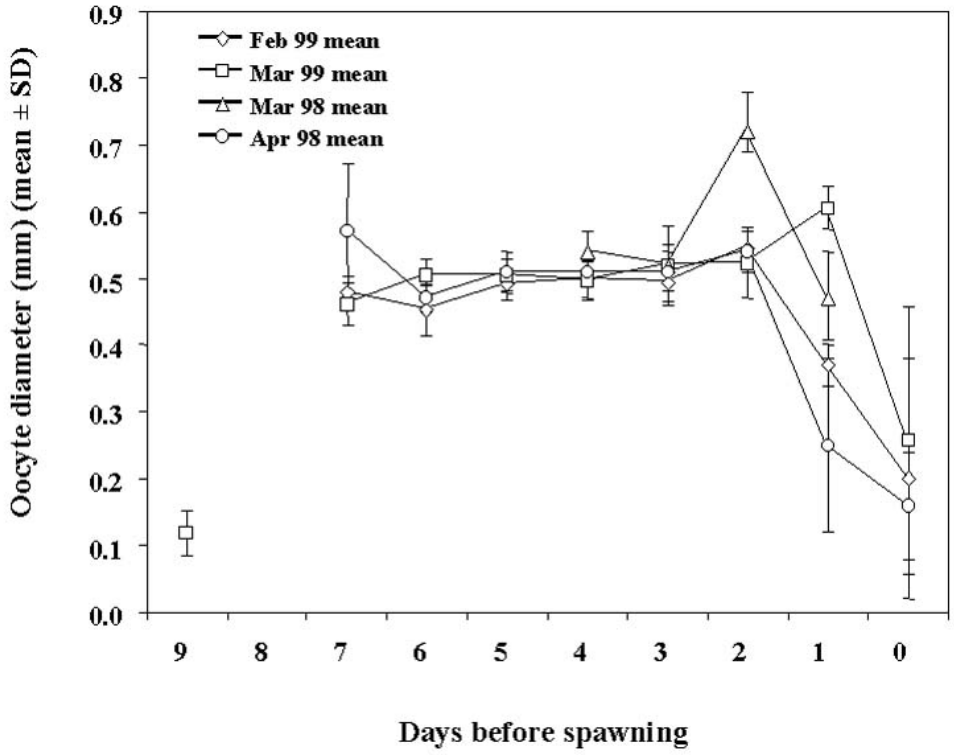


Figure 28. Oocyte diameter measurements for female** Epinephelus polyphekadion **taken from the spawning aggregation area relative to the time of spawning (redrawn from Rhodes and Sadovy, 2002).

Measurements of oocyte diameters (using unfixed material and a microscope) can be used as a simple means to track oocyte maturation and identify, indirectly, when spawning occurs (Fig. 28). As eggs mature in the days leading up to spawning, they increase in diameter and when they are released, mean egg diameter will decline reflecting the smaller diameters of the oocytes remaining in the ovary. More precise estimates, needing subdaily (preferably hourly) sampling, are possible if oocytes can be measured for changes in oocyte diameter (of the largest oocytes present) over time (Fig. 29). If gonads can be prepared histologically, the presence of post-ovulatory follicles (the follicles surrounded the mature egg until egg release and collapse, remaining for a short time after spawning – they therefore represent a useful indicator of very recent spawning) can be used to detect recent (i.e. within the previous 1-2 days) spawning (Fig. 29b). As always, thought must be given, however, to ensuring that sufficient samples are taken to be scientifically meaningful.

If fish are to be returned alive to aggregations, see above for handling ripe fish and detection of hydrated eggs. Regular, hourly or so, sampling of aggregating fishes will be necessary to pinpoint time of spawning. The presence of running sperm is not necessarily an indication of imminent spawning in males, although the release of clear, hydrated, eggs does signal imminent spawning in females.

DNA Samples

Molecular genetics can now be done using a small sample of tissue from which DNA is extracted. Tissues can be preserved in ethanol (the higher concentration the better, 95% being preferable) or other buffers in small o-ring sealed vials of 1-2 ml volume. Tissue samples can be taken from freshly fish caught by fishermen, and just about any tissue will do. Samples can also be collected by fin clip of live fish, cutting a piece of the membrane between fin spines (usually the dorsal), which does not hurt the fish and allows it to be returned alive to the water. It is also possible to take tissue samples by spear, using a modified biopsy type needle to collect a plug of tissue when the spear is shot into a fish. Fin clips have also been taken from large groupers such as jewfish (Goliath grouper) (*E. itajara*), that were docile enough to let a diver grab their dorsal fin membrane and quickly slice a small piece of tissue from the membrane. The fish hardly react to the taking of such a tissue sample.

DNA samples, if they can be analysed at a fine enough resolution, may be useful for looking at the population structure of aggregating fish and in some case might provide some insights into where an aggregating population is coming from. If samples are taken over a broad geographic region, the population structure of a species might be evident and allow some statements about the genetic interchange via planktonic larvae among a species. Care is needed in interpreting genetic information, however, since the absence of any apparent population structuring following analysis of DNA material, while it may indeed indicate that no structuring is present, could also be reflecting inability to detect such differences due to the resolution of the techniques used.

Gut Contents

The gut contents of aggregating fish may be of interest if it is important to determine whether or not fish of both sexes are feeding during the aggregation. In many cases aggregating fish take baited lines or enter traps which are baited with live fish or other dead bait; in others, however, aggregated males may not feed and can be difficult to sample by hook and line (Sadovy, pers. obs.). In most cases speared fish from areas where fishermen and traps are not present are to be preferred for examining natural gut contents. Hungry fish are the ones that tend to take baited lines or go into baited traps, and even if a fish is speared, if there has been such fishing around the fish speared may have gone into a trap, taken bait and exited or might have taken bait from a hook without being caught. Where traps are unbaited, gut contents may say something about the state of the aggregated fish, but again should be interpreted with caution, as other fish, better fed, may not enter the trap.

Sampling Otoliths

Otoliths are often collected from aggregating fish. They can potentially provide information on age and growth or may be used for fine microchemical analyses. Various otoliths have been reported to have "spawning marks" indicative of when the fish has spawned previously. While such marks have generally not been positively verified, it might prove useful to compare otoliths from fish captured at an aggregation with a number of individuals, tagged at the aggregation and collected later, for evidence of spawning marks. The collection, preparation and analysis of otoliths is not further within the scope of this manual and the reader is urged to consult appropriate references for these items. However, it should be noted that many reef fish can not easily be aged using otoliths so these should be checked for growth marks before a full-scale sampling programme is launched.

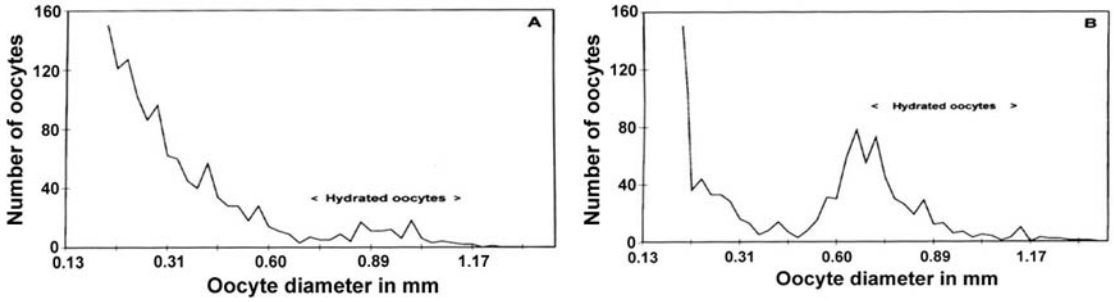


Figure 29. Oocyte diameter frequencies taken (A) in the morning (SL=508 mm) and (B) in the afternoon (SL=551 mm) of 22 December 1988 during the spawning of the Nassau grouper, *Epinephelus striatus****, from the Bahamas from two reproductively active and aggregating females. The appearance of a mode of larger oocytes in 29B reflects hydration reflecting imminent spawning (Sadovy and Eklund, 1999 – NOAA Tech. Rpt. NMFS 146).***

IV. E. Fishery-dependent Monitoring of Species that Aggregate to Spawn

Monitoring a fishery is critical for assessing its status over the long term, for determining appropriate management action and for evaluating the effects of management. Estimating catch is covered in Section VI. The current section explores how different types of catch data can be used to determine seasonality in fishing of aggregations and to identify trends in aggregation-focused fisheries over time. It also addresses important considerations for designing catch sampling programs.

Fishery monitoring **must** be done on a species by species basis. It is not valid to, say, monitor 'groupers' or 'snappers' as a general category that includes several or many species of grouper or snapper and hope thereby to understand the status of individual species. The reason for this is that ecological species interactions, especially among closely related species, mean that what happens to one species may influence what happens to another. As just one example, larger species of grouper tend to be more vulnerable, for a range of reasons, to most fisheries than smaller species. In the Caribbean, as larger species declined, it is likely that competition with smaller species was reduced and this allowed an increase in biomass of smaller species. If only the category 'groupers' was to be recorded from the fishery, there might appear to be no overall changes in landings even when larger species were declining significantly because an increase in the smaller species would compensate.

It is possible to determine a marked fishing season, which might reflect aggregating behavior, by appropriately designed monitoring of the fishery. A distinct fishing season exists if the majority of landings of a particular species is taken, or the catch per unit of effort (CPUE) of landings are particularly high, at certain and consistent times of the year. Such seasonality might help in identifying an aggregation period, because during such periods catches or CPUE are often higher than at other times of the year. It is important, however, to recognize that catches can vary considerably throughout the year due to factors that might have nothing to do with aggregating behavior. Examples include weather conditions that strongly affect fishing activity, preventing or allowing access, for example, of boats to particular places at particular times. Trends in the fishery whereby fishers change their activities due to factors such as market demand or regulations elsewhere may also be important. A simple set of questions for fishery officers of fishers should help you to determine what factor(s) might be involved when regular changes in CPUE or landings are noted for particular species.

It is essential to assess the landings or, more appropriately, the CPUE at different times of the year if the fishery is to be effectively managed and the response of the fishery to fishing is to be evaluated over the long term (note that even if aggregations are exploited, the species could also be heavily exploited at non-aggregation times). For example, if a standardized program monitoring the fishery of a particular species shows that CPUE or landings decline over time (i.e., over a number of years) this may signal that overfishing is occurring and that management might be needed. Note that CPUE is the value that is needed to obtain an idea of what is happening in the fishery if the fishing effort (such as number of boats or fishermen) is changing over time because changes in landings alone may not reflect anything about the species but reflect fisher behavior rather than fish numbers. In general, CPUE is preferred to landings data as an indicator of the status of a fishery and should be collected whenever possible (but see below the discussion on aggregation CPUE).

If the majority of the annual landings of a particular species occurs during its spawning (aggregation) period, then management attention might best be directed at the aggregation itself rather than on the species at other times of the year. If, on the other hand, declines in aggregating species occur and the species is fished all year round, then management of the aggregation may well not be sufficient to stop or reverse the decline. Aggregations are only part of the life history of aggregating species which may also be vulnerable to fishing at other times of their life cycle or moving to and from aggregations (Lindeman and Claro, 2003). It is important therefore to determine when and where, and how heavily, fisheries take place during the year to ensure appropriate management measures are developed for a given species in a given fishery.

Another important point to consider is that species that aggregate to spawn, and for which a large proportion of the landings is taken during the aggregation period, can be surprisingly difficult to monitor reliably based on aggregation landings alone. While total annual landings is the sum of aggregation and non-aggregation landings, landings or CPUE taken at the aggregation site may well not reflect the condition of the fished population over time. Given what we have already said about CPUE, why is this? The reason is that CPUE is considered to reflect abundance (since we can not measure fish abundance directly we have to have some parameter that indicates abundance and CPUE is commonly used for this since it corrects for fishing effort). In species that aggregate, however, aggregation CPUE may stay high, even as population levels decline. This occurs if aggregation fishing does not remove most of the fish each during aggregation period but fish continue to aggregate even as population levels decline substantially. CPUE from the aggregation will, as a result, remain constant until numbers become severely reduced, and at some point CPUE will start to drop rapidly and the aggregation may cease to form (Sadovy and Domeier, unpublished manuscript).

What this suggests is that CPUE should, for exploited species that aggregate to spawn, also be measured during the non-reproductive season, as an indicator of fish abundance, even if most of the landings occur during the aggregation season. Alternatively, well-designed fishery independent surveys, involving estimates of fish numbers would be recommended (see Section IV.B). Such considerations are critical for fully understanding trends in fished populations, are simple in concept and in most cases can effectively be built into current or new monitoring programs with careful planning.

In conclusion on fishery-dependent monitoring, it is essential, if a fishery is to be productive in the long-term, that monitoring programs be implemented. They do not have to be complicated. In fact, simple and clear monitoring programs are more likely to persist long-term than complex or expensive ones. Important decisions, however, do have to be made about when

and how to monitor and which species to monitor. When and how have been discussed for aggregating species (i.e. CPUE should be measured outside of the aggregating season if at all possible and body size information can also give indications of long-term changes if carefully assessed – refer to Section IV.C). Which species are to be monitored will depend on monitoring capacity but, if this is limited, then decisions might consider tracking trends in particularly important species, or particularly vulnerable species. For example, many of the larger reef fishes are likely to be more vulnerable than smaller species if targeted specifically. Finally, care is also needed that the monitoring of similar-looking species is not affected by misidentifications.

IV. F. Observing and Documenting Courtship and Spawning Behavior

We are always faced with the question of whether some behavior seen in fish is actually courting and spawning and how to "prove" this is the case? While documenting spawning is often an objective of a study, it is sometimes uncertain that some reports of spawning is actually what occurred. In most cases experience with the species of interest and with other fishes in general helps. If you have seen reproductive behavior in a number of fishes, particularly ones closely related to those now being watched, this provides background to interpret what is seen in another species. Many observers, including all of the present authors, have at one time or another believed they were watching courtship or spawning behavior when in reality they were really seeing something very different. In our cases, we were able to figure out what we were seeing was not courtship and spawning (by applying some basic criteria to verify spawning) before we prematurely published observations as reproductive when they were not.

Similar care is also needed in linking sex and coloration. In many cases, the relationship between differing coloration and sex can be easily observed, this does not mean that under different conditions the coloration is constant to one sex. A good example of this is the "bicolor" phase of the Nassau grouper. When a small group of fish is present at an aggregation site, preliminary courtship (an hour or more before the spawning at sunset) often has a male in bicolor phase courting females in a somewhat normal phase (see Fig. 30). This might seem simple that males are bicolor and females are not. In reality though, the bicolor phase is believed to represent a "submissive" or non-aggressive signal, not limited to a single sex. In group spawns, females are believed to also display the bicolor pattern, and during non-aggregation, non-spawning season encounters, either sex can flash the bicolor pattern in response to an approach by a larger fish, to indicate it is being submissive to the larger individual.

Courtship and Agonistic Behavior: How to Tell the Difference

More than one observer has been fooled into thinking that some type of agonistic behavior represents courtship in groupers and other reef fishes. In many groupers, males hold territories in the area of the spawning aggregation and often interact at the edges of their territories with other males (Fig. 31). There are a number of things to assess when you see something that might be either courtship or aggression. These include 1) do the two fish differ in color pattern, 2) do they repeatedly tend to threaten each other with a direct head on approach, often with the mouth open and gill covers flared, 3) after an encounter does one fish tend to swim off while the other remains near where it was. Is one fish swollen with eggs while the other is not? Generally there will be some color differences between males and females, although this can also occur in same

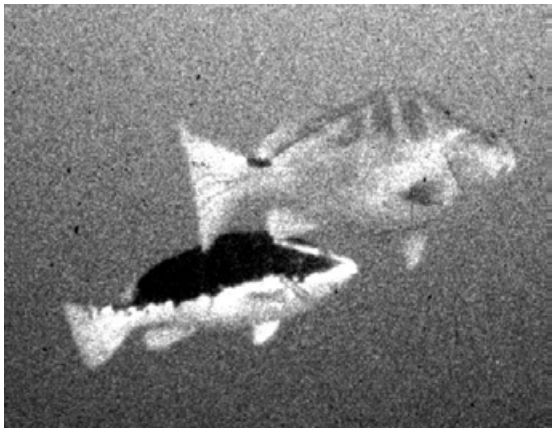


Figure 30. Pair of Nassau groupers, with male below, female above with big belly, engaged in courtship behavior in mid-water, Long Island, Bahamas (PLC).

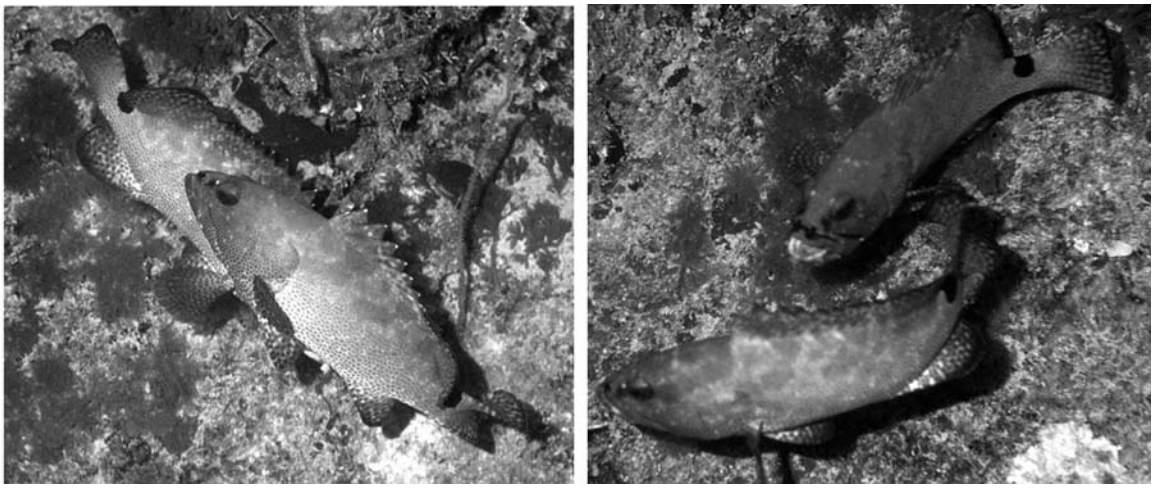


Figure 31. Two male** Epinephelus polyphekadion **engaged in a boundary dispute during an aggregation period, Palau (PLC)

sex fish if they are in different behavioral states or may only last for a short period of time. Where there are alternating aggressive thrusts, where fish are appearing to assess the other individual's strength, these are most likely males engaging in a territorial assessment. Females generally seem to be less likely to be aggressive, but not in all cases. In territorial disputes, the loser will usually retreat and the victor remains at the edge of the territory claimed.

Aggressive encounters may be related to spawning territories or have nothing to do with spawning at all. Usually the best way to separate such activities is to gain some knowledge of the behavior of the fish in question by observing their activities over a period of time. Without knowing the typical (day to day) behavior of a fish, it is difficult to determine when it is doing something different, like preparing to spawn (Fig. 32). Aggregation workers first need to be good fish observers.

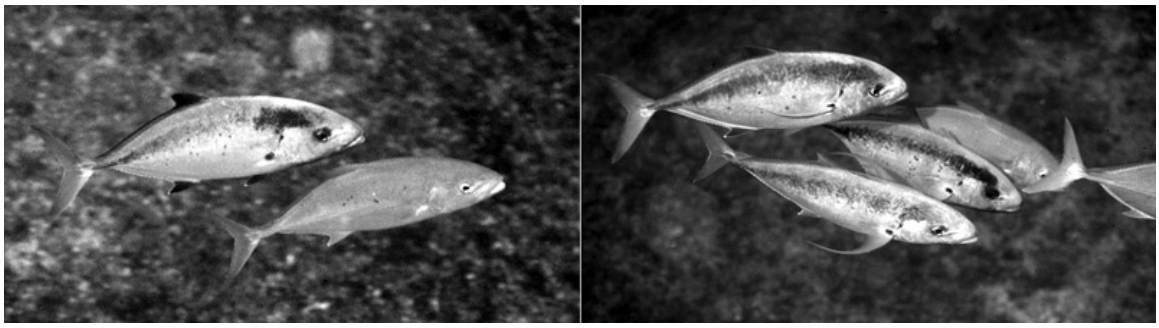


Figure 32. Probable courtship among *Carangoides ferdau* ***at Chuuk Atoll, Micronesia. The probable males have dark areas, typical of courting male carangids. Also in carangids males tend to trail behind females and attempt to keep other males from following the same female. (PLC)***

Determining Duration and Timing of Spawning Behavior

In most cases, observers have simply made a series of diving observations which over time (days to weeks) allow a general idea of spawning duration and timing. This can provide significant information and at present is the most feasible method of gathering spawning occurrence data. The method of starting a video camera at a known time and continually taping all activity at the spawning site is quite useful, since it allow the exact timing of documented events to be compiled later (Fig. 33). This technique is discussed in more detail under video documentation.

There are several methods to improve the gathering of this type of data. Most would involve some sort of electronic monitoring of spawning, once spawning behavior has been

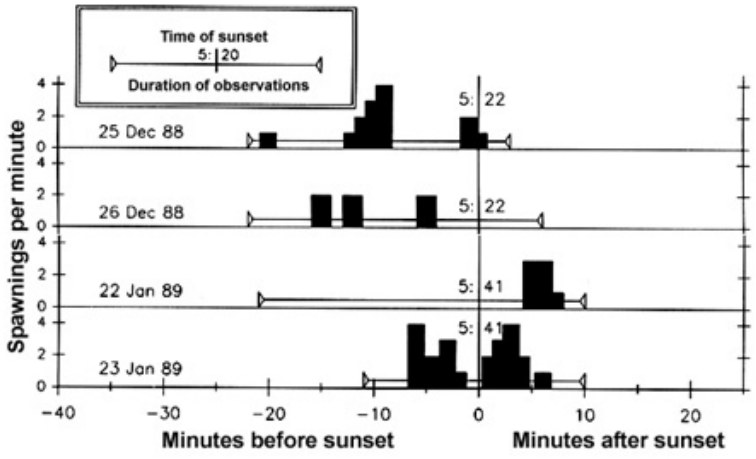


Figure 33. Timing of spawning relative to sunset for *Epinephelus striatus* ***at Little Harbor aggregation site, Long Island, Bahamas. Data from Colin, 1992***

observed and positively identified. For example, Lobel (1992) reported group spawning *Scarus iserti* to make hydrodynamic noise when spawning which could be detected. This allowed for counting and acoustic mapping of mating events using spawning-associated noises. Such sounds can often be heard by divers, if they are quiet and listen, as a "whooshing" as a group of fish ascends to spawn. Such information would allow the monitoring of spawning activity acoustically by remote stations not unlike those that presently are used to track sonically tagged fishes. Video techniques have great potential to monitor spawning activity, particularly in remote

areas where divers can not easily visit study sites on a regular basis. An AUVS system (described subsequently) might find utility in this regard, although the biology of the species must be understood to determine whether sound and spawning are always closely associated.

How to Prevent Disturbance of Spawning Fish

Observers quickly learn that many spawning fishes are disturbed by the presence of divers. The physical presence and movement of divers is disturbing, coupled with the desire of the divers to approach activity closely. Scuba gear generates noise and bubbles as does associated equipment used by the divers, such as dive scooters, lights, etc.

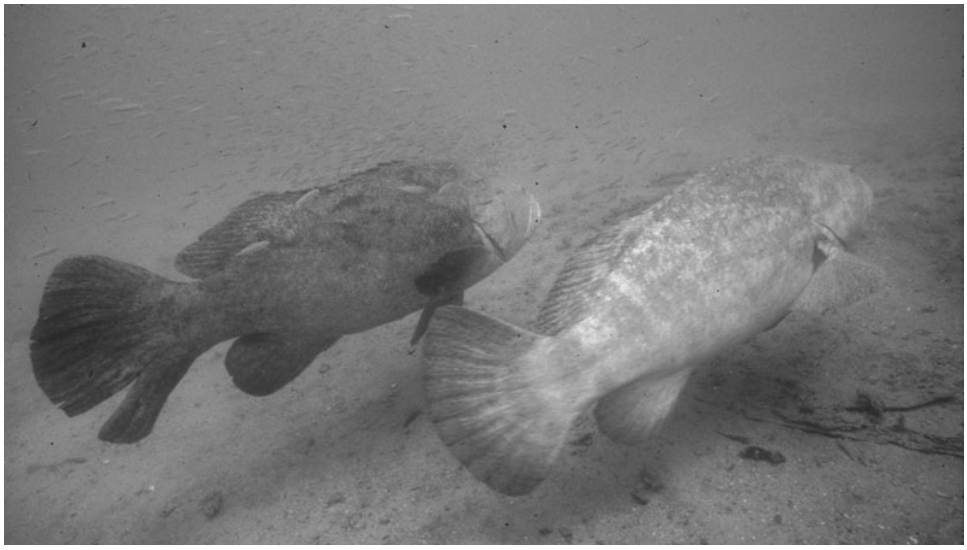


Figure 34. Probable courtship in the jewfish, *Epinephelus itajara.* ***The fish on the left is believed to be the male, with a dark body and light head, while the female is on the right in "normal" coloration. The presumed male approaches the female and displays, often producing a booming sound with its swim bladder. Spawning has not yet been observed in this species. (Photograph copyright by Doug Perrine)***

There are strategies for dealing with all of these problems. Disturbance from the physical presence of divers and their movements can sometimes be reduced by 1) moving slowly, 2) staying close to the bottom, 3) reducing the number of divers present, 4) reducing the motion of arms and legs while holding station, 5) not moving directly towards fish, and 6) approaching from a down current direction. As a general rule, try to avoid approaching too close by gradually moving towards the fish and if behavior is interrupted, stop approaching for awhile or even back off a bit.

One particularly useful technique for both short and long-term observations of aggregations is "tethering" (Colin and Clavijo, 1988). The idea of tethering is to make the observer into a stationary object floating above the bottom on a line attached to the bottom. Scuba divers should be equipped with a buoyancy control device, such as a buoyancy compensator. A light nylon line of suitable length is attached to the sea bottom and a small clip on the upper end is used to attach to some point on the diver. The diver inflates the BC sufficiently to achieve moderate positive buoyancy, so that the diver in essence floats above the bottom like a tethered balloon. Since no swimming is needed to remain above the bottom and the tether prevents the diver from drifting away, the observer can remain stationed above the bottom without moving

hands or feet. This greatly reduces the exercise load of the diver and reduces the breathing rate, distracting diver movements, and noise from inhalation and exhalation. In this manner, a tank of air lasts longer and the diver produces minimal disturbance. This also reduces the depth of the diver, compared to being on the bottom, and at depths below about 10 m can mean a significant increase in observation time without decompression being required.

For various types of long-term (day after day) observations, the use of the same tether point allows a directly comparable area (assuming stable water visibility) to be observed each day. At their option, two divers could be stationed sufficiently apart to separately survey two different areas, while remaining in contact visually or by sonic signals, such as banging on tanks, etc. Tethering was used in Puerto Rico to document spawning activity rates per minute for aggregations of two surgeonfishes, *Acanthurus bahianus* and *A. coeruleus* (Fig. 35) (Colin, 1985). Permanent tether lines were attached to the reef in 60 feet of water and occupied by an observer for an hour or more each afternoon.

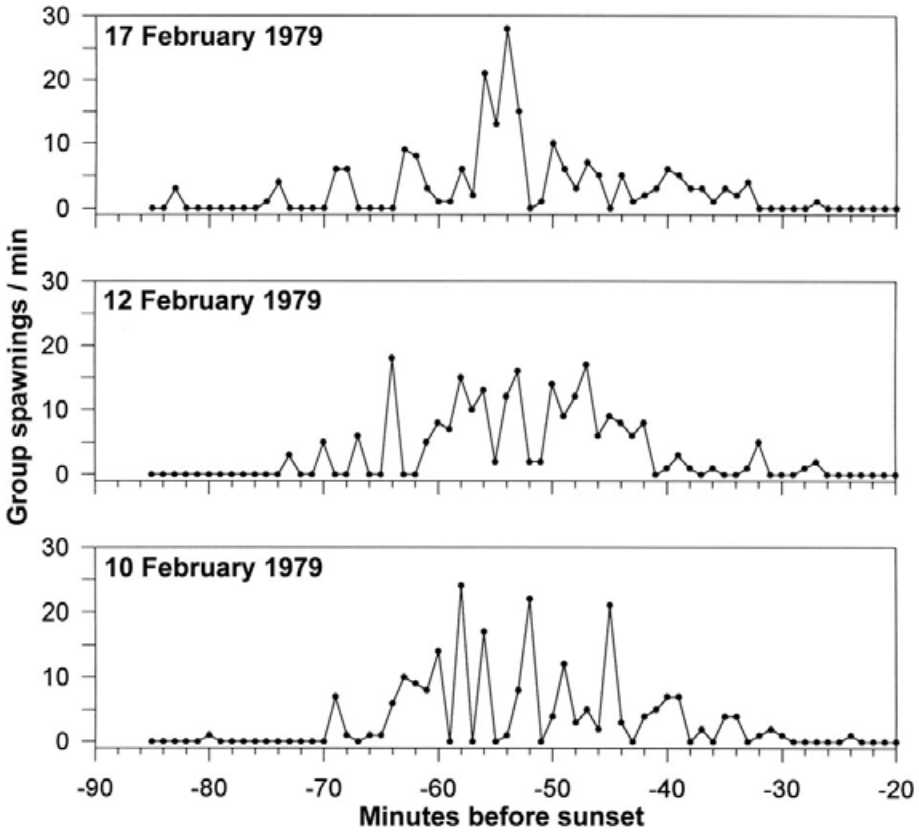


Figure 35. Daily spawning activity of an aggregation of** Acanthurus bahianus **off La Parguera, Puerto Rico. Data were taken at 1-minute intervals from within a consistent field of view monitored by an observer tethered above the bottom. (After Colin, 1985)

Tethering can also be done while on snorkel at the surface. Many times it is advantageous to be at the surface motionless, able to hold place in a current, for observations. This was true for the humphead wrasse, *Cheilinus undulatus*, which is very wary, despite it size, and readily disturbed by scuba divers. Since it spawns near the surface, in Palau it could be easily observed while snorkeling and a long line to the bottom allowed the observers to hold position over the drop off without swimming actively.

Where tethering isn't feasible, it might be useful to pick a single point from which to make observations (Colin, 1978). Fishes tend to become habituated to a diver's presence over a period of days. Look for a location where there might be a rock or coral head you can hide behind to reduce your visual presence.

Divers using open-circuit scuba find that the noise and bubbles of exhalation are particularly disturbing to fish. While no longer commonly available, some observers prefer the old-style "two-hose" regulators, which exhaust the bubbles behind the head and are somewhat quieter than single hose regulators. The benefits of diving rebreathers are an unknown factor when considering spawning observations. It might seem that having no bubbles or scuba noise would be a positive factor in reducing disturbance of fishes, but no one has actually used such equipment on any major spawning aggregation study, as far as we are aware.

Diving Techniques and Dive Safety

Many aggregations are located in remote areas, far from shore, and it is often necessary to dive at or near the time of sunset. Such dives may well last until well after sunset, particularly if the divers need to do safety stops or decompression at the end of the dive. Also it is often rough in areas of spawning aggregations, making it difficult for boats to anchor while divers are in the water, or currents may make it difficult to work from an anchored boat. Techniques need to be used that allow divers to work safely in such conditions.

One example we can cite, where it was difficult to work from boats was the Vieques tiger grouper aggregation (Sadovy *et al*., 1994). There the aggregation was deep (33-35 m) with no shallow water for anchoring is nearby. If the boat did anchor, the bottom was such that the anchor may easily foul. Recommended approaches to dealing with this were to either install a temporary mooring for the dive boat or else dive around a temporary shot line. The shot line should have an adequate lead weight, a line length perhaps one third more than the water depth (i.e. for a 30 m depth, the line should be at least 40 m long) and a large, visible surface float. The float should have a light or at the very least reflective tape, so the boat on the surface can find it easily in the dark. The shot line is dropped at the correct area and the divers are dropped by the boat at the shot line when ready to dive. The divers descend the shot line to the bottom and ideally work around it and ascend up the shot line at the end of the dive. For maximal safety, the divers could move out from the anchor weight using reels and lines, to prevent losing the location of the ascent line, or, a small chemical light could be attached at the anchor weight to help guide divers back to it in the dark after sunset.

The importance of divers not losing track of the ascent line or anchor line of the boat in the dark can not be overemphasized. In the case of an anchored boat, a diver surfacing some distance away may not be easily seen or recovered, since the boat probably must wait for other divers to ascend first. There is the possibility that currents may prevent a surfaced diver from swimming to the boat. Obviously each diver must have devices to indicate their location in the dark, such as lights and whistles (or other sonic device).

For any work on spawning aggregations at depths below about 10 m, it is almost essential to use decompression computers to track bottom time and the need for decompression. The dive computer will allow the observer to focus on the behavior of the fish, rather than constantly be scanning depth gauges to note any change in depth that would affect no-decompression limits when using dive tables. Spawning aggregation dives often involve multiple depth levels, which are well-suited to dive computers.

Nitrox (compressed air with additional oxygen added to reduce the percentage of nitrogen in the gas mix) diving has great potential for use in spawning aggregation work. In many cases, spawning aggregations occur over water depths where nitrox diving is most advantageous, generally about 20-40 m depth. For example, if a 32% oxygen mixture (rather than the 20.8% found in normal air) is used to dive to 30 m, this makes the effective air diving depth of about 24 m. with a no decompression bottom time at 24 m of nearly 30 minutes, this almost doubles the amount of no decompression bottom available on a first dive to a depth of 30 m. Repetitive dives on nitrox gain an increasing advantage, compared to air, so that more effective bottom time at a given depth is achieved using nitrox. The down-side of this is that the lower depth limit for nitrox is much less than for air. Workers should receive specialized training in using and handling nitrox before attempting to dive with such gas mixtures, as the dangers of oxygen toxicity and other factors are very real.

Photographic and Videographic Documentation

Still photography

Still photography using either film cameras or digital cameras can provide extremely useful information on both aggregations and spawning. In general if you wish to capture a large area with many aggregated fish, it is usually necessary to use available light (e.g., Fig. 36). Wide-angle lenses are useful for getting the overall scene. Ideally some feel for the bottom communities can also be obtained from wide-angle photography. For more detailed documentation of color patterns of fish, it is usually beneficial to use electronic strobe illumination for photography.

In the first study to publish any photographs of reef fishes spawning planktonic eggs Randall and Randall (1963) included stills taken from 16 mm motion picture shots of spawning ascents by groups of *Sparisoma rubripinne*. Myrberg et al. (1988) contains excellent photographs of spawning groups of surgeonfishes taken with Nikonos and super-8 movie camera. Colin (1978) had spawning sequences of *Scarus iserti* (=*croicensis*) taken from motion picture footage. Gilmore and Jones (1992) obtained excellent photographs of color patterns of deep-water groupers in Florida aggregations using the external still camera on a submersible. Such photographs, along with notes and sketches made of color patterns, can be used to prepare drawings showing color patterns associated with various behaviors (see Gilmore and Jones, 1992 for examples), illustrate spawning behavior or sequences, or directly show color changes associated with courtship (Fig. 37, 38).

In many instances photographing upward against lighter surface layers, particularly for aggregations in deep water where the fish move off the bottom at the time of spawning, such as Nassau groupers and some snappers, can provide a useful exposure whereas photographing downward would not have sufficient light for a decent exposure. Upward oriented photos can provide useful information on fish numbers, since they generally appear as silhouettes against a lighter background.

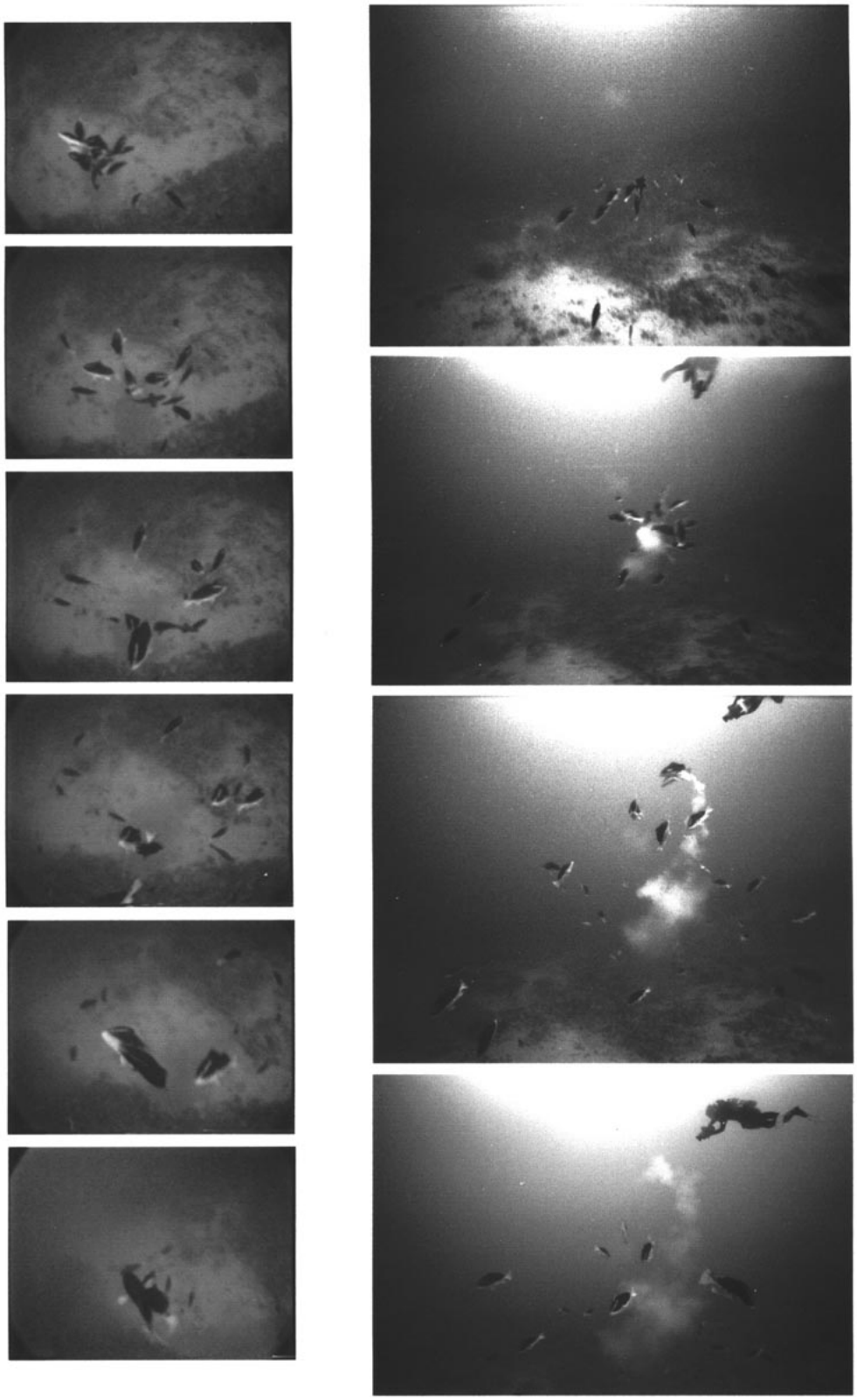


Figure 36. Spawning sequence of Nassau grouper, *Epinephelus striatus****, as recorded by video (left) and still photography (right). The left panel has stills taken from a 8 mm analogue video tape while the right photos of the same spawning sequence which were taken using a Nikonos camera with 15 mm lens on Tri-X black and white film and available light. (PLC)***

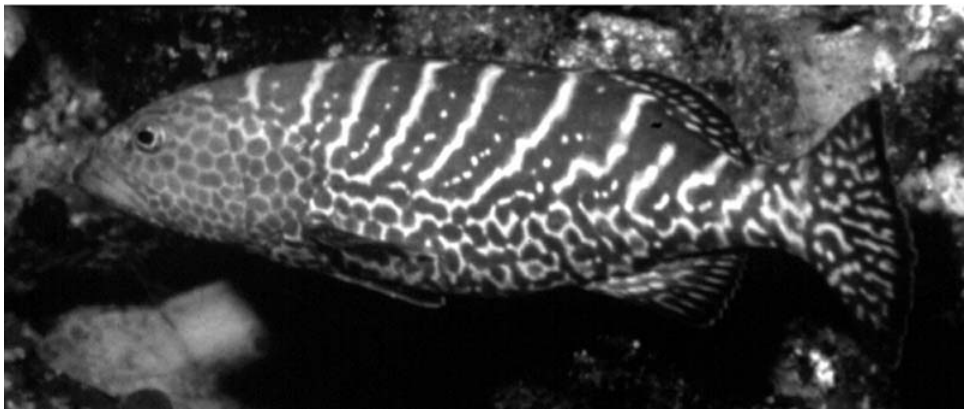


Figure 37. Normal color pattern in the adult tiger grouper, *Mycteroperca tigris.*

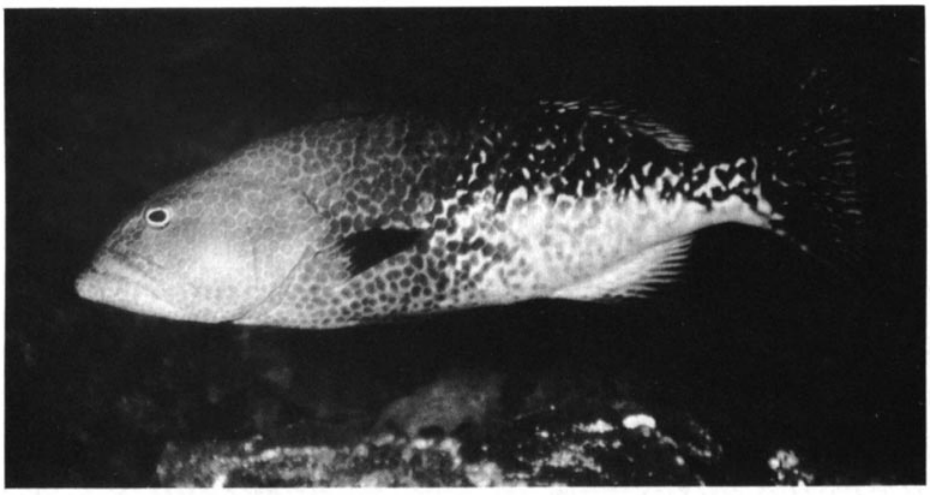


Figure 38. Male tiger grouper, *Mycteropeca tigris****, in courtship coloration near Vieques Island, Puerto Rico, February 1992 (from Sadovy et al. 1995) copyright M.L. Domeier.***

Color film can be used in low light situations, with ASA ratings being able to be push developed to over 1600 or so. However, when film is pushed, graininess increases and detail is lost. A fast black and white film, such as Tri-x, is perhaps preferable to color slide film for ambient light photos during late afternoon. During the period near dusk, it is almost always necessary to shoot with the camera aperture wide open and a slow shutter speed. This requires a steady hand on the camera. We feel the Nikonos series cameras with wide angle to normal angle lenses and high speed B&W film are the best way to get still photos of low light spawning sequences. Often still photographs and video are used to prepare a drawing showing the typical spawning behavior of a species (Fig. 39). This allows all the aspects of a spawning act to be shown in one picture.

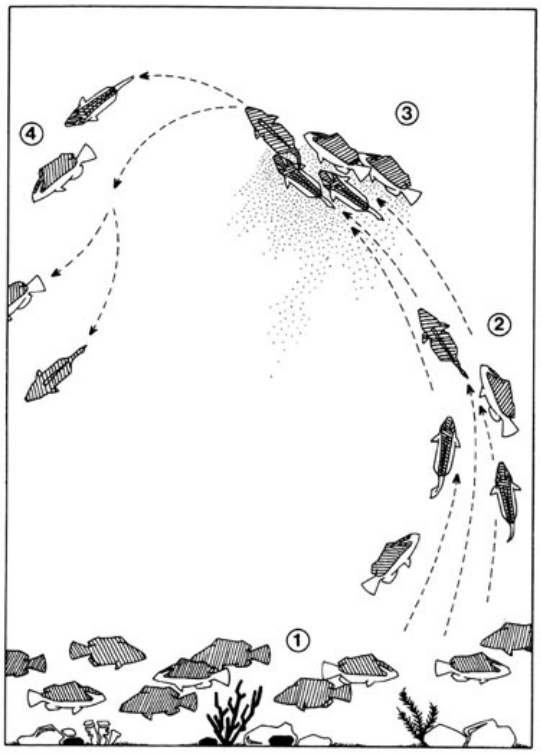


Figure 39. Generalized pattern of a spawning rush by the Nassau grouper (from Sadovy and Eklund, 1999 - NOAA Tech. Rpt. NMFS 146) indicating the color patterns, motion and gamete release point for the spawning. Numbers represent different stages of the spawning rush: 1. fish begin to move into the water column; 2. small group of fish rising; 3. sperm and eggs are released, and 4. fish return rapidly to the substrate.

Digital Still Photography

Digital photography is becoming increasingly common underwater and would seem to have great potential for documenting spawning aggregations. The light sensitivity of such cameras is generally better than high speed film, and the ability to quickly download the images taken, and manipulate their contrast and brightness, allows rapid use of such photos in the field. There are many underwater housings for the popular digital cameras, however, at this point we can not make any specific recommendations since our experience in this new field of photography is limited.

Useful still images can be obtained from digital video cameras by "grabbing" individual frames. This would be useful in analyzing a spawning sequence (see below).

Artificial Illumination

To obtain the details of coloration in aggregated or spawning fish it is usually necessary to provide strobe illumination for still photos. Artificial lighting is generally not useful in photographing overall aggregations as it can only illuminate a small area and disturbs the behavior and coloration of the fish.

Video Cameras and Underwater Housings

Underwater motion pictures for aggregation and spawning have pretty much been superceded by video camcorders, and only the most unusual circumstances would warrant shooting motion picture film of aggregations for scientific study.

The underwater video camera is perhaps the single most useful tool in documenting aggregations (Fig. 40); including the species present, numbers of fish present, and their behavior, including courtship and spawning. Before the mid-1980's, scientists were limited to still photographs and occasionally, super-8 or 16 mm film, to visually document aggregations. The range of video camera and housings available today is great and the selection of the right equipment can be important for optimal results.

Today the major choice is between digital and analogue video cameras. Each has its advantages and neither type is ideal for all situations. Digital video cameras provide better resolution, the ability to stop-frame with high resolution, the ability to make full resolution copies and export digital images. Their one drawback, however, is a lesser light sensitivity, compared to analogue video, and given that much spawning and courtship activity occurs in low light conditions around sunset, and in some cases sunrise, this can be a major disadvantage. Ideally a study should have both types of video cameras available, with the analog camera specifically capable of imaging in low light conditions.

Figure 40. Typical underwater video camera with the housing on the left and camcorder on the right. This particular model, a Sony VX-1000 is a high-end three chip digital camera. It produces excellent images, but is limited in its low-light level capabilities.(PLC)

With zoom lenses, a single video camera is capable of capturing the broad picture of an aggregation, then zooming in on specific behavior that might have to be recorded from some distance away (assuming clear water). Tape length becomes a factor if extensive recording needs to be made on a single dive. Digital cameras generally having a 60 min tape length while analogue cameras have up to 120 min tape length. Battery life also is important, as earlier video cameras often did not have the battery power to record an entire video-tape without recharging the battery. More recent video cameras have improved batteries and lower power consumption, so an entire tape could be recorded on one dive. However, if using or purchasing an earlier video

camera, the factor of recording time on one battery charge is important. Also, batteries used for underwater videos need to be in good condition and their recharging carefully undertaken to retain maximum battery length. There is nothing worse than having your video camera die in the middle of recording seldom seen and critical activity.

It is possible to do analogue to digital transfers, which can then be used for stop frame analysis not possible directly viewing the analogue images.

Video Lights

The use of external video lights is a subject that needs some consideration. In general, it is not advisable to use external video lights to try to document aggregations and spawning behavior. Lights usually constitute a disturbance beyond that of observers that can interrupt behavior or drive the fish away from observers. In mid-day periods, if an aggregation is large, lights are not powerful enough to provide significant illumination compared to ambient light. During low light periods, when they would be more useful, the disturbance factor increases greatly.

Garth (1991: 48) described what happened when video lights were used to try and film Nassau grouper spawning. He writes "they were all swollen with milk (sic) and eggs, so spawning was still to come. However, we noted that the lights from our cameras spooked them. Some even changed back to their normal color as fast as the light hit them. As it happens, the fishermen are still complaining that the catch has not been as good since Cousteau came several years ago. In an attempt to capture the spawning on film, the Cousteau team had set up lights that burned all night. Nothing of substance was obtained, presumably because of the lights".

If using still cameras, attempts to document spawning behavior can be done using high-speed film, either color or black-and-white, or flash photography. While fishes are usually disturbed by video lights, they are less likely to be disturbed by electronic flash. Consequently, some good photographs showing color patterns and such can be obtained by judicious use of electronic strobe photography. The flash may interrupt behavior, but this is usually just temporary. It can be argued that an electronic flash is something which is not totally alien to reef fishes, as they would be exposed to light from lightening flashes both day and night, whereas a continuous light emanating from a video or movie light is something they would not normally encounter, hence would view it as more disturbing.

Using Video Equipment

How the video equipment is to be used is important. It is advisable to tape a short section at the beginning of each recorded segment showing the time, date and location where the tape is to be recorded, for reference purposes. We believe it is extremely useful to adopt the strategy of continuously recording activity at the spawning site. In other words, the video camera is started at some point and run continuously until the end of a dive or observation period. This goes somewhat contrary to impulse, in that it would appear to "waste" video-tape, however, if the intention of the project is to extract maximum information from observations, it is a reasonable thing to do. First, by knowing the time the camera was started, this allows the time of any event occurring to be determined. Some of the more advanced digital video cameras have a time clock built into their recording system, that would allow this information to be obtained without continuously running the tape, but not all cameras have this feature. If the tape is constantly recording, then when something happens suddenly, the observer can tape it simply by pointing the camera towards it, rather than having to activate the record system, which may take several

seconds and valuable information will be lost. Also, the timing of rapid spawning events can be determined later by analysis of the recording, resulting in better data. Below is an example of spawning frequency information extracted from video-tapes.

One unusual advantage of using a video camera is that the activity of say a pair of fish in one direction, can be recorded by simply pointing the camera at them while the observer is actually looking at something else (a second pair of fish) in a second direction. Rather than twisting the head back and forth, the tape can be reviewed later to see what the first pair of fish was doing.

Disadvantages to this continuous taping approach are that unless the camera battery is well charged it may die before the end of the tape is reached. This is not a good thing to have happen. Also a lot more recorded video-tape is generated, with greater costs for tapes and need to store them.

It is also useful to consider mounting the video camera on a tripod, or simply setting it on the bottom in a safe place (where there is minimal surge, etc), then at some point starting the record function of the camera and swimming off to leave the camera to run for its tape duration. The diver starting the camera can go elsewhere, or totally exit the water. Fish very quickly adapt to the presence of the camera and behave as they would normally. This technique proved useful in studies of spawning behavior of Nassau grouper and especially the humphead (Napoleon) wrasse. Also because the camera does not move, changes in numbers of fish in its field of view can be quantified over time, giving perhaps insight into activity that would be impossible to obtain by direct diver observations.

We have used cheap standard camera tripods to mount video cameras underwater and these have proved satisfactory over periods of months. Try to avoid tripods with lots of easily corrodable metal parts, look for plastic legs, locks and other parts. Some lead diving weights can be taped to the legs to increase the weight and stability of the tripod underwater in currents and swell. In extreme conditions, or when you might leave the camera and tripod overnight or longer, it would be advisable to tie the legs of the tripod to structures on the bottom. Simple, non-adjustable tripods can be made from concrete reinforcing steel (rebar) welded together. Such camera mounts can be adjusted by actually bending the legs underwater once a particular site is selected.

The next step beyond a tripod mounted stationary video system would be a system which can be put in place at any time, with a self contained computer programmable timer/controller, that will turn the video camera on and off at times selected in advance. Such a unit, which we could call an "Autonomous Underwater Video System", or AUVS (Fig. 41) would prove, we think, exceptionally useful in documenting parameters of many spawning aggregations, particularly those in deep water or remote locations where regular diving observations are not feasible. Besides the underwater video system, an AUVS could include a pan and/or tilt mechanism to increase its observational view, controls for lights or other equipment. One of us developed a prototype system some years ago (Fig. 41), which was successfully used to document feeding

Figure 41. Autonomous underwater video system (AUVS) being programmed prior to deployment (left) and in use on the reef (right).

activity in garden eels, cleaning activity at a cleaning station and occurrence of fishes in a "ghost" fish trap. However, it has not yet been used to document aggregation presence and behavior, its originally intended role! A new generation of AUVS is under development, using digital video camera and lower power consumption components. Such a unit will find much use in aggregation studies and many other areas of marine biology and oceanography (Fig. 42)

There is also great potential in using stereo video in documenting fish aggregations. Harvey and Shortis (1997) have described a system useable to assess the accuracy and precision of measurements of fish length and distance from the cameras. This type of system has yet to be utilized on any spawning aggregation, but would seem a natural extension of this technology.

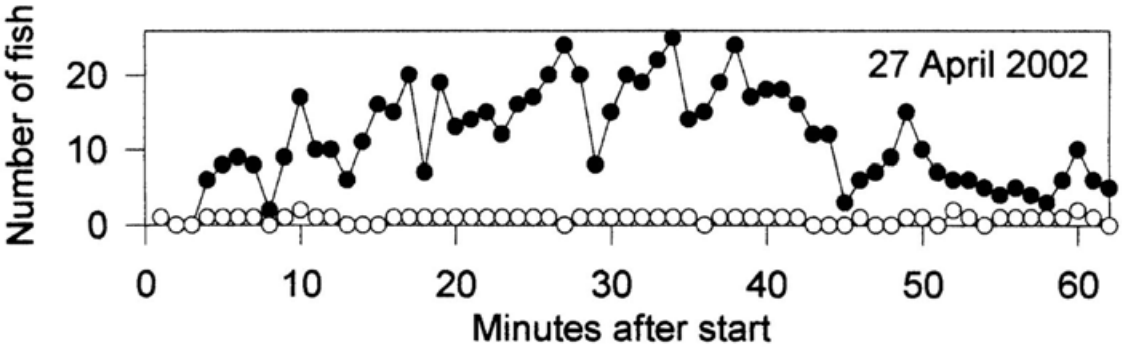


Figure 42. Appearance of** Cheilinus undulatus **at a spawning site in Palau, determined by unattended video camera on a tripod at the site. Number of females (dark circles) and males (open circles) within the field of view during a 10 period each minute (PLC)

Instrumentation on Aggregation Sites

Gathering additional information on physical parameters at aggregation sites is very useful and important, as we discuss below. The most important among these are probably temperature and current speed/direction.

Temperature measurement

Temperature is relatively simple to document since there are many data-logging instruments now available. Temperature monitoring at sites can range from making

measurements only during an aggregation period, to installing instruments to monitor temperature continuously over the year at the site. The latter option is best, since it would allow you to characterize the annual temperature cycle at the site and look at the temperature trends before and

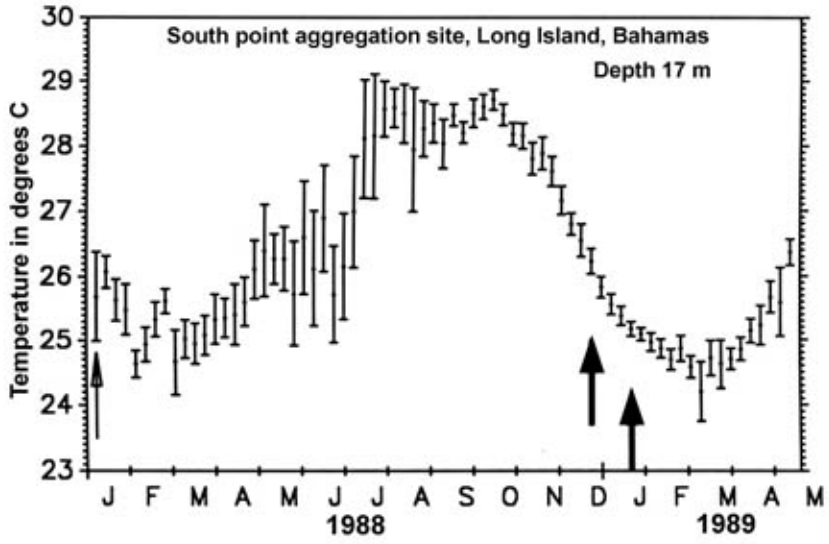


Figure 43. Annual temperature pattern at a Nassau grouper aggregation site, Long Island, Bahamas. Data are weekly means with range represented by the vertical bars. The times of aggregation and spawning are indicated by arrows. (after Colin 1996)

after the time of aggregation. Colin (1992) installed a thermograph at a Nassau grouper aggregation site in the Bahamas (Fig. 43). This study found that the aggregation occurred on two different months at temperatures of 25-25.5^{o}C. The aggregations occurred during the general downtrend of temperatures in winter, but were not at the time of the yearly minimum. If temperature had been measured only during the aggregation, there would have been no way to place aggregation occurrence in relation to the annual thermal cycle. It is also useful to monitor temperature at some sites not used for aggregation, to see if the aggregation site might possibly have some different temperature regime, say induced by upwelling, compared to non-aggregation sites. Where multiple instruments are involved, they should be carefully cross-calibrated to provide comparable numbers.

Current measurement

Currents involve both a current speed and a direction making them more difficult to measure than temperature. Their measurement can be approached as either a simple or complex undertaking. In most cases we would be focused on "why" a fish is using a particular site, so it is important to gather information on the currents at the site both during spawning and at other times and at other sites nearby. There are, unfortunately, no inexpensive current meters comparable to the recording thermographs now available. Recording current meters typically cost a minimum of a few thousand dollars, but if the project budget can afford them, they are well worth the investment. Sancho *et al*. (2000) used InterOcean S-4 current meters mounted on 1.5 m tripods in a channel with an average depth of 4.5 m. Colin (1992) used the General Oceanics tilt vane current meters successfully during the Nassau grouper work to document currents both at aggregation and non-aggregation sites (Fig. 44). This meter uses a special stand-off from the mooring wire that reduces meter oscillation due to swells.

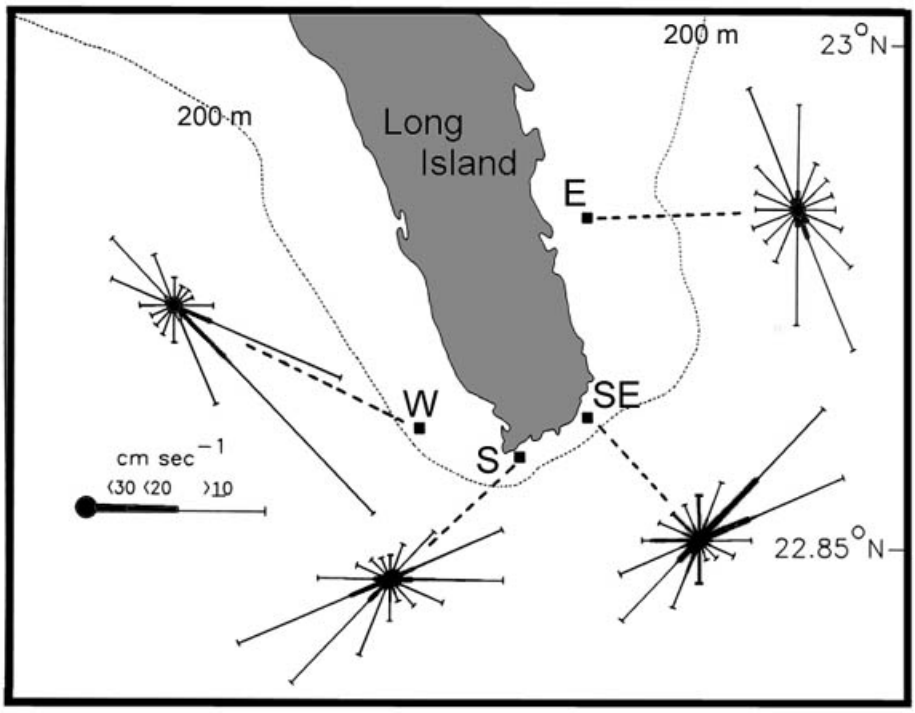


Figure 44. Currents around Long Island, Bahamas as recorded by stationary current meters, October 1988-March 1989 (redrawn from Colin, 1996).

In most cases, the currents at and above the level in the water column where the gametes are released are of most interest. This usually means the current meter needs to be moored above the bottom using a mooring line topped by a float capable of supporting the meter and mooring above the bottom in currents (which would tend to pull the mooring down) expected at the site. For anything other than just a short deployment, we would recommend using stainless steel wire rope for the mooring, with the ends made into loops, with stainless steel thimbles inserted, using either crimped nikopress sleeves or wire rope clamps. If properly crimped or clamped, there is little chance the mooring line will fail. The mooring must be anchored to the bottom, and we recommend a heavy (20-30 kg) lead weight with a solid eyebolt to which the mooring line is shackled. It is likely the mooring will be set up by a diver and in the case of the mooring anchor, the heavy lead weight is best moved into place using a lift bag. There are benefits in having the entire mooring (anchor, line, meter and float) prepared on the surface and put into place as a unit. It would help to have the site selected for the meter carefully located and buoyed at the surface so there is little need to swim any distance with the mooring. If possible the exact depth of the meter on the mooring can be adjusted after deployment through some type of moveable clamp on the mooring.

Flowmeters can be used for measuring currents at spawning sites, but have the disadvantage of having to be read manually, usually by a diver, at certain time intervals and provide no current direction information (although a diver can determine the direction of the flow meter using an underwater compass). General Oceanics Inc. makes an excellent plastic flow

*

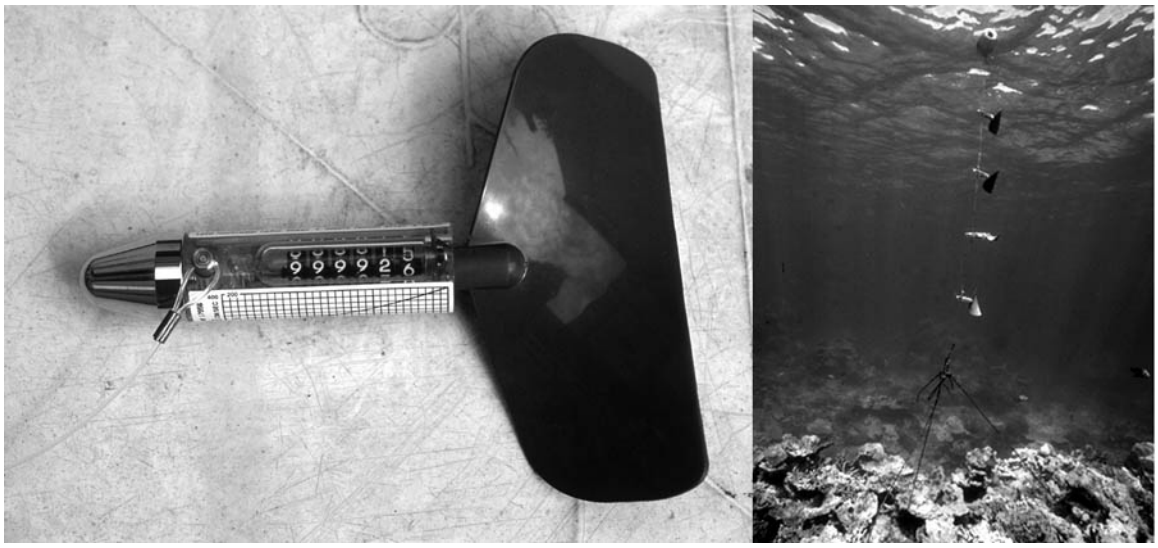


Figure 45. (Left) General Oceanics flowmeter, showing the low speed rotor and mechanical counter for revolutions. (Right) Vertical array of four flowmeters, tethered to reef by lines and held up by subsurface float.

meter (GO Model 2030 mechanical flowmeter) which counts the revolutions of a small propeller and is designed to face into the current. The version with the "low speed rotor" is probably most suitable for aggregation current measurements. A stacked array of flowmeters can be used to give a vertical picture of current distribution (Fig. 45), as in the illustrated case of the current coming off a shallow reef as it reached deeper water. The flow meter has to be manually read at intervals, and the current speed later determined by a revolutions/time determination. At a minimum, the flow meter could be read at the start of a spawning period and again at the end, with current direction measurements taken similarly (Fig. 46).

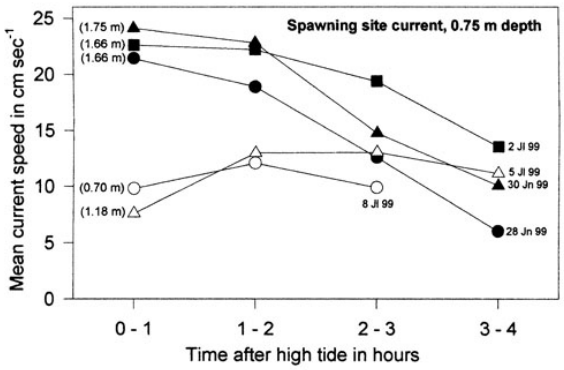


Figure 46. Currents flowing off Lighthouse Reef, Palau after high tide, as determined by moored flow meters. Black symbols are spring tide days while open symbols are neap tide days (PLC).

Flow meters could also be something as simple as a model airplane propeller or some type of fan blade in which the revolutions are counted for some period of time, such as 1 minute.

The flow meter would need to be calibrated against some device for which relative current speeds are known, but would be an inexpensive alternative.

At the simplest extreme Shapiro *et al*., (1993) measured current velocity by releasing fluorescein dye from a syringe 1.5 m off the bottom and timing 150 cm of movement of the leading edge of the dye. Shapiro (pers. comm.) also used a simple device to estimate current speed. A neutrally buoyant object, like a small buoy or solid ball is attached to a string of known length. The object is released by a hand also holding the end of the string, the time it takes the object to reach the end of the string provides an estimate of current speed. For example, if it takes the object 10 s to reach the end of a 1m line, the current is approximately 10 cm sec^{-1}.

Light Measurement

As far as we are aware, no investigators have measured light intensity quantitatively in connection the spawning aggregation studies. There is no reason why this could not be done, and might be interesting to do for species that aggregate and spawn in late afternoon, irrespective of tide, such as *Acanthurus coeruleus* and *A. bahianus*. Whether the time of spawning is affected by the amount of light on any given day, which can vary at the same time due to cloudy conditions, is unknown. Instruments to quantify light intensity could either be a self contained unit, or a unit with the sensor on a cable and the display portion on the surface, usually in a boat.

IV. G. Migrations and Tagging Documentation

In the past researchers who wanted to study fish movement could choose from a suite of conventional tags (spaghetti tags, Peterson disc, T-tags, various dart tags, internal anchor tags, dyes etc.), that all resulted in the same data: a location of tagging and a location of recovery. Although interesting, these data can be misleading. In many cases it gave us more information about where people were fishing than where the fish were actually spending time. For example, a species may have a wide ranging seasonal pattern of movement that passes through a popular fishing area, resulting in the tag to be recovered very near the spot it was originally tagged a year earlier, but giving no indication of the vast migration in between. Sampling strategies can be developed to avoid such potential bias.

Many aggregating fishes are known or believed to migrate to spawning aggregations. These migrations can be a daily event (typically for resident aggregations) or may occur only during a limited portion of the year (for transient aggregations). Often biologists want to know from what area does a known aggregation draw its members, and until recent times it was usually necessary to tag fish and hope for their recovery at a later date. This question can be approached in two ways. Fish can be tagged when they are dispersed, prior to an aggregation, with possible capture at the aggregation. Otherwise, they can be caught and tagged at an aggregation, then be captured later after they have dispersed. Ideally both strategies could be used, however the particular circumstances may favor one or the other. This passive tagging requires some way to physically tag the fish and often involves capture of the fish. Physical tags can be of many types, such as dart tags with a streamer or beads exposed externally, or other markings such as freeze branding by liquid nitrogen or acrylic paints. Internally fish can be tagged with sonic tags or micro-chip tags.

Among studies of spawning aggregations, Myrberg et al. (1988) tagged *Acanthurus nigrofuscus* by first herding the fish into large monofilament nets, then captured by hand net. They were tagged with "textile tags". The tags had no effect on the behavior of the fish, some tagged fish spawning the same day after capture and tagging. Myrberg et al. (1988) included

studies on migration, surveying fish swimming to spawning sites from the point along the shallow reef where they normally left the reef to proceed over sand to the spawning site. The site selected as the place to count the temporal occurrence of fish was critical and provided much interesting data in this study.

External tags can also sometimes be placed without capturing the fish. They can be emplaced by spear where the fish can be approached closely enough to accurately place the tag. Colin (1992) used this technique to tag dispersed Nassau grouper, while C. Koenig and others have used similar methods on goliath groupers (jewfish) in the Atlantic. Difficulties include getting accurate placement of the tags, and the limited time that can be spent underwater finding the fish and placing the tags. When the fish can be easily captured by hook and line or traps that do not negatively affect their survival, they can be tagged quickly and released with high quality placement.

Freeze branding is another marking technique that has been applied to the study of reef fish. Zeller and Russ (1997) freeze-branded adult *P. leopardus* using liquid nitrogen cooled numerals, tagged specimens remaining healthy with brands evident during visual censuses for a period of at least 2-3 months. This might be a useful technique to tag fish captured from an aggregation site and immediately released after branding. Their continued presence at the aggregation site would indicate the number of days an individual fish remains at the aggregation.

In general, tagging while dispersed is the most feasible way to determine the "capture area" of an aggregation. There are several reasons for this. If fish are tagged while dispersed, then the most concentrated fishing effort and observational effort can be focused on the aggregation itself, hence a greater chance of finding tagged fish when they are concentrated and often being caught. Also a very high reward can be offered to fishermen for capture of any tagged fish and this provides a modest economic incentive to cooperate with researchers. It also offers an opportunity to let a fishermen, who captures a tagged fish, know exactly where the fish was tagged (as long as the information is readily accessible to the field workers), something which again may pique their interest in the research. Also if a tagged fish is seen in an aggregation and the tag is externally identifiable (say by color coded beads), the fish can be searched for regularly over a period of days, providing extra information on duration of individuals at aggregations, etc.

If fish are tagged during an aggregation, there are several problems. First, the tagging operation, either through capture or underwater tagging, may disrupt the aggregation. Fish captured, say by fish traps, tagged and released quickly at the aggregation site may not behave normally and observations of such fish may distort ideas of normal behavior, etc. On the other hand, tagging of aggregated fish does offer the chance of doing tag-recapture studies to assess total populations, etc.

Johannes *et al*. (1999) tagged groupers caught by hook and line from aggregation sites in Palau. After capture, fish were held in a tub of seawater, then measured, sexed (if possible), excess gas removed from the swimbladder using a hypodermic needle, and then tagged. Ten-12 cm Hallprint dart tags were used, inserted so the barb was anchored beneath vertebral spines or pterygiophores. Although results from this study were mixed, with reluctance of fishermen to return tags having a large influence on success, recaptures showed that at least some individuals returned to the same aggregation sites from one year to the next.

Migration routes can be determined in a number of ways. Where there are large numbers of fish undertaking a daily migration, the route can be marked by dropping painted stones as the

fish are moving and later the route can be surveyed by compass and tape (Myrberg et al. 1988). Alternatively, if the migration path is in shallow water a snorkeler can swim the route of the migration above the fishes, and either have a GPS unit in a water proof housing, writing down the coordinates at regular intervals, or have a boat follow close by that has an observer recording GPS readings. In the latter case, it is more feasible to have the swimmer going in the direction opposite to the migrating fishes, in this way the person meets the migrating fishes head on and does not potentially disturb their choice of pathways by swimming along with them. This method was used successfully in tracking scarids in Palau that were migrating along a shallow reef towards a channel in the reef for a daily spawning (see Fig. 10). The migration path determined by GPS coordinates can be applied to other GPS determined maps of the spawning area, such as bathymetry, habitat maps and aerial photography.

Telemetry to Document Fish Movements, Behavior and Habitat Use

The last 10 years has seen the development of new technologies that have proven to be valuable tools in the study of fish movements and would have considerable application to spawning aggregations. Archival tags, acoustic tags and satellite pop-up tags have all revolutionized the study of fish movement, behavior and habitat usage. A data logger coupled to a pressure sensor, thermistor and a light receptor allows for the nearly continuous collection of data that permits us to determine the temperature, depth and geographic position (calculated from light levels) of the fish while carrying the data logger. An archival tag is simply a data logger that is placed in, or on, the fish and later recovered if the fish is recaptured. Pop-up satellite archival tags (PSATs) collect the same information as an archival tag, but the fish need not be recaptured because the tag detaches from the fish, floats to the surface and transmits the information to a satellite. Because of the relatively slow baud rate of the data transfer to the satellite, much of the data must be compressed into histograms. Because of this data compression, PSATs do not provide nearly as much resolution as an archival tag, but the rate of data return is usually much higher because the majority of archival tags are never recovered.

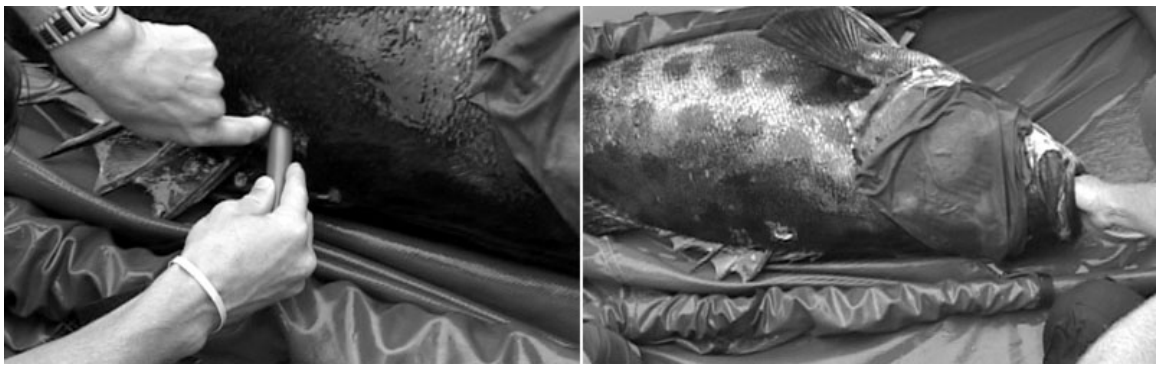


Figure 47. Handling of this large black sea bass is similar to that which any fish being implanted with an acoustic tag should undergo, if properly handled. In the left photo the tag is being inserted intramuscularly into the dorsal musculature. On the right, the fish is having its gills irrigated with fresh water from a hose and the eye is covered which calms the fish.

Archival tags have not yet been applied to the study of reef fish aggregations, but PSATs have been deployed on goliath grouper in Florida. One difficulty with both of these tag types is that the geo-location data that result from the light data are not very precise (resolution of 1 degree of latitude and longitude), so the fish must move a great distance to provide a useful track. Longitude is more accurately estimated than latitude from light data. In some cases where the coastline is running east/west, a combination of longitude and depth (if the behavior of the

species allows for confident determination of bottom depth) can be used for a fairly good position. For reef species that spend much of their time near the bottom this technique holds promise. Additionally, the location of the tag when it surfaces is pinpointed by the satellite with a great deal of accuracy, which provides an end point from which to interpret earlier position data.

Acoustic tags are different in that they do not usually collect data (the exception is the very large CHAT tag (Vemco) that is not presently not suitable for reef fishes), but instead they continuously transmit a pulse at a specific frequency that allows for very fine scale movement studies. Some have an integrated pressure sensor that allows for determination of the depth, or a thermistor that can transmit internal and/or external body temperature. They can be externally affixed via a leader and dart (can be done on a vessel, at the side of the vessel or in situ with a modified spear gun) or surgically implanted in the peritoneal cavity or intra-muscularly (Fig. 47). These tags can be monitored through either manual tracking or remote sensing. Manual tracking allows for extremely precise movement data but is usually limited to 24-48 hours due to the requirement that the researcher must follow the tagged fish with a boat and listening device. Eventually the researcher becomes exhausted and the boat needs fuel. For species that do not move much, individuals can be relocated and tracked many times over the life of the tag. Short term tracks are always suspect due to the fact that the trauma of capture and surgery can have an effect on behavior. Specimens that are followed over a period of weeks to years (by relocating the fish) result in more valuable data since the tracks are not influenced by the immediate trauma of capture and tagging.

One excellent example of manual tracking of individuals moving to and from a spawning aggregation (*Plectropomus leopardus*) was conducted by Zeller (1997). The Nassau grouper has also been the subject of studies using acoustic tags. Bolden (2000) tagged Nassau grouper with surgically implanted acoustic tags, held the fish for several days before release and successfully monitored their presence for several weeks thereafter. One fish was captured months later at a spawning aggregation, and the tag discovered when the fish was cleaned. This fish had migrated over 200 km from the tagging site to the aggregation site. Carter et al (1994) acoustically tagged two Nassau groupers prior to the spawning aggregation, and one was recovered two years later 150 miles north of the tagging location. They also tracked a number of non-aggregating fish to determine home ranges and daily activity patterns. One fish was captured months later at a spawning aggregation, and the tag discovered when the fish was cleaned. This fish had migrated over 200 km from the tagging site to the aggregation site.

Remote tracking can be done by mooring data logging hydrophones in the study area. Multiple hydrophones can be spaced to create a continuous array that gives complete coverage of the study area. If the tagged individual remains in the study area a continuous record of its movements is obtained. When depth coded tags are used the researcher gets both movement and depth data. Multiple specimens can be tracked simultaneously. When tags with long battery life are used (up to 5 years), a tremendous dataset can result. This technology has been applied to the study of spawning aggregations. One example exists at Anacapa Island, one of the southern California Channel Islands, where an array completely encircles the island. This array has been used to study giant sea bass, thought to form a spawning aggregation at the island, for almost 2 years. Individual fish have been documented to return to the island during the spawning season after an absence of 11 months. Other individuals have been shown to reside at the island year-round.

The technologies described here hold enormous potential for the study of spawning aggregations in the future. As the technology for PSATs evolves the tags will become smaller and therefore applicable to a wide array of species. At this time PSATs can only be used on the

largest species (we would not recommend tagging an individual weighing less than 25 kg). Acoustic tags and archival tags can be purchased in a variety of sizes and can be used to study most species of concern.

IV. H. Submersibles and Remote Operating Vehicles (ROV)

Submersible or ROV technology, although expensive, may be the only way to approach direct observations of aggregation and spawning in water below depths for scuba diving.

The only study that has used a submersible for observing spawning aggregations is that of Gilmore and Jones (1992) for deep-water groupers. They found their most productive observations were made when the submersible remained stationary on the bottom for at least an hour with unnecessary systems, such as external and internal lights and hydraulics, turned off. They remained on station for up to 7.5 hr and obtained unique observations in this manner. Low light level black and white video, which was more sensitive than their eyes, was used to watch behavioral interactions.

One of us (PLC) previously used a ROV to attempt to extend the duration of observations of a jewfish aggregation on a wooden boat wreck in relatively deep water (33 m, Colin 1994), but found the device was not especially useful. Despite the presence of up to 20 large groupers on a low profile debris field only 30 m in length, few fish were ever seen with the ROV. The ROV observations were unproductive, compared to diver observations, and on their own would have lead to a very distorted impression of fish abundance and activity. There may be some instances where a ROV is useful for observations, but, in general, any information obtained with such remote technology should be interpreted cautiously.

Section V. Eggs and Larvae and Their Fate

V. A. Obtaining Eggs and Larvae

The collection of a significant number of eggs in the field can be an important element of any study and almost always implies that spawning has actually occurred. Eggs can also be obtained by spawning fish caught from an aggregation, either naturally or artificially. If the eggs are fertile and undamaged, they would permit the description of the egg size and characteristics for species if this information is not already known. The ascent rate (most eggs are positively buoyant) and hatching times at a particular temperature can be determined using these fertile eggs. They can be hatched for larval rearing attempts. Finally eggs can be used for other experimental purposes. As just one example, large quantities of surgeonfish eggs from spawning aggregations to examine the effects of diesel fuel on hatching success (PLC).

Collecting Eggs - Net and Bag Techniques

A number of methods can be used to collect eggs in the field and there is a substantial literature available for reference. Here we discuss methods applicable to spawning aggregation work. The simplest is by straining the water where eggs have been released with a fine mesh net (Colin and Clavijo, 1988; Colin 1983, Colin and Bell, 1991). One of us (PLC) prefers a small hand net about 10 cm across at the mouth and made with plankton netting of about 250 micron mesh (Fig. 48). The net has a short handle, about 15 cm long. This net can be swept through the volume of water where eggs have been released and then the entire net put into a plastic bag, and

the netting everted as it is withdrawn, resulting in the eggs being deposited inside the bag. If desired the net can simply be left inside the bag until removal in the lab. Generally it is best to only about half fill the bag with water, then tie a knot in the neck, so the eggs are captured and unlikely to leak away (unless the bag is punctured). They can be returned to the lab in the plastic bags.

Figure 48. (Left) Quantity of reef fish eggs collected by moored plankton net in only one hour, Lighthouse Reef, Palau. The material on the bottom of the jar is almost 100% fish eggs. (Right) Typical fine mesh hand net used by diver for collecting eggs after spawning. Mesh is 220 micron and the net is about 15 cm across.

Similarly Kiflawi *et al*. (1998) collected eggs of *Acanthurus nigrofuscus* aggregations using a 100 micron mesh net with a 20 cm diameter frame and a 1 liter plastic bottle on the cod end from gamete clouds released by spawning. The bottle had two sides with 300 micron mesh so water would flow through the bottle. Samples were collected by sweeping the net through the area of gametes for 5-10 sec, then the bottle would be removed, capped and put in a zip-loc plastic bag. The net could be cleaned, presumably by everting it, a new bottle installed, and a new sample collected.

Plankton nets moved through the water by divers have also been used to collect eggs. For example, Samoilys (1997b) collected coral trout (*Plectropomus leopardus*) eggs at Scott Reef, Great Barrier Reef using a plankton net of 700 micron mesh towed by divers. While this will work and they usually have a larger mouth size than hand nets, they are hard to handle and often have to be brought to the surface with each sample. A diver-operated plankton net is not an efficient way to capture large numbers of eggs. A small net can be used over and over, and each sample deposited in a separate plastic bag.

Shapiro *et al*. (1994) used large plastic bags (about 67 by 60 cm holding up to 50 l of water) to collect spawns of *Thalassoma bifasciatum* in an effort to capture all eggs and sperm released by these relatively small fish. Using such large bags for collecting eggs has the advantage of having less effect on the normal process of fertilization, compared to collecting eggs with a net, but such bags are difficult (weights would be approaching 50 kg) to remove from and handle out of the water (Marconato *et al*., 1997). Given the documented effects of collection methods on fertilization rates (egg damage or collecting eggs too quickly-generally less than one minute after spawn) with a net, investigators are advised to choose their methods carefully with

consideration of what they want to do. If the purpose of egg collections is to document egg size or perhaps collect eggs for rearing work, net collection may be preferable, while studies of fertilization rates and sperm numbers would probably benefit from collecting eggs in large plastic bags. Some aspects of these are discussed further below.

The potential for damage to eggs collected by net techniques is real. Petersen et al. (1992) collected eggs using hand nets, and found damage to eggs by this technique. Kiflawi *et al*. (1998) found some evidence of damage to eggs by the net, but many eggs were not damaged, hence such samples could be used to document the size and characters of eggs. If fertilization rates are being examined, or some other aspect of spawning that requires all eggs be undamaged, then such net damage becomes an issue. The time between gamete release and collection with a hand net may be the critical factor in egg damage by the net. The longer the time elapsed after release, the less egg damage will occur. Also all "plankton mesh" is not the same. The nitex mesh normally used in plankton nets is fairly rough, particularly when new, and may cause abrasive damage to eggs. The mesh used in "brine shrimp nets", used in the aquarium trade, is softer and may not damage eggs as much (Colin, 1982)

It is likely that increasing the time between gamete release and net collection of eggs reduces the mechanical damage to eggs during collection. The delay needs to be balanced against the dispersion of the eggs after release. Fertilization must have occurred or at the least the sperm penetrate the egg before the eggs can be collected. This happens very rapidly in most reef fishes and generally if about 1 min has elapsed after spawning, the eggs will be fertilized and can be collected. If you jump the gun too quickly, though, the eggs captured will probably be infertile and fertilization rate can not be assessed. For many aggregation-spawning fishes, the gametes released remain visible, particularly with copious amounts of sperm released, for a minute or more, making it is easy to track the location the eggs. For those species that spawn as pairs out of an aggregation, or that do not have a highly visible gamete cloud, marking the water where the eggs were released with a dye is often useful. We have used both ink and fluorescein dye successfully, dispensing a bit of the marker just after egg release a short distance away from the actual focus of the gamete cloud. After waiting the required time, the hand net is swept through the dyed area and all the volume of water around it. Sweeping in a figure 8 motion is often effective in hitting the egg patch. The eggs are then deposited in a plastic bag as described previously. Fluorescein dye in high concentrations can have a detrimental effect on fertilization (PLC pers. obs.), however, so ideally the dye is released a short distance from the gamete cloud to minimize any effects.

Where there is a distinct current associated with spawning and the fish are releasing eggs near the surface, it is possible to use a moored plankton net some distance down current from the aggregation to collect eggs (Fig. 49). Colin and Hamner (in prep) did this at Lighthouse Reef, Koror, Palau and were able to capture many thousands of eggs about 80 m down current of the spawning area (Fig. 48). Disadvantages of moored nets include not being able to collect specific spawns and often the eggs captured have a mix of many species.

Obtaining Eggs - Artificial Fertilization - Spear and Strip Technique

It is also possible to strip eggs and sperm from fish speared or otherwise caught from the aggregated fish at the time of spawning, mix them and obtain fertile eggs (Fig. 50). Colin *et al*. (1987) used this "spear and strip" technique to acquire fertile eggs from red hind, *Epinephelus guttatus*, assembled in an aggregation. Fish were initially stripped underwater after spearing by placing them inside plastic bags, squeezing to expel the gametes and mixing them. Fish were also brought to the surface and stripped shortly thereafter. Finally they were held at room temperature

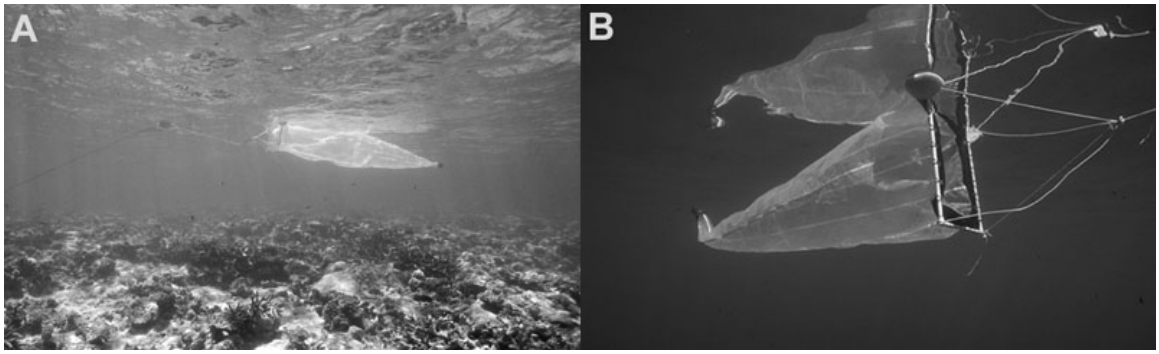


Figure 49. Moored plankton nets on Lighthouse Reef, Koror, Palau used to collect eggs of fishes spawning upcurrent (from Colin and Hamner, in prep). A. Plankton net moored over a shallow reef flat. B. Net moored over deep water. The frame has a four-point bridle, with the upper portion supported by buoys so the net fishes at the surface.

for a few hours after death, and successfully stripped and fertilized. All variations were successful, but stripping immediately produced a high fertilization rate with reduced rates after longer delays. Colin and Clavijo (1988) stripped gametes from a number of species of speared reef fishes, including aggregating acanthurids, to obtain fertile eggs, while others (Randall and Randall 1963) have done additional species.

Rimmer *et al*. (1994) obtained eggs and sperm from coral trout, *Plectropomus leopardus*, speared from an aggregation site at Scott Reef off Cairns, Australia. The sex of fish could be determined by color differences and the selected sex speared and brought to the surface. Gametes were stripped using firm hand pressure to the ventral surface of the fish. Milt was collected in disposable syringes and stored at 5-10^{o}C for up to 2 hours. If milt was not readily flowing, the testes of the fish were removed and macerated to obtain milt. Eggs were stripped into a plastic bowl and their volume measured in a graduated cylinder. Milt was added and sperm activated by the addition of 100 ml of seawater. The eggs, milt and seawater mixture was swirled for 15 min. Afterwards the eggs were placed in a clean plastic tray with a 120 micron screen base and washed in clean seawater to remove excess sperm. The clean eggs were then placed in a 2 l transfer container and aerated during transfer (3 h) to the hatchery. Fertile eggs were obtained on several occasions with these methods, with fertilization rates of 5-85%.

Obtaining Eggs - Artificial Fertilization Techniques - Live Fish

Kiflawi *et al*. (1998) also obtained fertile eggs by stripping gametes from live fish captured at an aggregation. They found that stripped eggs may be "aged" (not exposed to sperm) a few minutes without losing their ability to be fertilized, as long as fresh sperm were added. If both eggs and sperm are allowed to "age", fertilization capacity drops greatly 20-30 sec after stripping. For field collected samples, they found eggs collected from the water less than 20-30 sec after spawning had low fertilization rates (60-90%), while those taken after 25-30 sec had high success, typically near 100%.

When ripe fish are not available, or are needed for mariculture work, it is often expedient to induce maturation of gonads by hormone injections. While not generally within the scope of the present manual, there have been numerous instances where fishes caught from spawning aggregations have been artificially spawned. Since such fish are usually in advanced state of readiness for spawning, it takes little effort to fully induce them to gonadal maturation. Colin

(1992), Colin *et al*. (1996), Tamaru *et al*. (1996), and Head *et al*. (1996) used such techniques to obtain fertile eggs.

Figure 50. Stripping of gametes from Nassau grouper caught from spawning aggregation and held in tanks on board a vessel at the site. (PLC)

If fish are obtained from fishermen or by fishing, and you wish to retain them alive, it is often necessary to release gas from the swim bladder if the fish have been brought up from 10 m or more. This is best done with a large gauge hypodermic needle stuck into the swim bladder, releasing the excess gas pressure. In the Bahamas fishermen often simply stuck a thin bladed knife into the sides of Nassau grouper in the area of the swim bladder and twisted the blade 90^{o}, releasing the gas pressure. The fish were then put into live wells and survived for at least a few days. No matter what method is used, once stable, the fish can be held in tanks. For females, the disturbance of being captured, deflated and put into a tank often stops the process of egg maturation. It has been our experience with Nassau grouper and others that if nothing is done, the eggs will not mature and females will become "egg bound" and often die. A series of hormone injections are generally used to push the final maturation of the eggs. This might include hormone injections for both species, but often running ripe males need no further inducement, if stripped within a few days, while using hormones to cause females to mature ova. Testes removed from ripe males (such as those obtained from a fisherman who wishes to sell the catch) can often be stored for up to several days in a refrigerator or cryopreserved using liquid nitrogen for use when hydrated eggs are available. The testes of male red grouper, *Epinephelus morio*, obtained from fishermen during the spawning season were stored in plastic bags on ice for up to several days. When needed, a portion of the testes was macerated and mixed with seawater, then used to fertilize freshly stripped eggs from hormone-injected females (Colin et al. 1997).

The appropriate levels of hormones need to be injected and if excessive, can significantly shorten the life of the fish, an important consideration for mariculture broodstock. There is a large mariculture literature for appropriate materials and dosages that should be consulted for more details on hormonal induction of spawning.

V. B. Tracking Eggs and Larvae

In most cases attempts to track the movement of eggs and larvae involve some type of tagging of the water mass containing the propagules, usually by dye, drift cards/bottles or "current-following" drifters. The "tagging" of eggs is generally unfeasible, unless there is some special biochemical tag or genetic marker that might be used (e.g., Jones *et al*., 1999).

Dye has uses in some studies where a very "clean" start to the tracking is desired. Hensley et al. (1994) and Appeldoorn et al. (1994) used Rhodamine dye to track the transport of eggs after spawning. A saturated solution of dye (presumably in sea water) was placed in thick walled rubber balloons, then attached to anchored lines at a height above the bottom at selected spawning sites. When it was desired to release the dyed water, the balloon was burst from a distance using a long spear. Carter et al (1994) attempted to track the spawn of Nassau grouper using florescent dye, but unless the dye is tracked by sampling water using a fluorometer, it quickly becomes too diffuse to be visible to the human eye.

The drift card or bottle is another way to approach the question of where eggs go after spawning. Long a tool of physical oceanography, they can be used to address questions of where recruits resulting from a spawning aggregation might settle. One of us (Domeier, in prep) successfully used drift bottles to investigate the downstream dispersal from a known mutton snapper aggregation site (Fig. 51). A large number of scintillation vials ballasted with BB's (copper coated lead pellets) (so that they were barely buoyant) were released at the aggregation site at the presumed time of spawning to model potential recruitment pathways. A label placed inside instructed anyone finding the vial to contact the researcher. This technique is inexpensive, provides statistically meaningful sample sizes and is appropriate for use in populated regions (i.e., where returns are likely). The method may not be suitable or useful where there are few persons to find drifters, an inability to return information, or vast areas of open ocean, since relatively few drifters may eventually be grounded and found.

At their simplest level, current-following drifters are nothing more than an in-water object (sea anchor, vane or drogue) with a high resistance to lateral movement and held at a certain depth by a line and floats tethered to some type of marking device to allow it to be followed. For short-term use, up to a day or so, simple vane drifters with a pole marker ("high-flyer") can be used (Fig. 52). The drifter is tracked from a small boat, and its position determined by coming alongside it at intervals with a GPS receiver. The position data are then plotted on a map of the area and a recorded track of the drifter derived from that.

Current-following drifters work relatively well for the initial few days of the existence of a fish egg and larvae in the plankton. Eggs are buoyant and take about 24 hours or less to hatch into yolk sac larvae. This initial larval stage does not swim much, but may be able to control its depth to some extent. After larvae begin feeding (3-4 days after hatch), they may well move into water depths below the depth of the drogue, and consequently be transported differently from the drifter. Each day increases the chance that drifter tracks do not reflect the movement of larvae. As larvae approach settlement, they may well swim actively towards reefs, perhaps in response to sound or olfactory clues, rendering drifter tracks almost meaningless at that time. Drifter data should always be interpreted cautiously, that level of caution increasing as time after spawning increases.

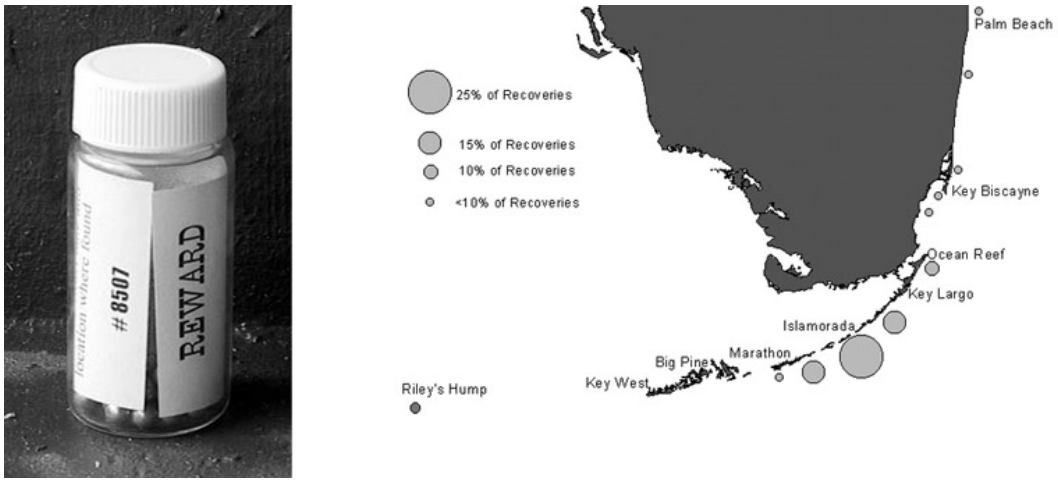


Figure 51. (Left) Drift bottle made from standard scintillation vial ballasted with BB's to the point of being only marginally positively buoyant. (Right) Locations of recoveries of drift bottles along the south Florida coast released at Riley's Hump (a spawning area) at the time of presumed spawning.

There is a need for low-cost current-following drifters that exceed the capabilities of the manually tracked drifter described above. Potentially basic GPS units could be integrated into the simple systems logging the track of the drifter at regular intervals. These stored data could be

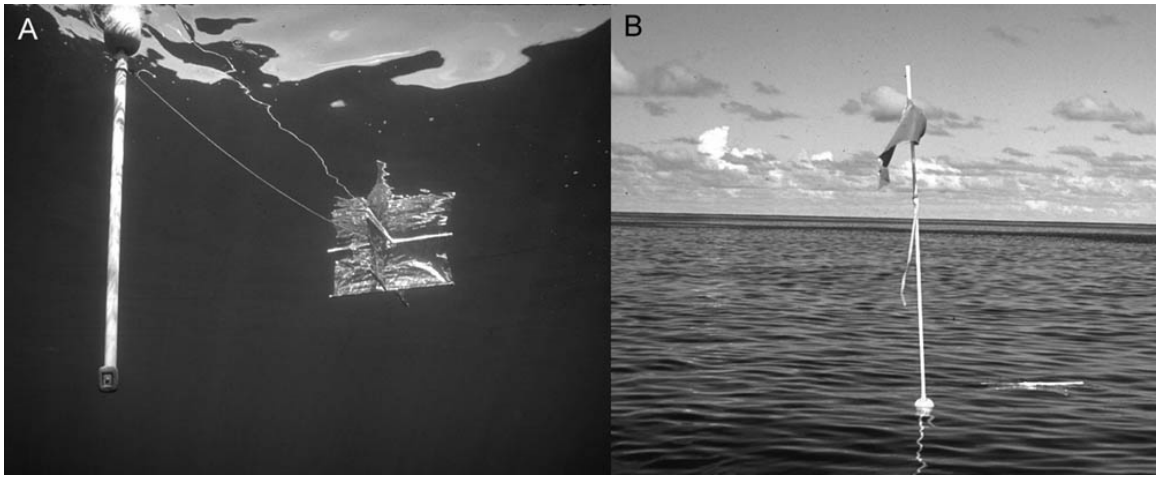


Figure 52. Simple current-following drifter. A. Underwater view of the drogue or vane is to the right, 1 m by 1m extending downward to 50 cm depth, attached by a line to the pole and float. B. Surface view of the marker pole, with colored flag to aid in locating from a boat.

accessed after several days by either recovering the drifter or by some sort of electronic transmission. It is likely that some sort of locating means would be needed to make such a system work, such as VHF radio beacons or something similar.

For longer duration tracking, satellite-tracked drifters are the only viable option. They are similar to the manually tracked drifter, with a drogue set at a depth of interest, but the surface float has a satellite transmitter and battery pack (Fig. 53). Position information is acquired several times per day by satellite, and can be accessed by the user via modem. The data are near

real time (most recent positions are often only a few hours old), but like other data, the track of the drifter between points must be estimated.

V. C. Monitoring Spawning Success

Monitoring the spawning success (i.e., number of eggs produced) of reef fishes is not easy. Monitoring numbers of fish in aggregations is largely intended to allow the determination of whether populations of fish in aggregations are stable, declining or increasing, but seldom deal with the actual number of eggs being produced by an aggregation. A number of suggested methods for monitoring the numbers of fish at an aggregation site have been included in Section IV.C. The methods for determining the numbers of eggs being produced by spawning fishes are even more tenuous and difficult than counting numbers of aggregated fish. The number of spawning events in a given area would be one way to potentially compare spawning success at different times, but we would have to make the assumption that roughly equal numbers of eggs are produced from each spawning. However, this will not be true if there are marked differences in female size (body size is related to fecundity), while we already know that there can be much variation in the number of eggs released per spawning event (or egg batch) even by individual females. The numbers of individual fish spawning would also be a useful way to look at possible spawning success, but again doubts arise concerning the actual number of propagules produced. Finally some method of monitoring the eggs produced within an aggregation directly would be very useful, such as the "moored plankton nets" described in Section V.A. Moored nets would likely produce similar results at different times, differences in egg numbers collected reflecting differences in spawning activity, if other factors such as currents, wave action and weather remained constant. Of course, they don't and may well change greatly the number of eggs collected versus those spawned.

We would suggest that a variety of approaches would have the best change of monitoring spawning success and detecting changes over time. Numbers of spawners, number of spawns and number of eggs captured can be assessed independently, and hopefully the trends among the three are similar, giving some confidence. Where there are differences among the three measures and perhaps among data collected at different times (months, years), the reasons for the differences might be assessed.

In all cases where a given aggregation site is intended for long-term monitoring, be certain reports written about the present status of sites include adequate information for someone to locate and repeat surveys of the site in future years. While it has been emphasized before, this fact should not be forgotten for any aspect of aggregation-related studies, and should be a guiding principle in the gathering of information.

It may some day be feasible to attempt to estimate spawning success by the number of fish recruiting from the plankton to benthic habitats. However in this regard our knowledge is so rudimentary that to make an assumption without reliable data on spawning activity, transport of eggs and larvae, and recruitment levels would be reckless. Indeed, for most fishes, the relationship between the number of spawners and the number of recruits is unknown and, given the substantial mortality that must occur in the planktonic phase, may never be clearly estimated. Nonetheless, the relationship between spawning as a source of eggs and recruitment as the ‘sink’

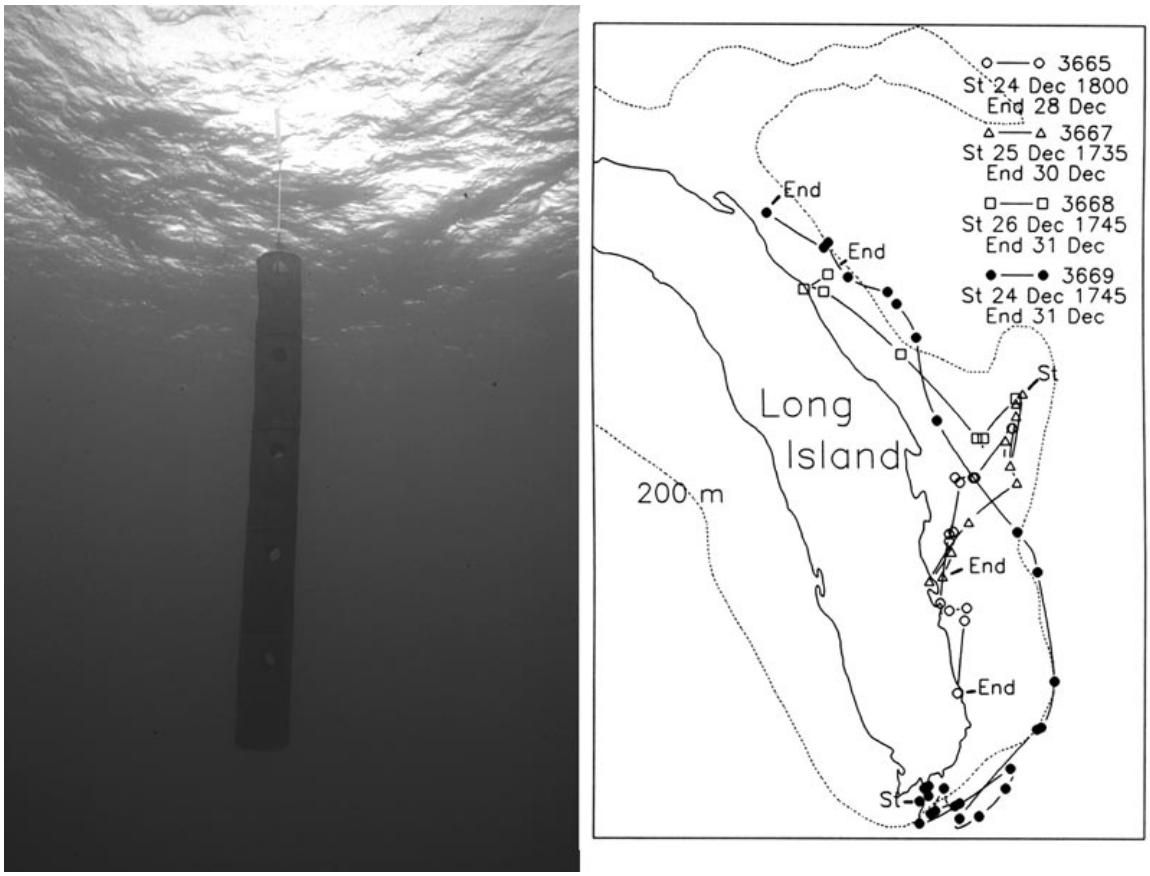


Figure 53. (Left) Typical satellite tracked current drifter, with 10 m long drogue ("holey sock") with 5 m line to surface float that contains transmitter and batteries (PLC). (Right) Tracks of current drifters released at or near Nassau grouper spawning sites (after Colin, 1996).

is at the core of the efforts to design and best place marine protected areas to provide for downstream seeding of recruits. Ideally studies should attempt to gather data on all of the factors related to these questions, so that we can begin to understand the relationships between spawning and recruitment for those fishes with aggregation spawning. Most work on reproductive success has utilized demersal fishes, particularly damselfishes (Pomacentridae), and while their early life history has many similarities to planktonic aggregation spawners, there may be major differences.

Monitoring recruitment can often provide information regarding the timing of spawning through back-dating to the time of spawning using otolith daily increments in juvenile fishes (Colin *et al*., 1997). In species for which the spawning period is not known, this might provide clues as to when to look for aggregations or spawning in the field. It can also help if a large number of juveniles are sampled and aged to determine the range of the spawning period and lunar/seasonal periodicity. Whenever working back from otolith ages, it must remembered that the fish captured and aged may not reflect the population as a whole, having been only those individuals which survived their planktonic life and happened to be captured for study. Methods for capturing juveniles for otolith work include light traps, plankton trawls for advanced state pelagic juveniles and channel nets. Channel nets, for example, have been used for monitoring recruitment of groupers (Keener *et al*., 1988, Colin *et al*., 1997, Shenker *et al*., 1993). Both light traps and channel nets can capture large pelagic juveniles alive, that can then be used for other experimental work, if desired (Doherty, 1987).

Section VI. Documenting the Fishery

Nearly all known aggregations of the larger food fishes will have some type of fishery associated with them; no published studies of spawning aggregations of food fishes have been conducted in the absence of a fishery. Studying an unfished aggregation could lead to some valuable comparative data, but will likely require traveling to a very remote location, requiring substantial resources. Because most aggregations are going to have a fishery, documenting fisheries that are targeting spawning aggregations has become the most important task for field workers. While the behavioral ecology aspects of spawning aggregations are typically more interesting to researchers, there is a need to have every available aggregation that is exploited documented, which inevitably includes the level of exploitation. In this era of global fisheries markets and efficient fishing technologies, aggregation fishing that goes undocumented and unchecked could lead to the rapid extirpation of targeted aggregations. All researchers who venture into the field to study spawning aggregations must understand their responsibility to place a high priority on collecting fishery information.

Several approaches can be taken to document a fishery, including government catch statistics, fishery-dependent surveys and interviews. The interviewing skills discussed in Section III are also important for documenting aggregation fisheries. Much of the information needed to write a summary of a particular fishery can be collected at the dock through interviews and observation. Observations made in the field are valuable for confirming information obtained through interviews or collecting data independent from interviews. Through these interviews it may be possible to arrange for a trip on a fishing vessel to observe the fishery directly.

VI. A. Types of Information on the Fishery and Sources

The types of information typically gathered from fishers or obtained from examining the catch include the following.

Fishery Catch Statistics

Of course, many fishery departments collect records and it is hoped that annual or monthly landings data are collected with a properly designed sampling program. It is critical that any sampling protocol is properly documented and consistently applied over time for the data to be of value in assessing trends in a fishery. When referring to departmental records for historic datasets, it is important to refer carefully to the methodology applied to determine to what extent past and present data might be comparable. For example, often landings are collected with no reference to effort. Knowledgeable fishery officials could be consulted to determine whether important changes in effort might have occurred that could significantly have affected available data in departmental records. Ideally CPUE data should be used (refer to Section IV.E). If there is no reason to suppose significant changes in effort over time, landings data can still be useful.

Fishery Gear

Describing the method of take for the aggregation fishery is important and relatively easy. Common gear types include hook and line, fish traps, and speargun. In some cases nets (gill net or other) may be used. Each gear type has many variations so details are necessary to adequately describe the fishery. For example, hook and line fisheries can vary from a single hook

on a handline to a large vessel putting out miles of longline with thousands of hooks. Fish traps vary in size and design as well as methods for baiting, deploying and retrieving.

Fishing Vessels

Record the type and size of boats that are fishing the aggregation. Information on what ports/villages the boats originate from is valuable as well as the place they offload their catch. Often aggregation fisheries are based from small boats (Fig. 54) making it difficult to monitor a large number, but as many as possible should be examined. The number of vessels fishing the aggregation is important for estimating catch and effort.

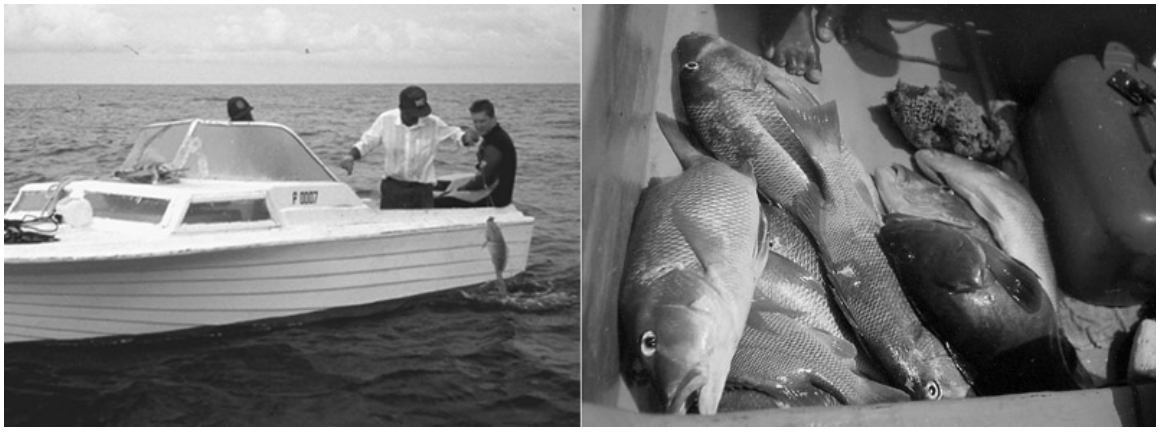


Figure 54. (Left) Handliner fishing from a small outboard boat capturing mutton snapper, *Lutjanus analis****, from a spawning aggregation. (Right) Catch of mutton snapper along with one grouper taken from the area of a spawning aggregation (MLD).***

Sampling the Catch

Measurements taken from a random sample of fish being landed can provide biological and fishery information. Of course sampling can only be done with the permission of the fisher or buyer. Getting permission can be difficult when the fish are landed/shipped live. Even in these circumstances there may be mortalities that you will be allowed to examine. By examining gonads fish can be sexed and if gonad samples can be taken, data can be collected for gonadal somatic index (GSI) and ovaries can be examined for the presence of hydrated eggs and/or post ovulatory follicles (see Section IV.D.). Recording lengths and weights is important for converting numbers of fish caught to weight of catch. Although it is tempting to use these samples to characterize the aggregation (sex ratio, size frequency etc.) one must be careful to consider the selectivity of the gear.

Even crude estimates of catch and effort are better than no data. Information gathered simply from interviews can be used to estimate catch. Fishers may give rough numbers for how many fish they take per day or per year from the aggregation. Average catches per boat can be expanded by the number of boats known to be fishing to estimate total catch. The buyers may know the approximate biomass of the catch. More detailed catch and effort data are extremely valuable if they can be obtained. For example, CPUE expressed as a fish per unit gear (e.g. number/weight of fish per trap or per hook) or per unit time (number/weight fish caught per hour fished) can be obtained through observation and interview. These data are critical for documenting trends in the fishery. Below are specifics on collecting catch data.

Fish Market Surveys

Where there are just a few markets and all or the majority of the catch is landed at these markets, market surveys can provide a good indication of seasonality in landings (although as already noted above, landings can vary because of fisher behavior which can assessed by speaking to fishers directly). Regular visits to markets to carry out a pre-determined sampling protocol is advised but careful decisions need to be made concerning how many fish to sample, how many stalls or shops to sample, and how often and which species to sample. How will the data be organized and what is the objective of collecting the data? These are the kinds of questions that must be asked, and answered, in order to develop an appropriate sampling program. There are plenty of examples of this kind of sampling and it is not difficult to plan a program, but if there is no planning then the data may mean very little and much time, money and valuable information may be lost. CPUE data could be estimated by calculating the number of fishers and some measure of their effort such as number of days or hours they fish per day. Standardized forms should be developed so that different workers are sure to sample in the same way and over the long term. This means that forms should be clear and simple, with as little detail as possible.

Market Records

Some large and well-organized markets keep records of sales. These may or may not be useful depending on how they are taken and whether species are reliably and **individually** recorded (i.e., each fish is recorded to species level). Again, an estimate of fishing effort will be needed unless it can reasonably be assumed that effort is not changing over time. In some cases, market surveys might not be of any use for evaluating catches in local waters. For example, in Hong Kong, although all chilled fish caught by Hong Kong vessels must, by law, be sold through local markets, since Hong Kong vessels largely fish outside of local (Hong Kong) waters, market records tell us nothing about local Hong Kong catches (also, there is much fish sold outside of legal channels). Be aware when inspecting market records (as well as for other types of market surveys) what the numbers are actually telling you. For example, chilled, filleted and live fish may well be handled by different market sectors so be sure to check how your species of interest are marketed. Some might be sent directly to restaurants without ever passing through a retail market, for example, or culturally important species may go directly to the local community.

Port Surveys

In some fisheries it is possible to sub-sample the catches of boats as they come into port. There are well-established methods for doing this and the usual considerations of which fish species to sample, how many boats and how often to sample, etc., must be made. As for any types of surveys, planning is essential and knowing your fishery is important. For example, port surveys have long been practiced in Puerto Rico, providing valuable fishery data. It was discovered, however, that a couple of species were under-sampled because they were particularly preferred by fishers and taken home to their families. These species had gone largely unrecorded in port-collected samples but, realizing the situation enabled suitably cautious interpretation of data.

Fisher Interviews

Interviews of fishers can also provide valuable information on both catches and effort (see also Section III). Again, the basic questions of how many, how often and standardization of the approach are essential. It is also important to have some means of verifying responses. This

could be achieved by including, amongst survey questions, questions with answers known to the interviewer but not to the fisher. Often interviews can only be undertaken sporadically but even more qualitative information can provide useful indications of trends in catches within the year and over the long term and how and where a particular fishery is conducted. One particular advantage of fisher interviews is that questions can be asked regarding whether fish were releasing milt or eggs at capture, thereby identifying possible spawning aggregations.

Logbooks

In some fisheries, ship captains are required to keep logbooks and these can be used to determine catches for a known unit of time for a given vessel. Note, however, that it is essential that such logbooks be cross-checked periodically (for example by assigning on-board observers) and, in analyzing the data, it is essential that the resolution of the data is known. For example, it may be easiest for a captain to simply make an estimate of his monthly landings. This might provide a useful seasonal pattern of landings within an annual cycle but would not identify shorter-term patterns such as spawning aggregations that may occur for just a few days in a month. An interesting example of problems in interpretation occurred in the fishery of the coral trout, *Plectropomus leopardus*, on the Great Barrier Reef of eastern Australia. Logbooks provided monthly information that indicated an increase in catches during the known months of aggregation. However, the data were of insufficient resolution to detect whether the aggregations themselves were being specifically targeted during these months. Although reports by some fishers indicated that indeed aggregations were being targeted, the logbook data were used to suggest that there was no evidence of targeting of aggregations. This conclusion had consequences for the types of management measure subsequently considered. Again, as we have emphasized elsewhere, know your data and their limitations and interpret accordingly.

Export Records

In some countries there is an important export trade in reef fishes that aggregate to spawn. Aggregations may provide particularly attractive bounties for exporters. If export records are complete enough they may be suggestive of seasonality in catches. This will depend very much on the local situation. Since exports are very likely to be an amalgamation of fish caught from many places and, moreover, only represent part of the catch, they may show little trend even when there may be seasonality in some of the fishing areas involved. However, if much of the catch of certain species is exported and mostly through one or two ports or airports, inspection of export records might be of value. This will only be the case, of course, if exports are noted by species and these identifications are reported accurately.

Restaurant Surveys

If restaurants are a major purchaser of certain fish species (often the higher value ones), then periodic restaurant surveys might provide an idea of seasonality, at least in the availability of such species.

VI. B. Documenting Local Management

Determining the fishery management in effect for a given species would seem to be a relatively straightforward exercise. Often it is, but sometimes it can be difficult to obtain reliable information on local fishery related laws. This brief section covers several considerations that may need to be made when seeking such information; we do not consider it to be comprehensive but the examples should alert a wary reader to some of the possible problems that might arise.

The obvious place to start when seeking current local legislation is with government offices, most likely fishery departments or divisions or those that deal with fishery issues. Local fishery coops might also be helpful. Whatever approach is used or most convenient, it is advisable to obtain a paper copy of the relevant legislation. The reason for this is that often people who should know the law may not be up to date or may not be fully familiar with all the fine details of the law. How well do you know your own country's fishery laws? Note also that different jurisdictions may have different laws (e.g., State and Federal laws in the United States), or that some widely respected regulations may not be officially documented such as those where there is traditional marine tenure.

To determine the effectiveness of fishery-related regulations, one must determine the level of enforcement, numbers of enforcement personnel, and it might be valuable to ask about the number of convictions that have been effected. It is also important to determine how information on regulations is disseminated, how familiar local fishers and local judiciary are with the laws and how management might be modified as new information becomes available (i.e., the approach to co-management)..

In some regions fisheries are managed at a local level though historical or cultural traditions. Documenting the history and effectiveness of these traditions could be useful for long-term management or allocation disputes as outside fishery influences change the dynamics of the fishery.

All of this information is important, especially if management recommendations are to be made, for understanding what kind of management might be effective, what is likely to be socially acceptable and how robust the recommendations might have to be. Summarizing and documenting management practices is helpful to other scientists/resource managers not familiar with the area you are studying. Finally, summarizing the level of enforcement is important to understand the effectiveness of any management.

Section VII. Spawning Aggregation Conservation Methods and Long Term Monitoring

VII. A. The Need for Conservation

Spawning aggregations are predictable in time and space and are particularly vulnerable to fishing. Moreover, many of the more vulnerable reef fish species (i.e., long lived, late maturing) are the ones that aggregate to spawn and are also particularly valued for food. The World Conservation Union (IUCN) has rankings for the threat posed to various species by human activities. Among reef fishes that aggregate to spawn, several are presently listed as endangered or vulnerable, or are being considered for such a listing by the IUCN. These include the Nassau grouper (*Epinephelus striatus*), the humphead wrasse (*Cheilinus undulatus*), and several other groupers. Their inclusion in the Red List has much to do with their tendency to form spawning aggregations that are targeted by fishermen. In the Indo-Pacific, threatened or vulnerable species have been listed because of declines associated with demand for them in the Live Reef Fish Trade (LRFT). The LRFT is discussed below.

Most effort going into studying reef fish spawning aggregations is focused ultimately on their preservation through complete protection or management. Intelligent conservation decisions

require good biological knowledge. Although it would be shortsighted to attempt to plan detailed conservation and management initiatives without the appropriate scientific underpinning, we do already understand enough to know that all exploited aggregations must be protected or managed in some way, otherwise there is a good chance that they will decline and ultimately cease to form. **With very few exceptions, it is irresponsible not to manage an exploited aggregation**. To fine-tune conservation and management measures, however, to ensure the very best possible protection, solid knowledge regarding the timing, duration, location, migration distances, physical oceanography of sites, and a dozen other aspects of spawning aggregations are essential, knowledge to be gained through sound science.

So, should aggregations be fished at all? Although aggregations have been exploited for long periods at very low levels of fishing in the distant past, and although there are sizeable social and economic incentives to continue to exploit spawning aggregations, we do not know what are sustainable levels of exploitation for any aggregation. Most importantly, it is patently clear that aggregations can not withstand modern levels of fishing pressure or modern techniques of fishing. Until we understand more about aggregation dynamics and the effects of the total sum of fishing pressure on a species both on their spawning aggregations as well as at non-aggregating times, the literature and experiences elsewhere strongly suggest that unmanaged aggregations should not be fished at all. All aggregations that are being monitored are showing probable decreases in abundance of fish, increasing bias in sex ratios (e.g., Koenig *et al*., 1996) or other changes, although unfortunately relative few are currently being monitored over the long term, or in a consistent and standardized way.

If aggregation sites are known, it might be feasible to close only the aggregation sites to fishing with no other management measures needed. However, this does little to protect fish that might be migrating to the site (e.g., lane snapper, *Lutjanus synagris*; Claro *et al*., 2001), or fish occurring at an aggregation that is not widely known. In such cases, a seasonal closure on the species may be appropriate, so that all aggregation sites (known and unknown) are protected. In some extreme cases, where a species does aggregate to spawn but overall its populations are under heavy fishing pressure, it might be advisable to close the entire species to exploitation. This was the case for the goliath grouper (jewfish), *Epinephelus itajara*, in Florida, where in 1990 the species was declared a no-take fish. Incidental fish caught on hook and line had to be released. For possible management options other than seasonal and spatial closures see Domeier *et al*., (2002).

In some locations regulations have been put in place that have not fulfilled their objectives due to insufficient scientific information. In Palau, western Pacific, a closed season was initially instituted for various groupers, extending from April through the end of July. Subsequent observations of aggregation presence indicated, however, these fishes continued to aggregate until at least the end of August, leaving them open to fishing during a portion of their aggregation period (Johannes *et al*., 1999).

Rhodes (1999) detailed a number of management steps that were taken in Pohnpei (Federated States of Micronesia) to modify existing regulations protecting grouper aggregation after a survey indicated they were not adequately protecting the aggregations. This sort of active revision of regulations in light of new biological information is important to make part of any management program. In the case of Pohnpei, various fishers were able to legally access much of the total aggregation (up to one third of the aggregation in 1999) at time and locations outside of those protected by regulations (Rhodes, 1999).

Such examples illustrate the importance of having sound information on which to base management action if it to fulfill its intended protection effectively. They also illustrate why regulations always need to be open for amendment, to continually improve protection and management initiatives as new information becomes available or as new markets, and hence new pressures, open up.

Figure 55. This male humphead wrasse, *Cheilinus undulatus****, is a prime target for the Live Reef Fish Trade (PLC).***

The Live Reef Fish Trade (LRFT) represents a relatively new and heavy pressure on reef fish resources of the Indo-Pacific (Fig. 55). Prior to its emergence, fishing on aggregations was limited to subsistence and modest freezing or export levels. However LRFT has been growing steadily since the early 1990s and is now big business, currently worth over a billion US$ annually (Sadovy and Vincent, 2002). The LRFT involves the capture of a relatively small number of reef fish species, predominantly groupers, that are maintained alive until they reach

Figure 56. A large humphead wrasse, *Cheilinus undulatus****, freshly dead on a sidewalk in Hong Kong. Taken from a coral reef somewhere in the vast Indo-Pacific, shipped alive from the point of capture to Hong Kong, held in a tank until someone willing to pay the premium price (>$100 a kg), then dispatched and quickly cooked and eaten as a special meal to impress business clients or celebrate special events, the Live Reef Fish Trade threatens survival of fishes such as these in many parts of their ranges (PLC).***

demand centers such as Hong Kong and mainland China where they are sold as food. Consumers are prepared to pay a premium price for these fish and so business can be extremely lucrative for traders and importers. As wealth increases in China, so demand for these live fish is predicted to increase (Fig. 56). There is growing evidence that fishers and traders are looking for reef fish at spawning aggregations for these are lucrative and efficient to target. In most places in the Indo-Pacific there is no management of spawning aggregations at all, and, given the high demand for live fish and the large size of the live fish transporter vessels (Fig. 57), very large numbers of fish are likely to be removed if a spawning aggregation should be targeted (Fig 58). Indeed, there are already several examples where aggregations have been fished out as a result of fishing for live fish, and mortality of fish removed from aggregations can be high maybe because they are stressed at this time (Johannes, 1997; Johannes *et al*., 1999; Johannes and Lam, 1999). They are not recommended for reef fish aggregations.

Mapstone et al. (2001) felt that *Plectropomus leopardus* populations were at relatively low risk from targeted harvesting for the live reef fish trade because of the relatively small size of its spawning aggregations, their scattered locations and the limited percentage of the total population present at aggregations at any given time. They felt, however, that species with larger, more predictable aggregations may be more vulnerable to fishing pressure on aggregations.

Ideally conservation organizations and fishery management authorities should adopt and promote the concept that reef fish aggregations should be protected from nearly all direct fishing effort, at least as a default position in the absence of effective management and appropriate biological information. Aggregating fishes are often easily disturbed and until we have a clear understanding of the effects that fishing (and other human) activities have on reproduction, we need to reduce overall fishing. This requires, at the minimum, that all known aggregation sites not effectively managed already be protected and/or that all relevant species are not fished during their reproductive season(s). Concepts, such as "pulse fishing" (Graham 2001) or other more complex forms of exploitation that require tight compliance and heavy enforcement are unlikely to be feasible in most fisheries and may, instead, be detrimental to aggregations overall.

VII. B. Archiving of Information

It is important to be able to archive and consolidate information concerning reef fish spawning aggregations. This can include anecdotal information and observations, "hard" data from catches from aggregations, photographs, film and video footage, and other types of information.

The Society for the Conservation of Reef Fish Aggregations has a database on reef fish aggregations world-wide and intends to serve as a repository for other types of information. In connection with the overall database, certain criteria have been established that observations must meet before a given aggregation of fish is included in the database as a spawning aggregation (see Section II.C). The intention of these criteria is not to exclude information, but rather to put confirmed and tentative information into different tables, so that what is confidently known is not confused with the tentative and unconfirmed. If an observation does not meet these criteria, it is still possible to include the observation as "probable", "possible" or some other relative estimation of the confidence associated with the observations or data and efforts can later be made to confirm or otherwise such reports. The instructions for making additions to the SCRFA database are available on the SCRFA website (www.SCRFA.org).

Figure 57. A Live Reef Fish Trade vessel, photographed in North Male Atoll lagoon, Maldive Islands. Copious water pours from the tanks inside the vessel, indicative of the amount of effort required to keep its cargo of reef fish alive prior to steaming to a distant port such as Hong Kong (PLC)

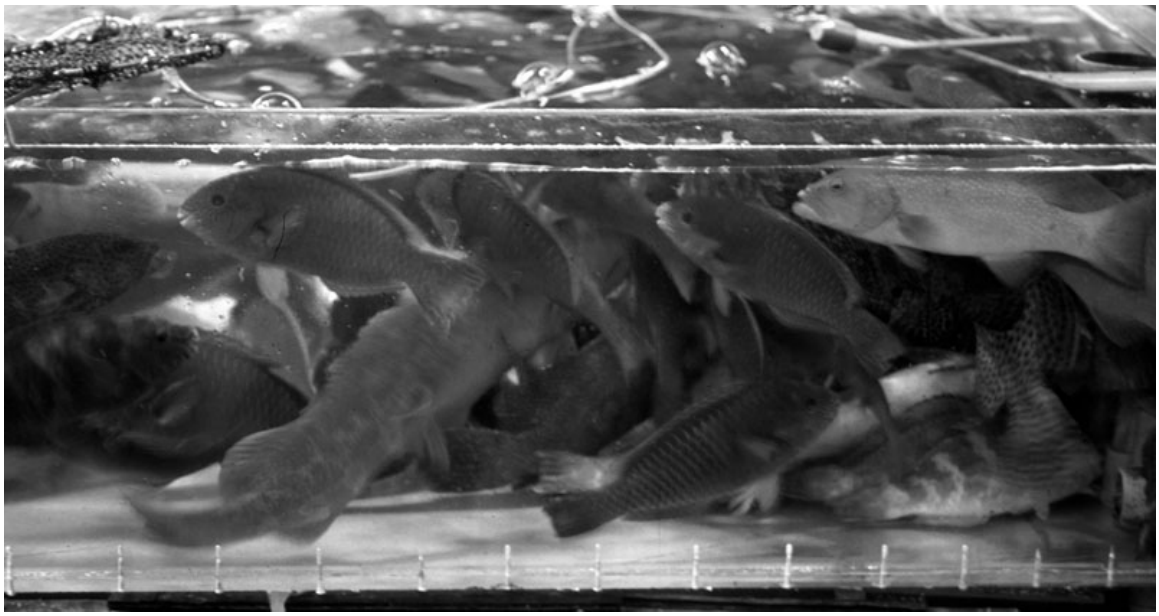


Figure 58. Live reef fishes, mostly parrotfishes and small groupers in a streetside aquarium in Hong Kong (PLC)

VII. C. Conservation of Spawning Aggregations

Does Protection of Aggregations Help?

In a number of places, various types of protection have been put in place to conserve aggregations. Do they help and what means can be utilized to show that they help? While it is not within the scope of the present manual to cover these protections in detail, we would like to comment on the types of protection put into place in some areas. Protection can include a prohibition of fishing in a given site, usually the aggregation area and nearby reefs, and protection of species by closed seasons during the spawning period. A combination of both types of restrictions seems most likely to have a positive effect and has been implemented in several places. The former seems the most obvious when fish(es) at certain sites are rapidly being wiped out. Closed seasons are particularly relevant where aggregations can not be patrolled or site protection readily enforced. The latter type of regulation covers aggregations that are unknown to anyone, except perhaps a few fishermen, but may have great importance in reproductive success.

Various methods can be used to prevent overexploitation of aggregations. Johannes and Kile (2001) recommended banning the catching and holding of live groupers (for the live reef fish trade) during the months of spawning aggregations, instead of trying to protect the aggregation sites which are widely dispersed and far from settlements. The type of protection implemented depends on the local social and cultural contexts as well as species and fishery conditions involved (Domeier *et al*, 2002). Presently the most widely applied approaches to protecting aggregating fishes are seasonal (no fishing and/or no commercial sale of the species during the spawning season) or spatial (the spawning site itself is protected during the aggregation period or is incorporated into a marine protected area).

Figure 59. These fishermen in the Cayman Islands have made a good catch of Nassau groupers from the spawning aggregation, but how long will this last. This photo was taken in 1977, a time of no regulation of the spawning site and declining catches. In earlier years, many more fish were caught, but those days were already gone. At present the fishery is closely regulated, but catches have yet to improve.

There are some signs that protection can result in increased fish size when non-aggregated populations are still fished. Beets and Friedlander (1998) documented a case where declining catch and size, plus a highly skewed sex ratio (in favor of females) among red hind, *E. guttatus*, in the U.S. Virgin Islands reversed after protection was put in place. They reported an increase from 295 mm SL to 395 mm SL for red hind after seven years of spawning site closure to fishing, while the sex ratio change from 1:15 males to females to 1:4.

The protection of aggregation sites also tends to provide some protection for spawning population other species of fishes. Johannes (1998) says that "more than 40 species of reef fish spawn at the three aggregation sites" of groupers he studied. The spawning of these additional species is not limited to the grouper aggregation site, but protection of a site, as opposed to a species, means that other fishes derive secondary protection.

Fostering (the right type of) Protection

Many instances of disappearances of spawning aggregations are known (Sadovy and Domeier, in prep). In most cases fishers of a given aggregation have been aware of the declines leading to the extirpation. Sometimes the loss is rationalized by saying the fish have moved elsewhere or were disturbed and don't come anymore. While these are possible, most likely the aggregation has been fished out and most fishermen know it. In many cases, calls for protection come from those who suffer most from the loss, the fishers themselves. This is particularly true of older fishermen, who have a longer experience and have more likely seen an aggregation fishery in its prime.

In the last decade, the importance of spawning aggregations to fishery success and the level of exploitation in many areas have raised awareness, at least among monitoring agencies, of the importance of spawning aggregations in many species used for human food. Awareness needs to be raised at the level of government and the individual fisher. Without the support of fishers, it is considerably more difficult for government to put in place the controls needed to prevent loss of aggregations and ultimate decline of the fishery.

To encourage protection, communities need to identify with local aggregation sites as "theirs" and something to protect for their own sake. Certainly any community fishing the area where fishes are drawn from to spawn at the aggregation has a stake in the future of that aggregation. This is why tagging studies can be particularly important for making people realize that the fate of the aggregation affects both their short-term ability to catch fish (none will be left if the entire aggregation is fished out) and the long-term health of the fishery of the aggregating species. Such studies are also important to illustrate how far fish can move and that overfishing an aggregation in one community can affect catches in the non-reproductive season in a distant community.

Spawning aggregations within marine parks have potential value added components. Ruitenbeek (2001) reported that the value of spawning aggregations within the Komodo National Park, Indonesia was roughly $600,000 USD at 100% protection, a level similar to the direct recreational value of the park. However, care is needed before blindly converting an aggregation into a tourist attraction in case uncontrolled disturbance should have a negative impact on reproduction. Studies can be conducted to determine such potential impacts.

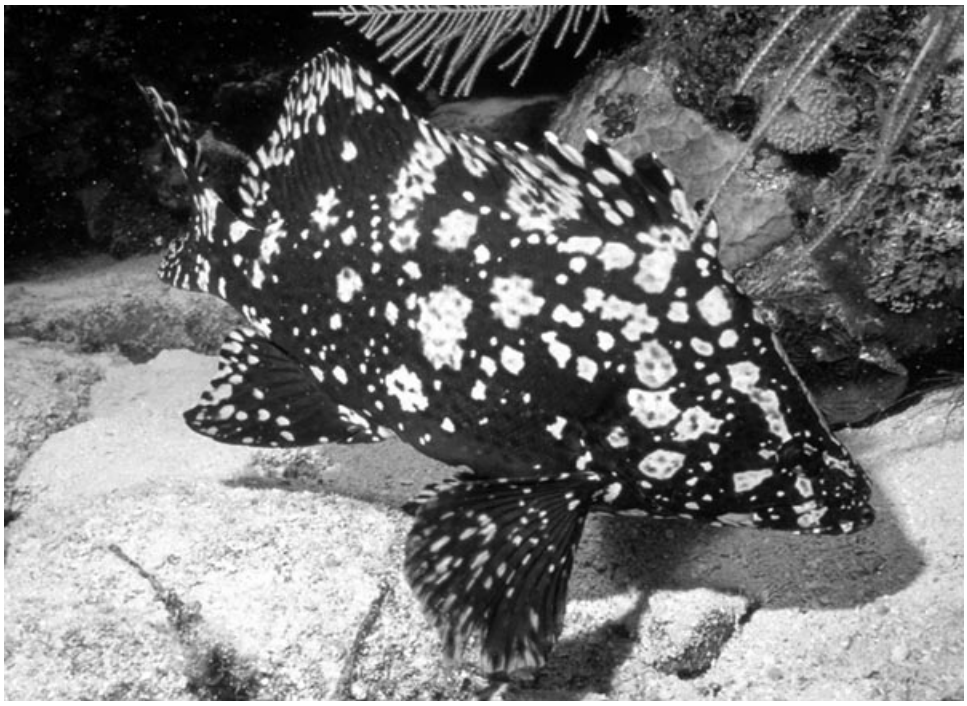


Figure 60. Groupers such as this marble grouper, *Dermatolepis inermis****, from the Florida Keys are often thought to be rare, because they are seldom seen. They may just occur in an environment that is seldom fished or visited by divers, but they may also have had their populations driven down by fishing pressure. Determining such things is one of the hardest aspects of working on species of fishes that aggregate to spawn.***

It is also essential to look at fisheries of those species that aggregate in its entirety and not to solely focus on the aggregation. In many cases fishing pressure occurs on both aggregated and non-aggregated individuals, both of which may be important to consider when examining population status and determining appropriate management. For example, managing an aggregation of a species that is also heavily fished at other times may ultimately contribute little to the overall problem of overfishing of the species. As just one possible example, in Palau, aggregations of *Plectropomus* and *Epinephelus* were found to be predominantly males, to the point that any females in the aggregation were continuously harrassed by the many males (Johannes *et al*., 1999). This may have disrupted spawning, although the spawning behavior of the species involved is not well known. The question also arises over how such a distorted sex ratio could occur. Johannes *et al*. (1999) did not have an explanation for this, but speculated perhaps that this might have resulted from heavy fishing of females at non-aggregated times in more built-up, and therefore, heavily exploited areas. This emphasizes a point that is often not explicitly considered: that changes to aggregation numbers, sexes and fish sizes at aggregations could also result from non-aggregation fishing pressure. This means that the entire fishery needs to be considered for management, research or other interventions.

Education Issues

Although historical data on aggregation status is critically important for understanding the effects of targeting these gatherings, the video is one of the most powerful way to convey the message of the fragility of spawning aggregations today. Videos could include interviews with fishermen telling of their experiences, particularly older fishermen who are likely to have seen many changes in their lifetime. Video should have underwater and spawning footage if at all

possible since most fishermen have probably never seen aggregations underwater and are unlikely to have witnessed spawning. It is, of course, important to explain scientific work in a simple manner but everybody understands the reproductive imperative and so spawning aggregations represent an excellent opportunity to teach of the importance of allowing fish to reproduce to replenish their kind.

A second video might be prepared to assist fishermen or divers who want to help on a project. One of us (PLC) previously used a video showing people how to remove gonads and otoliths for grouper work in the Gulf of Mexico.

Sociological Issues

Support from fishers is of the utmost importance to the long-term success of protecting aggregations. It is important to communicate the results of studies on aggregation to the fishers involved. This is usually not easily done, but when it can be shown clearly that aggregations are of benefit to retaining local populations, this may drive home the message that is in the fishers own best interests to conserve the aggregation.

VII. D. Publication of Information

It is important for scientists to get technical information out into the general scientific literature as a basis for sound conservation action. It is also important that some of this information is also made available to a wider public as well as to the fishing community. On the other hand, certain kinds of information, such as the specific locations of aggregations should not be released unless for the express purpose of management and conservation to ensure that it can not be misused.

To get information into the hands of practitioners, it might be more useful to make it available in video form, rather than printed documents, although brochures and posters are also very important. The video is a powerful tool and even the most remote islands, if they have a human population, almost always have a video system for watching movies.

VII. E. Long Term Monitoring

In one sense we really don't know how to do long term monitoring of spawning aggregations, as we do not necessarily know what is the essential information to acquire now that we will want in the future. It is better to document as many things as possible now, in a thorough, quantitative and repeatable manner, in the hopes we are doing the right things now.

If a firm basis for monitoring is established, then long term monitoring becomes feasible. Maps, GPS location information, other "hard" documentation is crucial. Our technology of obtaining this information may improve significantly in the future, but today we need to do the best job we can with the tools available.

Since we are seeing a general decline in spawning aggregation worldwide (there are relatively few that are growing or recovering), we should work to gather as much baseline information as possible now. This is generally true for all shallow water tropical environments, particularly coral reefs.

We know little about the recovery of aggregations, once fished out. Protection has been applied to some areas that no longer support aggregations, however we can learn some very important lessons from watching what happens to such areas. Do they never recover? Of, once protection is in place, they will come back. We have evidence of one grouper site in Palau which is beginning to recover, but because there is only qualitative information concerning its demise, its "recovery" is may be distinct. This could be a case where there always has been an aggregation, but its demise documented by observations made at the wrong time of year, month, or day. The negative is often not easy to prove, so we need to be careful in saying something is gone, when it just may be "hiding". The monitoring of "extinct" sites is useful and important, particularly in areas where other aggregation sites have never been known. It might seem like a futile exercise (the authors have all been through it many times), but the importance in the medium to long term might be great

VII. F. Concluding Comments

If we really want to set up global long-term monitoring of aggregations, it is going to be necessary to continue the "discovery component" of aggregation work at as fast a rate as possible. At present there are only a handful of sites that could even remotely be considered as adequately quantified to serve as true long-term monitoring sites. We need to be establishing such sites now, and many of the sites needed are not yet even known to scientists or managers. The sites that are known need to be studied and quantified in more detail, one of the major reasons we have gone to the trouble of writing this manual.

VIII. References Cited

Aguilar-Perera, A. 1994. Preliminary observations of the spawning aggregation of Nassau grouper, Epinephelus striatus, at Mahahual, Quintana Roo, Mexico. Proc. Gulf and Carib. Fish. Inst. 43: 112-122.

Appeldoorn, R.S. D.A. Hensley, D.Y. Shapiro, S. Kioroglou and B.G. Sanderson. 1994. Egg dispersal in a Caribbean coral reef fish, *Thalassoma bifasciatum*. II. Dispersal off the reef platform. Bull. Mar. Sci. 54(1): 271-280.

Beets, J. and A. Friedlander. 1998. Evaluation of a conservation strategy: a spawning aggregation closure for red hind, *Epinephelus guttatus*, in the U.S. Virgin Islands. Env. Biol. Fish. 55: 91-98.

Bell, L.J. and P.L. Colin. 1986. Mass spawning of *Caesio teres* (Pisces: Caesionidae) at Enewetak Atoll, Marshall Islands. Envir. Biol. Fishes 15(1): 69-74.

Bohnsack, J.A. and S.P. Bannerot. 1986. A stationary visual census technique for quantitatively assessing community structure of coral reef fishes. NOAA Tech. Rep. NMFS 41:1-15.

Bolden, S.K. 2000. Long-distance movement of a Nassau grouper (*Epinephelus striatus*) to a spawning aggregation in the central Bahamas. Fish. Bull. 98: 642-645.

Carter, J, G.R. Marrow and V. Pryor. 1994. Aspects of the ecology and reproduction of Nassau grouper, *Epinephelus striatus*, off the coast of Belize, Central America. Proc. Gulf and Carib. Fish. Inst. 43: 65-111.

Claro, R., and K. C. Lindeman. 2003. Spawning aggregation sites of snapper and grouper species (Lutjanidae and Serranidae) on the insular shelf of Cuba. Gulf and Caribbean Research 14(2): in press.

Claro, R., K. C. Lindeman, and L. R. Parenti (eds.). 2001. Ecology of the marine fishes of Cuba. Smithsonian Institution Press, Washington, D. C., pp. 253.

Colin, P. L. 1996. Longevity of some coral reef fish spawning aggregations. Copeia 1996(1): 189-192.

Colin, P. L. 1994. Preliminary investigations of the reproduction of the jewfish, *Epinephelus itajara*. Proc. 43rd Gulf Carib. Fish. Inst.: 138-147.

Colin, P. L. 1992. Reproduction of the Nassau grouper, *Epinephelus striatus* and its relationship to environmental conditions. Envir. Biol. Fish. 34: 357-377.

Colin, P. L. 1985. Spawning of western Atlantic surgeonfishes. National Geographic Res. Rpts. 18 (1977): 243-250.

Colin, P. L. 1983. Spawning and larval development of the hogfish, *Lachnolaimus maximus* (Pisces: Labridae). Fishery Bulletin 80(4): 853-862.

Colin, P. L. 1982. Aspects of the spawning of western Atlantic reef fishes. NOAA Tech. Mem.NMFS-SEFC 80: 69-80.

Colin, P. L. 1978. Daily and summer-winter variation in mass spawning of the striped parrotfish, *Scarus croicensis*. Fishery Bulletin 76(1): 117-124.

Colin, P.L. and L.J. Bell 1991. Aspects of the spawning activity of labrid and scarid fishes (Pisces::Labroidei) at Enewetak Atoll, Marshall Islands, with notes on other families. Env. Biol. Fish. 32: 229-260.

Colin, P. L. and I.E. Clavijo. 1988. Spawning activity of fishes producing pelagic eggs on a shelf edge coral reef, southwestern Puerto Rico. Bulletin of Marine Science 43(2): 249-279.

Colin, P. L. and I.E. Clavijo. 1978. Mass spawning of the spotted goatfish, *Pseudupeneus maculatus* (Pisces: Mullidae). Bull. Mar. Sci. 28(4): 780-782.

Colin, P. L., W.A. Laroche and C.C. Koenig. 1996. Development from egg to juvenile of the red grouper, *Epinephelus morio* (Pisces: Serranidae) in the laboratory: 409-422. in: Arreguin-Sanchez, F., J.L. Munro, M.C. Balgos and D. Pauly (eds.) Biology, fisheries and culture of tropical groupers and snappers. ICLARM Conf. Proc. 48, 449 pp.

Colin, P. L., W.A. Laroche and E.D. Brothers. 1997. Timing of ingress and settlement in the Nassau grouper, *Epinephelus striatus*, (Pisces: Serranidae), with relationship to spawning occurrence. Bull. Mar. Sci. 60(3): 656-667.

Colin, P.L., Douglas Y. Shapiro and D. Weiler. 1987. Aspects of the reproduction of two species of groupers, *Epinephelus guttatus* and *E. striatus* in the West Indies. Bull. Mar. Sci. 40(2): 220-230.

Doherty, P.J. 1987. Light traps: Selective but useful devices for quantifying the distributions and abundances of larval fishes. Bull. Mar. Sci. 41(2): 423-431.

Domeier, M.L. and P.L. Colin. 1997. Tropical reef fish spawning aggregations: defined and reviewed. Bull. Mar. Sci. 60(3): 698-726.

Domeier *et al*. 2002. Transforming coral reef conservation: reef fish spawning aggregations component. Spawning Aggregation Working Group Report, The Nature Conservancy, Hawaii, April 22, 2002. pp. 85 (http://www.scrfa.org/doc/FSAS.pdf).

English, S., C. Wilkinson and V. Baker. 1997. Survey manual for Tropical Marine Resources, Australian Inst. Mar. Sci., Townsville, 390 pp.

Garth, F.D. 1991. By the light of the grouper moon. Scuba Times 1991: 46-48.

Gilmore, R.G. and R.S. Jones, 1992. Color variation and associated behavior in the Epinepheline groupers, *Mycteroperca microlepis* (Goode and Bean) and *M. phenax* Jordan & Swain. Bull. Mar. Sci. 51: 84-103.

Graham, T.R. 2001. Advantages of pulse fishing in live product fisheries. Secretariat of the Pacific Community Live Reef Fish Inf. Bull. 9: 5-9.

Gronell, A. M. and P.L. Colin. 1985. A toroidal vortex for gamete dispersion in a marine fish, *Pygoplites diacanthus* (Pisces:Pomacanthidae). Animal Behavior 33(3): 1021-1023.

Harvey, E.S. and M.R. Shortis. 1996. A system for stereo-video measurement of sub-tidal organisms. Mar. Tech. Soc. J. 29(4): 10-22.

Harvey, E., D. Fletcher and M. Shortis. 2000a. A comparison of the precision and accuracy of estimates of reef-fish lengths determined visually by divers with estimates produced by a stereo-video system. Fish Bull. 99: 63-71.

Harvey, E., D. Fletcher and M. Shortis. 2000b. Improving the statistical power of length estimates of reef fish: a comparison of estimates determined visually by divers with estimates produced by a stereo-video system. Fish. Bull. 99:72-80.

Harvey, E., D. Fletcher and M. Shortis. 2002. Estimation of reef fish length by divers and by stereo-video: a first comparison of the accuracy and precision in the field on living fish under operational conditions. Fisheries Research 57(3): 255-265.

Head, W.D., W.O. Watanabe, S.C. Ellis and E.P. Ellis. 1996. Hormone-induced multiple spawning of captive Nassau grouper broodstock. Progressive Fish Cult 58: 65-69

Hensley, D.A., R.S. Appeldoorn, D.Y. Shapiro, M. Ray and R.G. Turingan. 1994. Egg dispersal in a Caribbean coral reef fish, *Thalassoma bifasciatum*. I. Dispersal over the reef platform. Bull. Mar. Sci. 54(1): 256-270.

Johannes, R.E. 1981. Words of the Lagoon, Univ. of California Press, 245 pp.

Johannes, R. E., 1997. Grouper spawning aggregations need protection. SPC Live Reef Fish Information Bulletin 3: 13-14.

Johannes, R.E. 1998. The case for data-less marine resource management: examples from tropical nearshore fisheries. Trends Ecol. Evol. 13(6): 243-246.

Johannes, R.E. and N. Kile. 2001. Protecting grouper spawning aggregations, a potential target of the live reef food fish trade in Ysabel and Wagina Islands. Secretariat of the Pacific Community Live Reef Fish Inf. Bull. 8: 5-9.

Johannes.R.E. and M.Lam 1999. The live reef fish trade in the Solomon Islands. Secretariat of the Pacific Community Live Reef Fish Inf. Bull. 5: 8-15.

Johannes, R.E., L. Squire, T. Graham, Y. Sadovy and H. Renguul. 1999. Spawning aggregations of groupers (Serranidae) in Palau. The Nature Conservancy Marine Research Series Publication No. 1, 144 pp.

Jones, G. P., M. I. Milicich, M. J. Emslie, and C. Lunow 1999. Self-recruitment in a coral reef fish population. Nature (London) 402: 802-804

Keener, P., G.D. Johnson, B.S. Stender and E.B. Brothers. 1988. Ingress of postlarval gag, *Mycterperca microlepis* (Pisces: Serranidae) through a South Carolina barrier island inlet. Bull. Mar. Sci. 42: 376-396.

Kiflawi, M., A.I. Mazeroll and D. Goulet. 1998. Does mass spawning enhance fertilization in coral reef fish? A case study of the brown surgeonfish. Mar. Ecol. Prog. Ser. 172: 107-114.

Koenig, C.C., F.C. Coleman, L.A. Collins, Y. Sadovy and P.L. Colin. 1996. Reproduction in gag, *Mycteroperca microlepis* (Pisces: Serranidae) in the eastern Gulf of Mexico and the consequences of fishes spawning aggregations: 307-323 in Arreguin-Sanchez, F., J.L. Munro, M.C. Balgos and D. Pauly (eds.) Biology, fisheries and culture of tropical groupers and snappers. ICLARM Conf. Proc. 48, 449 pp.

Lobel, P.S. 1992. Sounds produced by spawning fishes. Env. Biol. Fish. 33: 351-358.

Mapstone, B.D., A. Jones, C.R. Daview, S.J. Slade and A.J. Williams. 2001. The live fish trade on Queensland's Great Barrier Reef: changes to historical fishing practices. Secretariat of the Pacific Community Live Reef Fish Inf. Bull. 9: 10-13

Marconato, A., D.Y. Shapiro, C.W. Petersen, R.R. Warner and T. Yoshikawa. 1997. Methodological analysis of fertilization rate in the bluehead wrasse *Thalassoma bifasciatum*: pair versus group spawning. Mar. Ecol. Prog. Ser. 161:61-70.

Myrberg, A.A., W.L. Montgomery and L. Fishelson. 1988. The reproductive behavior of *Acanthurus nigrofuscus* (Forskal) and other surgeonfishes (Fam. Acanthuridae) off Eilat, Israel (Gulf of Aqaba, Red Sea). Ethology 79: 31-61

Pet, J. L. Squire, C. Subagyo and A. Mulyadi. 1999. Grouper and Napoleon wrasse spawning aggregation sites Komodo National Park, Monitoring Report 1998-1999. The Nature Conservancy, 43 pp.

Petersen, C.W. and Warner, R.R. 2002. The ecological context of reproductive behavior. :103-118. In: Sale, P.W. (ed) Coral Reef Fishes, Elsevier Science (USA).

Petersen, C.W., R.R. Warner, S. Cohen, H.C. Hess and A.T. Sewell. 1992. Variable pelagic fertilization success: implications for mate choice and spatial patterns of mating. Ecology 73: 391-401.

Randall, J. E., and Randall, H. A. 1963. The spawning and early development of the Atlantic parrot fish *Sparisoma rubripinne*, with notes on other scarid and labrid fishes. Zoologica (N. Y.) 48: 49-59

Rhodes, K. 1999. Grouper aggregation protection in proactive Pohnpei. Secretariat of the Pacific Community Live Reef Fish Inf. Bull. 6: 14-15.

Rhodes, K.L. and Y. Sadovy. 2002. Temporal and spatial trends in spawning aggregations of camouflage grouper, *Epinephelus polyphekadion*, in Pohnpei, Micronesia. Envir. Biol. Fish. 63: 27-39.

Rimmer, M.A., R.N. Garrett and M.A. Samoilys. 1994. In vitro fertilization of eggs of coral trout, *Plectropomus leopardus* (Serranidae), collected from an aggregation site on the Great Barrier Reef, Australia. Bull. Mar. Sci. 54(1): 356-358.

Ruitenbeek, H.J. 2001. An economic analysis of the spawning aggregation function in Komodo National Park. Indonesia. Secretariat of the Pacific Community Live Reef Fish Inf. Bull. 9: 13-16.

Russ, G.R., D.C. Lou and B.P. Ferreira. 1996. Temporal tracking of a strong cohort in the population of a coral reef fish, the coral trout, *Plectropomus leopardus* (Serranidae: Epinephelinae), in the central Great Barrier Reef. Can. J. Fish. Aquat. Sci. 53: 2745-2751.

Sadovy, Y. 1993. The Nassau grouper, endangered or just unlucky? Reef Encounter July 1993: 101-2.

Sadovy, Y. 1996. Reproduction in reef fishery species:15-59 in Polunin, N.V.C. and C.M. Roberts (eds) Reef Fisheries, Chapman and Hall, London

Sadovy, Y and P.L. Colin.1995. Sexual development and sexuality in the Nassau grouper, *Epinephelus striatus* (Bloch) (Pisces: Serranidae). J. Fish Biol. 46: 961-976.

Sadovy, Y. and Eklund, A. 1999. Synopsis of biological data on the Nassau grouper, *Epinephelus striatus* (Bloch, 1792), and the jewfish, *E. itajara* (Lichtenstein, 1822). U. S., Dep. Commer., NOAA Tech. Rep. NMFS 146; FAO Fisheries Synopsis 157, 65 p.

Sadovy, Y. J. and A. C. J. Vincent. 2002. Ecological Issues and the Trades in Live Reef Fishes. Chapter 18. In: Coral Reef Fishes: Dynamics and Diversity in a Complex Ecosystem, Peter F. Sale, ed., Academic Press, San Diego, pp. 391-420

Sadovy, Y, P.L. Colin and M. Domeier. 1994. Aggregation and spawning of the tiger grouper, *Mycteroperca tigris (Pisces: Serranidae)*. Copeia 1994(2): 511-516.

Sala, E., E. Ballesteros, R.M. Starr. 2001. Rapid decline of Nassau grouper spawning aggregations in Belize: fishery management and conservation needs. Fisheries 26(10): 23-30.

Samoilys, M.A. (ed.) 1997a. Manual for Assessing Fish Stocks on Pacific Coral Reefs. Dept. Primary Indust, Queensland, Training Series QE97009, 75 pp.

Samoilys, M.A. 1997b. Periodicity of spawning aggregations of coral trout *Plectropomus leopardus* (Pisces: Serranidae) on the northern Great Barrier Reef. Mar. Ecol. Prog. Ser. 160: 149-159.

Samoilys, M.A. and L.C. Squire. 1994. Preliminary observations of the spawning behavior of coral trout, *Plectropomus leopardus* (Pisces: Serranidae), on the Great Barrier Reef. Bull. Mar. Sci. 54(1): 332-342.

Samoilys, M. A. and G. Carlos. 2000. Determining methods of underwater visual census for estimating the abundance of coral reef fishes. Env. Biol. Fish. 57(3): 289-304

Sancho, G., A.R. Solow and P.S. Lobel. 2000. Environmental influences on the diel timing of spawning in coral reef fishes. Mar. Ecol. Prog. Ser. 206: 193-212.

Shapiro, D.Y. 1981. Size, maturation and the social control of sex reversal in the coral reef fish *Anthias squamipinnis*. J. Zool., Lond. 193: 105-128

Shapiro, D.Y., A. Marconato and T. Yoshikawa. 1994. Sperm economy in a coral reef fish, *Thalassoma bifasciatum*. Ecology 75(5): 1334-1344.

Shapiro, D.Y., Y. Sadovy and M.A. McGehee. 1993. Size, composition , and spatial structure of the annual spawning aggregation of the red hind, *Epinephelus guttatus* (Pisces: Serranidae). Copeia 1993(2): 399-406.

Shenker, J.M., E.D. Maddox, E. Wishinski, A. Pearl, S.R.Thorrold and N. Smith. 1993. Onshore transport of settlement-stage Nassau grouper *Epinephelus striatus* and other fishes in Exuma Sound, Bahamas. Mar. Ecol. Prog. Ser. 98: 31-43.

Sluka, R.D. 2001. Grouper and Napoleon wrasse ecology in Laamu Atoll, Republic of the Maldives: Part 2. Timing, location, and characteristics of spawning aggregations. Atoll Res. Bull. No 492: 1-15.

Smith, C.L. 1972 A spawning aggregation of Nassau grouper, *Epinephelus striatus* (Bloch) Trans. Amer. Fish. Soc. 101:257-261.

St. John, J., G. R. Russ and W. Gladstone. 1990. Accuracy and bias of visual estimates of numbers, size structure and biomass of a coral reef fish. Mar. Ecol. Prog. Ser. 64(3): 253-262

Tamaru, C.S., C. Carlstrom-Trick, W.J. Fitzgerald, Jr. and H. Ako. 1996. Induced final maturation and spawning of the marbled grouper *Epinephelus microdon* captured from spawning aggregations in the Republic of Palau, Micronesia. J. World Aquacult. Soc. 27(4): 363-372.

Thresher, R.E. 1984. Reproduction in Reef Fishes. T.F.H. Publ., Neptune City, N.J.

Watson, R. A., G. M., Carlos, and M. A. Samoilys. 1995. Bias introduced by the non-random movement of fish in visual transect surveys. Ecol. Model. 77: 205-214

Vilaro Diaz, D.J. 1884. Corrida y arribazon de algunos peces cubanos. Manuel Gomez de la Maza, La Habana, 15 p.

Zabala, M., A. Garcia-Rubies, P. Louisy and E. Sala. 1997a. Spawning behaviour of the Mediterranean dusky grouper *Epinephelus marginatus* (Lowe, 1834)(Pisces, Serranidae) in the Medes Islands Marine Reserve (NW Mediterranean, Spain). Sci. Mar. 61(1): 65-77.

Zabala, M., P. Louisy, A. Garcia-Rubies and V. Garcia. 1997b. Socio-behavioural context of reproduction in the Mediterranean dusky grouper *Epinephelus marginatus* (Lowe, 1834)(Pisces, Serranidae) in the Medes Islands Marine Reserve (NW Mediterranean, Spain). Sci. Mar. 61(1): 79-89.

Zeller, D.W. 1997. Home range and activity patterns of the coral trout *Plectropomus leopardus* (Serranidae). Mar. Ecol. Prog. Ser. 154: 65-77.

Zeller, D.W. and G.R. Russ. 1997. Marine reserves: patterns of adult movements of the coral trout *Plectropomus leopardus* (Serranidae)). Can. J. Fish. Aquat. Sci. 55: 917-924.