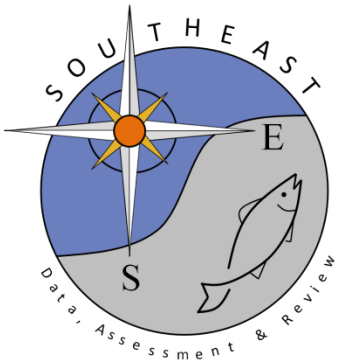
Overview of sampling gears and standard protocols used by the Southeast Reef Fish Survey and its partners

Tracey I. Smart, Marcel J.M. Reichert, Joseph C. Ballenger,
Walter J. Bubley, and David M. Wyanski

SEDAR55-RD05

7 August 2017



Overview of sampling gears and standard protocols used by the Southeast Reef Fish Survey and its partners

Tracey I. Smart, Marcel J.M. Reichert, Joseph C. Ballenger, Walter J. Bubley, and David M. Wyanski

Marine Resources Research Institute
South Carolina Department of Natural Resources
P.O. Box 12259
Charleston, SC 29412

MARMAP Technical Report # 2015-005

Research described in this report is part of the Marine Resources Monitoring, Assessment, and Prediction (MARMAP) program contract (NA11NMF4540174) and the South East Area Monitoring and Assessment Program (SEAMAP) contract (NA11NMF4350043) sponsored by the National Marine Fisheries Service (Southeast Fisheries Science Center) and the South Carolina Department of Natural Resources.

Overview

Fishery-independent measures of catch and effort with standard gear types and deployment strategies are valuable for monitoring the status of stocks, interpreting fisheries landings data, performing stock assessments, and developing regulations for managing fisheries resources. Inevitably, tighter management regulations result in fishery-dependent catches reflecting the demographics of a restricted subset of the population, affecting the utility of fishery-dependent data when assessing the current status of the stock. When fisheries are highly regulated, fishery-independent surveys are often the only method available to adequately characterize population size, age and length compositions, and reproductive parameter distributions, all of which are needed to assess the status of stocks. The Marine Resources Monitoring, Assessment and Prediction (MARMAP) program has conducted fishery-independent research on the continental shelf and shelf edge between Cape Hatteras, North Carolina, and St. Lucie, Florida, for over 40 years to provide information for stock assessments and evaluation of management plans. Housed at the Marine Resources Research Institute (MRRI) of the South Carolina Department of Natural Resources (SCDNR) in Charleston, SC, the overall mission of the MARMAP program has been to determine the distributions, relative abundances, and critical habitats of economically and ecologically important fishes off the southeast United States' coast (SEUS), and to relate these features to environmental factors and exploitation activities.

Although the MARMAP program has used various gear types and methods of deployment since its inception, the program has strived to use consistent gears and sampling methodologies throughout extended periods to allow for analyses of long-term changes in relative abundance, length frequencies, life history parameters and other information. This report outlines standard deployment protocols for the gears either currently in use or for which use is likely to resume in the near future. Gears included in this document are the chevron trap, the short bottom longline, the long bottom longline, hook and line, and video. For a full description of many gears previously used by the survey, see MARMAP (2009).

Until recently, the MARMAP program was the only long-term fishery-independent program that collected the data necessary to develop indices of relative abundance for species in the South Atlantic Fisheries Management Council's (SAFMC) snapper-grouper species complex. In 2008, with a first field season occurring in 2009, the Southeast Area Monitoring and Assessment Program's South Atlantic component (SEAMAP-SA) provided funding to complement MARMAP efforts. A particular goal of the SEAMAP-SA Reef Fish Complement is to assist with the expansion of the geographical sampling coverage of the current fishery-independent surveys, focusing on either shallow or deep potential live-bottom areas. In addition, the SEAMAP-SA complement funding allowed for expanded sampling in marine protected areas (MPAs).

Beginning in 2010, NOAA Fisheries made funding available to create the Southeast Fisheries Independent Survey (SEFIS) program housed at the Southeast Fisheries Science Center (SEFSC) laboratory in Beaufort, NC. This fishery-independent survey was designed to further complement the historical MARMAP/SEAMAP-SA reef fish monitoring efforts, again aimed at extending the geographical range and increasing the sampling volume of the surveys. SEFIS activities were closely coordinated with those of MARMAP/SEAMAP-SA staff, who trained SEFIS personnel and have participated in SEFIS monitoring cruises. SEFIS uses gear and methodologies identical to MARMAP/SEAMAP-SA to maintain the

integrity of the long-term data set. The combined efforts of MARMAP, SEAMAP-SA Reef Fish Complement, and SEFIS to conduct fishery-independent reef fish monitoring in the US South Atlantic region are now referred to as the Southeast Reef Fish Survey (SERFS).

Survey Design for Live-Bottom Sampling

The standard SERFS sampling area includes waters of the continental shelf and shelf edge between Cape Hatteras, NC, and St. Lucie Inlet, FL, although over the years the majority of sampling has occurred south of Cape Lookout, NC (Fig. 1). Throughout this range, we sample stations established on confirmed live bottom (monitoring) from May through September each year, though cruises have occurred as much as two weeks prior to and after these months in some years. Live-bottom habitats are hard-bottom areas that have been colonized by attached flora or fauna. These can range anywhere from flat pavement to rock ledges and pinnacles with either attached invertebrates such as sponges or corals or attached algae. Gear deployments on suspected live bottom in a given year (reconnaissance) are evaluated based on catch and video or photographic evidence of bottom type for inclusion in the sampling universe the next year.

Two types of reef habitat stations are available for sampling in a given year: low to moderate relief for chevron traps and moderate to high relief for the short bottom longline (SBLL). Each year, a subset of stations are selected randomly from known live-bottom stations identified for monitoring in a manner such that no station selected in a given year is closer than 200 m to any other selected station, though the minimum difference typically is closer to 400 m. Currently, there are approximately 3,500 reef habitat stations in the sampling universe for chevron traps, ranging in depth from 9 to 109 m, although the vast majority of stations are generally shallower than 100 m. Currently, there are approximately 330 SBLL stations, with depths ranging from 35 to 360 m, with most stations found deeper than 100 m. Chevron stations and SBLL stations are randomly selected separately, as there is very little spatial overlap between these sampling universes.

Hook and line deployments generally occur over live bottom, but are not restricted to stations or a given type of relief. Locations of hook and line deployments are decided upon by the expertise of the ship's crew based on conditions and the needs of the scientific sampling as these deployments are often targeted toward collecting species for directed projects.

Survey Design for Soft-Bottom Sampling

Long bottom longlines (LBLL) are deployed over soft-bottom areas (sand or mud). Rather than stations, LBLL deployments are conducted within 15 blocks of confirmed soft bottom (each block covers between 50 and 65 km^2; Fig. 1). No more than 2 LBLL monitoring deployments occur within a given block in a given year and the minimum distance between these deployments is 200 m. Exact locations of deployments within a block are determined by weather conditions and current direction and speed. LBLLs are deployed at depths between 160 and 300 m.

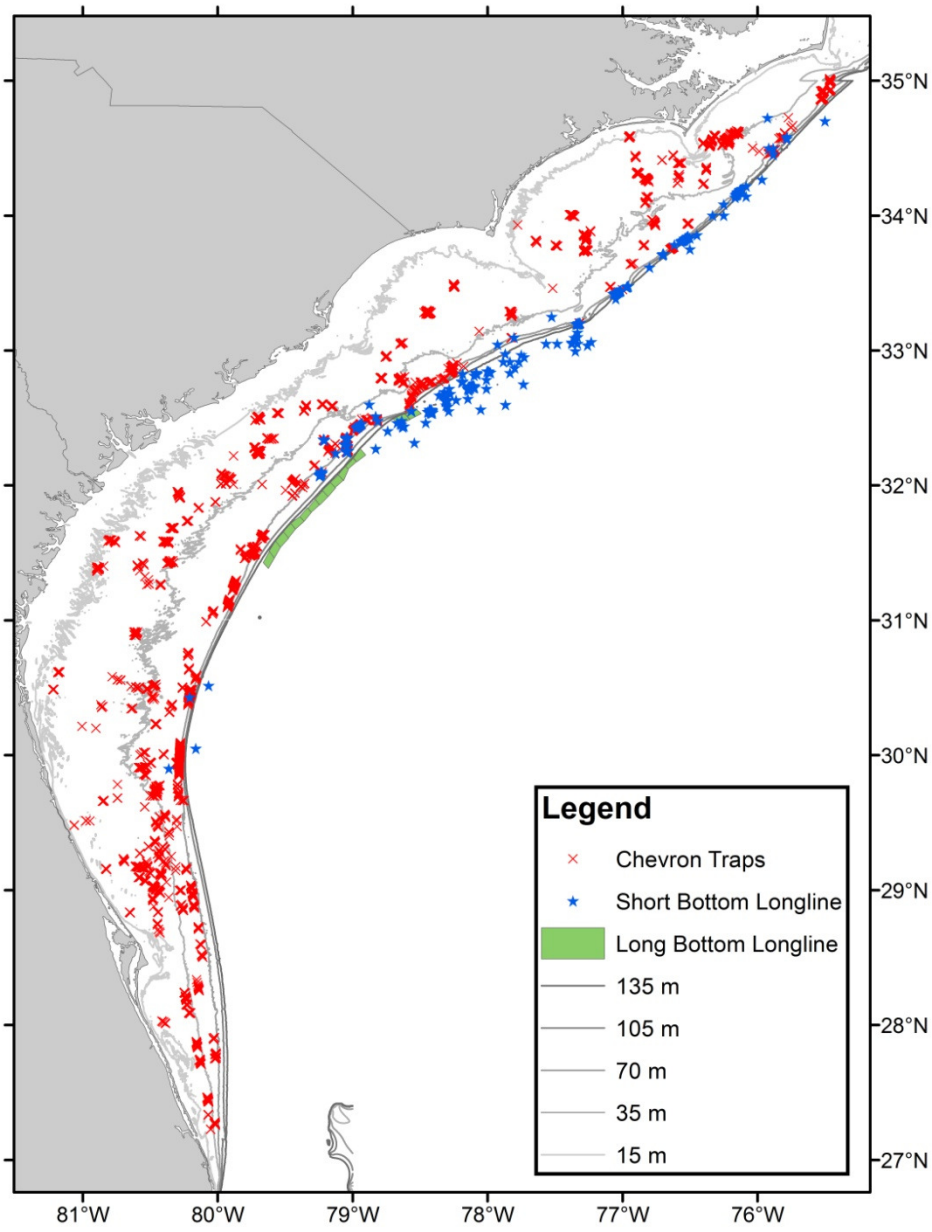


Figure 1. 2014 reef habitat and soft-bottom sampling universes. Both universes were developed over time based on exploratory or reconnaissance sampling, previous surveys, and information provided by other researchers or fishermen. The reef habitat universe is divided into stations appropriate for chevron traps (low to moderate relief) and short bottom longline (moderate to high relief). The soft-bottom (mud/sand) universe is divided into blocks is used by the long bottom longline survey.

Chevron Traps

MARMAP began using chevron traps in 1988 after a commercial fisherman introduced the use of this trap design in the SEUS region (Collins 1990). Subsequently, in 1988 and 1989, chevron traps were used simultaneously with blackfish and Florida Antillean traps to compare the efficiency of the three different trap designs at capturing

reef fishes on live-bottom habitats (Collins 1990). Results indicated that the chevron trap was most effective overall for species of commercial and recreational interest in terms of both total weight and numbers of individuals captured (Collins 1990). Based on these results, the MARMAP program has used chevron traps for reef fish monitoring purposes in the US South Atlantic since 1990, using this single gear to replace both blackfish and Florida Antillean traps. Currently, all three fishery-independent monitoring programs composing SERFS continue to utilize the chevron trap as their primary monitoring gear.

The chevron trap time series has been continuous from 1990 to present, although the distribution and extent of sampling has changed over time. The spatial coverage of the survey has expanded over the time series as we have added stations and sampling effort in the northern and southern ends of the survey. Figure 1 shows the extent of the survey for the 2014 sampling year.

Chevron traps are arrowhead shaped, with a total interior volume of 0.91 m^3 (Fig. 2, Collins 1990, MARMAP 2009). Each trap is constructed of 35 x 35 mm square mesh plastic-coated wire. Each trap possesses a single entrance funnel ("horse neck") and release panel to remove the catch. Prior to deployment each chevron trap is baited with a combination of whole or cut clupeids, with *Brevoortia* spp. most often used. Four whole clupeids on each of four stringers are suspended within the trap and approximately 8 clupeids, with their abdomen sliced open, are placed loose in the trap. An individual trap is attached to an appropriate length of 8 mm (5/16 in) polypropylene line buoyed to the surface using a polyball buoy. We attach a 10 m trailer line to this polyball buoy, with the end of the trailer line clipped to a Hi-Flyer buoy or another polyball. Generally, traps are deployed in sets of six when a sufficient number of stations are available in a given area. Traps are retrieved in chronological order of deployment, using a hydraulic pot hauler, after an approximately 90-minute soak time. All chevron trap deployments occur during daylight hours (no earlier than 30 minutes after sunrise and retrieved no later than 30 minutes before sunset).

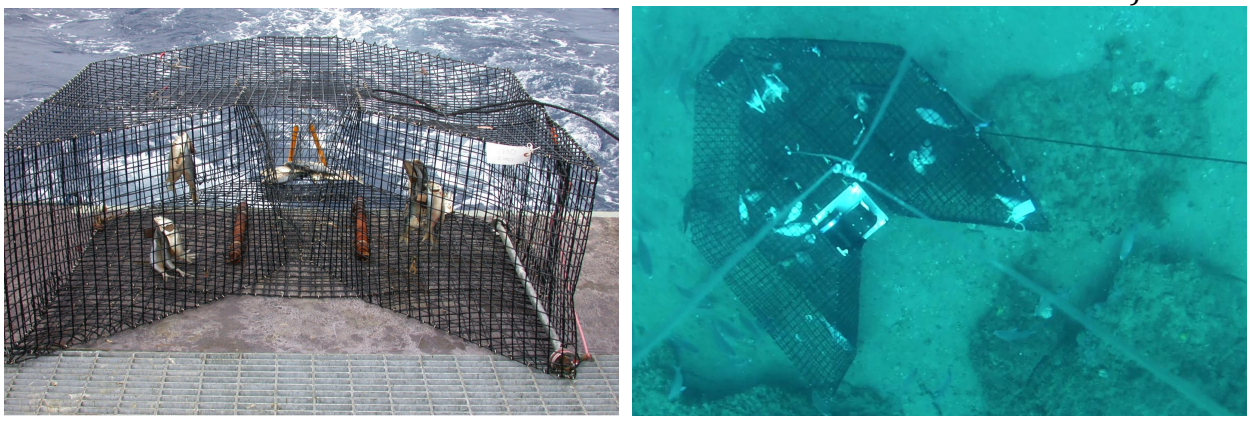


Figure 2. Chevron trap baited prior to and after deployment. Note video cameras on the trap in the right panel (see below). Photos courtesy of SERFS.

Short Bottom Longline (SBLL)

SBLLs are bottom longlines that are deployed over moderate- to high-relief live bottom and are shorter than our other type of longline gear (see below). SBLLs were first deployed by the MARMAP program in 1979, with a standard methodology since 1996. This gear was originally called "vertical longline" and replaced the use of Kali Poles, which were used for only 1983-1986 (MARMAP, 2009).

The SBLL consists of 25.6 m of 6.4 mm diameter treated solid braid Dacron (polyester) ground line dipped in green copper naphthenate (Fig. 3). We attach twenty gangions to this ground line at intervals of approximately 1.2 m. The gangions consist of an AK snap, 0.5 m of 90 kg monofilament and a non-offset circle hook (almost exclusively #5 Eagle claw size) baited with a whole squid (*Illex* sp. or *Loligo* sp.). The line is deployed by stretching the ground line along the vessel's gunwale with 2-3 sash weights (ca. 4.5 kg each) attached at each end of the line. The ground line is attached to an 8 mm (5/16 inch) polypropylene line buoyed to the surface using a polyball buoy. The buoy is attached to a hi-flyer buoy using a 10 m trailer line. Soak time is approximately 90 minutes, after which the gear is retrieved utilizing a hydraulic pot hauler. The SBLLs generally are deployed in sets of six, with a minimum distance between longlines of 200 m. Each longline is attached to its own surface polyball and hi-flyer buoy (note: one buoyline per ground line) and not connected to any other longline or the ship. All SBLL deployments occur during daylight hours (no earlier than 30 minutes after sunrise and retrieved no later than 30 minutes before sunset).

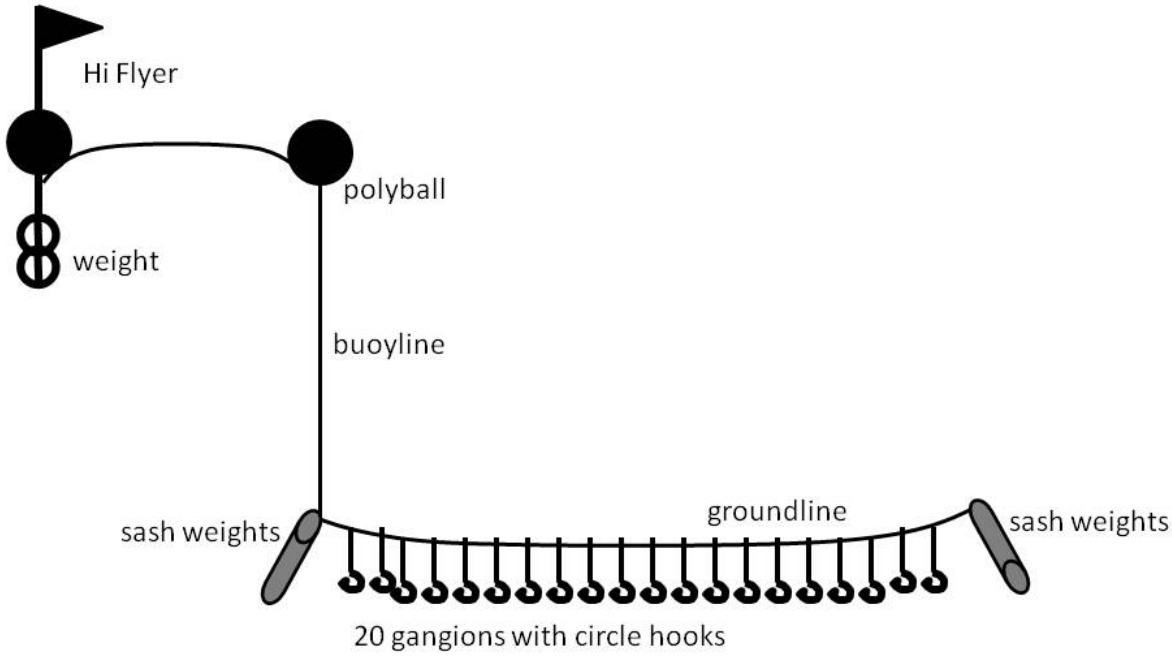


Figure 3. Short bottom longline illustration.

Long Bottom Longline (LBLL)

The LBLL was initiated in the early 1980's to sample the snapper-grouper species in the tilefish grounds (in particular Golden Tilefish, *Lopholatilus chamaeleonticeps*), which typically are areas of smooth mud or sand bottom (Low et al. 1983). This gear type was traditionally called "horizontal longline" by MARMAP, since it was a bottom longline deployed over relatively flat bottom. We amended the name of this gear to long bottom longline in 2011 to better capture the nature of this gear and distinguish it from the short bottom longline (see above). Potential LBLL sampling areas were identified from Kali Pole surveys conducted during 1985 and 1986, input from commercial and recreational fishermen, fathometer data, and previous exploratory surveys in 1980-1981 (Low et al.

1983). Sampling locations were divided into sampling blocks based on the LORAN grid and converted to GPS coordinates in 2009. This gear is deployed at two locations within each block with a minimum distance of 200 m between locations. Sampling generally is conducted from August through October.

Since 1996 LBLLs have been constructed of 3.2-mm galvanized cable (1,525 m long), deployed from a longline reel, with 1,220 m of the cable used as the ground line and the remaining 305 m buoyed to the surface (Fig. 4). Two to three sash weights (10-11 kg each) are attached to the leading end of the ground line and 100 gangions are attached in 12 m intervals to the ground line. Gangions consist of an AK snap, approximately 0.5 m of 90 kg monofilament, and a non-offset circle hook (almost exclusively #5 Eagle claw size). Another 2-3 sash weights are attached at the terminal end of the ground line (buoy end) and the remaining 305 m of cable is buoyed to the surface with 1 or 2 polyball buoys and a hi-flyer buoy attached to a 10 m trailer line. Hooks are baited with whole squid (*Illex* sp. or *Loligo* sp.). LBLLs generally are deployed while running with the current at a speed of 4-5 knots and LBLLs are deployed in sets of two. Each line soaks for 90 minutes and is retrieved using a hydraulic pot hauler. All LBLL deployments occur during daylight hours (no earlier than 30 minutes after sunrise and retrieved no later than 30 minutes before sunset).

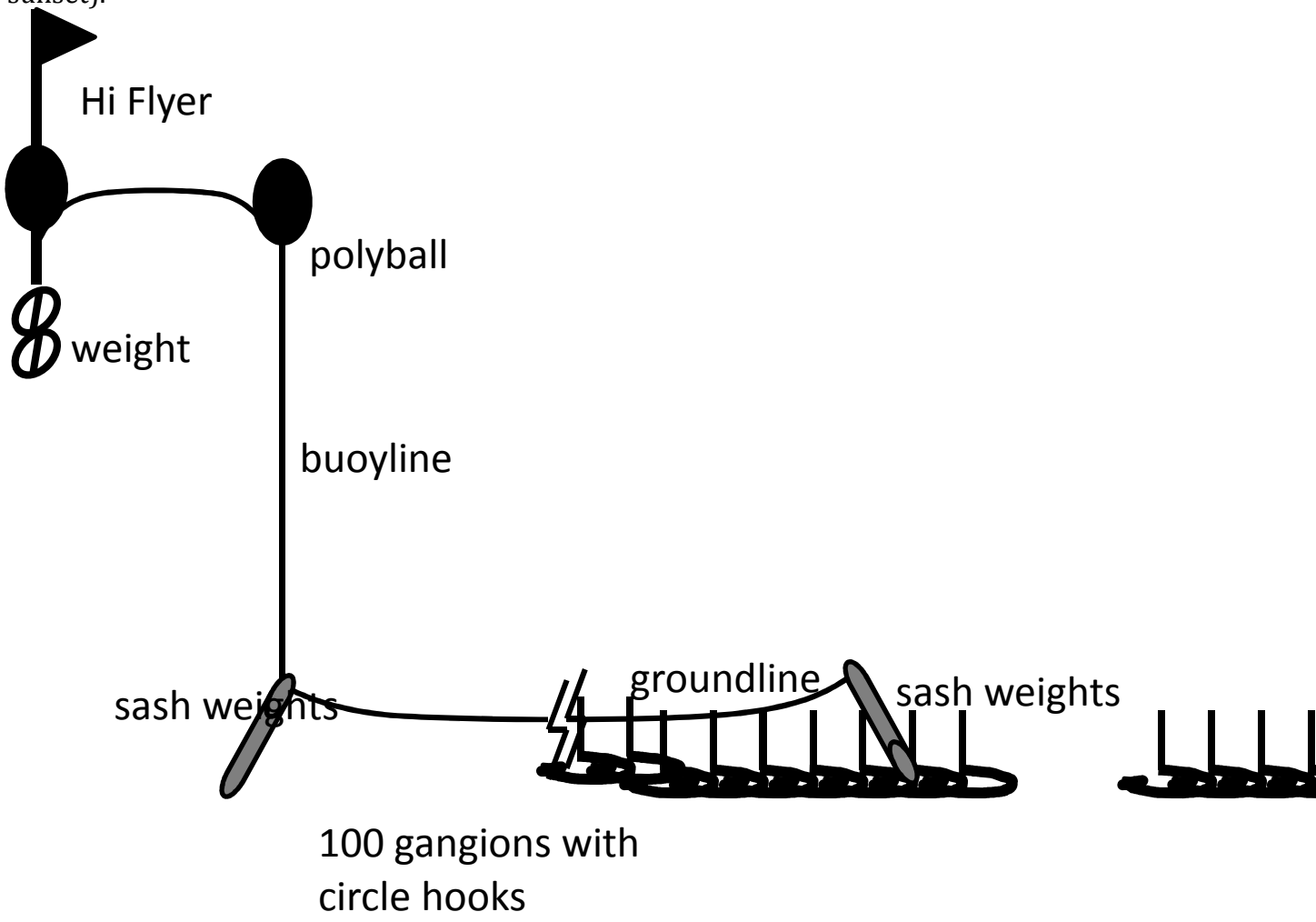


Figure 4. Long bottom longline. The ground line is equipped with 100 gangions with circle hooks (not all illustrated here).

Hook and Line

Sampling for reef fishes on live bottom occasionally occurs by haphazard gear deployments. There are two primary objectives addressed by these deployments: stomach content and increased sampling of relatively rare species. Two hook and line gears are the most commonly employed by the program currently, the personal rod and reel and the bandit reel. Personal rod and reel casts can be done with a variety of rods, but are generally baited with a combination of cut cigar minnow and squid on three hooks per drop (or deployment). Bandit reel drops are baited with a mix of cut fish cigar minnows or sardines and squid on generally three hooks and the bait portions are often larger than for personal rod and reel. Hooks are re-baited as needed between casts. Hook size and weights used are variable depending on focal species and sea state. Deployment duration is estimated as the length of time an individual is fishing by one of the above methods, rather than the soak time of a single cast or multiple casts, at a single anchored location. Hook and line fishing can occur during daylight or nighttime hours.

Videos

Video cameras were added to the chevron trap survey in 2010 on board SEFIS-led cruises. Starting in 2011, all traps deployed at depths appropriate for cameras were equipped with at least one video camera. In 2010, GoPro Hero®[*1] cameras in underwater housings rated to 40 m depth were attached to traps over the mouth and facing away from the trap. In 2011, a Canon Vixia HFS200[*1] camera replaced this GoPro[*1] over the mouth and a GoPro[*1] was attached over the nose of each trap, looking away from the trap, on SEFIS-led cruises (positions A and C, respectively, in Fig. 5). Canon[*1] underwater housings are rated to depths of 137 m. On MARMAP or SEAMAP-SA cruises, prior to 2011, still cameras set to time lapse (5-min intervals) were placed over the trap funnel, looking away from the trap in several years in the 1990s and 2000s. On MARMAP or SEAMAP-SA cruises, in 2011, Canons[*1] were placed over the mouth, and still cameras were moved slightly offset from the nose of the trap (positions A and CO, respectively, in Fig. 5). In 2012, all still cameras were replaced with GoPros[*1]. Prior to 2015, most GoPro[*1] housings were rated to 60 m depth. Both cameras are turned on and set to record as the vessel approaches a sampling station. Both video cameras record high-definition video for most or all of the time the trap soaks (approximately 90 minutes). After a trap is retrieved, memory cards are removed from video cameras and downloaded onto external hard drives for transport to the laboratory for processing.

Two supplemental video cameras were deployed on traps starting in 2013. An Internal GoPro[*1] is mounted below position C (Fig. 5), angled downward and facing the funnel entrance of the trap to allow for examination of entry/exit rates and fish behaviors in the traps. The Long camera is a floating housing containing a GoPro[*1] camera tethered to a trap by a gangion and 1,000 lb. monofilament attached halfway between positions A and C (Fig. 5). The Long camera floats about a meter above the trap and provides information about habitat and nearby fish.

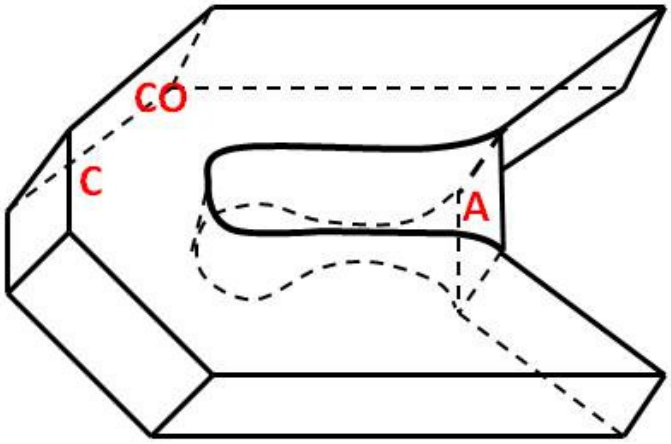


Figure 5. Diagram of video locations on SERFS chevron traps (based on Collins, 1990). **A** refers to current placement of Canon*1 cameras in all partner programs or 2010 placement of GoPro*1 cameras, **C** refers to current placement of GoPro*1 cameras on SEFIS vessels, and **CO** refers to current placement of GoPro*1 cameras or former placement of still cameras on MARMAP/SEAMAP-SA vessels.

Oceanographic Data

Oceanographic data is collected via a conductivity, temperature, and depth instrument (CTD) to complement most gear deployments. SeaBird*1 SBE 19, 25, and 25Plus CTDs have been used at various time points within the time series, with the 25Plus being the primary instrument in the most recent sampling year. All CTDs measure depth, temperature, and salinity, but the 25 and 25Plus are equipped with additional sensors such as dissolved oxygen, chlorophyll, nitrates, and phosphates. CTD casts are conducted while sets of either chevron traps or SBLLs are soaking and prior to or following each LBLL deployment. Only data from downcasts are archived.

Length Frequency Work-up

Immediately after each gear deployment is retrieved, collected fish are placed on ice in bins labeled for that deployment. Once a set of gear deployments is completed, all fish caught in each gear deployment undergo Length Frequency work-up (LF). A collection is defined as a single gear deployment and can refer to any collection from traps, longlines, hook and line, or other gear types. LF occurs during the day, shortly after collection and consists of identifying all fish in each collection to species level or the lowest possible taxon, then weighing and measuring the fish. If identification to species level is uncertain, fish are photographed and usually frozen after work-up for later species ID verification. An aggregate weight for each species per collection is recorded in g wet weight. The total number of fish per collection also is recorded for each collection or derived from the number of fish measured. Lengths (to the nearest cm prior to 2010 or nearest mm since 2010) of all individual fish per species per collection are recorded using a Limnoterra digital fish measuring board and custom processing software (FMB, starting in 1989). If a FMB is not available, length measurements are determined using a measuring cradle and recorded by hand on a paper datasheet. Either fork length (FL) or total length (TL) was measured prior to 2012 depending on the species. Beginning in 2012, lengths of all species

were recorded in TL. Note that TL is measured using the "pinch method" in which the caudal fin lobes are pinched together (also referred to as maximum TL). During LF, specimens of priority species are retained for additional life-history processing (see Age/Growth section below). Any fish not retained for the age-growth work-up are degassed as necessary and released.

Life History Work-up (At-Sea)

Fish designated for Life History (LH) work-up on-board the vessel are tagged with the collection number and stored on ice (not frozen) until processing begins, normally during night-time hours. In recent years, the priority species included Black Sea Bass, groupers, snappers, Red Porgy, White Grunt, tilefishes, Greater Amberjack, and Gray Triggerfish. Other species are kept for life-history sampling based on the SouthEast Data and Review (SEDAR) schedule, ancillary research projects, and as time allows. All individuals of most priority species are retained for LH work-up. However, the four species with the highest catches (Black Sea Bass, Red Porgy, Vermilion Snapper, and Gray Triggerfish) have been sub-sampled in many years. Prior to 2008, sub-sampling was based on length categories and latitude. See Appendix A for details as methodology changed among years and species. From 2008 through the present, a random selection of a given percent of each species was retained for LH work-up. In 2009, the total number of randomly retained specimens for each of the four species was based on numbers of fish captured and kept during the 2000-2007 MARMAP sampling seasons. The percentages of kept specimens for the four species with the highest catches were: Black Sea Bass 33%, Red Porgy 75%, Vermilion Snapper 50%, and Gray Triggerfish 80% in 2009-2012. In 2013, these percentages were again adjusted in response to several power analyses that indicated that only 20% of Black Sea Bass but 100% of Gray Triggerfish needed to be retained. Similarly, in 2014 all Red Porgy and Vermilion Snapper were retained. In addition, all very small (< 150 mm FL for Red Porgy, Vermilion Snapper, and Gray Triggerfish or < 150 mm TL for Black Sea Bass) and very large specimens (>400 mm FL for Red Porgy and Vermilion Snapper, > 450 mm TL for Black Sea Bass, and > 500 mm FL for Gray Triggerfish) of these species have been kept for LH if not selected randomly in years of random sub-sampling. These specimens were not included in development of fishery-independent age composition estimates, but kept to provide additional information for growth model development and for estimating reproductive parameters and natural mortality. In 2009, the random selection was tracked manually on board the vessel. In 2010, the fish measuring board software program was adjusted to accommodate the random sub-sampling, allowing for electronic tracking of the randomly selected specimens.

On-board, the LH work-up consists of verifying identification, weighing and measuring individual fish, and removing otoliths or dorsal spines, gonadal tissues, and possibly other tissues such as stomach and intestinal tract (for diet studies) and tissues for genetic studies. Starting in 1991, a Limnoterra[*1] Fish Measuring Board (FMB) is used to measure TL, FL (if applicable), and standard length (SL) of individual fish to the nearest mm. If no FMB is available, lengths are measured and recorded manually. Individual fish weight to the nearest gram is determined by an electronic wave-compensating scale or a manual triple beam scale if the wave-compensating scale is not available. Otoliths (generally sagittal) or dorsal spines (for age determination) are dissected from each fish and stored dry in coin envelopes. Gonad tissues (to investigate reproductive parameters)

are placed in Tissue Tek®[*1] cassettes fixed in 11% seawater-buffered formalin. If fish specimens are selected for fecundity studies (stage-2 and stage-3 yolked oocytes, sensu Hunter et al., 1992), the wet weight of the whole ovary is measured to the nearest g for ovaries > 50 g, and the whole ovary is fixed and stored in 10% seawater-buffered formalin. If ovary weight is ≤ 50 g, the whole ovary is fixed and stored in formalin. In the case of very large ovaries, a sub-sample of the ovarian tissue is fixed and stored in formalin. For diet studies of some species, stomachs are removed by excising the digestive tract from the esophagus through the pyloric caecum at the start of the intestine. The stomach is then placed in cheesecloth or a fine-mesh bag and either placed in 10% seawater-buffered formalin or frozen. Fin clips (generally the left pectoral fin) from several priority species, including snappers and groupers and White Grunt, are removed from fish during A/G work-up and preserved in 1.0% sarcosyl urea. Other tissues are treated and stored using appropriate methods for various tissues as requested by collaborators and students. All samples of individual fish are labeled and stored, and later processed and analyzed in the MARMAP/SEAMAP-SA Reef Fish Laboratory in Charleston, SC.

Age/Growth Studies (in Laboratory)

In the laboratory, spines (for triggerfish) and sagittal otoliths (all other species) collected in the field are processed for examination and age determination. The level of post-collection laboratory processing of the sagittal otolith depends on the species in question. For several priority species, including Gag and Black Sea Bass less than 6-years of age, there is no additional laboratory processing prior to examination, and age determinations are made through examination of whole otoliths using a dissecting microscope with reflected and/or transmitted light. Whole otoliths generally are examined in water to improve optical quality.

Otoliths of most species, as well as of Black Sea Bass and Gag older than 6-years of age, are sectioned. Sections subsequently are mounted to microscope slides before examination. Prior to sectioning, whole left otoliths (or the right if the left was broken or unavailable) are embedded in an epoxy resin (currently West System Resin). Then, using a low speed saw (currently an Isomet® 1000 precision saw (Buehler®[*1]), transverse sections are cut along the dorso-ventral otolith axis just off the core of the embedded otolith (generally 1-3 sections 0.4-0.7 mm thick with at least one section containing the core area). Thickness of the sections is species-specific and often based on the recommendations from aging workshops held for individual species. Subsequently, the resulting sections from an individual are mounted to one labeled glass microscope slide using Cytoseal™[*1] XYL mounting medium. Most often, whole otoliths and otolith sections are examined with transmitted or reflected light under a dissecting microscope equipped with a color digital camera and monitor, a personal computer, and image analysis software.

For Gray Triggerfish, the first dorsal spine is used for age determination, as triggerfish otoliths are small and brittle, and spines historically have been used to determine age in various geographical regions. Once removed, the spines are cleaned in the laboratory by scraping off soft tissue prior to sectioning. A series of 2 to 3 transverse sections are cut immediately distal to condyle groove. Sections are approximately 0.4 mm thick as recommended by a recent aging workshop for Gray Triggerfish (Kolmos et al. 2013). The sections are mounted and examined in the same manner as otolith sections.

From whole sagittal otoliths, otolith sections, and spine sections, increment counts are determined by counting the number of alternating translucent and opaque bands. In general, at least two independent readers assign increment counts independently without any knowledge of fish lengths, dates and locations of capture, and possible prior age estimates. Each reader assigns an edge type (opaque zone, narrow translucent zone, medium translucent zone or wide translucent zone) if applicable, and a readability index for each specimen (A-E, where A is no confidence in increment count and E is absolute confidence in increment count). Upon completion of independent reads, increment counts are compared and in cases where readers disagree, readers simultaneously view the ageing structure and attempt to reach a consensus. If consensus cannot be reached, the otolith or spine is coded as quality A and no increment count or edge is assigned. In some older individuals and difficult to age, long-lived species (e.g. tilefish), edge types and quality scores are difficult to assign and therefore may not be recorded.

Aging samples may be divided and examined by only one reader, without overlap of samples with other readers, when assessment schedules or other activities force the examination of large numbers of samples in a relatively short period of time. In those cases, a random subsample of no fewer than 100 samples is examined by all readers to provide a measure of error and check for potential bias (calibration set). In some cases, sets of samples are exchanged with other laboratories that age the same species. In recent years, preparations for SEDAR stock assessments often included an age and reproductive workshop in which researchers from state, federal, and academic institutions discuss methods and examination of structures and perform inter-laboratory calibration exercises.

Reproduction Studies (in Laboratory)

Following capture and dissection, the posterior portion of the gonads was fixed for 7–14 d in an 11% seawater–formalin solution buffered with marble chips and transferred to 50% isopropanol for an additional 7–14 d. Three transverse sections (6–8 μm thick) were cut from each sample with a rotary microtome, mounted on glass slides, stained with double-strength Gill hematoxylin, and counterstained with eosin-y. Sections of gonad tissue were viewed under a compound microscope at 20-400X magnification, and sex and reproductive class were determined without knowledge of capture date, specimen length, or specimen age. Usually two readers independently determined sex and reproductive state using histological criteria (Wyanski et al., 2006; Brown-Peterson et al., 2011). When assignments differed, the readers re-examined the section simultaneously to determine reproductive state. Each reader is trained in examination prior to independently reading using a training set. Upon completion of independent assessments of sex and reproductive class, assessments are compared and in cases where readers disagree, readers simultaneously view the gonad sections and attempt to reach a consensus. If a consensus cannot be reached, no sex and/or class is assigned.

To determine the fecundity type (indeterminate vs. determinate) of a species, oocyte size distribution and total fecundity are examined monthly during the spawning season. The size distribution of mid- and late-stage yolked oocytes (Stages 2 and 3 of Hunter et al. 1992) is determined using one 25 to 30 mg subsample of ovarian tissue from a random location in 5-10 specimens with developing gonads. Samples are weighed with a digital analytical balance ($\pm$ 0.01 mg). Image analysis software is used to measure the average diameter of each oocyte in a subsample of 300-500 whole yolked oocytes per

specimen. The counts of stage-3 oocytes from these same subsamples can be used to estimate total fecundity, but one additional random subsample per specimen should be processed. To increase the monthly sample size for the estimate of total fecundity, two 25 to 30 mg subsamples are taken from 10 additional specimens of a representative size range per month. Total fecundity is calculated by multiplying the preserved ovary weight by oocyte density (number of stage-3 oocytes/g of ovary). A regression equation (usually simple linear) is computed for each month and the effect of each month on total fecundity is examined using analysis of covariance (ANCOVA). If the pattern of yolked oocyte size distribution does not vary with time and total fecundity does not change with time, then the fecundity type is indeterminate and it is necessary to estimate batch size monthly over the course of the spawning season. Nearly all reef fish species studied to date exhibit indeterminate fecundity.

Prior to estimating batch fecundity, it is necessary to assess whether the oocytes that represent the batch are randomly distributed in the ovary. This step requires taking one 75-mg sample from each of three fixed locations (anterior, middle, and posterior) in one or both lobes. All oocytes undergoing maturation (migratory nucleus or hydrated stages) are counted and oocyte density is calculated (number of maturing oocytes/g of ovary). A 2-way analysis of variance without interaction is used to test for the effects of location and individual fish on oocyte density. If the effect of location is not significant, then subsamples for the estimate batch fecundity can be taken from fixed or random locations. Batch fecundity is calculated by multiplying the preserved ovary weight by oocyte density. A regression equation (usually simple linear) is computed for each month and the effect of each month on batch fecundity is examined using ANCOVA. Samples from all months can be pooled if the test results show that batch fecundity does not change with time (i.e., slopes and elevations of monthly equations similar).

Diet Studies (in Laboratory)

After 14 days, the stomachs preserved in formalin, are rinsed with tap water and all contents from each stomach are extracted, transferred to individual containers, and stored in 70% ethanol. The contents are sorted and examined in the laboratory under a dissecting microscope and identified to the lowest possible taxon. All prey items are then counted and weighed. For stomachs that have been frozen for possible use in DNA barcoding, contents are sorted and examined in the laboratory under a dissecting microscope and identified to the lowest possible taxon. Any unidentified prey items are preserved in 70% ethanol for subsequent DNA extraction.

The 2014 target species for diet studies were Black Sea Bass (*Centropristis striata*), White Grunt (*Haemulon plumierii*), Squirrelfish (*Holocentrus adscensionis*), Blueline Tilefish (*Caulolatilus microps*), and all encountered grouper/hind species (Family Serranidae). Documented in-trap predation and gorging on bait can confound diet analyses and obfuscate the natural diet for many species of fish. For these reasons, hook and line is the preferred gear for collecting specimens for diet studies. Therefore, stratified hook and line collection protocols are designed for species caught in large numbers (i.e. Black Sea Bass and White Grunt), ideally targeting twenty specimens in each of 24 strata defined by latitude and water depth. Each stratum consisted of one of three depth zones (0-20 m, 21-50 m, and >50 m) and one of eight 1-degree latitudinal zones (from 27° N through 34° N).

For rarer species, specimens collected by chevron traps also are examined for stomach content. Past target species includes Red Snapper (*Lutjanus campechanus*), Red Porgy (*Pagrus pagrus*), Vermilion Snapper (*Rhomboplites aurorubens*), and Gray Triggerfish (*Balistes capriscus*).

[*1]: Mention of trade names or commercial companies is for identification purposes only and does not imply endorsement by the South Carolina Department of Natural Resources or the National Marine Fisheries Service, NOAA.

References

Brown-Peterson, N.J., D.M. Wyanski, F. Saborido-Rey, B. J. Macewicz, S.K. Lowerre-Barbieri. 2011. A standardized terminology for describing reproductive development in fishes. Marine and Coastal Fisheries: Dynamics, Management, and Ecosystem Science 3:52-70.

Collins, M. R. 1990. A comparison of three fish trap designs. Fisheries Research 9: 325-332.

Hunter, J. R., B. J. Macewicz, N. C. Lo, and C. A. Kimbrell. 1992. Fecundity, spawning, and maturity of female Dover sole, *Microstomus pacificus*, with an evaluation of assumptions and precision. U.S. National Marine Fisheries Service Fishery Bulletin 90: 101–128.

Kolmos, K., J. C. Ballenger, and V. Shervette. 2013. Report on age determination and reproductive classification workshops for Gray Triggerfish (*Balistes capriscus*), September 2011 and October 2012. SEDAR32-DW-03. SEDAR, North Charleston, SC. 42 pp.

Low, R.A., G.F. Ulrich, and F. Blum. 1983. Tilefish off South Carolina and Georgia. Mar. Fish. Rev. 45(4-6): 16-26.

MARMAP. 2009. Overview of sampling gear and vessels used by MARMAP. (Brief descriptions and sampling protocol).

Wyanski, D.M., H.S. Meister, O. Pashuk, A.E. Williams, and P.P. Mikell. 2006. Reproductive classification of Gray Triggerfish (*Balistes capriscus*) and female blackbelly rosefish (*Helicolenus dactylopterus*). 3rd Workshop on the Gonadal Histology of Fishes, New Orleans.