

Manual for the Study and Conservation of Reef Fish Spawning Aggregations

by

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



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. The methods described in this manual have been based on the experiences of the authors and others, however 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. The authors are grateful to Ken Lindeman and Melita Samoily for their comments on sections of the manuscript, and to Environmental Defense for funding the first print run of the manual.

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.

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

ISBN 0-9729113-0-8

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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 were collected 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 retained 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 (has a plastic fastener that seals tightly). 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, Samoily (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" 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 taken 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

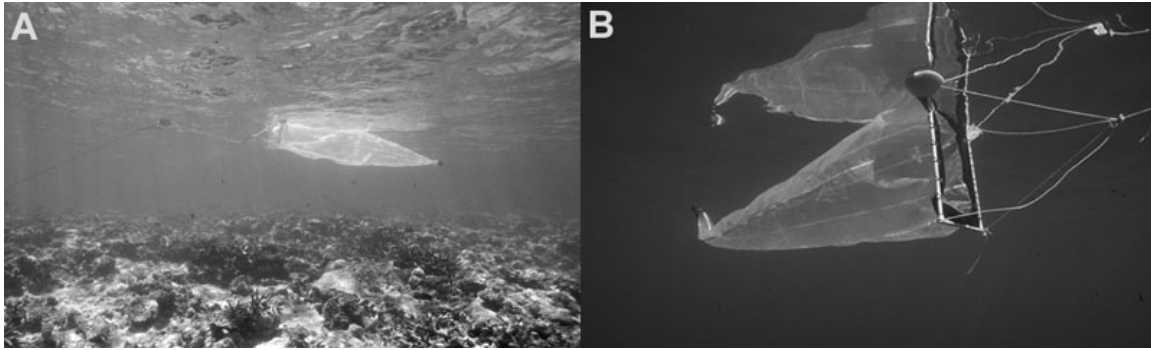


Figure 49. Moored plankton nets on Lighthouse Reef, Koror, Palau used to collect eggs of fishes spawning upcurrent (from Colin and Hamner, in prep). A. Plankton net moored over a shallow reef flat. B. Net moored over deep water. The frame has a four-point bridle, with the upper portion supported by buoys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

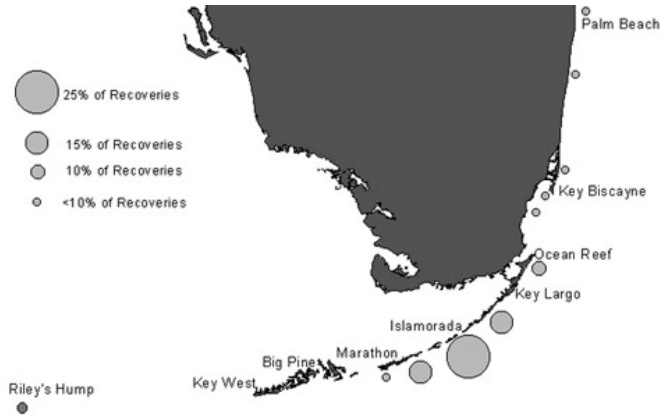


Figure 51. (Left) Drift bottle made from standard scintillation vial ballasted with BB's to the point of being only marginally positively buoyant. (Right) Locations of recoveries of drift bottles along the south Florida coast released at Riley's Hump (a spawning area) at the time of presumed spawning.

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

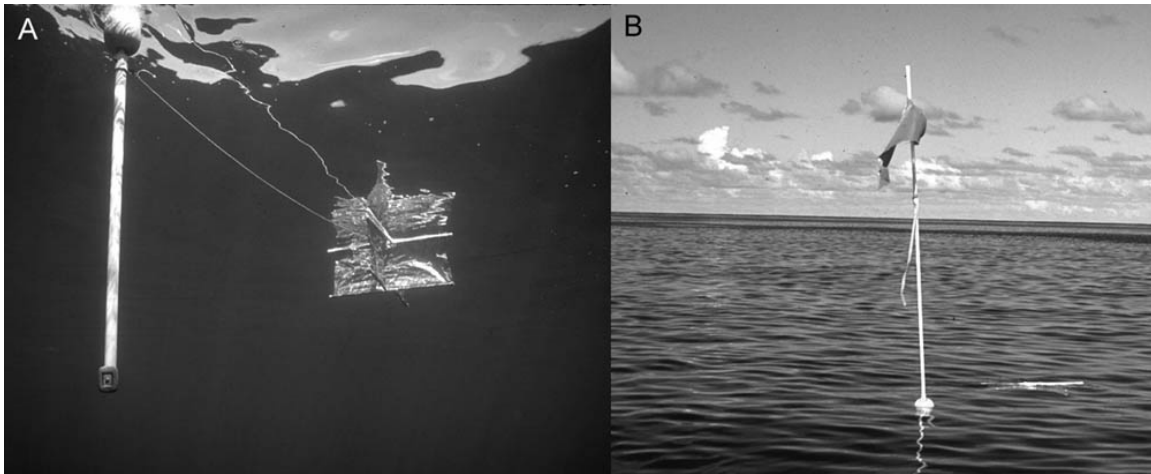


Figure 52. Simple current-following drifter. A. Underwater view of the drogue or vane is to the right, 1 m by 1m extending downward to 50 cm depth, attached by a line to the pole and float. B. Surface view of the marker pole, with colored flag to aid in locating from a boat.

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 chance 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'

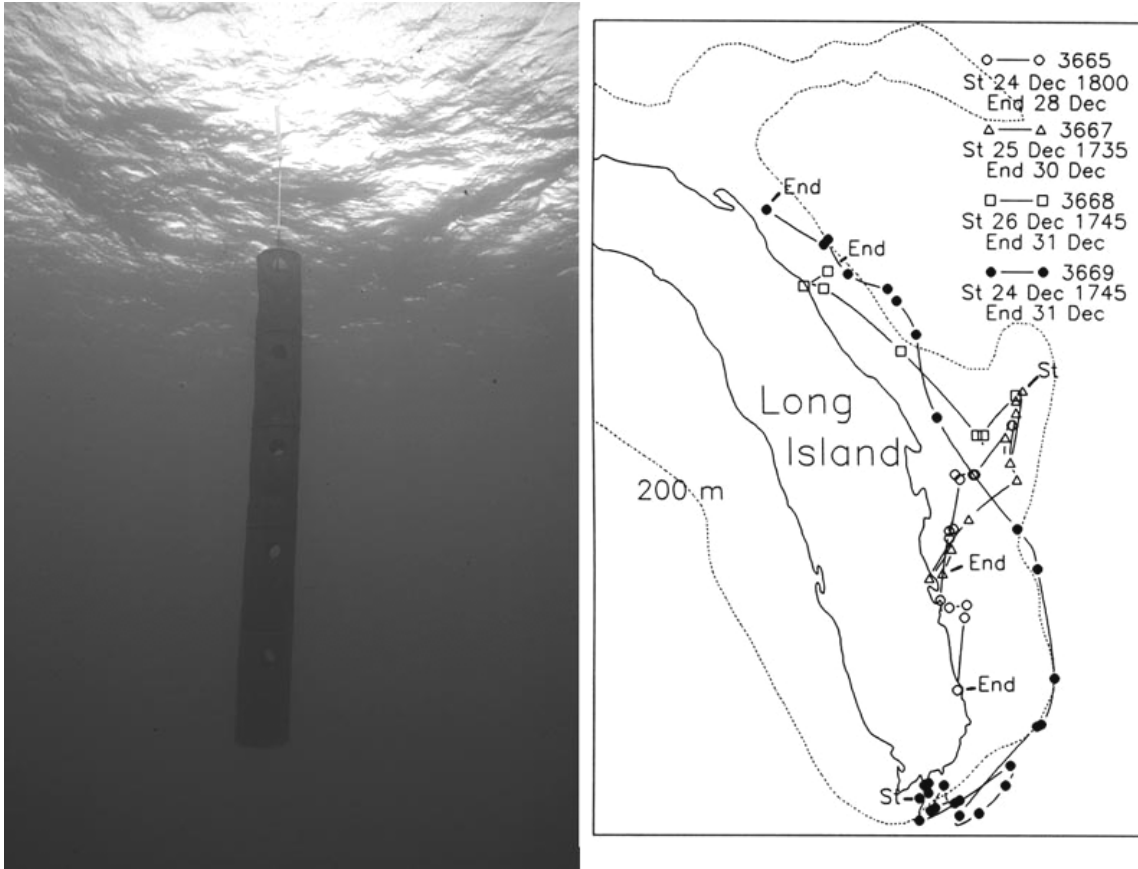


Figure 53. (Left) Typical satellite tracked current drifter, with 10 m long drogue (“holey sock”) with 5 m line to surface float that contains transmitter and batteries (PLC). (Right) Tracks of current drifters released at or near Nassau grouper spawning sites (after Colin, 1996).

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 be 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 stage 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).