

Marine Resources Monitoring, Assessment, and Prediction (MARMAP) Program

Protocols for Length, Age, and Reproduction for Fishery-Independent Sampling

The Marine Resources Monitoring, Assessment, and Prediction (MARMAP) Program is a cooperative fisheries project of the Marine Resources Research Institute (MRRI) of the South Carolina Department of Natural Resources (SCDNR) funded by NMFS. The program conducts fishery-independent surveys and provides data on relative abundance estimates, lengths and age compositions, and growth and reproductive parameters from fish sampled from Cape Hatteras, North Carolina to the St. Lucie Inlet area in southern Florida. This document explains the protocols for sampling and processing the collected fish samples.

A. Biomass, Abundance, and Length Frequency

On-board the vessel, all fish caught in each single-gear deployment (i.e. collection) are processed in two steps. First, shortly after collection all fish are processed following a so-called Length Frequency (LF) procedure. Specimens selected for further life history studies are subsequently processed using a so-called Age/Growth work-up (see below).

In the LF procedure, all individual fish in each collection are identify to the lowest possible taxon (mostly to species), measured to a standardized length measurement, and a total weight of all individuals by species is obtained. If identification to species level is uncertain, fish are kept frozen after work-up for later species ID verification. The aggregate weight for each species per collection is recorded in kg wet weight (biomass; Total Wt.). Lengths (total length or fork length to the nearest mm) of all individual fish per species and collection are recorded using a Limnoterra digital fish measuring board (FMB). If an FMB is not available, length measurements are determined using a measured 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 to maximize the TL. During the LF work-up, 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.

Data produced by the LF work-up include species composition, species weights per collection (biomass), fish counts per species per collection (abundance), and fish counts per 1-cm length bin per species per collection (length composition or frequency). These parameters can be matched to collection information through a Project-Collection-Gear (PCG) code.

B. Life history processing and examination

Fish designated for life history or Age/Growth (A/G) work-up on-board the vessel are tagged with the collection number and stored on ice (not frozen) until processing can begin,

normally during night-time hours. In recent years, the priority species included black sea bass, gag, scamp, and other grouper species, snapper species, red porgy, white grunt, tilefish, blueline tilefish, greater amberjack, and gray triggerfish. Other species were kept to collect life history samples based on the SouthEast Data and Review (SEDAR) schedule, special research needs, and as time allowed. All individuals of most priority species are retained for A/G work-up. However, the four species with the highest catches (black sea bass, red porgy, vermilion snapper, and gray triggerfish) are sub-sampled. Prior to 2008, sub-sampling was based on length categories with the first 15 fish per 1-cm length bin encountered in each collection kept. From 2009 through the present, 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 average percentages of kept specimens were: black sea bass 33%, red porgy 75%, vermilion snapper 50% and gray triggerfish 80%. In addition, all very small (< 150 mm fork length [FL] or total length [TL]) 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 are kept for A/G work-up if not selected randomly. These specimens were not included in development of fishery-independent age composition estimates, but kept to provide additional information for growth model development and estimating reproductive parameters. In 2009, the random selection was tracked manually on board the vessel. In 2010, the FMB software program was adjusted to accommodate the random sub-sampling, allowing for electronic tracking of the randomly selected specimens. The changes in subsampling procedures over time for the four species are described in several SEDAR working papers.

The A/G 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 stomachs (for diets studies) and DNA samples for genetic studies. A Limnoterra 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 using an electronic wave-compensating scale or a manual triple beam scale if the wave compensating scale is not available. Otoliths or dorsal spines of triggerfish are extracted from individual fish (for age determination) and stored dry in individually labeled coin envelopes. Gonad tissues are extracted (to investigate reproductive parameters) and fixed in 11% sea-water buffered formalin. Other tissues (e.g. stomachs and DNA samples) may be taken and are treated and stored using appropriate methods for specific tissues. All samples collected from individual fish are uniquely labeled and stored for later processing and analysis in the MARMAP/SEAMAP-SA laboratory in Charleston, SC. A unique PCGSS code pertaining to each specimen allows the linkage of samples collected from individual fish to associated length, weight, sample location, sampling date, and other information pertaining to that individual fish.

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 otoliths depends on the species in question. For gag and black sea bass less than 6-years of age, there is no additional laboratory processing prior to examination as age determinations are made through visual inspection of whole otoliths. Whole otoliths generally are examined in water to improve optical quality, using a dissecting microscope with reflected and/or transmitted light.

Otoliths of other species, as well as of black sea bass and gag older than 6-years of age, are sectioned. 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 an Isomet® 1000 precision saw (Buehler®), ~0.5 mm 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 an individually labeled glass microscope slide using Cytoseal™ XYL mounting medium. 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 triggerfish age in various geographical regions. Once removed, the spines are cleaned in the laboratory by scraping off excess tissue prior to sectioning. A series of 2 to 3 transverse sections are cut just distal to the condyle groove along the anterior-posterior axis of the spine. Sections are approximately 0.4 mm thick as recommended by a recent aging workshop for gray triggerfish. The sections then are mounted and examined in the same manner as otolith sections.

Increment counts in whole sagittal otoliths, otolith sections, and spine sections are determined by counting the number of alternating translucent and opaque bands (increments). In principal, at least two independent readers assign increment counts independently to all otoliths or spines without any knowledge of fish lengths, dates of capture, and possible prior age estimates. Each reader assigns an edge type (Edge: opaque zone, narrow translucent zone, medium translucent zone, or wide translucent zone) and a readability index for each specimen when applicable (Quality). In cases where readers disagree, readers simultaneously view a specimen to reach a consensus. If consensus cannot be reached, the otolith or spine is removed from analysis. 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.

When assessment schedules or other activities force the examination of large numbers of otoliths, spines, or gonad samples (see below) in a relatively short period of time, otoliths and gonad preparations may be examined by only one reader, and more than 2 readers may be involved in determining age or reproductive state. In those cases, a random subsample of no less than 100 otoliths, spines, or gonad samples is examined by all readers to provide a measure of error and check for potential reader bias.

Each reader is trained in examining otoliths, spines, or gonads using a calibrated training set and calibration set. Sets of otoliths, spines and reproductive preparations are routinely exchanged with other laboratories that examine life history parameters of the same or similar species.

In recent years, preparations for SEDAR stock assessments often included species specific age workshops. Researchers involved in ageing fish specimens from both fishery dependent and independent sources discuss methods and age structures, and perform inter-

laboratory calibration exercises. If needed, similar issues related to examination of gonad tissues are discussed at these workshops as well.

Data produced during the A/G work-up includes several length measurements and weights for individual fish of many species, increment counts for individual fish, and information about age and reproductive parameters. These parameters can be matched to individual fish, collection information by the specimen ID (PCGSS). In many species, collection information and edge types can be used to convert increment counts into calendar ages, fractional ages, year classes, and age composition data.



Individual fish that undergo the on-board Age/Growth work-up also are used to investigate reproductive parameters. On-board the vessel, transverse sections of gonad tissue are placed in Tissue Tek® cassettes and fixed in 11% seawater-buffered formalin. If fish specimens are selected for fecundity studies (stage-2 and stage-3 yolked, migratory-nucleus, and/or hydrated oocytes, sensu Hunter et al., 1992), the gutted weight of the fish is recorded (Gutted Wt., g), the wet weight of the whole ovary is determined (Gonad Wt., g), and a sub-sample of the ovarian tissue is weighed, wrapped in cheese cloth, and fixed and stored in 11% seawater-buffered formalin. Prior to each sampling season all field staff receive instructions and training as to the proper dissection of gonad tissues and sample treatment of all priority species.

After collection, the gonad samples remain in formalin for at least 7 days, after which they are transferred to 50% isopropanol in the MARMAP laboratory. The samples remain in this solution for at least another 7 days. Gonad samples then are processed in an automated, self-enclosed tissue processor and blocked in paraffin. Three transverse sections (6-8 μm thickness) are cut from each sample with a motorized rotary microtome, mounted on glass slides, stained with double-strength Gill hematoxylin, and counterstained with eosin-y, and covered with a cover slip. At least two readers independently examine sections under a compound microscope and assign sex (Sex) and reproductive state (Maturity) applying standard criteria. Specimens with developing, spawning-capable, or regressing gonads are considered sexually mature. Slides are re-examined simultaneously by both readers if assignments of sex or reproductive stage differ and a consensus is reached.

For fecundity studies, sub-sampled ovary sections are further sub-sampled randomly to produce two 25-30mg samples and the number of stage-3 yolked oocytes in these samples is counted. These counts are converted to total fecundity using the weights determined on-board

the vessel. To estimate batch fecundity, two 75-mg samples are sampled from random locations in the collected ovary and the number of oocytes undergoing maturation (i.e., lipid yolk coalescence, migration of nucleus, and/or hydration) is counted. These counts are extrapolated to the preserved gonad weight to generate batch fecundity estimates.

Data available for reproductive parameters include sex (ratio), age and size at sexual maturity, spawning season and location, age and size at sexual transition for hermaphroditic species, and if available fecundity estimates for individual fish. These parameters can be matched to individual fish and collection information, lengths, and increments or ages by the specimen ID number (PCGSS).

