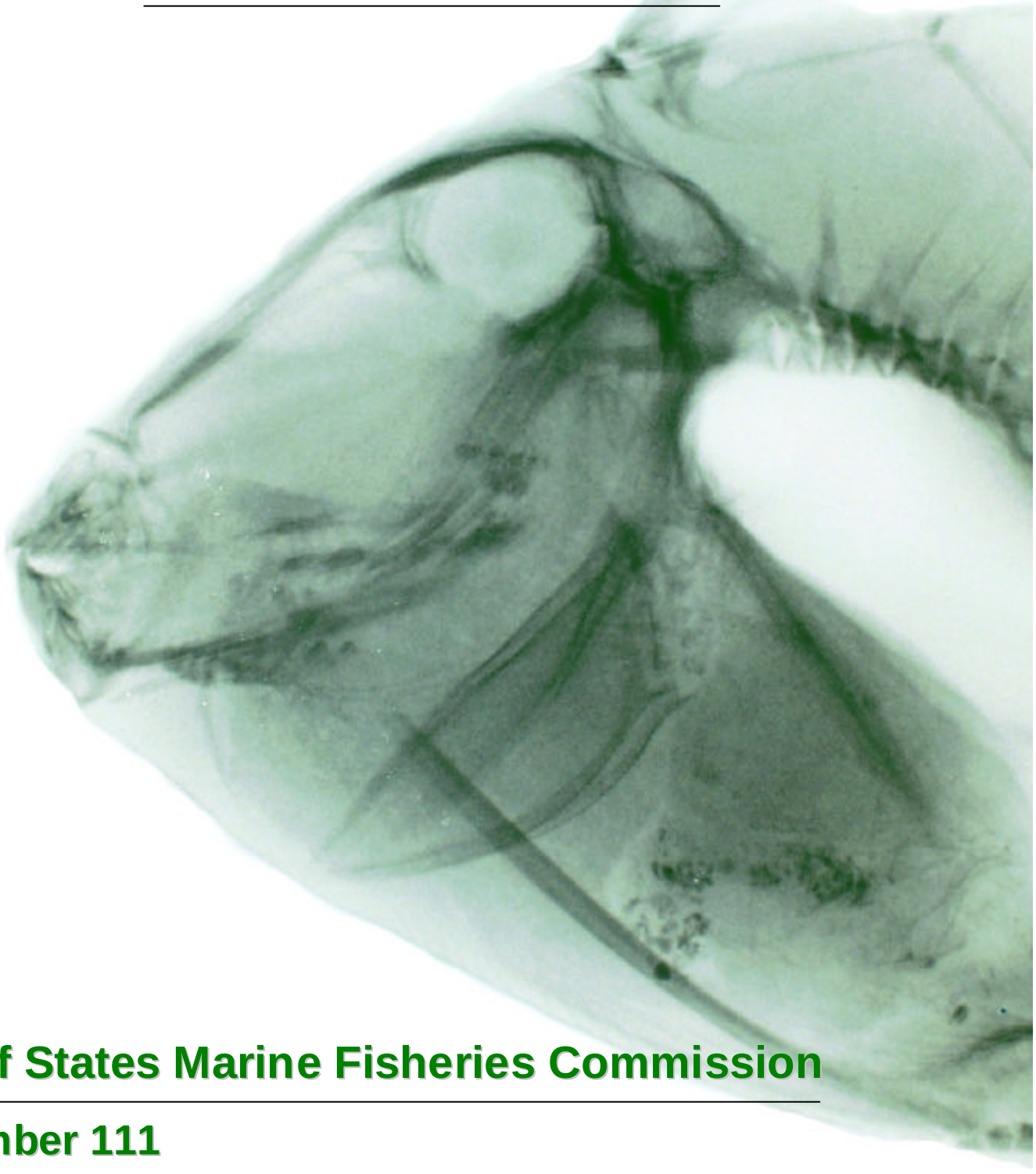
A Practical Handbook for Determining the Age of Gulf of Mexico Fishes



Gulf States Marine Fisheries Commission

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A Practical Handbook for Determining the Ages of Gulf of Mexico Fishes

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Abbreviations and Symbols

AMRD/MRD	Alabama Department of Conservation and Natural Resources/Marine Resources Division
DMS	Data Management Subcommittee
FFWCC	Florida Fish and Wildlife Conservation Commission
FMRI	Florida Marine Research Institute
g	gram
GCRL	Gulf Coast Research Laboratory
GSI	gonadosomatic index
GMFMC	Gulf of Mexico Fisheries Management Council
GSMFC	Gulf States Marine Fisheries Commission
IJF	interjurisdictional fisheries
kg	kilogram
km	kilometer
lbs	pounds
LDWF	Louisiana Department of Wildlife and Fisheries
LSU	Louisiana State University
m	meter
MIA	margin increment analysis
mm	millimeters
MDMR	Mississippi Department of Marine Resources
MFCMA	Magnuson Fishery Conservation and Management Act
MRFSS	Marine Recreational Fisheries Statistics Survey
mt	metric ton
n	number or sample size
NMFS	National Marine Fisheries Service
SAT	Stock Assessment Team
SD	standard deviation
SE	standard error
SEM	scanning electron microscope
SL	standard length
TL	total length
TPWD	Texas Parks and Wildlife Department
TW	total weight
VIMS	Virginia Institute of Marine Sciences

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Preface

This manual is the culmination of the expertise existing in the Florida Fish and Wildlife Conservation Commission, the Alabama Department of Conservation and Natural Resources Marine Resources Division, the Mississippi Department of Marine Resources, the Louisiana Department of Wildlife and Fisheries, and the Texas Parks and Wildlife Department. Additional expertise was provided by members of the National Marine Fisheries Service, University of Florida Gainesville, Louisiana State University, and other age and growth specialists.

Because the majority of fish ages in the Gulf States are determined by otolith interpretation, this manual focuses primarily on otoliths. Techniques using other hard parts are provided but in less detail. We have tried to provide information on various techniques that have proven to be useful or unsuccessful for each of the species covered in Section 5.0. We have also provided the agencies which utilize these techniques to provide the reader with a source for additional information. When new species are added to the manual in the future, these techniques will be expanded where appropriate. As additional methodologies are developed for marine species common to the Gulf States, updates will be available on-line or through the Gulf States Marine Fisheries Commission office. In an effort to provide timely updates efficiently, we suggest routine checks of the GSMFC website (www.gsmfc.org) where new or improved sections of this manual will be available for download. When requesting the document from the website, please provide your e-mail when prompted. This will place you on an updated distribution list ensuring your receipt of an electronic announcement when updates become available.

1.0 Introduction

Fisheries science has been at the forefront of studies on animal growth and population dynamics in part because the age of individual fish can be determined. The original technique used for estimating ages of fishes involved following modal progressions of fish lengths as they changed through time (Petersen 1892). Later, marks on the animal's hard parts (calcified structures) were found to be formed on a regular and sometimes annual basis (Hoffbauer 1898, Reibisch 1899, Heinke 1905). These hard parts include scales, bones, spines, vertebrae, and otoliths. Of these, otoliths appear to be the least sensitive to changes in fish condition (Campana and Neilson 1985). Otolith growth is allometric and enough material is continuously deposited on its medial surface that marks in the form of rings are distinguishable throughout the life of most fishes. This provides a reliable source that permanently records temporal features.

The significance of determining age is that it allows fishery scientists to relate their observations to a time frame and estimate various biological rates for various species. Ages of individual fish are required to estimate growth rate, age at recruitment, maturity schedules, and age-specific fecundity for a specific species. In addition, the calculation of natural and fishing mortality rates and age-specific sex ratios also require age data. In the simplest sense, this time frame may involve estimating the number of years a fish spends in a particular life stage or habitat or determining the number of years that fishes are available for harvest.

Age determination has become such an integral part of the analysis of exploited fish populations that most agencies responsible for fisheries management have begun to routinely collect and process otoliths taken from fish sampled using fishery-dependent and fishery-independent methods. The technical skills and equipment needed for 'production ageing' are variable depending on the type of fish and the information to be derived for the study.

Numerous publications have been written that describe these techniques for sampling, processing, and analyzing otoliths for age determination. Pentilla and Dery (1988) documented age determination techniques used by the staff at the Woods Hole Laboratory, National Marine Fisheries Service to process samples from Northwest Atlantic fishes and mollusks. Other reports have targeted the interpretation of daily growth increments from larval and juvenile fishes using equipment and techniques similar to those used for adult fishes (Secor et al. 1991, Stevenson and Campana 1992). In addition, the use of otoliths as records of age, stock identification, pollution exposure, and various environmental conditions during the life of a fish has developed into an inter-disciplinary scientific field (Secor et al. 1995).

In 1995, the Stock Assessment Team (SAT) of the Gulf States Marine Fisheries Commission (GSMFC) proposed a manual to facilitate consistent, quality age determination of exploited Gulf of Mexico fishes and outline methodologies employed in the processing of hard parts. The SAT recognized that its charge to integrate state-specific stock assessments for GSMFC fishery management plans would require consistent criteria for age determinations of fishes throughout the Gulf. Therefore, a work group of experienced fisheries professionals was assembled to develop and expand this manual. The work group is comprised of two individuals from each state agency along with contributors from academia and the National Marine Fisheries Service.

The purpose of this publication is to provide a practical guide for ageing marine fishes from the Gulf of Mexico. Current methodologies and techniques are generally described by species. Although we emphasize the use of otolith sections for age determinations, we also provide information on alternative processing and ageing techniques for particular species when appropriate. This manual should serve as a valuable training tool for new laboratory personnel and as a guide for ageing species of common interest to the Gulf States.

The intent of this document is to be a dynamic resource, one that changes as species specific processing nuances are developed. Documentation of these new and changing procedures can be posted in this manual as they occur. Standardization of techniques is a cornerstone of fisheries science, and we believe that this manual will facilitate the adoption of these techniques and standards for the same and similar species beyond the Gulf region. Moreover, adopting standardized ageing criteria for each species will provide comparable information necessary for age structured stock assessments both at state and regional levels.

2.0 Otolith Structure and Function

Most lower vertebrates utilize inner ear elements to process sensory information regarding movement, momentum, spatial orientation, and sound. The dorsal portion of the teleost inner ear includes three semicircular canals each with their own ampullae, a fluid filled chamber for sensing inertia (Figure 2.1A and B). The canals are oriented in such a way as to include the horizontal, lateral, and vertical planes allowing detection of pitch (head up or down), roll (rotation on the head-tail axis), and yaw (head side to side). Movement of the fluid (endolymph) within the ampulla impinges on sensory hair cells lining the walls of the chamber allowing the sensory system to process directional acceleration and deceleration. The dorsal portion also includes the utriculus and the utricular otolith, or the lapillus, which is used primarily to detect gravitational force as well as sound (Popper and Lu 2000).

The ventral portion of the teleost inner ear includes the sacculus and lagena which in turn contain their own otoliths, the sagitta and the asteriscus, respectively. This area of the inner ear appears to be used for both sound detection and acoustic transduction. Sound vibrations differentially affect the otoliths due to their higher density relative to the fluid filled chambers they occupy. As sound waves are intercepted, the otoliths move independent of the surrounding chamber causing mechanical stimulation of the hair cells. This process results in an auditory signal allowing the fish to "hear."

The sagittae, described here in detail, are typically the largest otoliths in most fishes and are therefore the most often used for ageing. Please note, however, that some researchers strongly recommend the use of other otolith pairs (Secor et al. 1991).

The sagittae lie within the sacculus and are attached to a noncellular, olithic membrane. Along the medial surface of the otolith lies a gelatinous pad within an area of the otolith known as the sulcus acousticus and the nervous tissue called the macula acoustics. This nervous tissue extends from the auditory nerve. Innervation of the gelatinous pad functions to receive stimuli due to angular accelerations, gravity, and sound. Surface features that can be distinguished on some sagittal otoliths include the rostrum and the anterostrum on the

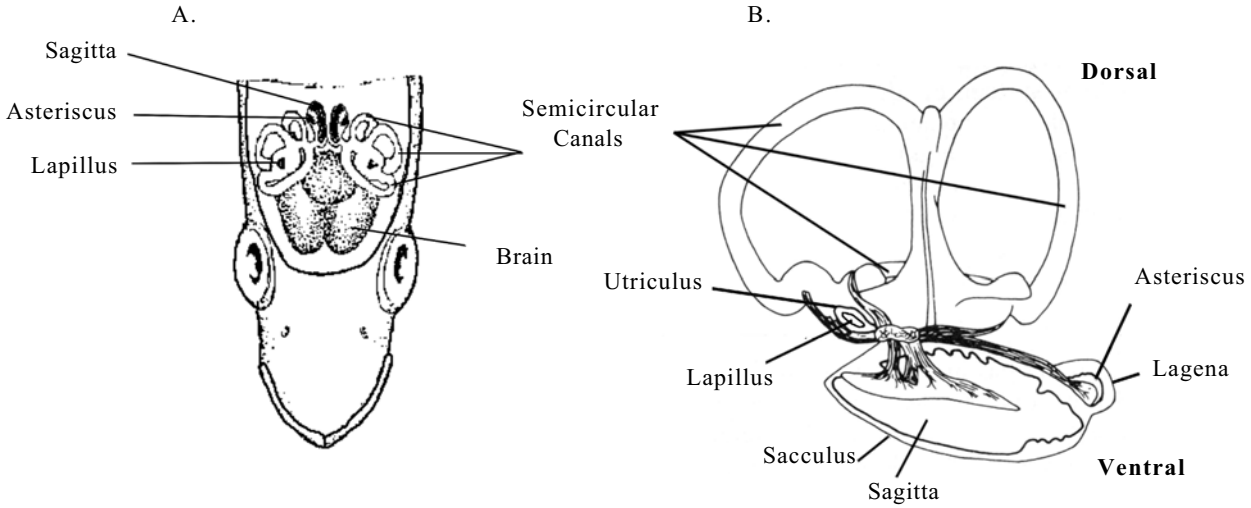


Figure 2.1. A). Location of the otolith pairs within a generalized fish (modified from Secor et al. 1991) and B). medial view of the inner ear (modified from Moyle and Cech 1988).

anterior end of the otolith and the sulcus acousticus that forms a furrow (sulcal groove) along the medial surface of the otolith (Figure 2.2). The sulcus acousticus can be divided into an anterior ostium section and a posterior cauda section. In some otoliths (e.g., those of certain sciaenid species) a marginal groove is present near the dorsal side of the inward facing surface of the sagitta.

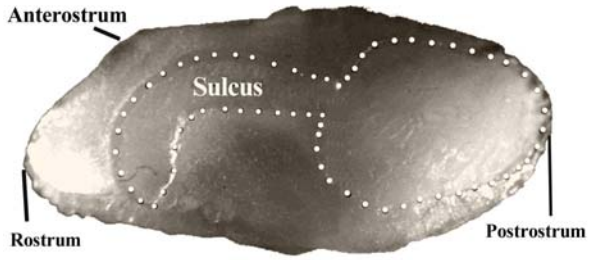


Figure 2.2. Photomicrograph of left sagittal otolith (medial side up).

Otoliths are crystalline in nature and are built up around a primordium/core region outward by the process of biomineralization, where calcium carbonate, mainly in the form of aragonite, is precipitated on a protein matrix of otolin. The otolin layers are generally oriented parallel to the outer surface of the otolith and are most densely aligned during periods of slower growth (usually associated with cooler months), thus forming characteristic, concentric **opaque rings** in otolith cross sections (Blacker 1974). Layers that are less densely spaced during periods of faster growth during warmer months make up the **translucent ring** (Figure 2.3). When the formation of successive opaque and translucent rings occur on an annual basis, they are collectively referred to as an annual growth zones. The winter growth zones, represented by opaque rings, are frequently called annuli (singular: annulus). Otolith growth in the linear dimension is usually greatest on the axis facing the sagittal midline of the fish.

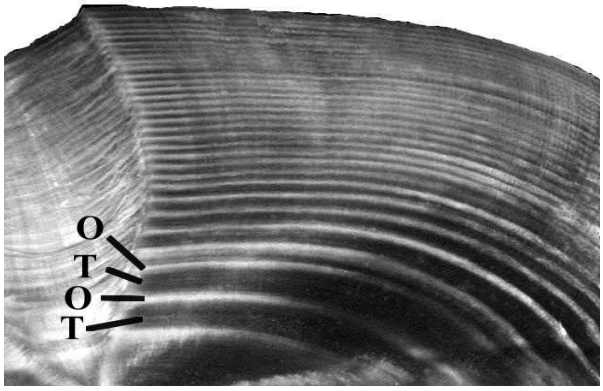


Figure 2.3. Close up of alternating opaque (O) and translucent (T) rings in a sectioned black drum sagittal otolith under reflected light.

When the alternating bands or rings of an otolith cross section are viewed under magnification, the opaque rings lying along a 'reading' or 'counting' axis, described by a line on one side or the other of the sulcus extending from the core to the outer edge (Figure 2.4), are conventionally the ones tallied for age estimates. The counting of presumed annuli for the purpose of assigning age estimates is analogous to the practice of dendrochronology, the ageing of trees using tree ring counts from a cross section of the trunk.

Daily growth microincrements in otoliths, first described by Pannella (1971) and later reported by Pannella (1974), Brothers et al.

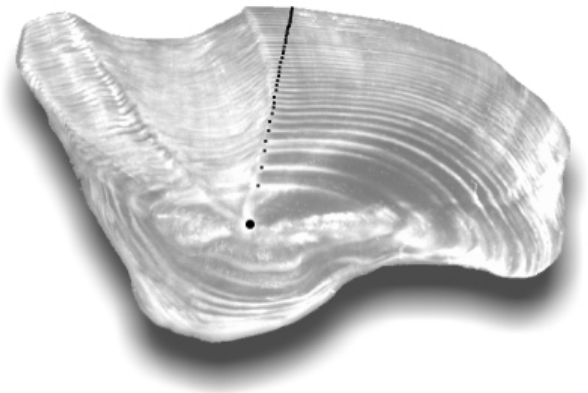


Figure 2.4. Transverse section of a black drum sagittal otolith including location of the core and rings along the sulcus.

(1976), Brothers (1984), Campana and Neilson (1985), and Radtke (1989) are used to infer growth events during the first year of life and during specific intervals later in the fish's life. Lapilli also have also been shown to provide daily growth increments or rings (Wenner et al. 1990). The astericii are not typically used for daily growth because they are formed later in life than the other two pairs of otoliths.

Otolith morphology differs by species (Figure 2.5). Otolith shape analyses use information extracted from digitized images for species identification (by matching archived key shape descriptors) and, in some cases, to resolve fish populations for the purpose of stock discrimination (Castonguay et al. 1991, Campana and Casselman 1993, Friedland and Reddin 1994, Colura and King 1995, Stransky 2001).

In summary, otoliths are anatomical structures that accrete recognizable layers as the result of differential deposition of organic and inorganic material. These layers may correlate with fish growth that varies with time and season and may provide a cumulative historical record of changes in climate, nutrition, hydrographic environment, and other ecological parameters. Their value are as biological and ecological information storage units (akin to "CD-ROMs of fish biology") that record the temporal signatures of various environmental conditions to which a fish has been subjected from hatching to time of death (Radtke 1990, Kingsmill 1993, C. Wilson personal communication). When comparing otoliths to other fish hard parts such as vertebral bones, scales, fin rays, and spines, otoliths often provide more accurate ageing data due to their continuous accumulation and limited resorption whereas other hardparts tend to underestimate age.

The successful application of techniques to enhance the detection of age marks in the otoliths of Gulf finfish species is of vital importance in estimating growth and mortality rates, population age structure, and other parameters needed for understanding the population dynamics of important fish stocks and their response to natural phenomena and exploitation.

Figure 2.5. Variation in sagittal otolith size and shape by species. From left to right: black drum (*Pogonias cromis*), red drum (*Sciaenops ocellatus*), spotted seatrout (*Cynoscion nebulosus*), gray snapper (*Lutjanus griseus*), sheepshead (*Archosargus probatocephalus*), southern flounder (*Paralichthys lethostigma*), and striped mullet (*Mugil cephalus*).

3.0 General Processing Techniques

3.1 Otolith Removal

Age data alone is not generally useful to fishery managers unless accompanied by some morphometric, meristic, or other descriptive feature about that fish. Some of these features include: length, weight, sex, and reproductive condition. Otoliths should be removed (post-mortem) only after these data are recorded since the otolith removal process will often physically alter the fish making these data impossible to accurately assess after dissection.

Sagittal otoliths lie inside the otic capsule located toward the posterior end of the ventral surface of the skull. Several methods may be employed to extract otoliths and depend on fish size, shape, and whether or not the whole fish is to be displayed in a market.

In the first method (Figure 3.1A), useful for small fish or when the external appearance of the fish must be maintained, the otolith can be excised by cutting into the dorsal junction between the operculum and the body to allow the operculum to be flared open exposing the gills and gill arches. The dorsal attachment of the gill arches and associated tissues to the skull are then cut and the gills and their arches flared forward to expose the tissue surrounding the base of the skull (Figure 3.1B). Under this muscular tissue and lateral to the midline is the outer wall of the otic capsule (Figure 3.1C). Its location and shape varies by species and is described in greater detail in the species accounts in Section 5.0 of this manual.

Using a stout knife or chisel (depending on the thickness of the capsule wall), remove layers of the otic capsule wall until the sagitta with its surrounding membrane are fully exposed (Figure 3.2B and C). Use appropriately sized forceps to gently remove

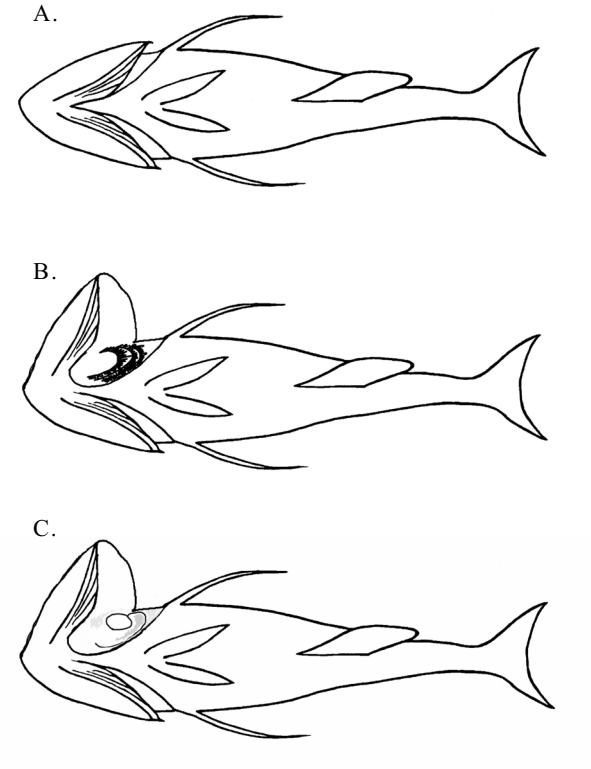


Figure 3.1. Otolith removal through the gill arches under the operculum; ventral view.

the sagitta (Figure 3.2D). Both sagittae can often be extracted through the single opening in the otic capsule. If not, simply repeat the process on the opposite side. If the external appearance of the fish is not a consideration, the gills and gill arches can be removed to expose the otic capsule. The otic capsule can then be scored transversely near its center and broken open along the score to reveal the otoliths.

The second method useful for larger fishes or when the external appearance must not be maintained in marketable condition, involves sawing through the dorsal surface of the head, down into or just above the otic capsule (Figure 3.3 Line A). Care must be taken in this method not to shatter the otolith or cut too deep during the initial incision. A hacksaw, heavy knife, bonesaw, or meatsaw is then used

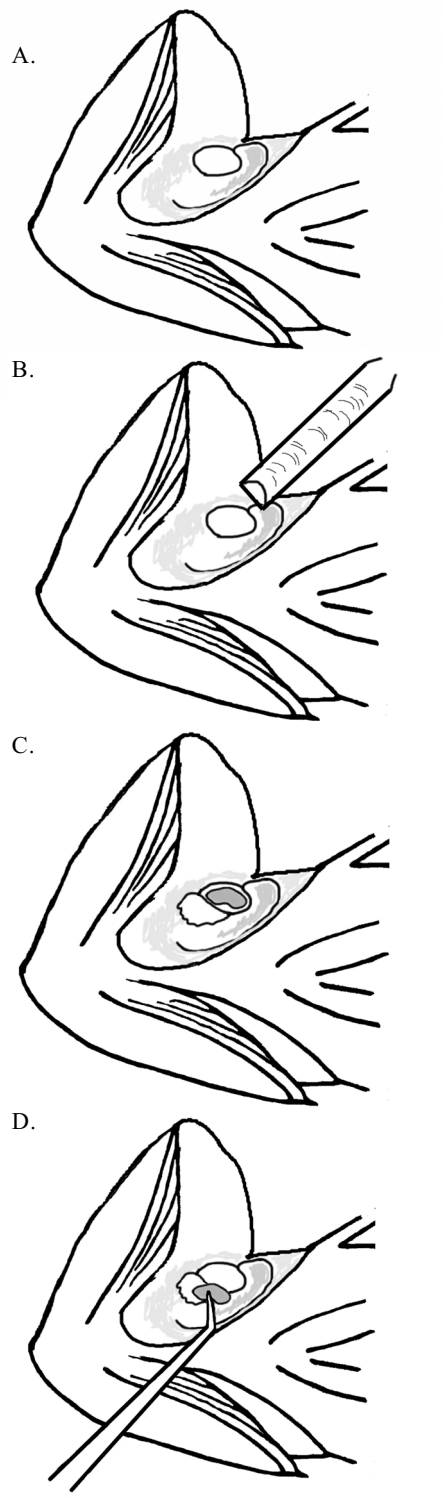


Figure 3.2. Removal of the otolith by exposing the otic capsule through the gill cavity using a sharp chisel. (A) Gill cover flared with gills removed exposing otic capsule. (B) Utilization of a chisel or other sharp object to scrape or shave off capsule surface. (C) Open otic capsule with otolith exposed. (D) Otolith removal.

to make a transverse cut (Figure 3.3 Line B) from the dorsal side of the head starting just anterior of where the operculum joins the body (roughly directly above the posterior edge of the preopercular margin). The cut is made deep enough to reach the otic capsule. If the left and right dorsal junctions where the operculum and body meet are cut sufficiently deep, the head can be flexed as if hinged near the snout, exposing the braincase and otic capsule. The otoliths are then removed using forceps.

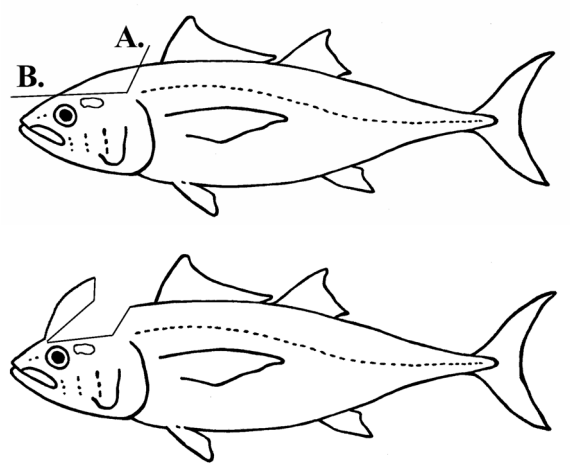


Figure 3.3. Cutting planes A and B for excision of the sagittal otolith through the upper neurocranium.

A third method is the butterfly technique which is useful on small and medium-sized fishes. This method requires a vertical cut parallel to the long axis of the fish's body (Figure 3.4A). A sharp knife is inserted into the top of the body behind the head and the entire neurocranium is split from posterior to anterior. Once the head is pryed opened exposing the split otic capsule, the otoliths are removed using forceps (Figure 3.4B). Note: it is important to make the cut down the center of the head to prevent damaging the otoliths.

3.2 Cleaning and Storage

Otoliths have been traditionally used for ageing fish; however, analysis of otolith

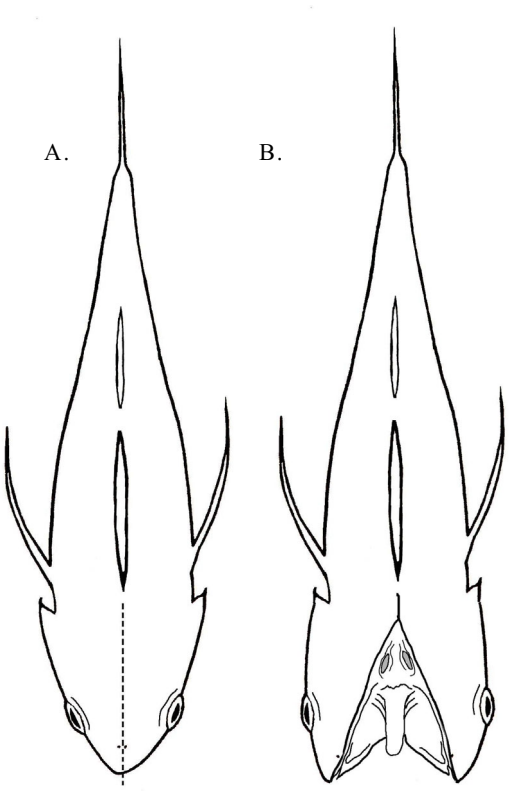


Figure 3.4. The butterfly method.

microchemistry has recently become widespread in fisheries ecology. In order for archived otoliths to be useful for both ageing and microchemistry studies, it is essential that otoliths be properly cleaned and stored to prevent alteration of their chemical composition. Following extraction, otoliths should be cleaned of any remaining tissue or fluids with water (distilled is preferred). Bleach should not be used because it will dissolve the aragonite matrix and may alter an otolith's chemical composition. Likewise, alcohol should not be used to rinse or store otoliths because it contains trace elements that may penetrate the aragonite matrix of the otolith. Once cleaned, otoliths should be air-dried completely before storage. Accurate weights (e.g., nearest 0.1 mg) may be determined using an analytical balance. Both left and right otoliths should be stored together in properly labeled paper envelopes or glass/plastic vials and archived for later use. Care should be taken when storing fragile otoliths in paper envelopes. **Note: Storage of specimens in formalin will degrade otoliths by reacting with the protein matrix and should be avoided.** Although left and right otoliths are collected, it is generally agreed that only one side is typically sectioned for ageing. Alternating between left and right for a species could lead to inconsistencies in the ageing process. A comparative analysis between left and right otoliths is recommended for each species since at times the non-designated otolith may need to be used, and there may be a lack of agreement between the left and right otoliths.

Archived otoliths must be assigned unique identification numbers consisting of a species code, a code for the sampling area, and a unique serial number for each individual from the sampling area. This identification number can also include a unique code for the date of capture. In addition, the following information and morphometric data must be recorded for each fish: collection date; location; source (fishery-independent, roving creel, fish house); gear type; length (total, standard, or fork); weight (total or gutted); and sex.

3.3 Sectioning Preparation

The techniques chosen for sectioning otoliths will depend on individual laboratory preferences, budgets, and otolith morphology. Three methods of preparation for sectioning are currently used in the Gulf region: embedding whole otoliths in an epoxy resin, mounting a whole otolith to a glass slide, and free hand cutting of whole otoliths followed by mounting on a slide for sectioning.

3.3.1 Embedding Otoliths

Embedding media are ideal for small or fragile otoliths; however, vapors from these

compounds are a potential health hazard so proper lab safety techniques should be followed. Resin mixing, pouring, and processing should be conducted under a fume hood or while wearing a respirator in a well-ventilated area. All individuals exposed to these products should read and have the MSDS sheets available. Several embedding media are available and are widely used throughout the Gulf States. The two most common, Spurr and Araldite, will be generally discussed, although Loctite (requires UV light to cure) has also been used in a few states to embed small otoliths in bullet type molds (Figure 3.5).

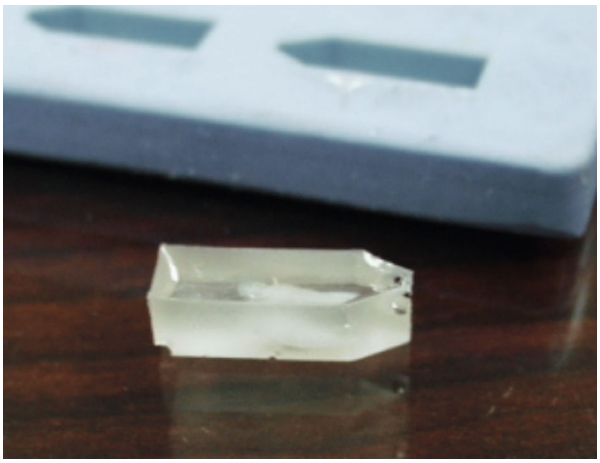


Figure 3.5. Small otolith embedded in a block of resin or embedding media that has been removed from the flexible, reusable bullet mold.

3.3.1.1 Embedding Whole Otoliths in Spurr's Low Viscosity Embedding Media

Spurr's Low Viscosity Embedding Media is easy to prepare and has been used historically for embedding otoliths, but its popularity has waned due to its carcinogenic ingredients. Spurr has excellent penetration qualities that provide thorough and rapid infiltration of tissues. Its hardness can be adjusted by changing the amount of the flexibilizer, one of the resin's four ingredients. Spurr is useful in sectioning small or fragile otoliths for annuli determination or grinding and polishing larval and juvenile otoliths for daily ageing.

Spurr is water sensitive to the point that local humidity may impact the final consistency of the mixture. Therefore, it is important to make sure no moisture exists on the inner surfaces of the pipets, pipet bulb, and beaker used to prepare the resin. Surface moisture can be eliminated by heating equipment in an oven or microwave.

Add the first three ingredients in the amount specified on the Spurr's Kit technical datasheet for a normal (firm) consistency. Next, cover the beaker and place it on a magnetic stirrer for approximately ten minutes on low until mixed. Stirring too fast can create bubbles in the mixture and poor final results. Finally, add the last ingredient (the hardener) and stir for another ten minutes on low.

Once the Spurr is thoroughly mixed for the second time it is ready for the mold. The empty mold should be slightly warmed in the oven to dessicate all moisture from the cells and to allow easy removal of the blocks of cured Spurr. As with all resins, Spurr may be applied to a mold in two steps, as a single pour placing the otolith directly on the bottom of the mold or with two pours. The first pour creates a false bottom and allows the otolith to be raised up in the mold and center it within the resin block.

Using forceps, carefully position each otolith longitudinally approximately ¾ of the way from the square end of each mold. This will allow easier processing later.

Apply Spurr until it has reached the top edges of each cell. If the otolith floats, use a probe or pipette to reposition the otolith. Once all otoliths have been properly repositioned, carefully return the mold to a level oven to ensure that the Spurr cures evenly. Cure time at

70ºC (158ºF) is eight hours. It can be left in the oven overnight, but no more than 16 hours or it may be rendered unusable. To dispose of unused Spurr, pour the remaining mixture into a small foil container, cure, and throw away in the regular trash.

3.3.1.2 Embedding Whole Otoliths in Araldite

Araldite, the more commonly used embedding media, has similar qualities to Spurr without the carcinogenic properties. In addition, this two part epoxy requires less time to combine the components for use. To ensure the correct 5:1 ratio of Araldite resin (Araldite-D-US) and hardener (Hardener HY 956 EN/US), mix the contents of each container in a separate container. If only a small amount of epoxy is needed, resin and hardener should be mixed in a disposable plastic beaker at a 5:1 ratio by weight. Araldite should be prepared under a fume hood or in a well ventilated area while wearing respiratory protection. Avoid skin and eye contact with the resin, hardener, and uncured mixture. As with any potentially hazardous chemical, MSDS should be reviewed and posted in a place accessible to all users.

Araldite may be poured into molds in two steps: a small amount is initially poured into a mold to create a false bottom and left to harden for a day. Next, the sample number is written on the false bottom. An alternative method is to use a permanent ink marking pen to label the inside of each mold with the unique otolith identification number (Figure 3.6). Once labeled, the otolith is placed in the mold and covered with a second batch of Araldite. After all the molds on a tray are filled, reposition each otolith as required (correct position is longitudinal; centered with the long axis of the otolith parallel to the sides of the mold) and roll them from side to side to release trapped air bubbles. Embedded otoliths should stand for

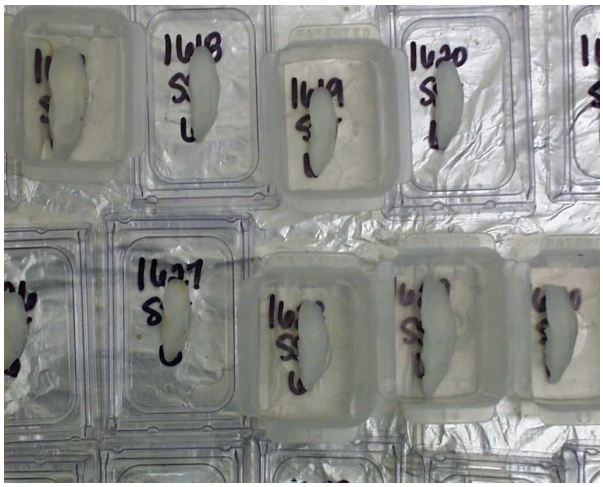


Figure 3.6. Embedding molds labeled with identification information.

one hour to allow the reaction heat to dissipate and then be placed in an incubator at 37°C for at least 16 hours while the resin cures. After the resin has completely cured, the otolith blocks are removed from the molds. If a label was applied to the mold or written on the false bottom, it transfers to the resin, and the blocks do not need to be relabeled. If sample numbers were written on the outside of the embedding mold, this number must be written on the block before it is removed from the mold.

3.3.1.3 Embedding Small Otoliths in Bullet Molds

Bullet molds are recommended for small, fragile otoliths such as king mackerel and Spanish mackerel. Epoxy is mixed as described above and then added as a thin layer into each cell of the mold with a small metal spatula before the otolith is introduced. The layer of epoxy on the bottom ensures that the entire otolith is covered and helps to prevent chipping or breaking during sectioning. The otolith is then placed into the cell, centered with the long axis of the otolith parallel to the sides of the cell. This placement ensures adaquate material for mounting the block into the saw's chuck. Since the epoxy is still tacky when the otolith is

placed into the mold, you can give the otolith a slight push to fix it into place so the otolith does not move when the remaining epoxy is added. The blocks should be completely cured before attempting to section.

3.3.1.4 Marking the Core

Regardless of the embedding media or mold type used, marking the otolith core on the resin block is essential for ensuring a traverse cut through the center of the otolith. After a block is removed from a mold, place it under a dissecting scope to locate the otolith core. Though embedded, the otolith should be clearly visible. With an ultra fine point pen or pencil, place a mark over the core of the otolith (Figure 3.7). On one side of the mark, a reference line can be drawn in the transverse plane of the otolith to assist in aligning the blade for sectioning.

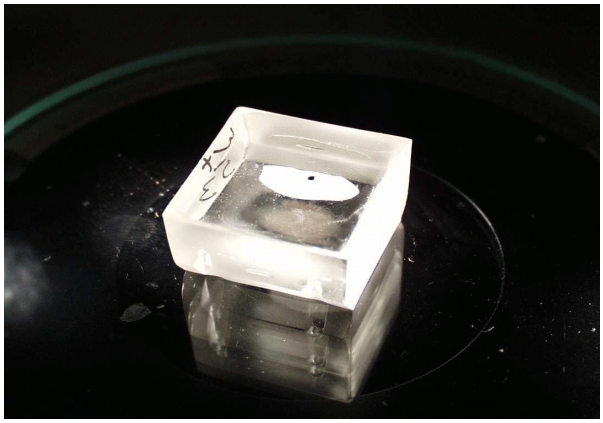


Figure 3.7. Embedded otolith with core region marked.

Occasionally, the embedding medium will adhere to the sides of the mold and the block will not be flat on the top side due to the capillary action of the medium. These raised areas can be flattened by sanding them with a small, 1-inch wide belt sander using 100 grit sanding belt, if desired.

3.3.2 Mounting Whole Otoliths on Slides

Otoliths to be sectioned should be clean and dry. Prior to sectioning, two slides are made for each otolith. One slide is frosted and contains pertinent information such as species, collection number, and sample number on which the otolith sections are permanently mounted. The second slide is a plain slide that holds the entire otolith during sectioning and is eventually discarded. It is generally necessary to mark each plain slide only with the sample number.

The whole otolith should be adhered to the plain slide using thermoplastic cement which melts easily. To begin, place the plain slide on a hot plate set at medium to high heat and apply a small amount of thermoplastic directly onto the slide and allow it to melt. Remove the slide from the hot plate. Work quickly as the thermoplastic will remain malleable for only a few seconds. Scrape the melted thermoplastic into a small pile toward one end of the heated slide using a broad flat instrument. Keep in mind, the slide will have to fit into the saw chuck so it is necessary to leave adaquate space at one end of the slide. While the thermoplastic is still soft, place the posterior end of the otolith into the pile of thermoplastic on the slide and pile some over the end of the otolith. If it cools before this can be done, simply return it to the hot plate for a few seconds and then pack. Next, turn the slide around and return it to the hot plate being careful not to melt the adhesive just packed on the opposite end. Repeat the above steps while packing thermoplastic around the anterior portion of the otolith; **remember to leave the core region free of plastic, as this is the area from where the sections will be cut**. When finished, the otolith should be securely fastened to the slide leaving enough room to place the slide in the saw chuck and ample room to cut sections from the middle of the otolith (Figure 3.8). Note that it is important to affix each end of the otolith separately. Do not try to save time by making a single pile of thermoplastic and splitting it into two smaller

piles. This will only make things more difficult later, because the core region may become adhered to the slide as well. This can be especially troublesome with smaller otoliths.

Figure 3.8. Otolith mounted to a glass slide using thermoplastic on each end. The central portion of the otolith must remain clear of adhesive.

3.3.2.1 Marking the Core

As each otolith is mounted, a line just anterior to the core can be drawn on the otolith in the transverse plane using a pencil. The line is made slightly posterior to the junction of the ostium and sulcus and is used as a guide during sectioning. Experience will show where to place the reference mark for a given species. An alignment mark may not be necessary on small otoliths, which will have the majority of midsection removed during sectioning.

3.3.3 Free-Hand Sectioning Preparation

Because this technique requires that the otolith be cut prior to mounting, it is described in greater detail in Section 3.4.3 as no pre-sectioning preparation really exists.

3.4 Sectioning Techniques

Otoliths are sectioned typically using rock and gem cutting (lapidary and metallurgical) saws. Three saw types are currently used throughout the Gulf States: the high speed wafering saw; the low speed wafering saw; and the thin sectioning saw. Thin circular saw blades coated with diamond particles are passed through the otolith in serial cuts to achieve thin sections which allow the transmittance of light. A tray located directly under the blade is filled with coolant solution. These solutions may be supplied by the saw manufacturer. Alternative saw lubricants include baby oil, mineral oil, glycerin/water solution, water with a surfactant added, or water only.

The saw and blade should always be checked prior to turning the saw on. It is important to make sure the blade is free of any imperfections that will interfere with sectioning or ruin the blade. After repeated use each blade should be dressed according to the manufacturer's directions to expose the cutting surface of the diamond particles. A Dremel tool equipped with a fine wire wheel can also be used to clean the flat portion of the blade.

Never start the saw with the resin block resting on the blade. Allow the saw blade to achieve target speed before making contact with the sample. Failure to do so could result in a broken blade or in the case of whole mounted otoliths, stripping the sample from the slide. Make sure to read all directions provided by the saw's manufacturer.

3.4.1 High Speed Wafering Saw

A high speed saw (Figure 3.9) has several advantages in terms of production; however, it is one of the more expensive saws, and blades are costly (see Appendix 8.2). Some problems with electrolysis or corrosion between the aluminum saw blade flanges and the copper-coated saw blades have been encountered but do not appear to impact saw operation or blade life. Saw blade flanges may have to be replaced every three years.

A high speed sectioning saw with a maximum speed of 5000 rpm (in 100 rpm

Figure 3.9. High speed wafering saw (cover opened).

increments), a 1000 g loading capacity (in 10 g increments), and chuck crossfeed adjustments in 0.005 mm increments can be used with four or six inch diamond-coated saw blades to produce 0.5 mm thickness otolith sections. Kerf size on a 6"saw blade is about 0.5 mm and 0.35mm on a 4" blade. The saw blade is lubricated and material residue is flushed away by a recirculating lubricant stream from a submersible pump (Figure 3.10). Loss of lubricant as spray is prevented by a cover. A safety switch prevents blade or pump operation with the cover in a raised position. Sample sections are retained in a metal basket over the lubricant reservoir but can occasionally be difficult to locate, as they will sometimes spray off the blade and adhere to the interior surface of the cover.

Cuts through a resin-embedded otolith usually range from 15 to 45 seconds, depending on block size (whole mounted otoliths can not be cut with this saw due to the cover which must remain closed during sectioning). Cutting speed, load, and chuck position are controlled by pressure pads and settings for all three are displayed digitally. A safety switch prevents manual sectioning or blade dressing, and also shields the operator from the high-speed blade, airborne material particles, and lubricant

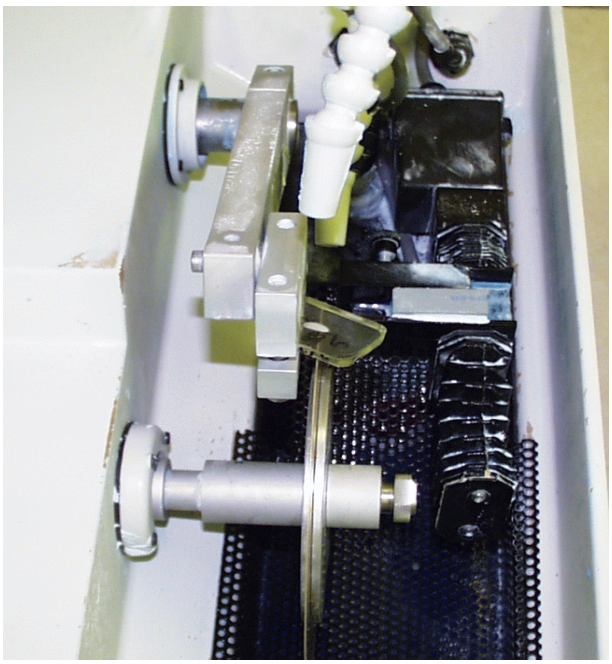


Figure 3.10. High speed wafering saw showing blade, cutting arm, coolant reservoir, and pumps with resin block ready to be sectioned.

aerosols from the cutting operation.

3.4.1.1 Embedded Otoliths

The resin block containing the otolith is placed in the chuck of a high speed (max 5,000 rpm) metallurgical saw equipped with a four or six inch diamond blade. Water-soluble oil diluted to label recommendations is used as a cutting lubricant. The block is oriented so the long axis of the otolith is perpendicular to the saw blade and the anterior end of the otolith is nearest the chuck (Figure 3.10). Sectioning begins just posterior to the otolith core, and sequential sections are made approaching the core region until a good section is obtained. The otolith block is advanced approximately 1 mm toward the saw blade after each cut which produces about a 0.5 mm thick section. Sectioning is typically done at 3000 rpm with a 1000 g load, and typically takes less than 30 seconds for all otolith sizes. Sections are examined under a dissecting microscope to identify the section containing the otolith core,

which is then affixed to a labeled glass slide.

3.4.2 Low Speed Wafering Saw

There are several benefits associated with low speed wafering saws. Simultaneous operation of several of these sturdy saws allows for a high production rate for sectioning. Low speed wafering saws are less expensive than the higher speed models. They are relatively safe, require no safety shield, simple to operate, and are relatively quiet. The low speed saws have a maximum speed of 300 rpm and generally use four inch diamond wafering blades with a 0.3 mm kerf.

Most of the saw manufacturers provide repair services and technical support and will recommend appropriate sized chucks for various sizes of resin blocks. Finally, the small size of these units allows for transfer between laboratories. Disadvantages are that the diamond wafering blades are fragile (brittle), expensive to replace, susceptible to bending and chipping, and processing time can be relatively long for extremely large or embedded otoliths.

3.4.2.1 Embedded Otoliths

A resin block containing a single otolith is positioned in the saw chuck so the cut will result in a 90° cross sectioning of the otolith. The chuck may be adjusted to orient the block by loosening the Phillips head screws (or thumbscrew if the saw has a vise-type sample holder) on the specimen arm. The operator should view the block from the top or bottom as well as from the front to check for alignment. When the block is correctly aligned, the screws are tightened (Figure 3.11). Failure to do so may result in a ruined blade. Every effort should be exercised during preparation to have the otolith properly aligned in the block to avoid having to make substantial adjustments to achieve the correct orientation in the saw chuck.

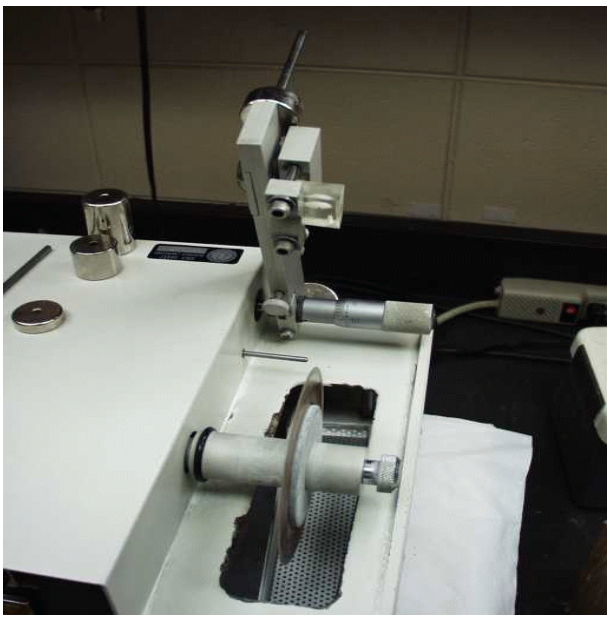


Figure 3.11. Embedded otolith mounted in low speed saw.

For otoliths embedded in small bullet molds, it may be necessary to first mount the block onto a slide using thermoplastic and then align the slide in the chuck.

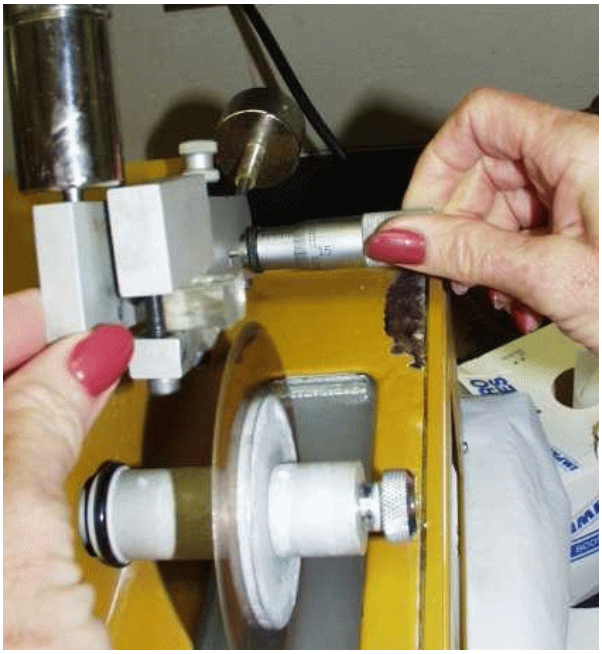


Figure 3.12. Adjusting the alignment of the block with the micrometer.

Sectioning begins posterior to the otolith core near the junction of the ostium and sulcus and sequential sections are made approaching the core until a good section is obtained. The block is moved across the blade after each cut using the micrometer cross feed to adjust the desired thickness of each section (Figure 3.12). Depending on the type of otolith, the saw speed is adjusted using the speed control, and weight may be added or removed from the specimen arm to achieve the best cut. With practice, a section containing the core region should be reached within two to three cuts.

Sections are removed from the specimen tray, rinsed in water, dried, and viewed under a low-power microscope to verify that a good core section has been obtained. If the core was missed, the block may be rechucked in the saw to attempt another core section.

The best core section or series of sections is then mounted on the final slide containing the relevant information for the specimen (Figure 3.13). **Note: If sections are embedded in Spurr, ethanol should not be used to rinse sections after sectioning as it may dissolve the Spurr.** The sections may then be covered with a mounting medium and set aside to dry or cure.

Figure 3.13. Mounting of otolith core section on final slide.

3.4.2.2 Whole Mounted Otoliths

When sectioning whole mounted otoliths (Section 3.3.3) using a low speed saw, check the recommended arm weight and blade speed for that species (examples in Section 5.0). This may require some trial and error with new species. Secure the slide in the chuck with an Allen wrench so that it does not slip during sectioning, but do not overtighten. Also check the angle of the chuck to ensure that the blade will section the otolith in the transverse plane. Line the blade up based on the core which should have been marked with a pencil. Although it is not necessary, it is often easier to begin sectioning with the micrometer crossfeed scale at the zero position.

To begin sectioning, turn the saw on with the otolith raised above the blade (specimen arm in the up position). Do not start the saw while the otolith is resting on the blade. Gently lower the otolith onto the turning blade and begin sectioning. Depending on the species,

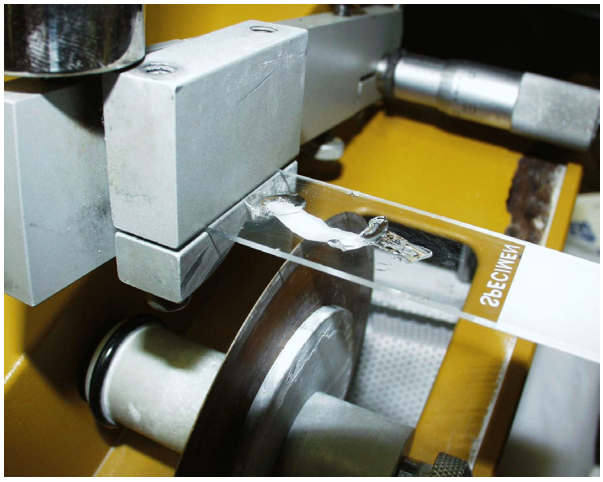


Figure 3.14. First transverse cut of a whole mounted otolith.

size of the otolith, weight, and saw speed, it can take anywhere from 30 seconds to several minutes to cut through the otolith (Figure 3.14).

It is practical to cut three or four sections from the otolith to ensure a section that includes the core. When the blade has passes through the otolith and begins to cut the glass slide, lift the specimen arm off the blade and advance the saw blade through the core (Figure 3.15). Sections are generally cut in 0.5 mm (500 um) increments. However, this can be altered depending on the species (Section 5.0).

Figure 3.15. Serial cuts from a whole mounted otolith.

Once all sections have been cut, lower the specimen tray and rotate it out from under the blade. Pull the specimen basket out of the cutting solution and remove all otolith sections with forceps. Rinse the sections in 95% ethanol or water and allow them to dry. Examine the sections under a low-power microscope to ensure that a good core section has been obtained. Permanently affix the section or sections to the slide using a mounting medium (Figure 3.16). It is best to position otolith sections on the final slide in a consistent manner for easier ageing.

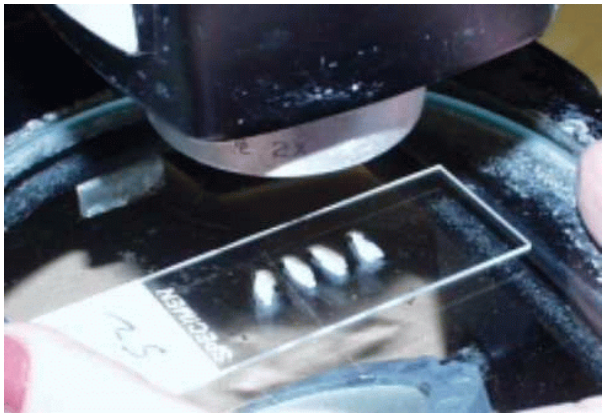


Figure 3.16. Four otolith sections prepared for final mounting and ring enumeration.

3.4.3 Thin Sectioning Machine

The thin sectioning machine is used to section unembedded whole otoliths. The procedure, which borrows petrographic techniques from geology, reduces sectioning time by eliminating the time-consuming steps of embedding and polishing. In addition, the apparatus allows the technician to prepare a large number of otoliths at one time. The sectioning process is quite loud so ear plugs or other protective ear wear is recommended.

The following is a method for the rapid processing of large otoliths first described by Cowan et al. (1995) with some minor modifications.

The water-cooled, thin sectioning machine is equipped with a 20-cm, diamond blade cut-off saw and a precision grinder (Figure 3.17). The grinder is equipped with a 20-cm vertically mounted, 320-mesh, metal-bonded-diamond grinding lap. The grinding lap is fitted with a precision dial controlled thickness gauge allowing the technician to vary the section thickness. Both have aluminum guide arms for feeding slides to the blades.

Otoliths are hand held and cut along the transverse plane near the core using the cut-off

Figure 3.17. Thin section machine containing a high-concentration-diamond, continuous-rim-blade cut-off saw (left) and a precision grinder (right).

saw before mounting onto slides (Figure 3.18). To ensure a high quality section, it is imperative to cut as close to the core as possible without actually cutting through it so that the core is contained at the transverse plane edge of the otolith half to be mounted. The cut surface of the otolith half is then pressed against the precision grinder to remove any rough edges or scratches. Additional polishing may further reduce scratches. This will provide a readable surface on both sides of the finished section.

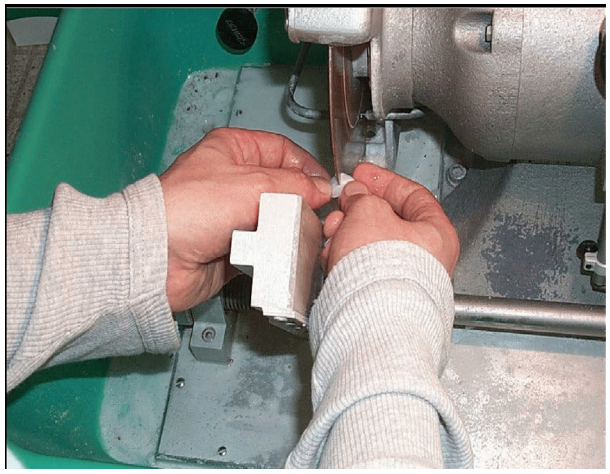


Figure 3.18. Hand cutting an otolith on the high speed thin sectioning saw.

Allow the otolith half containing the core to dry and mount it, cut side down, onto a final microscope slide. For ease of processing, two otoliths can be mounted per slide with identification numbers written under each using a water-proof marker (Figure 3.19).

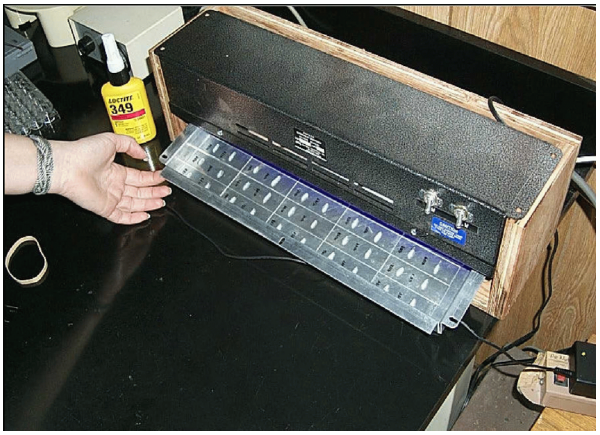


Figure 3.19. Otolith halves mounted on microscope slides with Loctite which is cured under ultraviolet (UV) light.

After curing, the slide containing the otolith halves is placed in the guide arm of the cut-off saw and guided past the saw to remove all but approximately a 100 µm section of each of the otolith halves. The slide is then placed into the precision grinder guide arm and fed past the grinding lap to remove any rough edges or scratches (Figure 3.20). Once the slides are dried, the otolith sections on each slide may be covered with a few drops of mounting medium which may eliminate the need for polishing. The otoliths are then ready to be read.

The following technique can be used for fragile (e.g., flounder) or small otoliths (e.g., mullet) and is similar to processing larger otoliths but requires greater manual dexterity as all processing is done on the precision grinder. Marking the core is essential in achieving a quality section using this technique. Otoliths are handheld by the posterior end and ground down to the transverse plane near the core. Again, it is imperative to get as close to the core

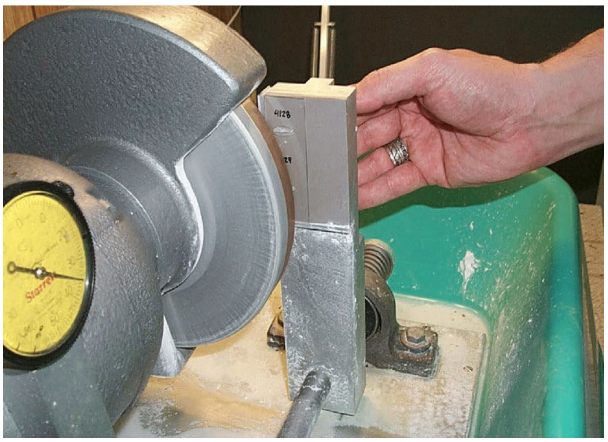


Figure 3.20. Final polish of otolith sections using grinding arm.

as possible. The otolith half is mounted cut side down onto a labeled microscope slide and cured. After curing, the slide is handheld and pushed against the grinder until remaining material is removed to approximately 1 cm. The slide is then placed into the precision grinder guide arm and fed past the grinding lap to reduce the section down to the desired thickness.

3.5 Permanently Mounting Sections

Section mounting, or adhering the otolith sections to a glass slide, can be done in several ways. The two most common mounting media used in the Gulf region are thermoplastic cement and Flo-Texx. Hand or machine polishing to remove saw abrasions or other imperfections from the section surface can take place before and/or after mounting. Following mounting, it is useful to apply a coat of commercially available permanent coverings such as Flo-Texx or temporary coverings such as immersion oil, glycerin/water solution, or plain water to increase clarity when reading. **Note: The use of Histomount for slide preparation is strongly discouraged due to its tendency to discolor and crack over time.**

3.6 Alternative Techniques

3.6.1 Break and Burn

As an alternative to thin-sectioning sagittal otoliths, fish ages can be determined using the "break-and-burn" method (Christensen 1964). With this method, the sagittal otolith is literally broken in half through its nucleus (core), and the exposed surface is heated over an alcohol flame to enhance the contrast between the organic and inorganic components of the matrix. Manual manipulation of an otolith half using fine-tipped forceps is required so this method is usually limited to larger otoliths (>8-10 mm in length). This does not preclude using this technique on smaller otoliths, but it does require more skill and care in the burning process. This method is successfully being used on white grunt (*Haemulon plumieri*) and red porgy (*Pagrus pagrus*) and may be appropriate for other species when rapid production ageing (i.e., to year class) is required, rather than specific ageing for growth.

To break a sagittal otolith, the transverse plain of the otolith is scored through the nucleus using a diamond-edged pen and then snapped in two using finger pressure. The broken surface of one-half of the otolith is then held at an angle and back and forth above an alcohol flame. **Note: When burning the surface it is important to keep the flame evenly distributed over the otolith's surface to get an even burn.** The otolith should not touch the flame directly or it will burn too quickly and char the surface making ageing impossible. The time required to burn a surface depends on the species and size of an otolith but is usually no more than 10-15 seconds. Care should be taken with smaller otoliths as they will require less time.

This process differentially burns the organic matrices within the annuli of the otolith, with the translucent bands of slow growth burning dark relative to opaque bands of faster growth

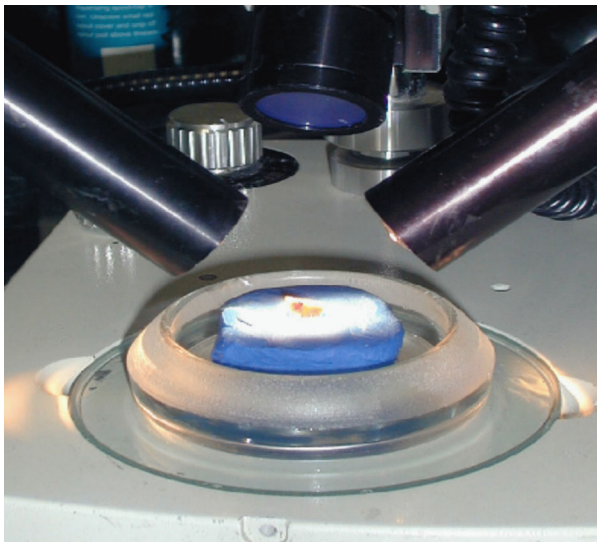


Figure 3.21. A break-and-burn otolith pressed in a plasticine block under a reflected light source.

(when viewed under reflected light). The otolith half is cooled (usually less than 30 seconds) and pressed into a dark-colored plasticine block (blue or green works well) with the burnt surface upright and tilted slightly (Figure 3.21).

Bands are counted using a stereomicroscope fitted with a fiber optic light source (reflected light) positioned to reduce glare while providing a source of focused light when using high power (Figure 3.22). The contrast among the bands can be enhanced using a drop of vegetable or canola oil on the burnt surface.

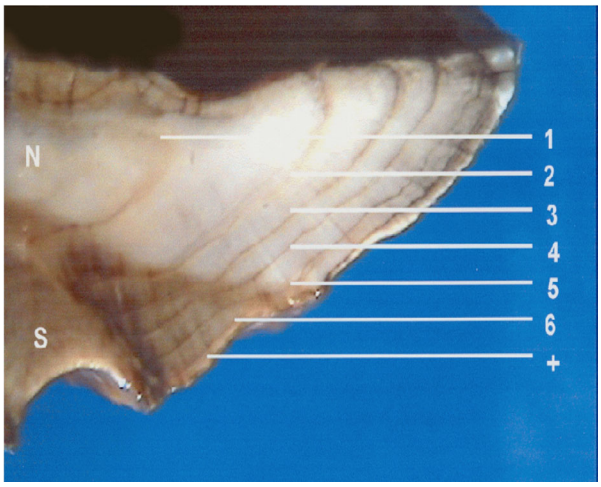


Figure 3.22. Broken-and-burnt surface of a sagittal otolith from an age-6+ white grunt under reflected light (N=nucleus and S=sulcus) (from Murie and Parkyn 1999).

Age estimates for a series of white grunt otoliths were processed with this method as well as sectioning and were almost 99% in agreement at least up to 16 years of age (Murie and Parkyn 1999). The primary advantage of the break and burn technique over thin-sectioning is the greatly reduced amount of time required for processing otoliths (minutes rather than hours). In addition, long-term storage of burnt otoliths does not appear to result in the fading of bands (D. Murie personal communication). Otoliths can be re-burnt to enhance visibility of bands or in most cases, the other half of the otolith can be used.

3.6.2 Scales

Scales were used to age carp, *Cyprinus carpio*, as early as 1898 (Carlander 1987), and during the early 1900s the use of scales for ageing fish and separating fish populations led to seminal research in ecology and fisheries management (Sinclair 1988). By the early 1920s, Welsh and Breder (1924) reported age and growth information for fish from southwest Florida using scales. Age determination using scales was so common that Lee (1920) reviewed their successful use for a variety of species. Lee noted, however, that difficulties could arise when using scales to age fish, namely 1) counting false annuli, 2) compaction of annuli near the edge, and 3) geographic variation in scale patterns. The purpose of this section is to discuss the general methodology and applicability of using scales to age fishes, particularly species of the southeastern United States.

One of the advantages of using scales in favor of other anatomical parts is that samples can be obtained without affecting the appearance of a

fish in the market or sacrificing the fish in the field. When removing scales from live fish, however, the collection area should be 're-slimed' to aid healing the fish's epidermis; it is recommended to use a wet and bare finger to spread the fish mucus back over the area where fish scales are collected.

Scales are often removed from the middle of the body, below the dorsal fin (Figure 3.23), but many species have precedent for removing scales from other locations. It is necessary to collect scales from a region of the body where scales first form. Because some scales are unsuitable for ageing, it is recommended that one collects 6-10 scales per fish. A typical problem arises from regenerated scales that are missing the interpretable ridges (i.e., circuli and radii) that define the annuli in the central portion (i.e., around the focus) of the scale.

Figure 3.23. Six to ten American shad (*Alosa sapidissima*) scales are taken from an area below the center of the dorsal fin and above the lateral line.

Another general advantage of using scales is that they are easily collected and stored. Scales can be removed quickly by using forceps or a knife and stored in inexpensive envelopes. If long-term storage is anticipated, it is recommended that scales are cleaned and stored in a cool, dry area and that moth balls are included to prevent mites from damaging the scales. Cleaning the scales when initially collected can save time later. A small brush, such as a toothbrush, and a cleaner (e.g., a mild soap solution, alcohol, or diluted bleach) will be necessary to apply to the scale once the fish slime has dried. No further processing is necessary if the raw scales are examined directly, although some additional effort to mount the scale, either dry or wet, can enhance the details of it for viewing and interpretation.

There are also methods for making scale impressions on plastic slides which can improve production ageing fish using scales (Dery 1983). The sculptured side of a fish scale can be imprinted on laminated plastic by using pressure, such as with a roller press. Cellulose acetate can also be used, but this medium requires heat, heavy pressure, or softening chemicals for impressions. Making impressions is a more laborious technique, but the time and cost can often be justified and provide several advantages over raw scales. First, impressions can enhance the details of scales with delicate features. Second, the impression will be flat, even if the scale is curved. A flat image reduces problems associated with light diffraction and minimizes the focal depth of field necessary for recording good photographs or digital images. Third, larger scales may be too thick to be transparent enough for direct viewing while impressions can be viewed using transmitted or reflected light. Fourth, multiple scale impressions on a single slide can be easier to handle than many small, loose scales in an envelope, and the best scales can be easily selected for reading. Fifth, impressions can be archived indefinitely.

Scales or scale impressions can be viewed with a light microscope, a microfiche reader, or a microprojector. The generalized criteria for counting annuli are to examine the patterns of cutting over, discontinuity, or crowding of the circuli. Cating's (1953) criteria for determining age of American shad (*Alosa sapidissima*) from scales were validated by recaptures of marked fish (Judy 1961) and stand as a good guide for ageing fish using scales. True annuli appear as lines on the scale surface and follow the contour of the scale periphery (Figure 3.24). They are most evident along the lateral fields of the scale. False

annuli are generally faint in comparison to true annuli but one important exception is the fresh-water zone mark laid down when juvenile shad move from fresh to salt water at the end of their first summer. The approximate locations of the true annuli during the pre-spawning period of a shad's life can be found by counting the number of transverse grooves that follow the baseline groove (Figure 3.24). The transverse grooves are unreliable for ageing the fish. The anterior edge of each true annulus generally falls between a narrow range of transverse grooves for virgin fish. In the south, American shad are semelparous, so spawning marks do not appear, but spawning marks need to be identified correctly for iteroparous populations of American shad (i.e., populations north of the Carolinas). Spawning marks are more jagged in appearance than true annuli because they arise from both eroding and regenerating processes of the scale margin. As a fish ages, the space between consecutive annuli becomes narrower, and the erosion caused by spawning can obliterate the recent annulus. Thus, after maturation the spawning mark is the annulus mark in species like American shad that spawn immediately following annulus deposition in its northern range. Spawning marks in American shad do not occur on the otolith, only on the scales, thereby offering a specific advantage over otoliths (i.e., these spawning marks indicate the age and size at maturation and the number of years a fish has spawned).

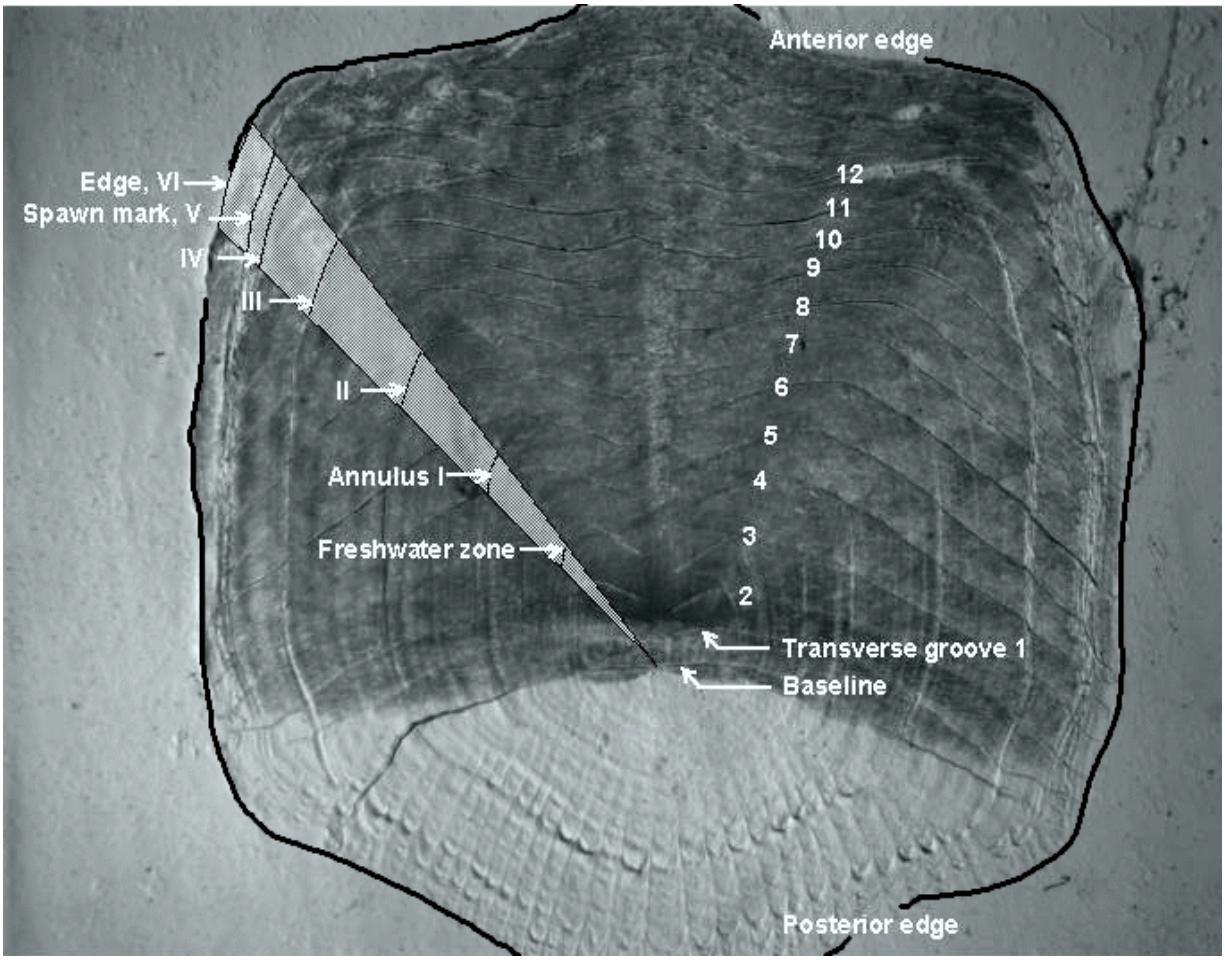


Figure 3.24. An acetate impression of a scale from an age-6 American shad (*Alosa sapidissima*) collected in the York River, Virginia, depicting annuli (Roman numbers), tranverse grooves (Arabic numbers), and other features.

Several other species in the southeast United States have been successfully aged using scales; Atlantic menhaden (*Brevoortia tyrannus*), ballyhoo (*Hemiramphus brasiliensis*), bluefish (*Pomatomus saltatrix*), dolphin (*Coryphaena hippurus*), tomtate (*Haemulon aurolineatum*), knobbed porgy (*Calamus nodosus*), whitebone porgy (*Calamus leucosteus*), black drum (*Pogonias cromis*), red drum (*Sciaenops ocellatus*), southern kingfish (*Menticirrhus americanus*), and striped mullet (*Mugil cephalus*). In these species, scale annuli were validated using margin increment analyses from recaptured fish or were judged to be valid based on the appearance of continuous growth of the scale and circuli patterns.

Validation of scale annuli is essential because scales may not always be useful for ageing fishes. Beamish and McFarlane (1987) demonstrated that the scale method provided erroneous ages for 16 freshwater and marine species. In general, maximum scale ages underestimated validated ages or ages determined by some alternative method (i.e., otoliths). Otoliths continue to grow as a fish ages. Problems can arise using scales, however, as they do not continuously grow in older fish and the calcium in the scales can be resorbed in stressed fish. Scales are regarded as unsuitable for ageing large pelagic fishes, namely tunas, billfishes, and sharks (Casselman 1983). Lowerre-Barbieri et al. (1994) published a good example of how to compare scale and otolith methods, and they noted that crowding of annuli on the scale margin was problematic in older weakfish (*Cynoscion regalis*). They concluded that sectioned otoliths provided more accurate ages and more precise indications of annulus location. Secor et al. (1995) concluded that scales were suitable for ageing striped bass (*Morone saxatilis*) younger than 12 years. They noted, however, that most stock assessments for this species are still based on scale ages to avoid sacrificing the oldest and largest females which serve as broodstock. To compensate for the use of scales instead of otoliths, they reported a linear equation that could correct the ages of older fish. These examples should make it clear that before expending time, energy, and funds to collect and use scales for life history studies or stock assessments, the issue of validating annulus formation on scales should be addressed.

In summary, scales are not appropriate for ageing many species, particularly slow-growing, long-lived species. However, scales may be useful for ageing faster-growing, short-lived fishes, and for ageing younger individuals of slower-growing species when mortality from scientific sampling needs to be reduced or eliminated. Using scales has some advantages over other hard parts such as their ease to collect, store, and process without sacrificing the fish. Validation of annulus formation is necessary, however, to make use of these advantages of scales for ageing fish. Although otoliths have been demonstrated to be quite reliable for determining age, scales may become more widely used in the future where non-lethal sampling is desirable or required. In addition, scale shape has been used for stock identification for several decades (Ihssen et al. 1981), and recently Moran and Baker (2002) demonstrated that archival scale samples are valuable for genotyping historical collections. The historical use of scales and the familiarity that most fish biologists have with scales have led to archived material at many labs, and these historic and newer collections can continue to play a part in understanding the population dynamics of fishes.

3.6.3 Spines and Rays

Using spines and fin rays for age and growth studies offers certain advantages over otoliths and other hard parts. In most cases, these structures can be removed and processed more easily than scales and otoliths. Like scales, it is rarely necessary to sacrifice the fish or significantly mutilate the carcass when sampling, which may

reduce the market value of a commercially harvested species. Soft rays are especially useful because, like scales, they can be removed at the time of tagging and compared with the corresponding structure at the time of recapture. For more detail on these techniques see Casselman (1983). Unlike scales, the annuli in fin rays and spines remain prominent for older fish when scale annuli are no longer identifiable because the annuli on scales result from a different process than those on other hard parts. Therefore, there is no reason to assume that annuli on both structures will be equally prominent throughout the life of the fish.

Determining age from spines and fin rays requires that the structures be sectioned near their base in a precise transverse plane. The exact location of the section depends on the species. A Dremel saw, fine jeweler's saw, or other thin sectioning device is used to section spines and rays. The thickness of the transverse section must be adjusted to assure that annuli are visible. Sections may be soaked in solutions containing acetic acid or bleach to remove unwanted tissue from their surface to make annuli observation and quantification easier. Spine and fin ray sections are then mounted using any one of the techniques mentioned in Section 3.6.

Sections are best viewed using a compound microscope, although they can be projected with a microfiche projector or viewed using a microscopic video camera and monitor.

Although spines and fin rays can be useful in the estimation of age and growth in fish, there are disadvantages. In older fish the core can undergo resorption and become vascularized, thus obscuring or eliminating the first few annuli resulting in an underestimation of age (Figure 3.25). This is common in many of the oceanic pelagic species. Spines and fin rays from older fish are also similar to scales in that the distal translucent zones may be so close together that they appear to coalesce, making optical resolution difficult or impossible.

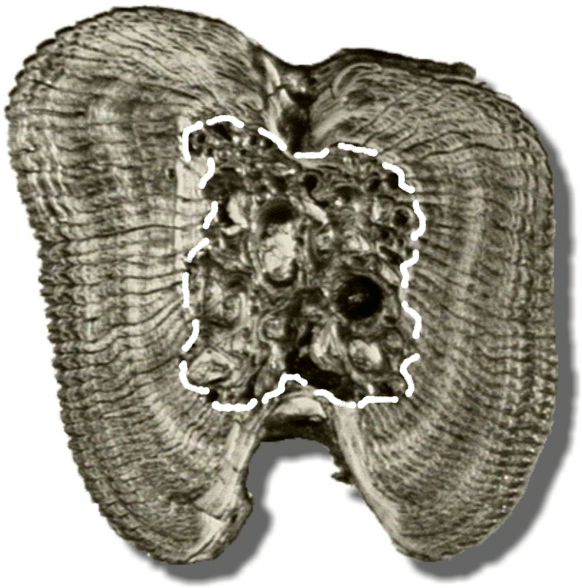


Figure 3.25. Resorption and deterioration in the core (indicated by dashed lines) of the first dorsal spine of a cobia (*Rachycentron canadum*).

False annuli, or pseudoannuli, appear similar to annuli but are associated with checks and zones that are often incomplete and irregular, and frequently found only in one region of the structure. Although they may be prominent, pseudoannuli are not associated with growth zones that form during the principal annual cessation or reduction in growth that produces annuli and should not be counted when ageing. Validation of the hard part for each new species is necessary to confirm that observed marks are in fact produced annually (See Section 4.2.1 and Section 5.6).

3.6.3.1 Sectioning Spines

While many different methods for sectioning fin spines exist, two techniques have been used successfully in the Gulf States using either the high speed, thin sectioning machine (AMRD, LSU) or a low speed wafering saw (FMRI). Differences in method between the two saws is detailed below.

3.6.3.1.1 Thin Sectioning Machine

The shaft of each dorsal spine is sectioned slightly above the condyle. The exact location in each species is determined by trial and error. A section too far up the spine (Figure 3.26, Line A) will result in more closely spaced annuli, and a section made too close to the condyle will result in annuli that are obscured by the convolutions in the condyle of the spine (Figure 3.26, Line C).

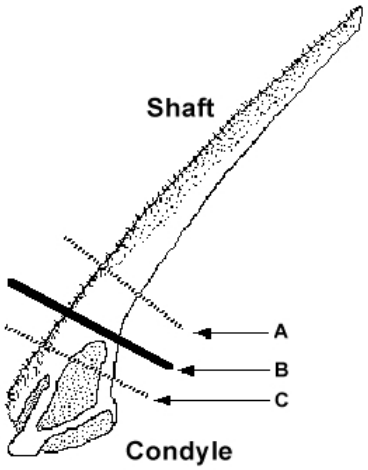


Figure 3.26 The first transverse cut (line B) provides the most widely spaced annuli with the best resolution when separating the condyle from the shaft (line A will result in more closely spaced annuli and line C will result in annuli which may be obscured by the condyle).

To make a transverse section, hold the spine horizontally and perpendicular to the saw blade. Then make the first cut while holding the spine as steady as possible (Figure 3.27). This will result in two portions of the spine – the distal portion and the proximal portion containing the condyle. Next, polish the cut surface of the distal portion and mount it to a final microscope slide with thermoplastic so that the plane of the cut is parallel to the plane of the slide (Figure 3.28). Place the slide into the guide arm of the cut-off saw. Make the second transverse cut using the guide-arm to pass the spine over the blade (Figure 3.29). The result is a spine section

Figure 3.27. Freehand transverse cut of spine on thin sectioning machine.

approximately 0.5-1.0 mm thick. The thickness can be adjusted by placing the slide in the guide arm of the grinding lap and feeding the section back and forth to polish it. Have a microscope set at 40x magnification nearby to monitor the clarity of the section as you adjust the thickness of it.

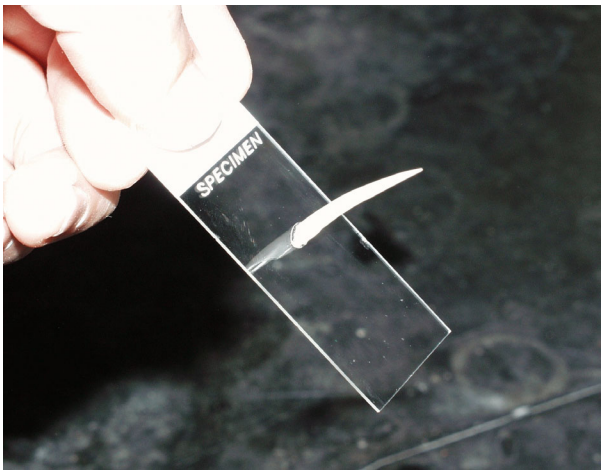


Figure 3.28. Distal portion of spine cemented to slide, ready for sectioning.

3.6.3.1.2 Low Speed Wafering Saw

The second technique for sectioning a fin spine uses a low speed wafering saw and is similar to the methods described for whole mounted otoliths (Section 3.4.2.2).

Figure 3.29. Removal of excess material from mounted spine.

The whole spine is attached laterally to a glass slide with thermoplastic at the condyle and the tip, making sure that the shaft itself is free from the slide (Figure 3.30).

Figure 3.30. Whole dorsal spine mounted to slide.

The slide is then placed in the chuck of the saw and lowered onto the blade to section the spine (Figure 3.31) in a transverse plane at 0.75 mm intervals beginning at the shaft base (just above the condyle). As many as six serial sections are then mounted on microscope slides with thermoplastic or Flo-Texx for reading.

3.6.3.2 Sectioning Fin Rays

Fin rays used for age determination are typically removed from the dorsal or pectoral fin. A modification of the method of Chilton and Beamish (1977, 1982) has been used successfully

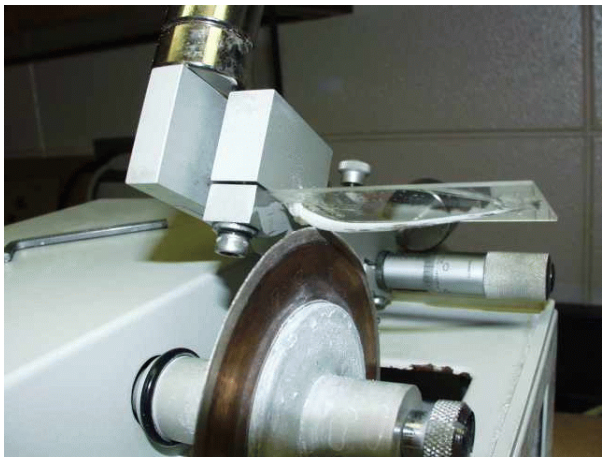


Figure 3.31. Whole spine mounted laterally to slide with thermoplastic and positioned for transverse sectioning on a low speed saw.

with fin rays to estimate ages for white grunt up to about ten years (Murie and Parkyn 1999).

Soft rays are removed from the dorsal fin (rays 4-7) by cutting across their base. For live fish, the rays must be removed as close to the dorsal surface of the body as possible to make sure that all annuli (especially the first) are present in the base of the ray. On dead fish, the rays can be removed down to their base ("knuckles"), which extends into the muscle of the fish. Fin rays should be trimmed of excess tissue and placed in a non-gummed manila coin envelope with the cut surface exposed to the air and the fin rays lying parallel to one another to dry for two to five days (Figure 3.32). **Note: It is important to arrange the fin rays in a parallel position so that they can be processed without having to be cut apart and realigned.**

Once dried, the fin rays may be embedded using a two-part epoxy resin (Figure 3.33). Though embedding is necessary to hold the fin rays in the saw chuck, the use of a mold is not necessary. The fin rays are placed on a piece of parafilm (to which resin does not adhere) and resin is applied over the basal surface of the dried fin rays. Finally, the distal portion of the fin rays

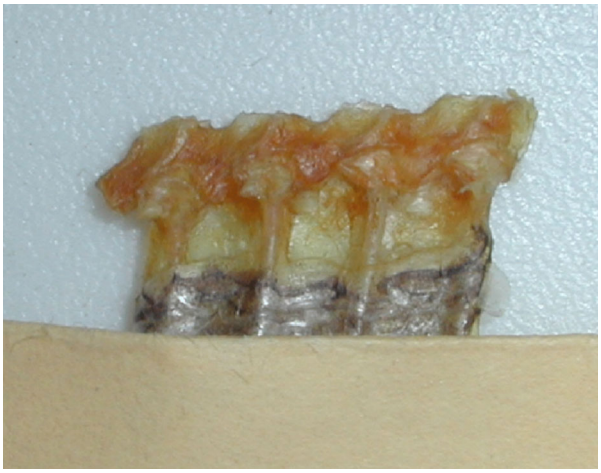


Figure 3.32. Dorsal fin rays from a white grunt arranged for drying in a coin envelope.

should be embedded in a large resin tear-drop.

Once cured, four to five serial sections (0.5-0.8 mm) can be cut from the distal end of the fin ray block. The sections are permanently mounted on a labeled slide for annuli enumeration (Figure 3.34).

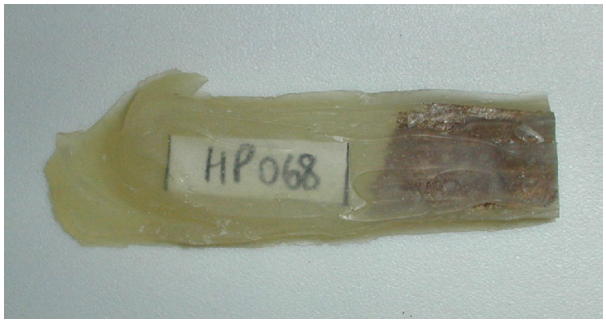


Figure 3.33. Embedded dorsal fin rays from a white grunt (rays are obscured by cured resin). Sections have been removed from the basal portion (right side) of fin ray block.

As with spines and scales, there is a problem of annuli accumulation at the edge of the fin ray structure, which can lead to an underestimation of the true age of the fish (Figure 3.35). For example, white grunt age estimates obtained from fin rays and sectioned otoliths agreed in 90% of the readings only for fish less than ten years old and decreased to 13% agreement for fish between

Figure 3.34. Sections of dorsal fin rays from a white grunt mounted to final slide.

11 and 18 years of age. Fin rays from these older fish do not display significant growth between consecutive annuli making it difficult to count individual annuli near the edge of the structure.

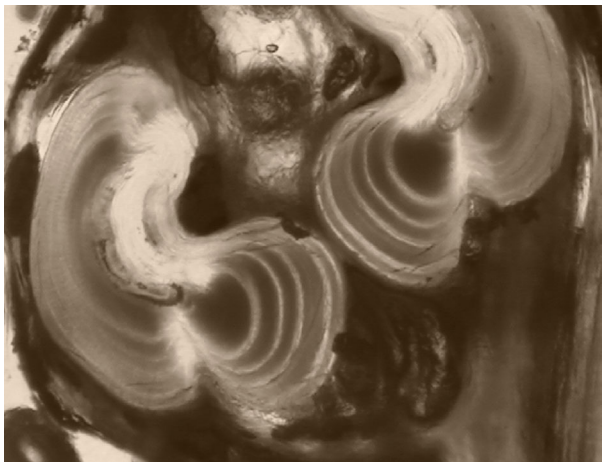


Figure 3.35. Magnified cross-section of the dorsal fin rays from an age-6 white grunt.

The degree of potential ageing bias due to underestimation should be evaluated for each species as the peculiarities of species-specific growth will affect the observed annuli pattern in the fin rays.

3.6.4 Whole Otoliths

Examination of a whole otolith using transmitted light can often reveal marks expressed on the surface (Figure 3.36). This technique has predominantly been used for otoliths taken from larval and small fish but has been used successfully to age older gag (*Mycteroperca*

Figure 3.36. Whole otolith from hardhead catfish, *Arius felis*. Rings are apparent from outer surface.

microlepis) and red grouper (*Epinephelus morio*). In general, marks observed from whole otoliths may correspond with opaque rings observed from sectioned otoliths, but this is not always the case. The use of whole otoliths requires less time and effort than sectioned otoliths, but validation must be undertaken to verify that rings counted on whole otoliths correspond with the "correct" number of rings observed in sections. Rings counted on whole otoliths for striped mullet in Mississippi were consistently one ring fewer than the ring counts from sections of the same otoliths (J. Warren personal communication). Inconsistencies have also been observed when comparing whole and sectioned southern flounder otoliths (A. Fischer personal communication).

Whole otoliths can be read using a dissecting microscope and either reflected or transmitted light. In most cases, 12x magnification is used, but 6x magnification may provide a "cleaner" image. **Note: Switching between the two magnification lenses while reading the whole otolith may produce better results.** Additionally, adjusting the angle of illumination or otolith position may increase the contrast of the rings versus the increment. Otoliths can be placed in a small watch-glass with enough water, oil, or glycerin/water to completely submerge the otolith to enhance the marks. An alternative to using a watch-glass would be to use a plastic, tissue culture tray. The advantage to culture tray cells is that multiple otoliths could be viewed simultaneously and the individual walled cells prevent the otoliths from "mixing" if the dish were to be bumped or moved accidentally. When reading whole otoliths, the younger fish are easier to age. The first annulus is generally clearer and whole otoliths from fish beyond age-5 become progressively more difficult to age as one gets further from the core. The ventral, posterior edge of the otolith is usually a better area to read; however, adjusting the angle of the light source or orientation of the otolith may produce better results (Figure 3.37).

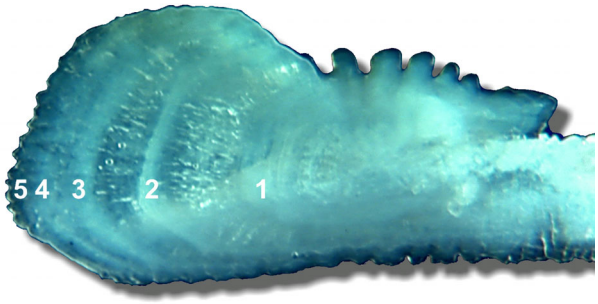


Figure 3.37. Ventral posterior edge of a whole sagittal otolith from an age-5 king mackerel.

3.6.5 Vertebrae

In some fish (i.e., elasmobanchs) which lack hard parts such as otoliths or usable scales, age and growth information is derived from marks observed on vertebral centra and spines (Caillet 1990). The current hypothesis is that thin, opaque bands are formed in the winter months and broad, translucent bands are formed in the summer months although this has only been

validated for a few species.

3.6.5.1 Extraction and Storage

Approximately ten vertebrae should be removed from just below the dorsal fin (Figure 3.38). This is accomplished by cutting straight through the body just anterior and posterior to the dorsal fin. The removed vertebrae can be cleaned of excess tissue and separated using a sharp knife or scalpel blade (Figure 3.39)

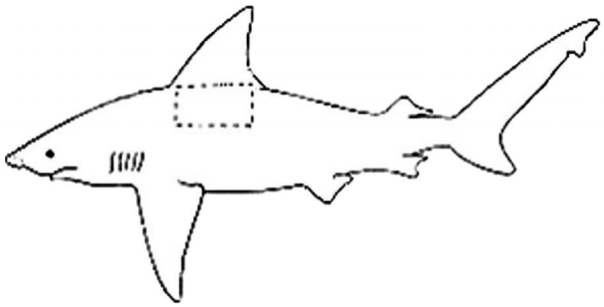


Figure 3.38. Depiction of shark with rectangular area denoting the section of vertebrae that should be removed.

The individual discs are soaked in a 5% sodium hypochlorite solution for 5-30 minutes or until all connective tissue has been removed. Once clean, vertebrae are stored in 70% ethanol until individual vertebrae are utilized for ageing.

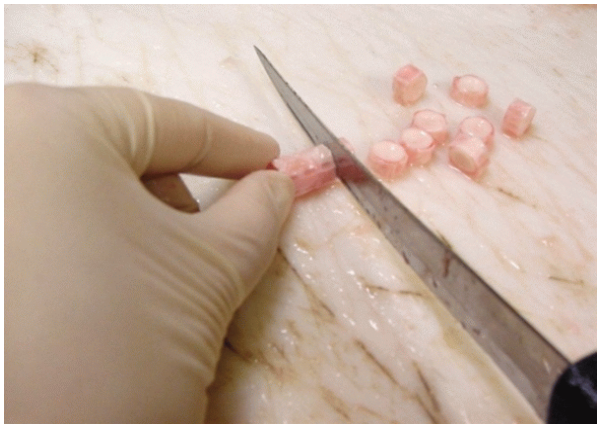


Figure 3.39. Separating and cleaning vertebrae of excessive tissue before sectioning.

3.6.5.2 Sectioning and Reading

Depending on the species of fish, vertebrae can be cut in half (perpendicular to the centrum face), read whole, or cut into thin sections. When cut into thin sections, the vertebrae may be stained to enhance the contrast of growth bands. The sections are then mounted to a slide and examined with a dissecting microscope (Figure 3.40).

Most coastal sharks are born in spring to early summer. When ageing sharks one must remember that the first band observed in a vertebral section is called the birthmark and is theoretically formed at the time the shark is born (Figure 3.40). The second band is formed six months later during winter, and a new band is formed every winter following. Therefore, a shark with two opaque bands is approximately 6+ months old but is still considered an age-0.

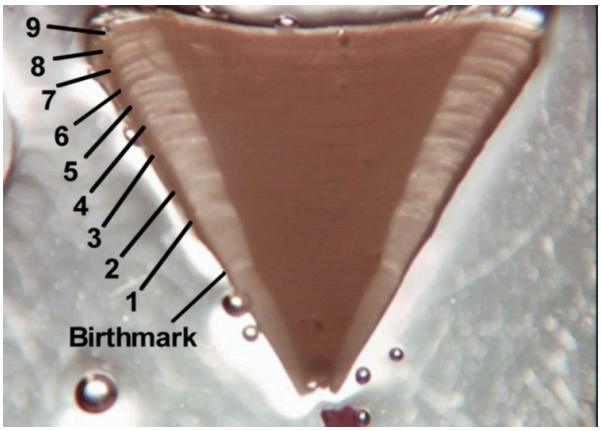


Figure 3.40. Sectioned vertebrae of an 8.5 year old shark with (birthmark indicated).

3.7 Section Enhancement

When reading otolith, fin ray, spine, or vertebrae sections, saw marks and other surface scratches can often reduce the reader's ability to see rings clearly. Optional techniques to enhance the readability of otolith sections include polishing, etching, staining, clearing, and baking. Other enhancement techniques may improve readability without directly

affecting the otolith section such as alternative lighting types, filters, polarizers, and light sources. The resolution on most otoliths can be improved using one or several of these techniques; however, a bit of trial and error must occur first. The species-specific sections (Section 5.0) will highlight enhancement techniques that have been used successfully in the Gulf region.

3.7.1 Polishing

Polishing involves using various grades of abrasive papers and polishing compounds to smooth the cut surface of the section. Electric polishers, gem polishers, buffing wheels, and hand polishing have all been used to remove saw marks and other surface imperfections. Alternatives to polishing include covering or coating the surface with clove, cedar, or immersion oil, glycerin, Flo-Texx, or Loctite (Figure 3.41). These solutions reduce light refraction making ring identification easier to the reader (Section 3.7.4).

Figure 3.41. First dorsal spine from a tripletail, *Lobotes surinamensis*, viewed in clove oil.

Note: Prolonged exposure to clove oil, cedar oil, or glycerin will result in reduced readability and should be used with caution (see Section 3.7.4).

3.7.2 Etching

Acid etching is a technique commonly used to enhance otolith microstructure, especially daily growth rings. This technique is also employed when otoliths contain growth zones or rings that are either too small or too faint to obtain accurate counts. This method takes advantage of the differing chemical composition of the opaque and translucent zones of the otolith by application of a chemical that will differentially dissolve the organic and inorganic components within the matrix (Pannella 1980). The chemical is most often an acid solution applied to an otolith thin section that will dissolve the regions of concentrated organic material (the translucent zone) more so than the calcified opaque zone. Three solutions used for etching by Davis et al. (1988) include immersion in 0.1 M disodium salt EDTA for 15 to 20 minutes, immersion in 1% HCl solution for 20 to 30 seconds, or immersion in 2% Histolab RDO for five minutes. The etched sections are then viewed under a Scanning Electron Microscope (SEM) (Figures 3.42A and 3.42B). An alternative to viewing the section is to create a replica of the etched surface using an acetate peel. The majority of otolith studies currently being conducted in the Gulf States focus on species that do not require the use of etching for analysis.

3.7.3 Staining

Similar to the application of oils or clearing substances, stains may be used to enhance the contrast between opaque and translucent growth zones, and more clearly define external and internal microstructure of the otolith. Dyes for this purpose generally act in one of two ways: 1) differential diffusion (uneven staining) of the protein and calcium matrixes or 2) reaction solely with the calcium carbonate portions of the otolith (Gauldie et al. 1998). Histological stains are most effective, and commonly used

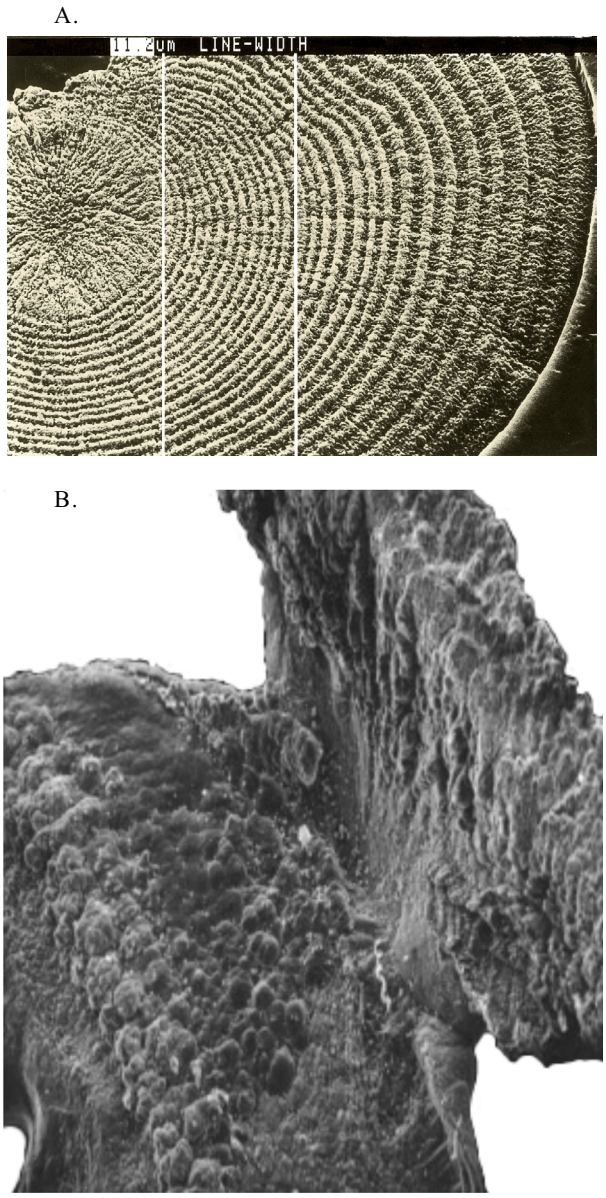


Figure 3.42. (A) Cross section of *Gymnothorax sp.* (moray eel) leptocephalus (larval) otolith, to show growth rings, SEM, X 4,930. (B). SEM micrograph of medial portion of a whole swordfish otolith, *Xiphias gladius*.

stains include Alizarin Red, Aniline Blue, Crystal Violet, and Toluidine Blue; the darker colors prove to be more effective (Richter and McDermott 1990). It is recommended that otoliths (whole sections) be exposed to the dyes from a minimum of one hour to as long as several days. Previous research by Richter and McDermott (1990) demonstrates that success in staining requires trial and error with different stains based on the properties inherent to the otolith of the individual species. Variance in the effectiveness of dyes between samples is likely due to interspecific differentiation in the otolith's proteinaceous otolin composition impacting the absorption of the stain and its reactivity with the section's surface. Staining works best when combined with other enhancing techniques such as acid etching (acidification of the stain), thin sectioning, and use of transmitted light, and has been demonstrated as an effective enhancement procedure (Gauldie et al. 1998, Richter and McDermott 1990, Albrechtsen 1968, Bouain and Siau 1988). Staining is often successful when used to aid in interpretation of otoliths that exhibit indistinct growth zones or annuli such as Florida pompano, *Trachinotus carolinus* (K. Guindon-Tisdel, FMRI, personal communication).

3.7.4 Clearing

Clearing an otolith section refers to the process of soaking a whole otolith or otolith section in a fluid medium that facilitates the passage of light through the specimen. It is used for: 1) a reduction in the appearance of saw marks and other surface imperfections with the application of an oil, glycerin, alcohol, or water (temporary) or 2) the perfusion of the clearing medium into growth zones within the section (permanent). Clearing, in this section, will refer to the perfusion of the clearing medium into otoliths microstructure by soaking the whole otolith in either clove oil, cedar oil, or glycerin. The duration of soaking is critical in achieving good contrast; however, once applied, the effect can continue and eventually render a section unreadable. Therefore, caution must be exercised when attempting this technique as time of soaking is dependent upon objective, species, and the otoliths size.

The soaking media effectively saturates the protein between the calcium carbonate crystals. Clearing usually affects the summer growth zone first. Continued soaking will eventually clear the opaque zones and eliminate any contrast optically washing out growth increments in the entire section/otolith. Therefore, careful removal of the clearing media must occur before long-term storage of a section.

3.7.5 Baking

Baking otoliths (whole and sections) is a technique adapted from the break and burn methodology (see Wischniowski and Bobko 2000 for a complete description). Although baking works very well for certain species, it may not with others, and considerable trial and error is involved initially. Variation of oven temperatures, baking times, and oven types will yield considerable differences in the outcome of the method. Baking time is generally a function of otolith size, desired final color, and is very subjective (Figure 3.43). The advantage of baking over burning is that the outer margin is not scorched beyond a readable state. At this time, baking has been used with limited success on a few species in the Gulf.

Figure 3.43. Baked otoliths in tray.

3.7.6 Filters

Several filters are available through microscope vendors and scientific suppliers that can alter the light source being used to interpret marks on otolith, spine, or fin rays. Polarization is commonly used throughout the Gulf States to enhance ring identification. Color filters have also been used with moderate success for particular species (Figure 3.44).

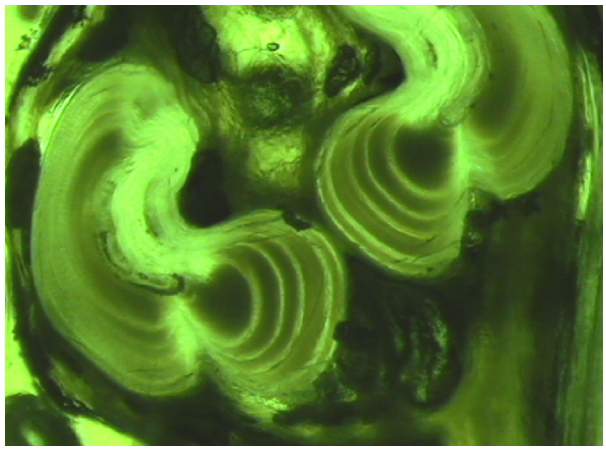


Figure 3.44. Cross-section of the dorsal fin-rays from an age-6 white grunt viewed with a green filter (540nm narrow-band).

3.8 Microscopy, Image Analysis, and Measurements

Otolith sections can be viewed under a low-power or stereomicroscope using reflected light, transmitted light, or a combination of the two. The choice of reflected or transmitted light is often made based on the preference of the reader, but subtle differences in readability may occur between illumination types (see Section 4.0 for discussion).

In recent years, the magnified image of otolith sections have been digitized, viewed, and analyzed using image processing software packages that utilize frame-grabbers and analog or digital cameras. This allows the scientist to acquire an image of otolith sections, view it on a video or computer monitor, recognize and mark the core and rings, and measure distances from the core to each ring, the core to the edge, and between rings (Figure 3.45). Ring counts

and distance measurements are then immediately stored in a computer file eliminating transcription errors that can occur if image measurements are manually recorded and

Figure 3.45. Image analysis station used to interpret an otolith section. System includes microscope, camera, computer, monitor, and interpretation software.

entered into a computer. Most of these software packages allow the reader to enhance the saved image making annuli recognition easier. Some of the more advanced packages can even automate the otolith reading process by guiding the reader through the entire process. Image analysis is also beneficial in that two or more scientists can discuss the features of otolith sections without having to look into a microscope. This allows for quick resolve of differences between readers.

4.0 Age Determination

This section is designed to give the reader guidance in age interpretations using otoliths. We have used a common sciaenid otolith as the model because its features are typically clear and obvious. Other species' otoliths can be more difficult to interpret and several species-specific accounts are listed in Section 5.0.

Throughout Section 4.0, an example data sheet is provided to track the procedure as the otolith is processed and an age determined for a fish with a July birthdate (Figure 4.1). This data sheet is purely for illustration but indicates the minimum data that should be recorded.

Fish Id.	Capture Date	# Rings	Margin Code	Biological Age	Age Group
ST00001	06/03/2001				
ST00002	06/03/2001				
ST00003	07/14/2001				

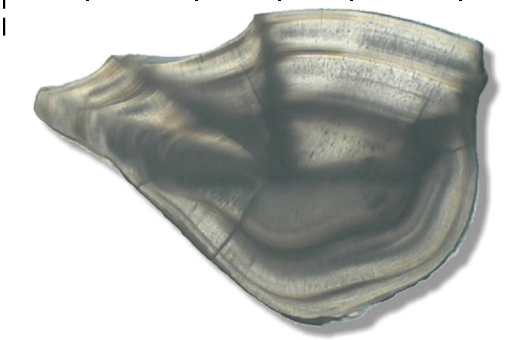


Figure 4.1. Example datasheet and section prior to assignment of rings, margin code, or age.

The appearance of structures used to age fish will vary under different illumination methods. Transmitted light (light from below passed upward through the section) and reflected light (light from above) will produce opposite contrasts in the observed ring patterns and the terminology used to describe the images can often be confused if the light source is not specified. That is why it is important to record the light source used when interpreting structures. Transmitted light (Figure 4.2A) makes the image appear as alternating wide (light) and narrow (dark or amber) rings while reflected light (Figure 4.2B) reverses the appearance. Either illumination method is useful and merely a personal preference. **However, for consistency in this manual, the use of transmitted light is assumed unless stated.**

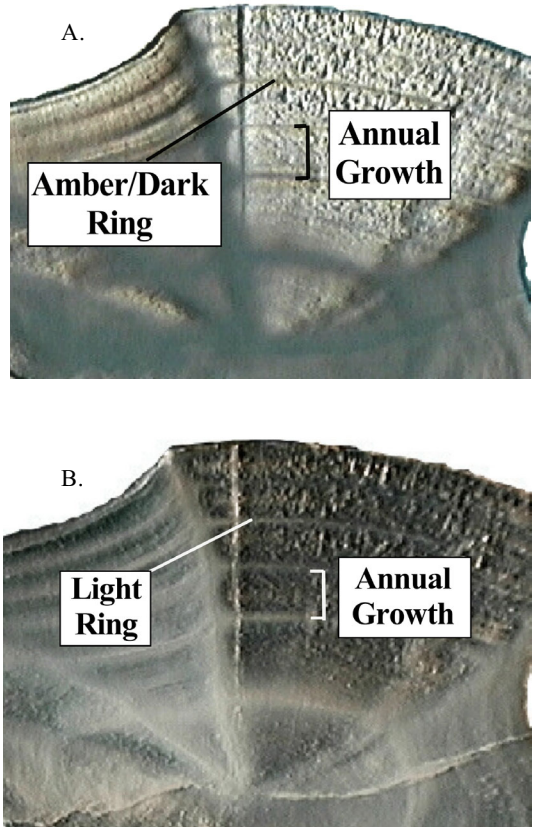


Figure 4.2. Otolith section viewed under A) transmitted light where opaque zones appear dark and B) reflected light where opaque zones appear light.

4.1 Otolith Development

A basic understanding of otolith development through successive periods of otolith ring formation is necessary to interpret the information contained in the structure. An otolith contains annual growth zones, each made up of a translucent and an opaque "ring"

or zone. In the southern U.S., the translucent ring is usually wider than the opaque ring and represents a period of faster growth (summer). The opaque ring is usually deposited during slower growth (winter) and is relatively narrow (see Section 2 for a detailed description on ring formation). This increment which includes a single translucent and opaque ring is an annual growth increment.

The exterior surface of a whole otolith may reveal observable rings. While some of these rings correspond with opaque rings observed in sectioned otoliths, it is **not** always the case (Section 3.5.4). The savings in time and effort of being able to enumerate rings on a whole otolith is obvious and tempting; however, validation is necessary to verify that rings counted on a whole otolith represent the number of rings that are observed in sections. For example, rings counted on whole striped mullet otoliths in Mississippi were consistently one ring fewer than the number counted on sections (Figure 4.3A and 4.3B).

While binocular dissecting microscopes yield the clearest view, more advanced image analysis systems can be used. An analog or digital video camera attached to a microscope and a television or computer monitor allow multiple individuals to view the same image at one time. By attaching the video camera to a frame grabber card installed in a computer the images can be saved, annotated, and cataloged or archived. This system can be further enhanced by installing image analysis software that gives the user the ability to enhance the otolith images and perform various analytical and quantitative tasks, such as measuring inter-annular distances on the otolith. Image analysis systems have also been used to rapidly enumerate measurements used to back-calculate the length at ring development and automatically determine number of rings on the otolith.

4.2 Ring Enumeration

While counting opaque rings in an otolith may seem straightforward, for some species separate opaque rings are not distinct. Two specific problems can be encountered: identifying the location of the first opaque ring near or within the core, and an opaque ring beginning formation very near or on the edge of the otolith. If the timing of opaque ring deposition is concurrent with or immediately following spawning, the first opaque ring may be hidden within the core region. If time of capture is concurrent with ring deposition, a distinct ring may or may not be observed at the otolith's margin. When rings are not particularly clear, techniques can be used to help discern rings and are discussed separately within each species account when they apply.

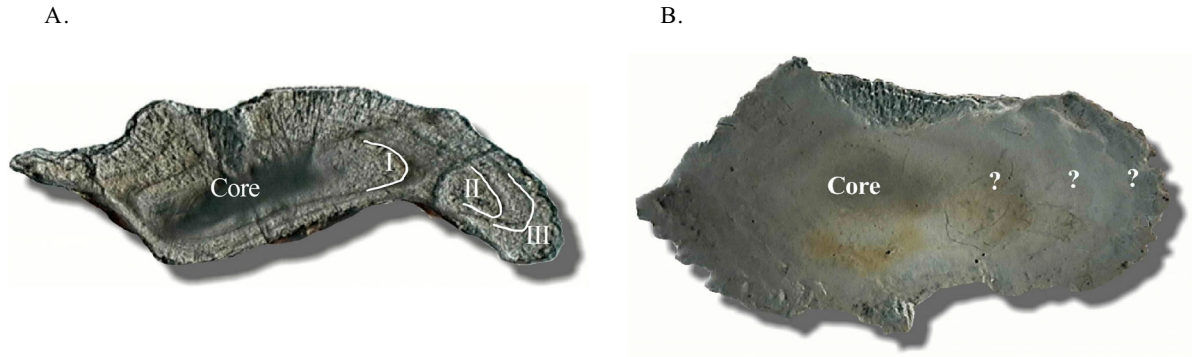


Figure 4.3. Rings observed in a mullet otolith using A) a thin section and B) a whole otolith.

Ring enumeration and edge development are typically made along the sulcus from the center of the core to a selected position on each ring, such as the midpoint, and to the otolith margin (Figure 4.4). The number of opaque rings are counted and recorded next to the corresponding fish identification number. These ring counts should be "blind readings" meaning without any knowledge of fish size or capture date. A second enumeration should be made by another, independent reader. This is commonly referred to as "verification." Consensus is achieved by revisiting enumeration disparities between readers or by a third party. A final ring count is then recorded for each fish.

Fish Id.	Capture Date	Rings	Margin Code	Biological Age	Age Group
ST00001	06/03/2001	3			
ST00002	06/03/2001				
ST00003	07/14/2001				

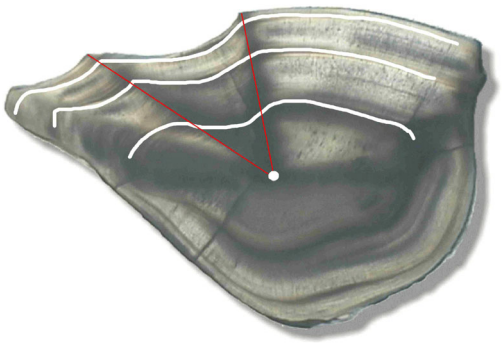
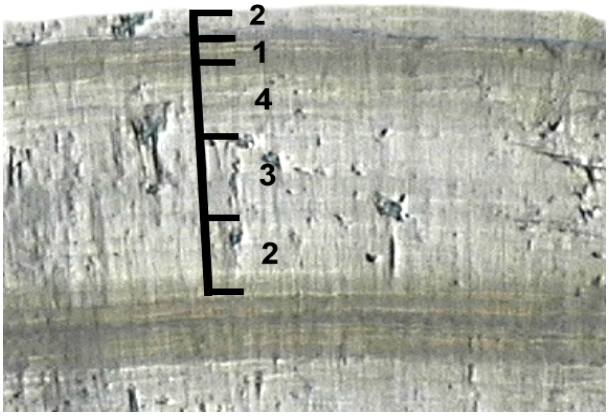


Figure 4.4. Highlighted core and subsequent opaque rings on an otolith section with the sulcus designated in red.

4.2.1 Margin Codes

Another necessary step when assigning ages to fish entails describing the relative stage of ring formation on the outer edge of an otolith's margin. Code 1 is assigned to the presence of an opaque ring at the edge and codes 2, 3, and 4 are assigned to progressive development of the translucent ring at the edge (Figure 4.5). Using the monthly frequency of occurrence of Code 1 through a calendar year can validate whether the formation of the opaque ring occurs on an annual basis (Figure 4.6). The determination of which 'third' the translucent



Code 1.	opaque zone present on edge
Code 2.	translucent zone forming to 1/3 complete on edge
Code 3.	translucent zone 1/3 to 2/3 complete on edge
Code 4.	translucent zone 2/3 to fully complete one edge

Figure 4.5. Codes identifying proportional margin development on sectioned otolith.

ring has completed is somewhat subjective; however, the presence/absence of the opaque ring is relatively straightforward. **The relative interval distance between rings changes as the fish ages owing to the geometery of the otolith and the rate of growth represented in a given annual growth zone.** Translucent and opaque rings usually become progressively narrower further from the core (Figure 4.7). The distances observed in the completed ring(s) closest to the edge are those used to judge the outer margin or proportion of completion of the outer ring being evaluated. Multiple codes can be observed in different fish captured at the

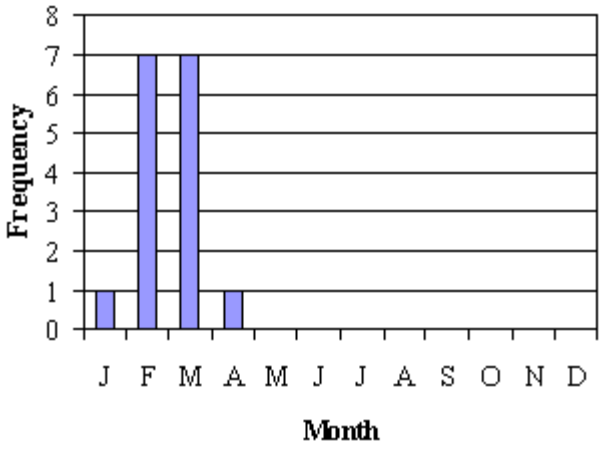


Figure 4.6. Frequency of occurrence of margin code 1 over twelve months or on an annual basis.

same time because the timing and duration of ring development can be protracted over several months.

Timing of initial deposition of opaque material at the edge of an otolith and subsequent completion of the opaque ring for a particular year may take a relatively short period of time (one to two months) for an individual fish (Figure 4.8). When observing this same process over a large population, the time between the first evidence of deposition in some fish until all fish are exhibiting translucent deposition (opaque deposition has ceased) may be as long as five to six months. In addition, the actual timing of formation is not necessarily concurrent with a birth date. Once determined, the margin code must be recorded (Figure 4.9).

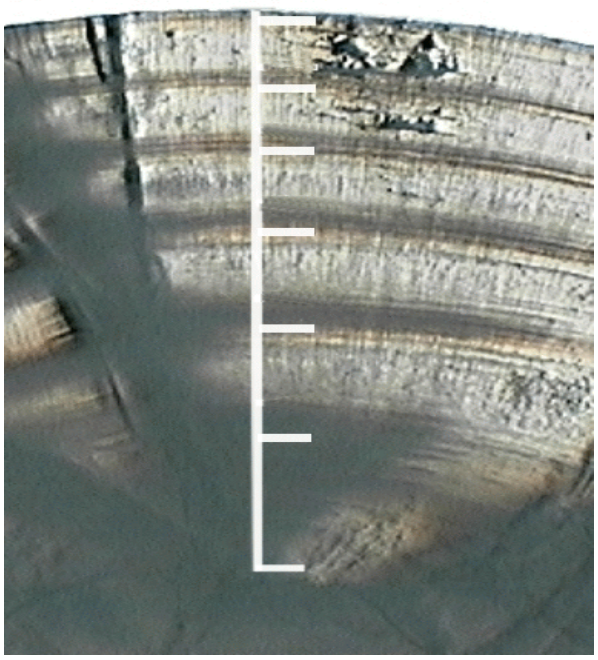


Figure 4.7. Change in relative distance, or narrowing of translucent area, for each progressive growth zone.

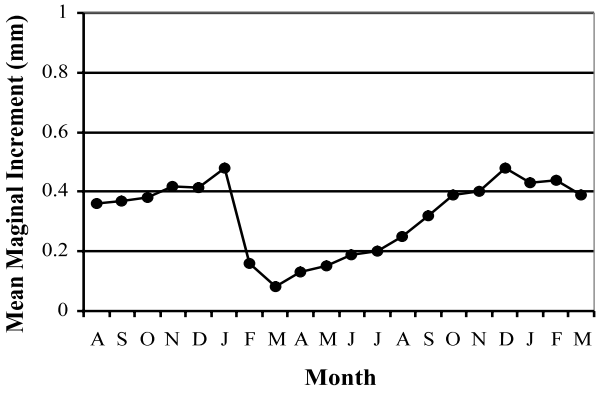


Figure 4.8. Mean margin increment distance plotted over a 20 month period indicating that opaque ring formation begins in February.

4.3 Assignment of Age

The analysis has now provided a **ring count** and a **margin code**. Both of these parameters have been obtained by physically viewing the otolith, understanding/recognizing what the rings are, counting the rings, observing the margin or edge, and recording that data.

Biological age and age group are then assigned from these data, taking into account the timing of opaque ring formation, date of capture and an estimated hatch date or birthday. The following discussion gives generalized examples to illustrate the concepts that are applied to these data to arrive at a useful age for each fish.

Fish Id.	Capture Date	Rings	Margin Code	Biological Age	Age Group
ST00001	06/03/2001	3	**2**		
ST00002	06/03/2001				
ST00003	07/14/2001				

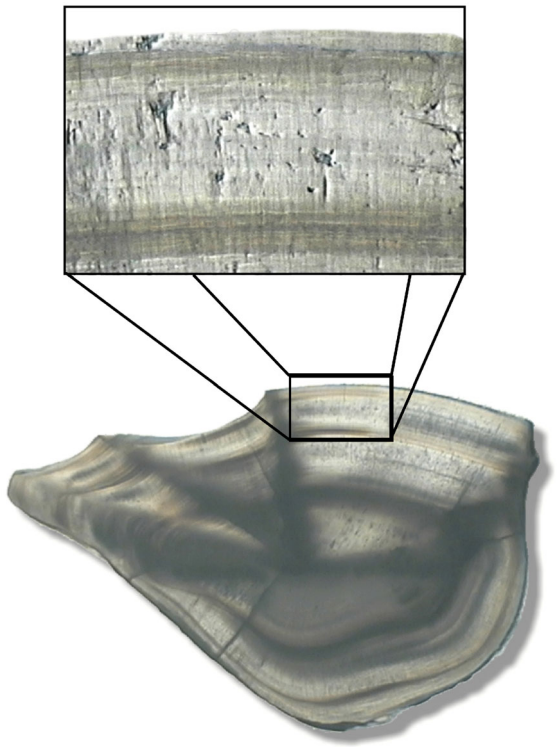


Figure 4.9. Break out section of otolith edge used for margin code assignment.

4.3.1 Biological Age

Because ring formation and birthdate may not coincide, the number of rings observed on an otolith is **not necessarily** the fish's age in whole years. In reality, the age of a fish in whole years and the number of rings coincide only during one month (time/period) per year. During all other months the age of the fish is the number of rings plus or minus the time (months) before or after its closest birthday.

An example would the fish with a July birthday that has just finished forming its third opaque ring in April and is captured June but will not become three years of age for another month. All of this makes assigning an age to a fish more than just using the number of observed rings as the age of the fish. The method used to assign an age is dependent upon the ultimate use of the age data (Figure 4.10).

Fish Id.	Capture Date	Rings	Margin Code	Biological Age	Age Group
ST00001	06/03/2001	3	2	**2.9**	
ST00002	06/03/2001				
ST00003	07/14/2001				

Figure 4.10. Example section with rings outlined ready to assign a biological age.

An age estimate and known length of the fish provides a basis for describing growth. Having age determined with the greatest resolution would, in most cases, yield the most accurate and reliable estimates of growth. The ages assigned to fish for use in determining growth are called biological ages. Biological age could be defined as the time elapsed from birth to capture and can be expressed in months or converted to the nearest tenth of a year (for ease of mathematical manipulation; Figure 4.10).

An average hatch date can be estimated from fecundity data or from peak densities of larval/post larval fish (Figure 4.11A).

Estimates of mean time of spawning can be calculated by dividing the mean size of postlarvae at capture by an estimated daily growth rate; thus back dating to the time of spawning. Mean timing of spawning can be calculated from an indicator of spawning such as maximum gonadal somatic index (GSI) values (Figure 4.11B).

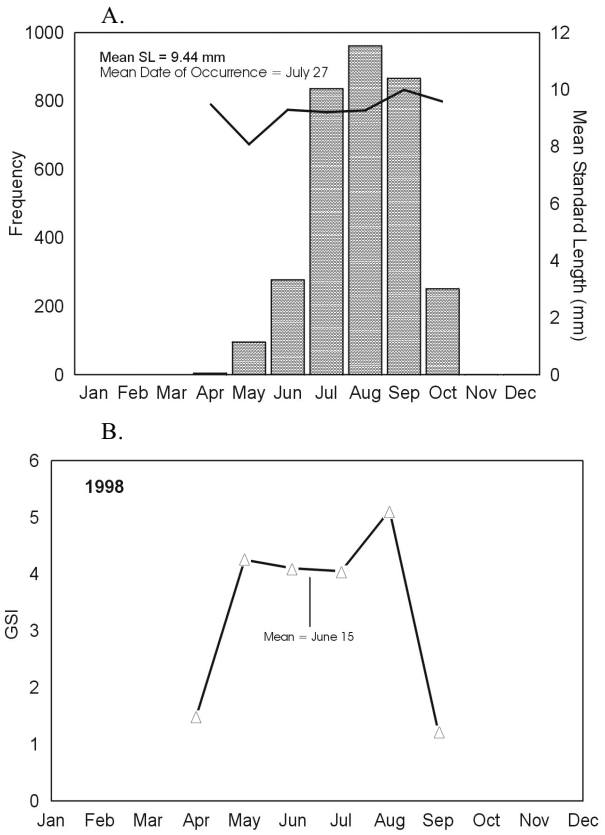


Figure 4.11. Birthdate determination using A. seasonal postlarval fish size and frequency data and B. seasonal Gonadal Somatic Index (GSI).

4.3.2 Assigning Age Groups

Stock assessments utilize cohort data as well as catch/population data grouped into ages. These data make up age groups representing single year classes or cohorts based on whole year ages. This grouping is needed to keep all fish sampled during a defined time period (calendar year, fishing year, etc.) together. While each year's offspring are considered a single cohort, there can be cohorts within the same year class as well. A good example of this is the bimodal spawning in spotted seatrout; two spawning peaks within one calendar year result in a spring cohort and late summer cohort. Therefore, we will use "age group" rather than cohort to define the age (in whole years) of a fish at the time of capture. This age reflects the greatest age that the fish would have attained during the selected time period, typically a calendar year (Figure 4.12). This means that all fish which would attain age-1 would be assigned an age group-1, regardless of the biological age (month) when captured. This ensures that all fish within a cohort remain together when analyzing the age structure of a population.

Fish Id.	Capture Date	Rings	Margin Code	Biological Age	Age Group
ST00001	06/03/2001	3	2	2.9	**3**
ST00002	06/03/2001				
ST00003	07/14/2001				

Figure 4.12. Example otolith section with all variables determined and biological age and age group assigned.

An illustration of assigning number of rings, a biological age and age group to an age-1 fish as it could be caught in any month over a calendar year is shown in Figure 4.13. Number of rings are normally assigned at the time of

reading. Biological age is assigned by evaluating the month of capture, number of rings observed relative to the month of opaque ring formation and an estimated month of birth. The year group or cohort is assigned by determining the largest whole year age a fish will attain during a calendar (fishing) year. The impact of using these two different "ages" on assembling an age structure is further illustrated in Figure 4.13. The age structures indicate a shift of younger fish into older age groups when using the year group method.

4.4. Quality Control in Processing

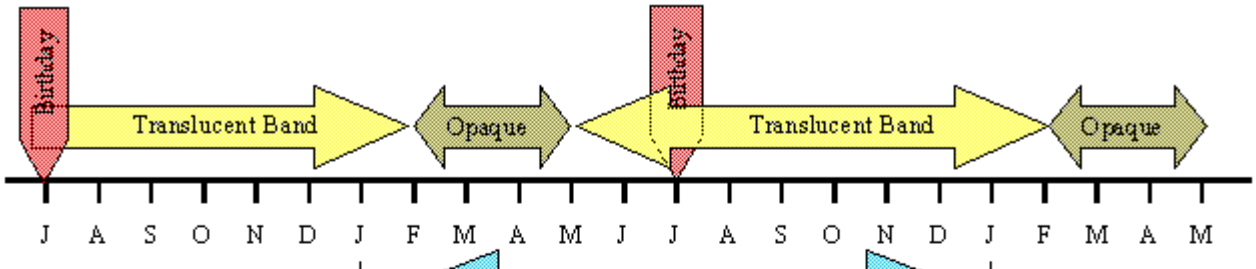
In production ageing of otoliths, several tests need to be conducted periodically to determine reader accuracy and precision of interpretation within individuals and between multiple readers. Additional training for processors in quality control should increase the acceptance of the science by managers and industry.

4.4.1 Validation

As a general rule when working with a new species, it should **not** be assumed that opaque rings are annuli. Annual deposition of opaque rings must be "validated" by any one of several methods.

4.4.1.1 Chemical Marking

The most direct method involves exposing a fish to tetracycline, calcein or some other chemical that incorporates a mark on the otolith through a physiological process. Through



Month	# Rings	Bio-Age	Year Group
Jan	1	1.5	2
Feb	1	1.6	2
Mar	1	1.7	2
Apr	2	1.8	2
May	2	1.9	2
Jun	2	1.9	2
Jul	2	2.0	2
Aug	2	2.1	2
Sep	2	2.2	2
Oct	2	2.3	2
Nov	2	2.4	2
Dec	2	2.4	2

Figure 4.13. Timeline illustrating birthdate of fish and periods of annulus deposition. Table illustrates the change in number of rings, biological age, and age class over one calendar year.

release and recapture of this marked fish over time, one had a direct method for validating whether one opaque ring is deposited on an annual basis. A problem with this approach is that the potential for recapture can be low in open marine systems making this method less practical. As an alternative, a marked individual can be held in captivity for an extended length of time for validation. However, the timing of opaque ring deposition of a fish held in captivity may not reflect natural conditions in the wild and should be interpreted with caution.

4.4.1.2 Margin Increment Analysis

Annual deposition of the opaque ring is more commonly validated by marginal increment analysis. The examination of the otolith edge condition for multiple fish captured over a time continuum (typically monthly) reveals the timing of formation of the last opaque ring. If opaque rings are found at the edge of the otolith only during one time period per year, it is inferred that the process is a yearly event (see Campana 2001 for review). Many times these data are presented as the monthly mean distance from the proximal edge of the last visible opaque ring to the margin of the otolith. Lowest monthly values of margin increments observed during a calendar year reveal the timing of opaque ring deposition and if the minimum value is observed only once per year it is inferred that the process is an annual event (Figure 4.5).

4.4.2 Accuracy

In practice, the accuracy of an age determination method may be known but the accuracy of a particular set of age estimates is seldom known (Beamish and McFarlane 1995). So age validation commonly refers to validation of the method used to determine age. Validation of absolute age is rarely done and has been primarily accomplished through age determinations of recaptured, tagged fish after a long interval of time or through the use of radiocarbon or radiochemical methods compared to growth increment estimates (Campana 2001).

Validation is critical for initial age and growth characterizations of a given species and validation of absolute age should be the preferred goal, but it is often exceedingly difficult and so two steps are recommended (Campana 2001). First, determine the time and age when the first increment forms. It is commonly overlooked because it can be problematic. Second, verify the increment periodicity across the entire age range of interest such that annulus formation/increment periodicity is determined for young immature individuals and old mature individuals (not necessarily every age class). See Campana (2001) for a recent review and critique of validation approaches.

Assuming for a given species that initial age and growth characterization is complete, validation of increment periodicity has been accomplished, and there is consensus on interpretation of ageing structures, ageing programs can move into the production phase whereby large numbers of samples are aged at regular intervals. At this stage, quality control monitoring becomes a very important component, including exchanges of age samples and cross-checking between laboratories (Boehlert and Yoklavich 1984, Morison et al. 1998).

4.4.3 Precision

As validation deals with error in accuracy, a second source of error that becomes critical in production ageing is precision or reader variability. Precision error is commonly reduced (improved) by resolving interpretation

differences among readers. Precision errors often result in "smeared" age distributions that tend to obscure strong or weak year classes. This interferes with attempts to track age-structure changes and to estimate mortality rates across time using an age-structured model, or when trying to compare age distributions with environmental or recruitment indices (Beamish and McFarlane 1995).

Some fish are difficult to age and precision errors are always inherent at some level, but experience is of key importance. There are a few, well documented approaches to quality control. Primarily they involve second readings or the use of a reference collection of resolved-age samples (Campana 2001). An example is the case whereby a primary reader may read all the otoliths and then an experienced secondary reader or tester may read a random sample of 20% without knowledge of the ages assigned by the primary reader. Examinations of bias and reader error (precision) estimates should be recorded and updated annually (Kimura and Lyons 1991).

4.4.4 Reference Collection

The use of reference collections serves many of the same purposes as reader-tester comparisons and has potential advantages. The dominant use of reference collections are to test precision among readers and to monitor consistency in age interpretations over time. A reference collection allows monitoring of long-term drift, an increase or decrease in counts over time based on subtle changes in a reader's interpretation of the ageing structure. This cannot be accomplished as well with a reader-tester approach using contemporary samples (Campana 2001). A reference collection is also useful for training purposes (Campana 2001). A subset of the reference collection can be imaged and annotated and used to illustrate ageing structures and characteristics during the training of new readers.

The reference collection must be a set of prepared ageing structures for which known or consensus-derived ages are recorded. The idea is to incorporate prepared otoliths (not necessarily textbook examples) that are representative of all age/size groups, regions and collection sources likely to be encountered by readers. Furthermore, building the collection using samples collected year-round is encouraged to show all stages of margin or edge development. If year-specific differences are suspected, consider including samples from several years. Dry storage of the otolith preparations is recommended for long-term archiving rather than storage in solutions such as glycerine (Campana 2001).

Although the size of the collection is arbitrary, Campana (2001) recommends about 500 age samples per stock. This number is large enough to prevent memorization and allows subsets to be exchanged among different groups of otolith readers. A particular subset (i.e., 100) may be thoroughly documented and used as a training set. Over time the collection should be augmented as new materials and processing procedures are updated.

Production ageing programs have shown that following initial orientation and training, periodic tests of precision and bias using the reference collection will enable several readers to age with consistency (Morison et al. 1998, Campana 2001). Consistency among readers and over time is important even if the consensus-derived ages, which serve as a basis for age interpretation, are later found to be inaccurate. If this happens, re-interpretation of the reference collection would allow age corrections to be readily made to the historical data sets (e.g., see Stanley 1986).

A "before and after" exercise is

recommended for each ageing session and is important for both experienced and novice readers. In the case of an experienced reader, perhaps some time has passed since a given species was last aged (at least a year or two) and a subset of the reference collection needs to be re-aged to tune the reader and prevent drift. For the novice reader, a training sub-set should be aged until a sufficient level of precision is achieved and reader bias is minimized (Morison et al. 1998). Near the end of the ageing session, a reader-tester exercise should be conducted, where another sub-sample of the reference collection should be read blind (without knowledge of previous readings, dates, or fish sizes), in order to generate an estimate of precision for the session (see below).

4.4.5 Reader Comparisons

When readers compare age estimates in order to achieve consistency, they need to examine any biases such that one reader may tend to under- or over-age another. A good approach for graphically detecting bias is to plot pair-wise age comparisons or age-bias graphs (Campana et al. 1995). For annual age comparisons, most workers estimate precision measures using either Average Percent Error (APE, Beamish and Fournier 1981) or percent Coefficient of Variation (CV, Chang 1982). Both approaches are valid and one may be preferred for various reasons. Regression analysis has shown that either measure can be easily predicted from the other (Campana 2001). Care should be exercised that comparisons are made for similar values; either raw increment counts or final assigned ages. Because it may be common for readers to have subtle differences in edge interpretations that are often hard to resolve and can affect the increment count, final assigned ages would tend to yield lower precision errors. Increasingly, these measures of reader error (precision) are being incorporated directly into stock assessment models in order to statistically correct age-structure estimates (Richards et al. 1992, Beamish and McFarlane 1995, Crone and Sampson 1998). In practice, a measure of reader error would be used to adjust or correct a single set of age determinations. This equates to what would have happened if several readers had come to consensus on each age in the set.

4.5 Other Parameters and Their Usefulness

Fish growth is usually derived from plotting length against age and/or fitting those data to an equation that can be used to estimate length for a given age. Many times only larger/older fish are available for examination (i.e., large specimens of fish from fishing tournaments or dockside sampling of commercial catch). Size and bag limits may hamper collections of fish representing the full size range of the populations when using fishery-dependent data. The growth rates of younger year classes of fish species that can grow quite old is of interest when smaller, younger specimens are rarely encountered. These estimates can be compared to observed lengths for each given age and provide insight into the overall growth and survival of fish in the population. In these cases, lengths at age can be estimated from a technique referred to as "back calculation." If the relationship of otolith radius versus fish length is linear then an estimate of fish length relative to a location (ring) on the otolith can be calculated.

The linear relationship of otolith radius and fish length is validated by regressing a series of otolith radiuses against the fish lengths for fish that cover as many ages/lengths as possible. Obviously, if no young fish are available, fish covering all ages may be non-existent. Assuming the relationship is linear, lengths are then estimated for each age by the following formula:

$$L_e = D_r/D_m * L_t$$

where L_e = estimated length,
D_r = distance from core to chosen ring,
D_m = radius of otoltih,
L_t = total length of fish at capture.

This formula gives an estimate of length for each chosen ring. If each ring represents an annulus (i.e., ring one represents age-1), estimates of length can be calculated for several ages on each otolith, given the number of rings present. This method is called the "direct proportion" method. Further refinement of the above formula includes the Y-intercept from the regression of total length and otolith radius, such that:

$$L_e = D_r/D_m * L_t + \text{Y-intercept}$$

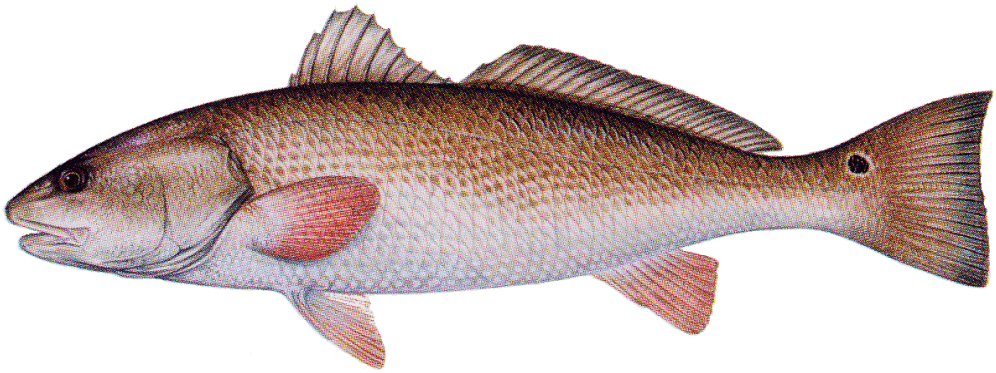
where L_e = estimated length,
D_r = distance from core to chosen ring,
D_m = radius of otoltih,
L_t = total length of fish at capture.

This technique is commonly called the "Fraser-Lee" or "modified direct proportion" method and is used when the regression of fish length and otolith radius does not pass through the origin. This method adjusts for any somatic length gained prior to otolith growth. Other similar methods have been used mainly with the intent of partitioning the variance into age effects and length effects. DeVries and Frie (1996) provide details of the above methods.

5.0 Species-Specific Otolith Characteristics and Processing Details

As noted in Section 3.3, the sectioning techniques used for each species will be determined by the equipment (i.e., sectioning saws) already available in a laboratory. Three saw styles are currently used around the Gulf region: the low speed wafering saw, the high speed wafering saw, and the high speed thin sectioning saw. Three methods of section preparation are currently used in the Gulf States: embedding whole otoliths in an epoxy resin, mounting a whole otolith to a glass slide, and free-hand cutting of whole otoliths followed by mounting on a slide for sectioning. Differences in fish shape and body size and otolith size among species require species-specific modifications to otolith extraction, preparation, and analysis. The following species accounts summarize these differences and highlight techniques currently being used in the Gulf region.

5.1 Red Drum *Sciaenops ocellatus*



Highlights

- Otoliths are large and relatively easy to locate and extract.
- Multiple sectioning techniques successful.
- Rings easily discernable.
- First distinct opaque ring forms at approximately 1.5 years of age.
- Long-lived species – up to 40+ rings.

Otolith Description

Red drum have large, stout sagittae that are thick enough to be opaque (Figure 5.1). The sagitta is slightly elongate and ovoid with a rather straight and slightly crenate dorsal margin and a convex ventral margin (Chao 1978). The anterior and posterior portions are about the same height forming a rectangular surface. There are often one or more knobby protrusions on the distal face.

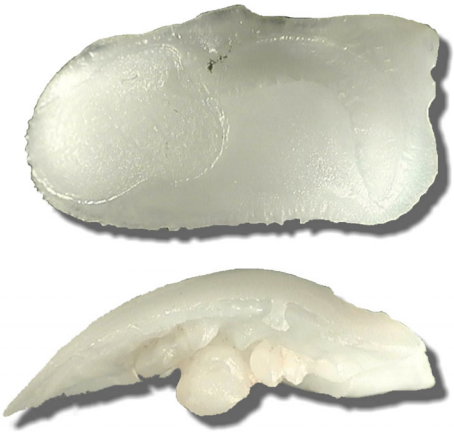


Figure 5.1 Red drum sagittal otoliths medial and top view.

The ostium of the sulcus is large and pear-shaped, and its expanded part does not reach the anterior margin. The 'J' shaped cauda of the sulcus acousticus is sharply bent, and its dorsal edge extends further into the ostium than its ventral edge. The rostrum and anterostrum are not distinguishable from one another. The core of the otolith usually lies just interior to the surface that faces outward from the midline of the fish. In the antero-posterior axis, the core lies adjacent to the junction of the ostium and cauda regions of the sulcus acousticus. The location of the otolith in the neurocranium is illustrated in Figure 5.2.

Otolith Extraction

Red drum otoliths can withstand expected impacts from otolith extraction devices without breaking. The otic capsule of red drum is somewhat convex making it easy to identify through the gill cavity near the posterior base of the skull above the gills. It is relatively easy to cut away the surface of the exposed otic capsule with a heavy knife. At larger sizes, otolith removal is best done using a hacksaw cut made

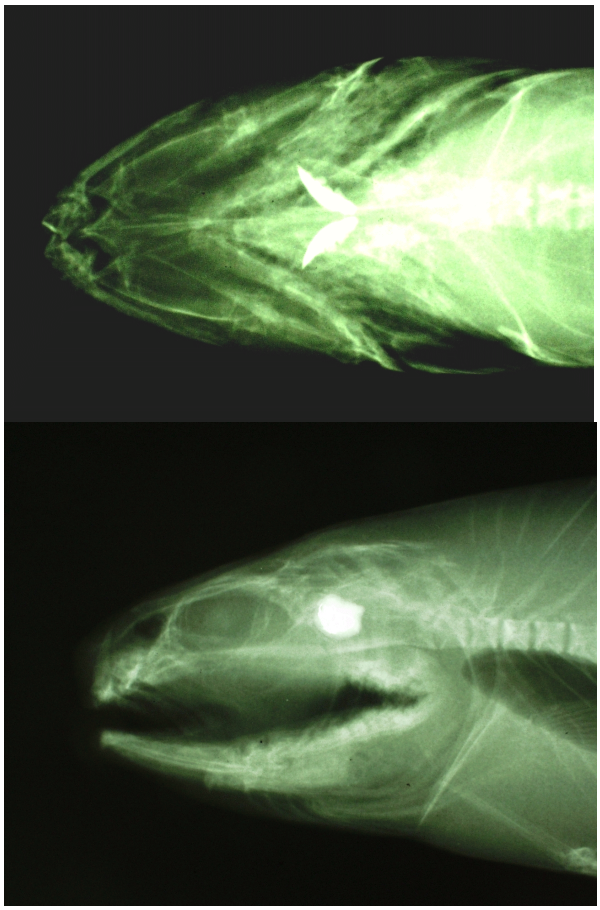


Figure 5.2 Location of red drum sagittal otoliths.

from the dorsal surface of the head to the otic capsule. Red drum otoliths are relatively robust across all life stages; however, due to the still fragile nature of young otoliths, extraction should be executed with care at smaller sizes. Several different techniques are effective; some may be easier than others on different sized fish.

Top Methods

Smaller Fish

1. Make a cut from the back of the skull to a point below and behind the eyesocket exposing the brain (Figure 5.3).
2. Remove brain to reveal the otoliths.
3. Remove the sagittal otoliths.

Figure 5.3 Extraction of red drum otoliths through the top of the neurocranium.

Larger Fish

1. Make a vertical cut in the skull at a point just behind the centerline of the opercle through the otic capsule (Figure 5.4).
2. Bend the head of the fish forward to reveal the sagittae.
3. Remove the sagittal otoliths.

Figure 5.4 Meatsaw technique for extraction of otoliths from red drum.

Bottom Method

This method causes minimal visible damage to the fish.

1. Pull open the opercle to expose the gills.
2. Pull the gill arches back to expose the otic

capsule (Figure 5.5).
3. Chisel away the otic capsule to expose the sagitta.
4. Remove the otolith.
5. Repeat for the other side.

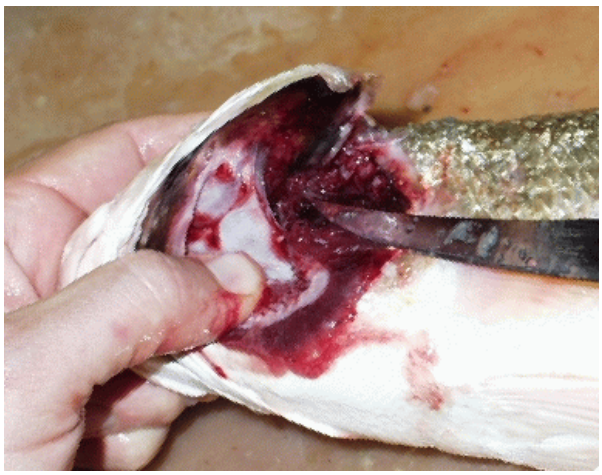


Figure 5.5. Extraction of otoliths from red drum through the operculum.

Otolith Processing

Due to the robust nature of this species, multiple techniques are acceptable and usually reflect available equipment. Generally, red drum sections are processed at approximately 0.5 mm. The following techniques have been used successfully throughout the Gulf.

Low Speed Wafering Saw Techniques

Embedded Whole Otoliths (Section 3.4.2.1)
LDWF, GCRL, MDMR, FMRI

1. Embed the otolith with the long axis parallel to the long axis of the mold.
2. Locate core and position block in chuck.
3. Adjust arm weight and speed. Make successive 0.5 mm cuts to obtain the core region.
4. Mount the core sections.

Mounted Whole Otoliths (Section 3.4.2.2)
FMRI

1. Mount whole otolith to slide, concave side down with the long axis parallel to the long side of the slide using thermoplastic.
2. Locate core and position slide in chuck.
3. Adjust arm weight (50-75 g) and speed (8-10). Make successive 0.5 mm cuts to obtain the core region.
4. Mount the core sections.

High Speed Wafering Saw Techniques

Embedded Whole Otoliths (Section 3.4.2.1)
TPWD

1. Embed the otolith with the long axis parallel to the long axis of the mold.
2. Locate core and position block in chuck.
3. Adjust load (1,000 g) and speed (3,000 rpm). Make successive 0.5 mm cuts to obtain the core region.
4. Mount the core sections.

Thin Section Machine

Free-Hand Whole Otolith Sectioning (Section 3.4.3)
LSU, AMRD

1. Firmly grasping both ends of the otolith, make initial cut adjacent to the core.
2. Hand grind additional material until core is visible.
3. Mount otolith half with core on labeled slide.
4. Place slide in chuck and section off remaining material.
5. Place slide into precision grinder arm and adjust caliper to 0.5 mm.

Age Determination

Red drum otolith sections call for special attention in the process of identifying the first annulus. Because red drum spawn in the late fall just before the time of opaque zone formation, a dark zone is often visible around the core. However, the first distinct opaque

mark is deposited during their second winter when the fish is about 14-18 months of age (Figure 5.6).

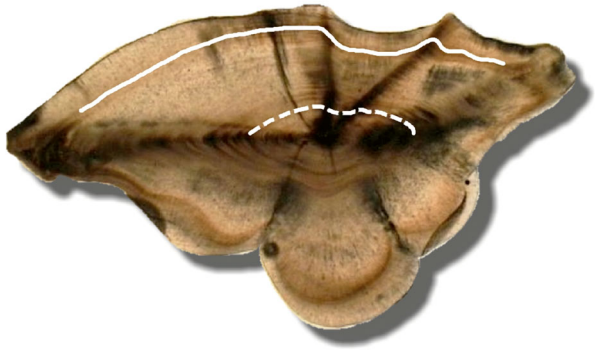


Figure 5.6 Sagittal otolith section from age-1 red drum. Mark near the core (dashed line) due to hatching just prior to ring formation and not counted.

Spawning in red drum is typically from August-November with annulus deposition occurring from February-April often reflected as a ring or dark smear present near or in the core region (Figure 5.7).

Other Ageing Methods

The vast majority of current red drum age and growth research utilize otoliths over other calcified structures to obtain age data. Age of an individual is most easily determined counting annuli visible on a mounted otolith section. Whole, uncut otoliths may also be used, but annuli are less discernable, and this method is therefore unreliable for the ageing of fishes age-3 or over (Theiling and Loyacano 1976). Other calcified structures in the fish are **NOT** recommended for use in obtaining age data in red drum. Scales have been demonstrated to be unreliable and inaccurate due to reabsorbtion of calcium, degradation with age, and exposure to the external environment (Prentice and Wilfred 1991, Summerfelt and Hall 1987). Similarly, the use of red drum spines and rays is discouraged, as researchers have determined they yield highly inaccurate age data (Rohr 1964; D. Tremain, FWC, personal communication).

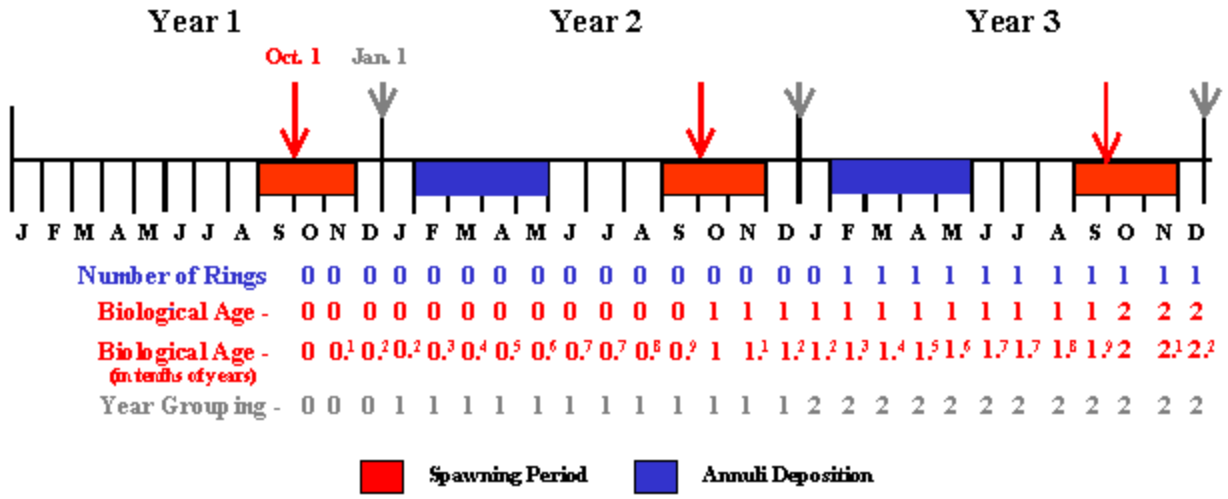
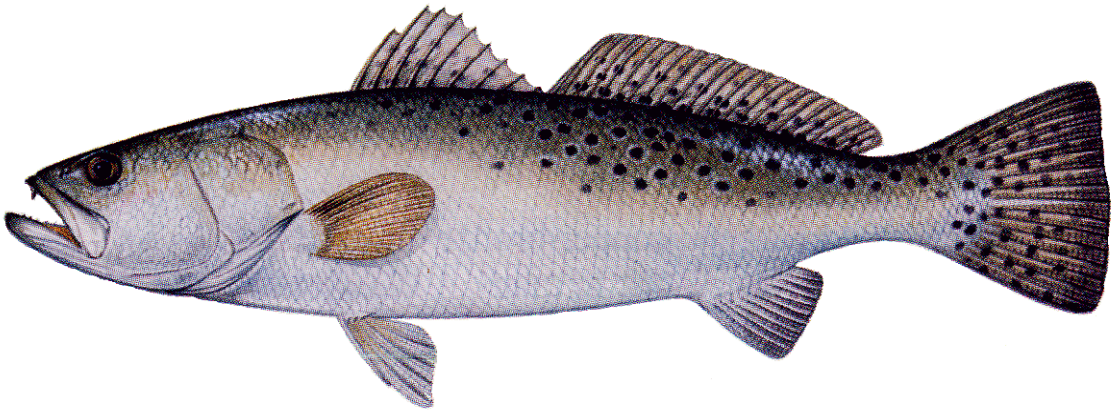


Figure 5.7 Birthdate assignment timeline for red drum. Age and year group based on biological birthdate (October 1), number of rings, and January 1 to December 31 year. A mark (ring or dark smear) generally occurs close to the core when the fish is 0.3-0.6 years old, however the first true annuli doesn't occur until the fish is actually 1.3 - 1.6 years old.

5.2 Spotted Seatrout *Cynoscion nebulosus*



Highlights

- Otoliths are large and relatively easy to locate and extract.
- Multiple sectioning techniques successful.
- Rings easily discernable.
- Distance from the core to the first opaque ring is variable.
- First ring formation occurs at <1 year.
- Generally fewer than 13 rings.

Otolith Description

Spotted seatrout have relatively large, elliptical, narrow sagitta that are opaque at most sizes (Figure 5.8). The dorsal margin is smooth and convex whereas the ventral margin is slightly concave and crenelate (Chao 1978).

Figure 5.8 Sagittal otoliths medial and top view from spotted seatrout.

The posterior portion of the sagitta is wider laterally.

The sulcus acousticus is elongate with the ostium ovoid and the cauda long and bent with a short distal end. The marginal groove is distinct, and the rostrum and anterostrum are not distinguishable from one another. The otolith core lies just interior of the midline of the distal surface of the otolith and beneath the juncture of the ostium and cauda of the sulcus acousticus. The location of the otolith in the neurocranium is illustrated in Figure 5.9.

Otolith Extraction

Spotted seatrout otoliths are strong enough to withstand expected impacts from otolith extraction devices without breaking. They are easy to identify through the gill cavity near the posterior base of the skull above the gills due to the strongly convex surface of the otic capsule which is easily cut away with a heavy knife.

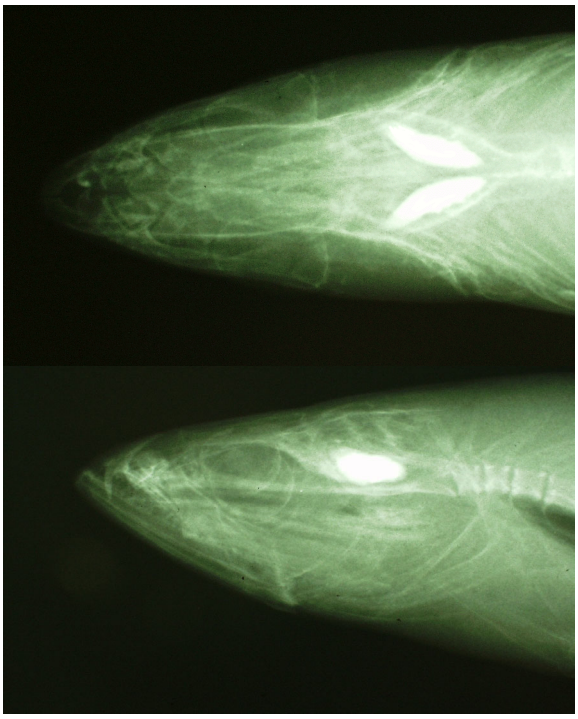


Figure 5.9 Location of spotted seatrout sagittal otoliths.

Several different techniques are effective; some may be easier than others on different sized fish.

Top Methods

Smaller Fish

1. Make a cut from the back of the skull to a point below and behind the eyesocket exposing the brain (Figure 5.10).

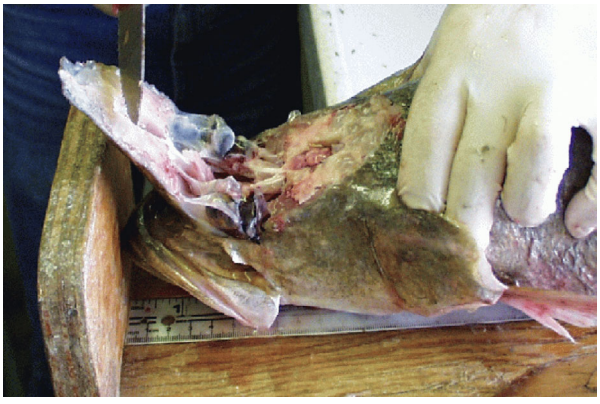


Figure 5.10 Removal of the top of the cranium in small spotted seatrout.

2. Remove brain to reveal the otoliths.
3. Remove the sagittal otoliths.

Larger Fish

1. Make a vertical cut in the skull at a point just behind the centerline of the opercle through the otic capsule.
2. Bend the head of the fish forward to reveal the sagittae (Figure 5.11).
3. Remove the sagittal otoliths.

Figure 5.11 Meatsaw technique for otolith removal in spotted seatrout.

Bottom Method

This method causes minimal visible damage to the fish (Figure 5.12).

1. Pull open the opercle to expose the gills.
2. Pull the gill arches back to expose the otic capsule.
3. Chisel away the otic capsule to expose the sagitta.
4. Remove the otolith.
5. Repeat for the other side.

Otolith Processing

Due to the robust nature of this species, multiple techniques are acceptable and usually reflect available equipment. Generally, spotted seatrout sections are cut to approximately 0.5 mm. The following techniques have been used successfully throughout the Gulf.

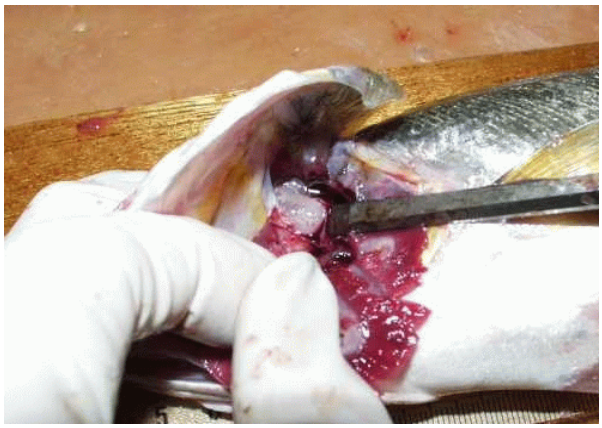


Figure 5.12 Removal of spotted sea trout otolith through the gill cavity.

Low Speed Wafering Saw Techniques

Embedded Whole Otoliths (Section 3.4.2.1)
LDWF, GCRL, MDMR, FMRI

1. Embed the otolith with the long axis parallel to the long axis of the mold.
2. Locate core and position block in chuck.
3. Adjust arm weight and speed. Make successive 0.5 mm cuts to obtain the core region.
4. Mount the core sections.

Mounted Whole Otoliths (Section 3.4.2.2)
FMRI

1. Mount whole otolith to slide, concave side down with the long axis parallel to the long side of the slide using thermoplastic.
2. Locate core and position slide in chuck.
3. Adjust arm weight (50-75 g) and speed (8-10). Make successive 0.5 mm cuts to obtain the core region.
4. Mount the core sections.

High Speed Wafering Saw Techniques

Embedded Whole Otoliths (Section 3.4.2.1)
TPWD

1. Embed the otolith with the long axis parallel to the long axis of the mold.
2. Locate core and position block in chuck.
3. Adjust load (1,000 g) and speed (3,000 rpm). Make successive 0.5 mm cuts to obtain the core region.
4. Mount the core sections.

Thin Section Machine

Free-Hand Whole Otolith Sectioning (Section 3.4.3)
LSU, AMRD

1. Firmly grasping both ends of the otolith, make initial cut adjacent to the core.
2. Hand grind additional material until core is visible.
3. Mount otolith half with core on labeled slide.
4. Place slide in chuck and section off remaining material.
5. Place slide into precision grinder arm and adjust caliper to 0.5 mm.

Age Determination

Spotted seatrout have a protracted spawning season which may extend from April to September, depending on annual variation in climate. Ageing is fairly straightforward even though the location of the first annuli can vary widely in its distance from the core (Figure 5.13) Due to the protracted spawning season there may be a corresponding variation in age (months) at first opaque zone formation,

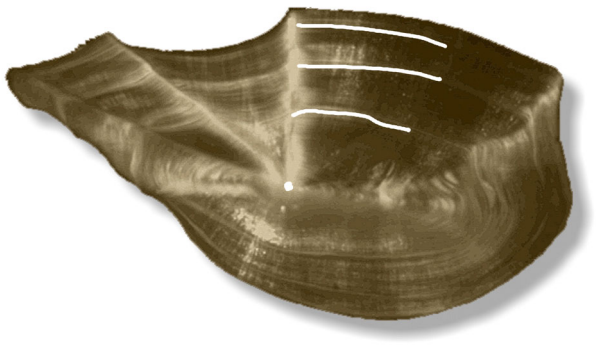


Figure 5.13 Sagittal otolith section from an age-3 spotted seatrout.

which may occur from October through May depending on geographic location (October-January in south Texas and February-May in Mississippi) (Figure 5.14).

For regional stock assessment purposes, three minimal parameters are recorded: number of rings, presence or absence of opaque ring at margin, and month of capture. Based on these three parameters, cohort and biological ages can be determined.

Other Ageing Methods

Whole spotted seatrout otoliths have not been used successfully in the Gulf region.

The usefulness of break and burn techniques for spotted seatrout has not been determined. However, this species (along with most of the sciaenids) may be a good candidate for break and burn.

Scales have been demonstrated to be useful in the first few years only. After age-4 annuli in scales become less consistent, resorption can occur at the core, and false annuli can occur due to spawning checks. See Wenner et al. 1990 for additional information.

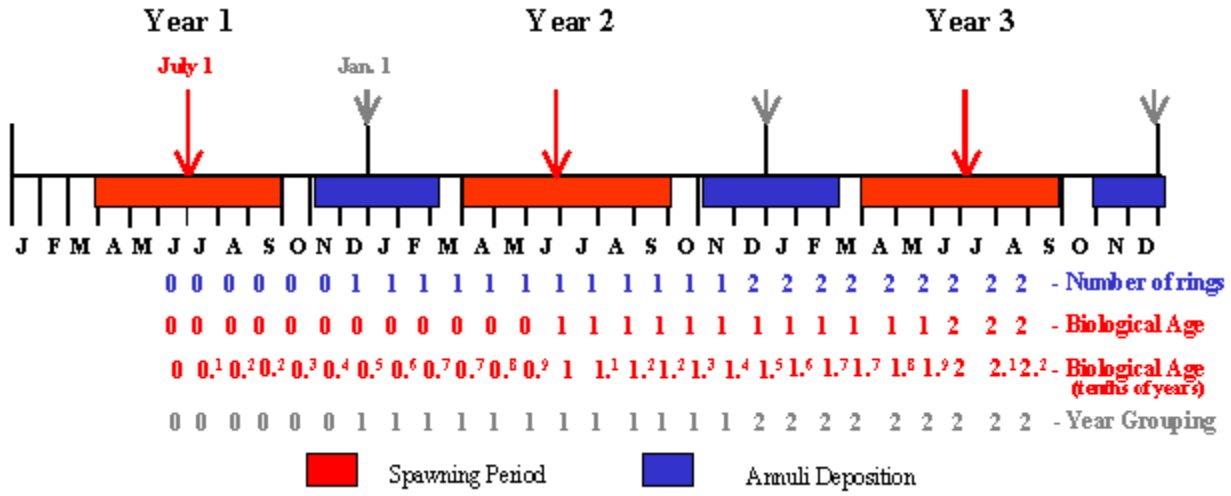
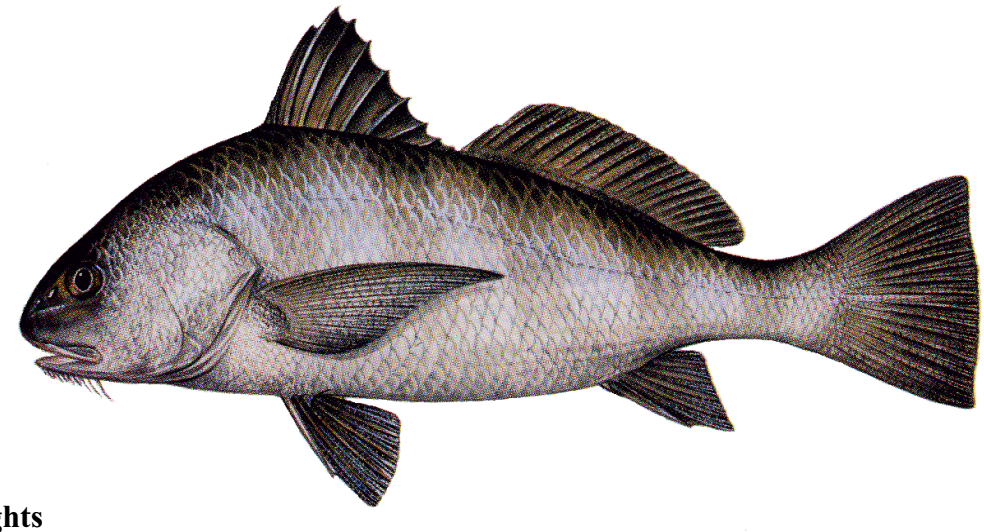


Figure 5.14 Birthdate assignment timeline for spotted seatrout. Age and year group based on biological birthdate (July 1), number of rings, and January 1 to December 31 year.

5.3 Black Drum *Pogonias cromis*



Highlights

- Otoliths large and relatively easy to locate and extract.
- Multiple sectioning techniques successful.
- Rings easily discernable.
- First distinct opaque ring forms at approximately 1 year of age.
- Long-lived species – up to 55+ rings.

Otolith Description

Black drum have a robust otolith that is semi-circular in juvenile fish and becomes somewhat rectangular in mature fish (Figure 5.15). The otolith is opaque with an oblong ostium and a crescent-shaped cauda. The rostrum and anterostrum are not distinguishable from one another. The otolith core lies just interior to the midline of the distal surface of the otolith. Black drum sagittae are opaque in older juvenile and adult fish. The location of the otolith in the neurocranium is illustrated in Figure 5.16.

Figure 5.15 Sagittal otoliths medial and top view from black drum.

Extraction

Black drum otoliths are strong enough to withstand expected impacts from otolith extraction devices without breaking. The ventral surface of the otic capsule of black drum is somewhat convex making it easy to identify through the gill cavity near the posterior base of the skull above the gills. It is relatively easy to cut away the surface of the exposed otic capsule with a heavy knife. A heavy bladed knife can also be used to cut from the dorsal skull base at about a 30 degree angle to the back of the ocular socket to open the

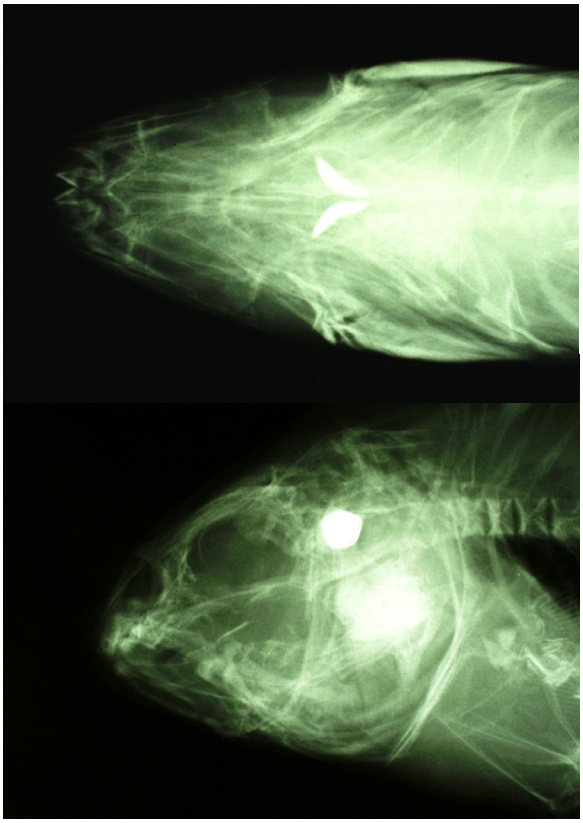


Figure 5.16 Location of black drum sagittal otoliths.

cranial cavity and expose the sagittae. At larger sizes, otolith removal is best done using a saw cut made from the dorsal surface of the head to the otic capsule. This method can also be performed on smaller fish but care must be taken that the cut does not extend through the otic capsule for risk of damaging the otoliths. Several different techniques are effective; some may be easier than others on different sized fish.

Top Methods

Smaller Fish

1. Make a cut from the back of the skull to a point below and behind the eyesocket exposing the brain (Figure 5.17).
2. Remove brain to reveal the otoliths.
3. Remove the sagittal otoliths.

Figure 5.17 Extraction of red drum otoliths through the top of the neurocranium.

Larger Fish

1. Make a vertical cut in the skull at a point just behind the centerline of the opercle through the otic capsule.
2. Bend the head of the fish forward to reveal the sagittae (Figure 5.18).
3. Remove the sagittal otoliths.

Figure 5.18 Meatsaw technique for otolith removal in black drum.

Bottom Method

This method causes minimal visible damage to the fish.

1. Pull open the opercle to expose the gills.
2. Pull the gill arches back to expose the otic

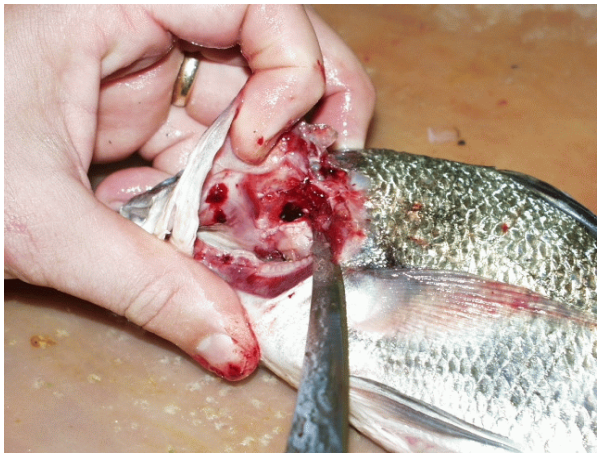


Figure 5.19 Extraction of otoliths from black drum through the gill cavity.

capsule (Figure 5.19).
3. Chisel away the otic capsule to expose the sagitta.
4. Remove the otolith.
5. Repeat for the other side.

Otolith Processing

Due to the robust nature of this species, multiple techniques are acceptable. The technique chosen will likely reflect your current equipment. Generally, black drum sections are processed at approximately 0.5 mm. The following techniques have been used successfully throughout the Gulf.

Low Speed Wafering Saw Techniques

Embedded Whole Otoliths (Section 3.4.2.1)
LDWF, GCRL, MDMR, FMRI

1. Embed the otolith with the long axis parallel to the long axis of the mold.
2. Locate core and position block in chuck.
3. Adjust arm weight and speed. Make successive 0.5 mm cuts to obtain the core region.
4. Mount the core sections.

Mounted Whole Otoliths (Section 3.4.2.2)
FMRI

1. Mount whole otolith to slide, concave side down with the long axis parallel to the long side of the slide using thermoplastic.
2. Locate core and position slide in chuck.
3. Adjust arm weight and speed. Make successive 0.5 mm cuts to obtain the core region.
4. Mount the core sections.

High Speed Wafering Saw Techniques

Embedded Whole Otoliths (Section 3.4.2.1)
TPWD

1. Embed the otolith with the long axis parallel to the long axis of the mold.
2. Locate core and position block in chuck.
3. Adjust load (1,000 g) and speed (3,000 rpm). Make successive 0.5 mm cuts to obtain the core region.
4. Mount the core sections.

Thin Section Machine

Free-hand whole otolith sectioning (Section 3.4.3)
LSU, AMRD

1. Firmly grasping both ends of the otolith, make initial cut adjacent to the core.
2. Hand grind additional material until core is visible.
3. Mount otolith half with core on labeled slide.
4. Place slide in chuck and section off remaining material.
5. Place slide into precision grinder arm and adjust caliper to 0.5 mm.

Age Determination

Ageing of black drum is relatively easy since opaque zones are normally very distinct (Figure 5.20). Black drum spawn in the winter at approximately the time of opaque zone formation; therefore, the first distinct opaque

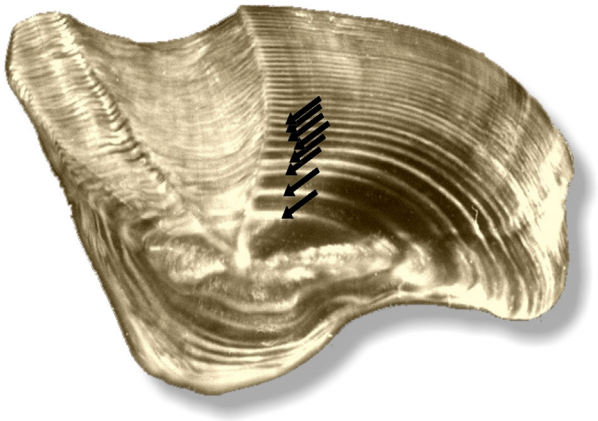


Figure 5.20 Sagittal otolith section of an age-30+ black drum. Arrows indicate the first eight annuli.

mark is deposited when the fish is about one year old (Figure 5.21).

For regional stock assessment purposes, three minimal parameters are recorded: number of rings, presence or absence of opaque ring at margin, and month of capture. Based on these three parameters, cohort and biological ages can be determined.

Other Ageing Methods

Whole black drum otoliths have not been used successfully in the Gulf region and the usefulness of break and burn techniques for black drum has not yet been determined. However, this species may be a good candidate for break and burn.

Scales have been demonstrated to be useful in the first few years only. After age-3 annuli in scales become less consistent and resorption can occur at the core (J. Moran, ASMFC, personal communication).

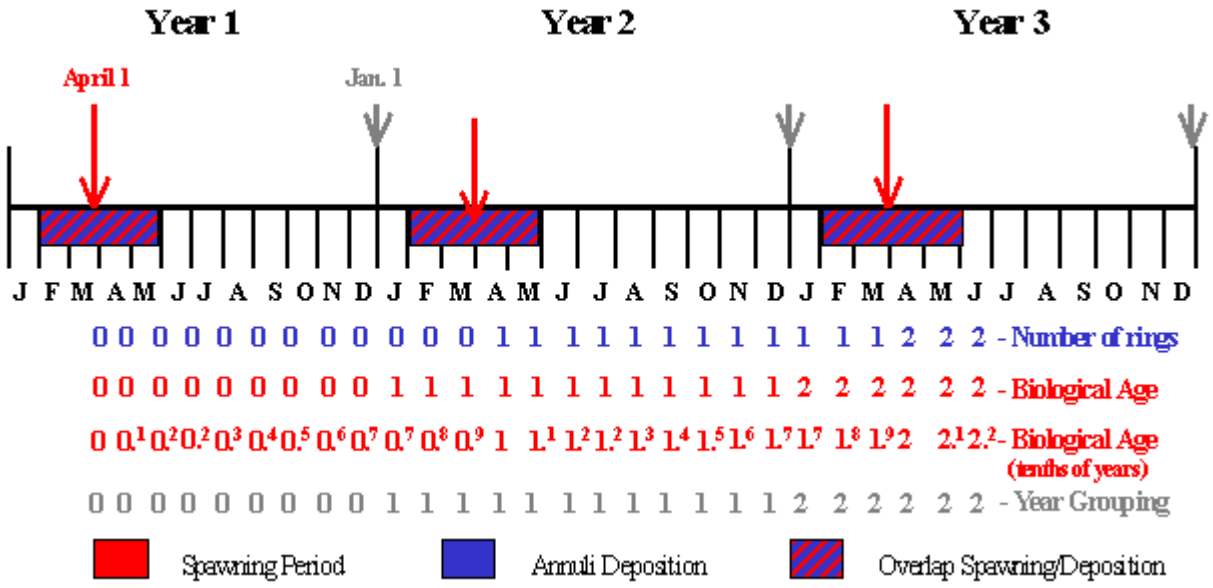
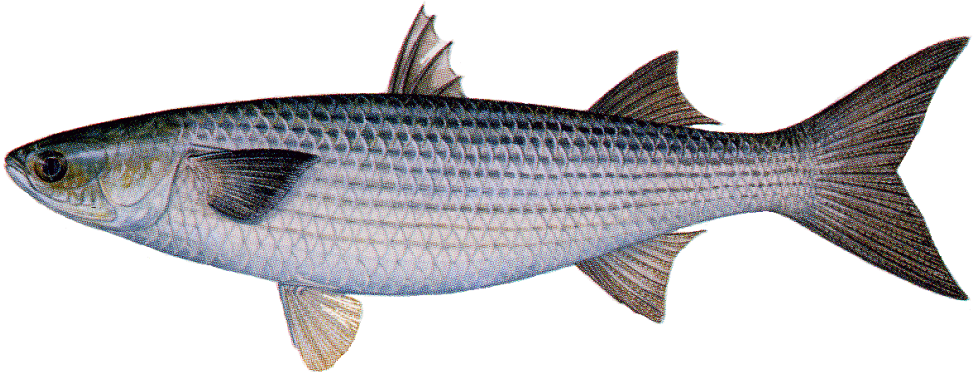


Figure 5.21 Birthdate assignment timeline for black drum. Age and year group based on biological birthdate (April 1), number of rings, and January 1 to December 31 year.

5.4 Striped Mullet *Mugil cephalus*



Highlights

- Otoliths are relatively easy to locate and extract.
- Otoliths are fragile; care must be taken in removal.
- Generally one removal technique practiced.
- Multiple sectioning techniques successful.
- Rings relatively faint but discernable.
- First distinct opaque ring forms at approximately one year of age.
- Generally <8 rings.

Otolith Description

Striped mullet have small, fragile sagittal otoliths, which may break during extraction. The ventral surface is moderately crenate (Figure 5.22). The distal side is concave with the visible core lying in the center of the otolith. The sulcus runs along the proximal dorsal half of the otolith.

Figure 5.22 Sagittal otoliths medial and top view from striped mullet.

The posterior margin is rounded. The location of the otolith in the neurocranium is illustrated in Figure (5.23).

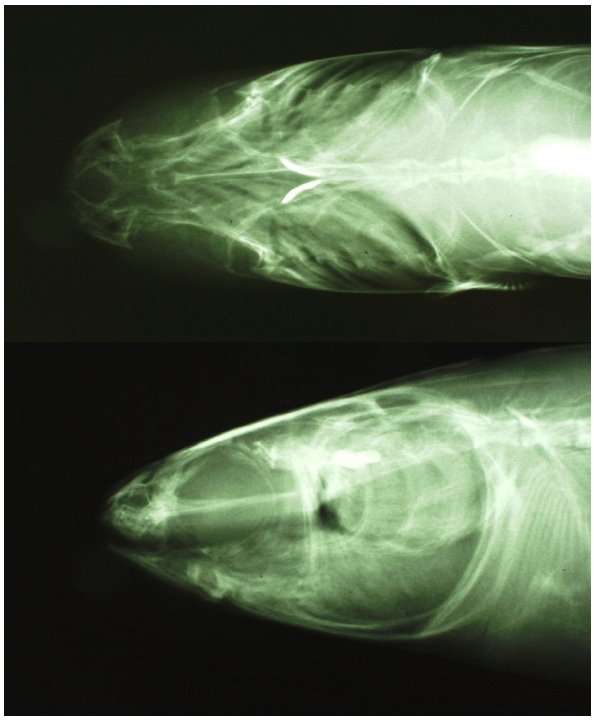


Figure 5.23 Location of striped mullet sagittal otoliths.

Otolith Extraction

Extraction begins by cutting the isthmus of the gill arch with a pair of angled head diagonal wire cutters (Figure 5.24). Next, gills may be

Figure 5.24 Cutting the striped mullet isthmus with wire cutters.

pushed aside or removed while bending the head back (dorsally) (Figure 5.25) and exposing the otic capsule (Figure 5.26). Caution should be taken on smaller specimens (>200 mm), because this action may rupture the otic capsule and expose or expel the sagittal otoliths. Insert a pair of wire cutters or chisel on the posterior section of the otic capsule and pry off the surface (Figure 5.27). Otoliths are small and

Figure 5.25 Striped mullet cranium forced upward exposing the posterior end of the otic capsule.

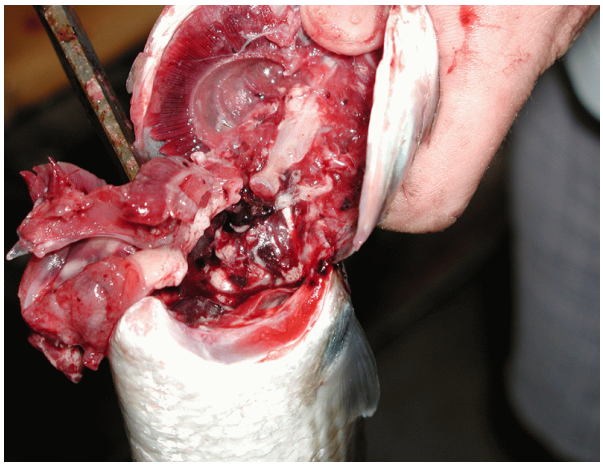


Figure 5.26 Removal of gill arches further exposes the otic capsule.

may become chipped or broken if care is not taken. For example, a striped mullet with a 280 mm fork length has an otolith 9 mm in length and 3 mm at its maximum width. Otoliths are removed with a pair of forceps and then rinsed with water (Figure 5.28). Samples are then dried and placed in coin envelopes or plastic zipper bags with pertinent information recorded on the outside.

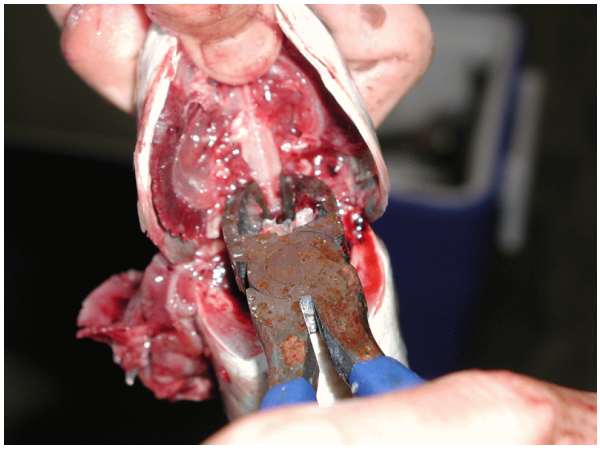


Figure 5.27 opening the otic capsule with wire cutters.

Otolith Processing

Although this species tends to have relatively thin and fragile otoliths, each of the sectioning techniques described in Section 3.0

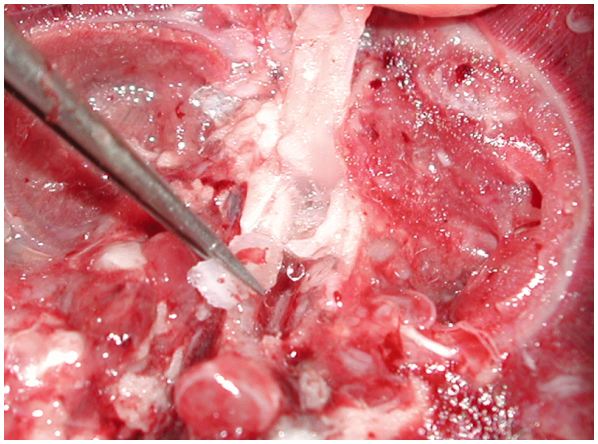


Figure 5.28 Removal of the sagittal otoliths.

can be used with care.

Low Speed Wafering Saw Technique

Embedded Whole Otoliths (Section 3.4.2.1)
LDWF, GCRL, MDMR, FMRI

1. Embed the otolith with the long axis parallel to the long axis of the mold.
2. Locate core and position block in chuck.
3. Adjust arm weight and speed. Make successive 0.5 mm cuts to obtain the core region.
4. Mount the core sections.

Mounted Whole Otoliths (Section 3.4.2.2)
FMRI

1. Mount whole otolith to slide, concave side down with the long axis parallel to the long side of the slide using thermoplastic.
2. Locate core and position slide in chuck.
3. Adjust arm weight and speed. Make successive 0.5 mm cuts to obtain the core region.
4. Mount the core sections.

High Speed Wafering Saw Technique

Embedded Whole Otoliths (Section 3.4.2.1)
TPWD

1. Embed the otolith with the long axis parallel to the long axis of the mold.
2. Locate core and position block in chuck.
3. Adjust load (1,000 g) and speed (3,000 rpm). Make successive 0.5 mm cuts to obtain the core region.
4. Mount the core sections.

Thin Section Machine

Free-Hand Whole Otolith Sectioning (Section 3.4.3)
LSU, AMRD

Note: Only use the grinder on small/fragile otoliths.

1. Firmly grasping the posterior end of the otolith, grind material until adjacent to the core.
2. Mount otolith half with core on labeled slide.
3. Holding slide in hand, grind down remaining material to approximately 1mm.
4. Place slide into precision grinder arm and adjust caliper to 0.5 mm.

Age Determination

Annuli in striped mullet are recognizable along the sulcus (Figure 5.29). The otolith radius and annuli are measured from the core at the base of the sulcal groove along a medial line adjacent to the sulcal groove. Striped mullet in the Gulf of Mexico are spawned around November and subsequently deposit a large opaque region around the core through

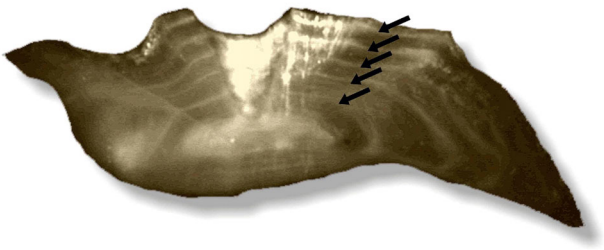


Figure 5.29 Sagittal otolith section of age-5 striped mullet. Black arrows indicate annuli. Note that the large opaque core is not counted.

February (Figure 5.30). This may be regarded as the first winter mark. The second winter mark or first true annulus is generally located further from the core, because it is deposited when the fish are approximately 12 - 14 months of age. Illuminated from below, the opaque rings in the section are relatively well defined.

Scales were originally used for mullet ageing from the 1950s and have been used through the 1970s. Ibanez-Aguirre and Gallardo-Cabello (1996) compared scales and otoliths for ageing purposes and reported that scales could be used for young ages, but otoliths provided better resolution for the older age classes.

Other Ageing Methods

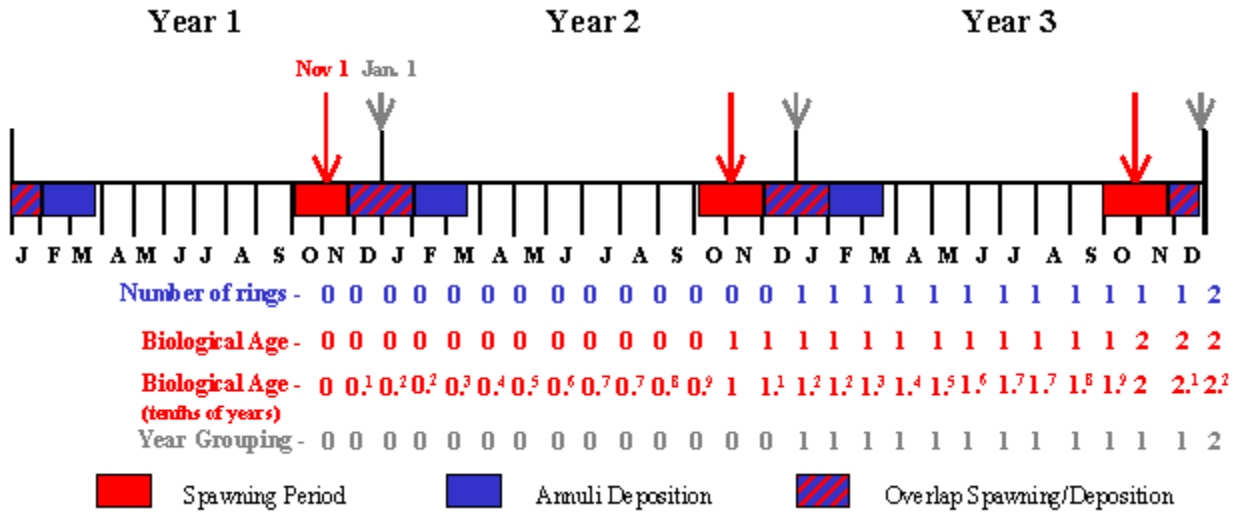


Figure 5.30 Birthdate assignment time line for striped mullet. Age and year group based on biological birthdate (November 1), number of rings, and January 1 to December 31 year.

5.5 Southern Flounder *Paralichthys lethostigma*

Highlights

- Otoliths small, fragile, and comparatively difficult to locate and extract.
- Otolith pairs asymmetrical to each other.
- Left otolith recommended for sectioning.
- Multiple sectioning techniques successful.
- Rings discernable.
- First distinct opaque ring forms at approximately one year of age.
- Differential growth in males and females.
- Maximum validated age of eight years.

Otolith Description

Southern flounder sagittal otoliths have a flat arrowhead shape. As in numerous flatfish, southern flounder display morphological differences between right and left saggitae (Figure 5.31). The core of the left otolith is located more posterior to center. Therefore, consistent use of the right or left otolith is recommended for ageing. The location of the otolith in the neurocranium is illustrated in Figure (5.32).

Figure 5.31 Left sagittal otolith medial and top view from southern flounder.

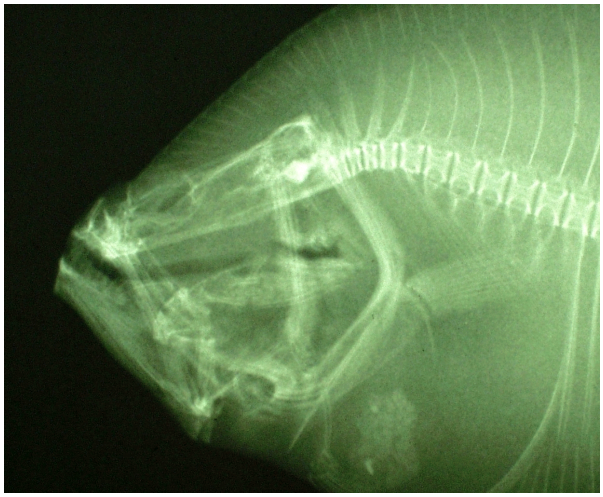


Figure 5.32 Location of southern flounder sagittal otoliths.

Extraction

Sagittal otoliths can be removed from southern flounder in two ways.

Top Method

This method requires the removal of the top of the neurocranium. The technique is the same as that used for other species even with the flounder's unusual anatomy (Figure 5.33).

1. Make a horizontal cut (parallel to the lateral line) just above the eye, back to the preopercle.
2. A vertical (dorsal) cut is then made intersecting with the first cut removing a triangular section of the fish's head, exposing the otic capsule and the otoliths within.
3. Right and left otoliths are easily removed with forceps.

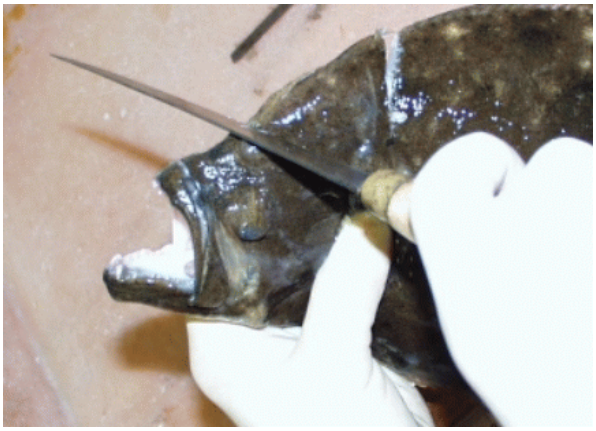


Figure 5.33 Pop-the-top method for otolith removal in southern flounder.

Bottom Method

This method requires going through the gill cavity and is preferred when sampling a commercial catch as it minimizes visible damage to the fish.

1. Pull open the left gill cavity exposing the gills.
2. Using a chisel, scrape the gills back to expose the otic capsule (Figure 5.34).
3. Chisel away the otic capsule to expose the otolith.
4. Remove the left otolith with a forceps.
5. Repeat steps on right side.

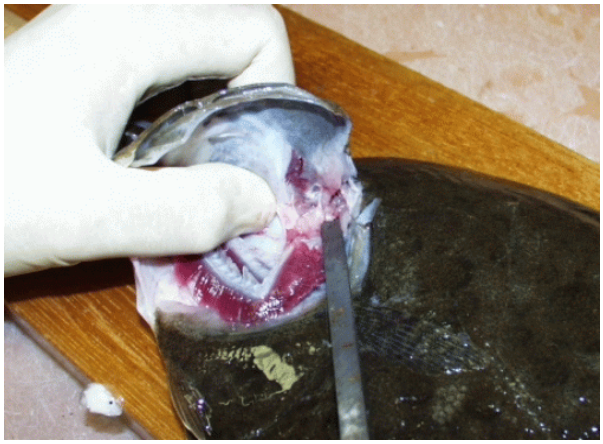


Figure 5.34 Otolith removal from a southern flounder through the operculum.

Otolith Processing

Due to the small size of southern flounder otoliths, the technique of sectioning whole embedded otoliths appears to provide the highest quality sections. Because of the differences in the left and right sagitta, it is suggested that the left be used for sectioning and the right catalogued and stored for possible future use. Southern flounder otoliths should be cross-sectioned at a thickness of approximately 0.5 mm to obtain the best results.

Low Speed Wafering Saw Techniques

Embedded whole otoliths (Section 3.4.2.1)

1. Embed the otolith with the anterior/posterior axis parallel to the long axis of the mold.
2. Locate core and position block in chuck.
3. Adjust arm weight and speed. Make successive 0.5 mm cuts to obtain the core region.

4. Mount the core section and label appropriately.

Mounted Whole Otoliths (Section 3.4.2.2)
FMRI

1. Mount whole otolith to slide, concave side down with the long axis parallel to the long side of the slide using thermoplastic.
2. Locate core and position slide in chuck.
3. Adjust arm weight (50-75 g) and speed (8-10). Make successive 0.5 mm cuts to obtain the core region.
4. Mount the core sections.

<u>High Speed Wafering Saw Techniques</u>

Embedded Whole Otoliths (Section 3.4.2.1)
TPWD

1. Embed the otolith with the long axis parallel to the long axis of the mold.
2. Locate core and position block in chuck.
3. Adjust load (1,000 g) and speed (3,000 rpm). Make successive 0.5 mm cuts to obtain the core region.
4. Mount the core sections.

<u>Thin Sectioning Machine</u>

Free-Hand Whole Otolith Sectioning (Section 3.4.3)
LSU, AMRD

Note: Only use the grinder on small/fragile otoliths.

1. Firmly grasping the posterior end of the otolith, grind material until core is visible.
2. Mount otolith half with core on labeled slide.
3. Holding slide in hand, section off remaining material.
4. Place slide into precision grinder arm and adjust caliper to 0.5 mm.

Age Determination

Opaque increments are easily distinguishable on both the dorsal and ventral sides of the sulcus in southern flounder otolith cross-sections (Figure 5.35) as spawning and annulus deposition overlap for the most part (Figure 5.36). Ages are assigned based on opaque increment count and edge condition recorded as opaque or translucent using the criteria of Beckman et al. (1991) and on a birth date of January 1 (Wenner et al. 1990).

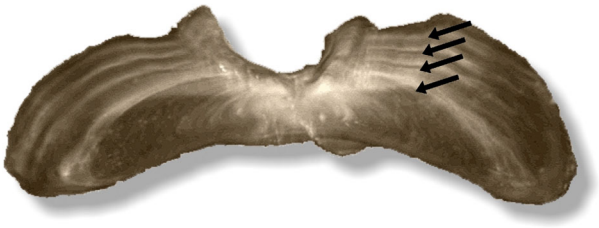


Figure 5.35 Sagittal otolith section from an age-4 southern flounder. Black arrows indicate annuli.

Other Ageing Methods

Whole otoliths – Fitzhugh (personal communication) indicates that young southern flounder (age-0 to age-4) may yield good ages when read whole but cautioned that corroboration with sectioned otoliths must be completed. MacNair et al. (2001) and Sipe and Chittenden (2001) both concluded that whole otolith ageing was adequate for young fish (to age-14 in California halibut, *Paralichthys californicus*, and age-4 in summer flounder, *Paralichthys dentatus*). Both of these studies compared whole otolith ages to sectioned ages in these two species of paralichthids.

Flounder otoliths may be too fragile and thin to achieve acceptable results using the break and burn technique.

Flounder scales were unsatisfactory for age determination due to a lack of consistent markings (Palko 1984).

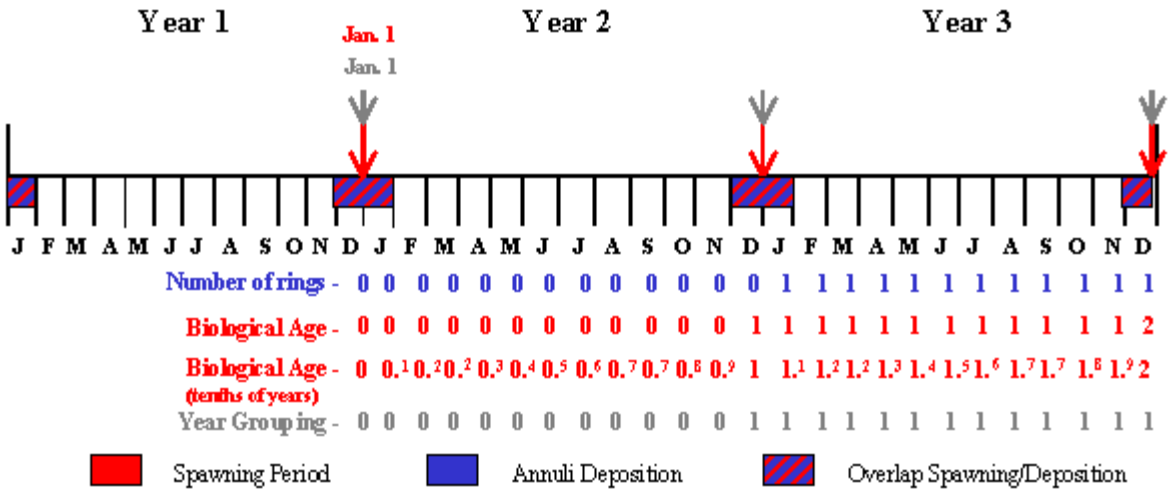
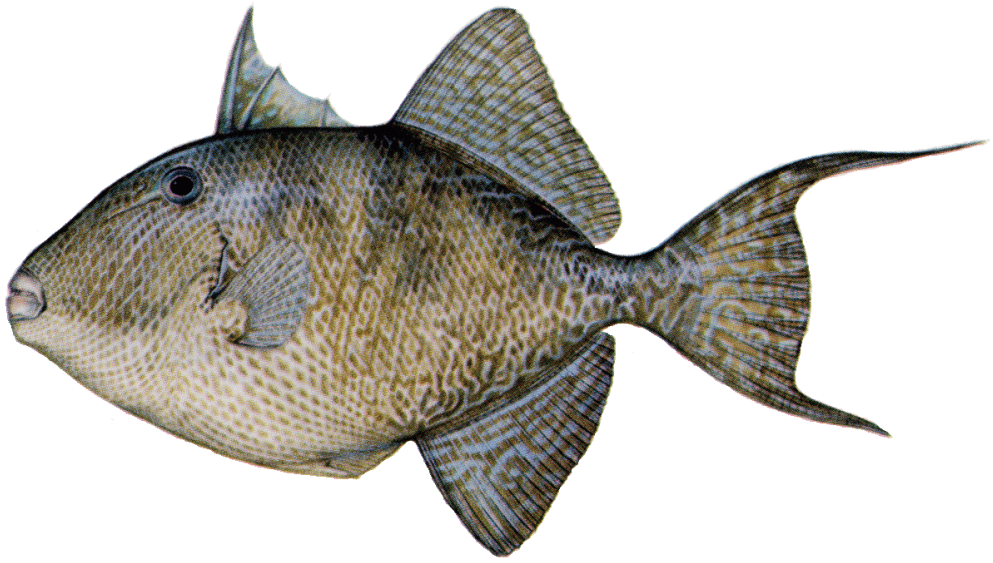


Figure 5.36 Birthdate assignment timeline for southern flounder. Age and year group based on biological birthdate (January 1), number of rings, and January 1 to December 31 year.

5.6 Gray Triggerfish *Balistes capriscus*



Highlights

- Otoliths very small and nearly impossible to locate.
- First dorsal spine commonly used for ageing.
- Spine stored frozen due to vascularization.
- False annuli can occur.
- Embedding not required.
- Focus deterioration in older fish can result in loss of early annuli.

Otolith Description

The otoliths of the gray triggerfish change their direction of accretion over time and do not contain annual marks (Ofori-Danson 1989, Johnson and Saloman 1984, Escorriola 1991, Wilson et al. 1995, Hood and Johnson 1997). In addition, the relative small size of the otoliths make them nearly impossible to extract. Therefore, estimates of age and growth in gray triggerfish have been reported by numerous scientists using annuli evident in the first dorsal spine rather than using otoliths. The location of the otolith in the neurocranium is illustrated in Figure 5.37.

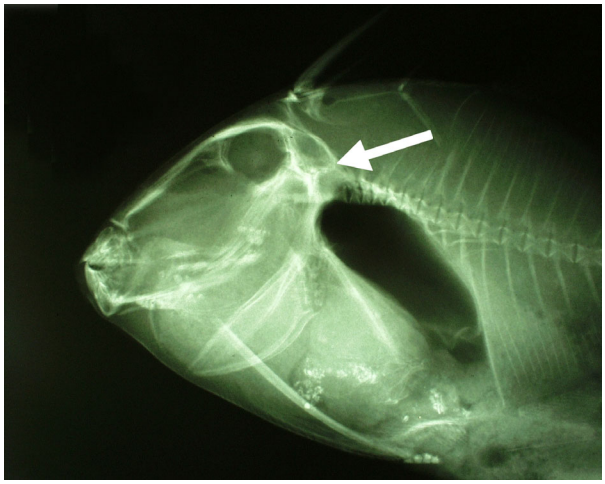


Figure 5.37 Relative location of the sagittal otoliths in a gray triggerfish.

Spine Extraction

Removal of dorsal spines from gray triggerfish is relatively straightforward and can be applied to many species. See Section 3.5.3 for a detailed description of the following methodologies. **Note: Due to the fact that spines are vascularized, failure to freeze spines will result in rapid deterioration!**

1. Cut the membrane between the first and second dorsal spine toward the joint (Figure 5.38, line A).
2. After the membrane is cut, insert the knife into the condyle socket behind the first dorsal spine, and remove any connective tissue holding the spine in place.
3. Applying pressure to the spine, pull it forward until it 'pops' out of the socket (Figure 5.38, line B).
4. Cut any remaining skin separating the spine from the fish.
5. Place the spine in a small, labeled envelope and store in a freezer **until ready to section.**

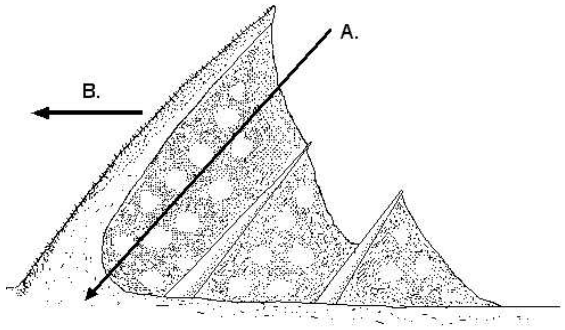


Figure 5.38 A) Cutting plane and B) direction of pull for removal of the first dorsal spine in gray triggerfish.

Spine Processing

As noted in Section 3.5.3, a modified combination of methods can be used to process the first dorsal spine of gray triggerfish. In order to ensure a definitive margin on the posterior lobes, remove the skin from between and covering the lobes. This will enable the production of a section with a smooth, readable, and measurable margin. Two techniques have been used in the Gulf for this species on both the high speed and low speed wafering saws, although any saw should suffice.

Thin Section Machine (Section 3.5.3.1)
LSU, AMRD

1. Cut the dorsal spine above the condyle freehand.
2. Adhere the distal portion of the spine to a slide on the cut edge.
3. Mount slide in chuck and cut remaining spine leaving a section adhered to slide.
4. Adjust thickness of section on the grinding wheel.

Low Speed Wafering Saw (Section 3.5.3.2)
FMRI

1. Adhere spine to slide attaching only the ends with thermoplastic.
2. Place slide in chuck and make successive 0.5 mm cuts.
3. Adhere sections to slide.

Age Determination

The summer and winter growth zones in a gray triggerfish spine section are translucent and opaque, respectively, opposite the pattern found in an otolith. These annuli radiate outward from the focus. The focus in a spine section is the main channel of vascularization for the spine. The spine radius is measured as the distance from the focus to the margin of one of the posterior lobes, as seen in Figure 5.39.

There are several occurrences of pseudoannuli or "false annuli" in gray

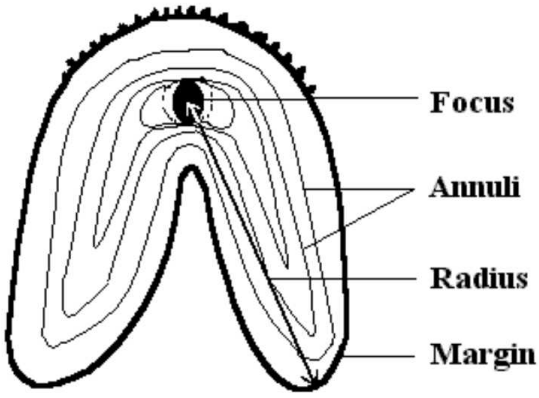


Figure 5.39 Generalized cross section of dorsal spine.

triggerfish spines (Figure 5.40). False annuli associated with checks and zones that are somewhat incomplete and irregular are usually found only in one part of the structure and often not in all structures. Although they are sometimes prominent, they are not associated with the growth zone that forms during the principal annual cessation or reduction in growth that produces the annulus (Casselman 1983). This problem can be corrected with the validation of the hard part. Although the cause is not known, it is believed they may be related to both larval settlement (false annuli near the

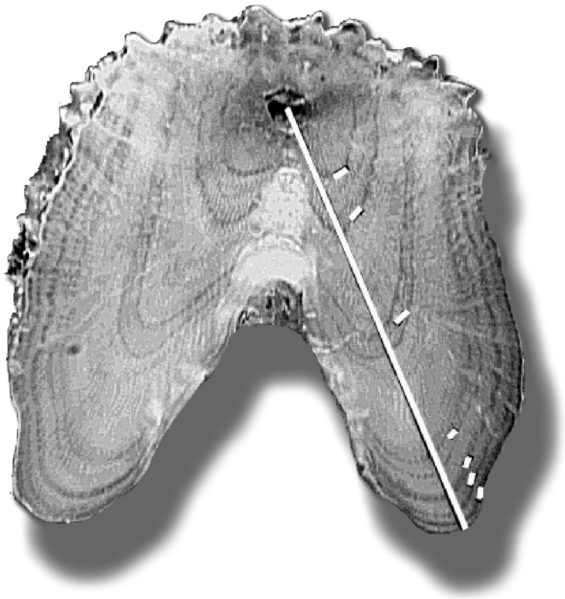


Figure 5.40 Cross section of an age-7 gray triggerfish spine indicating the core, radius, and annuli. False annuli occur where two annuli appear with a single dash.

focus) and adult spawning events (midsummer) (Ingram 2001). In addition, the first dorsal spine core can undergo resorption and become more vascularized, obscuring and even eliminating the first few zones in older fish (Figure 5.41) resulting in an underestimation of age (Casselman 1983).

After enumeration of the true annuli, estimate the biological age of the gray

Figure 5.41 Deterioration of the core region in the first dorsal spine of an old gray triggerfish.

triggerfish by adjusting for a June-July spawning date in the northern Gulf of Mexico (Wilson et al. 1995, Ingram et al. in prep); adjusting for an annulus formation date between January and April (Wilson et al. 1995) and adjusting for the date of capture (Figure 5.42).

For regional stock assessment purposes, three minimal parameters are recorded: number of rings, presence or absence of opaque ring at the margin, and month of capture. Based on these three parameters, cohort and biological ages can be determined.

Alternative Techniques

Since otoliths are not used to age gray triggerfish, break and burn would not be a useful alternative.

Scales have not been used in this species successfully due to the strong insertion of the scales into the triggerfish's tough skin (G.W. Ingram personal communication).

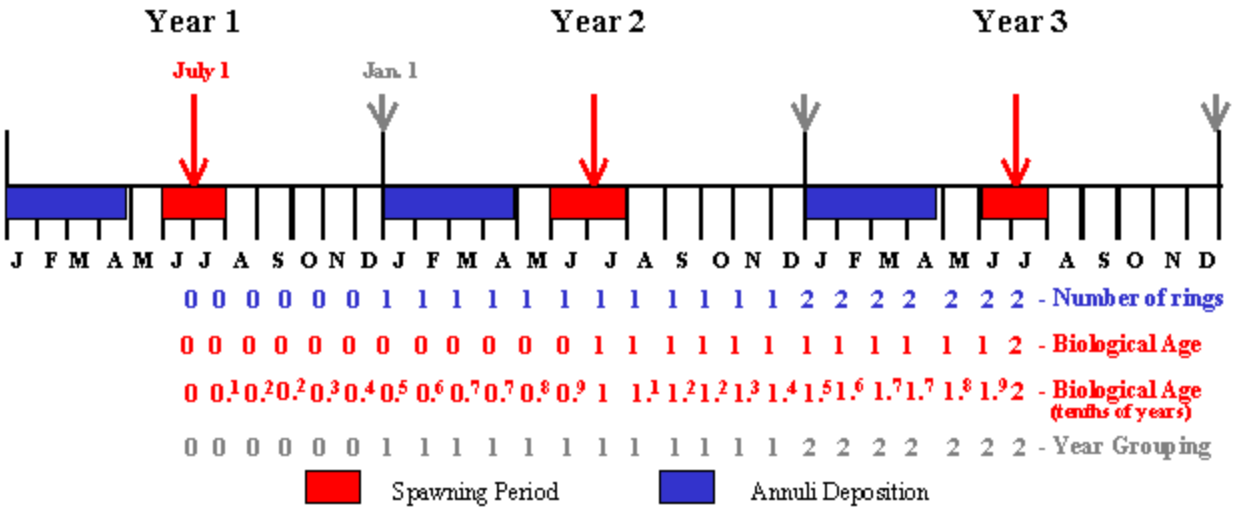
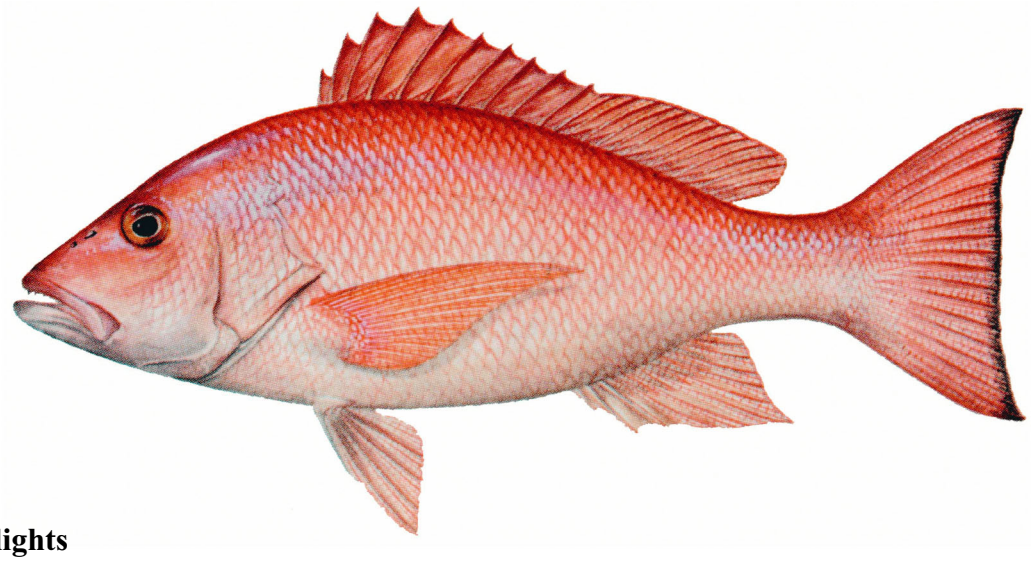


Figure 5.42 Birthdate assignment timeline for gray triggerfish. Age and year group based on biological birthdate (July 1), number of rings, and January 1 to December 31 year.

5.7 Red Snapper *Lutjanus campechanus*



Highlights

- Otoliths are ovate, laterally compressed.
- Otoliths are relatively easy to locate and extract.
- First increment can appear diffuse and difficult to discern.
- Opaque increment enumeration becomes increasingly difficult in older individuals.

Otolith Description

Red snapper otoliths (sagittae) are large, ovate, laterally compressed, and exhibit an indented sulcus on the proximal surface (Figure 5.43). The rostrum and anterostrum are distinguishable and quite fragile. The location of the sagittae in the neurocranium is illustrated in Figure 5.44.

Figure 5.43 Medial view of red snapper sagittal otolith.

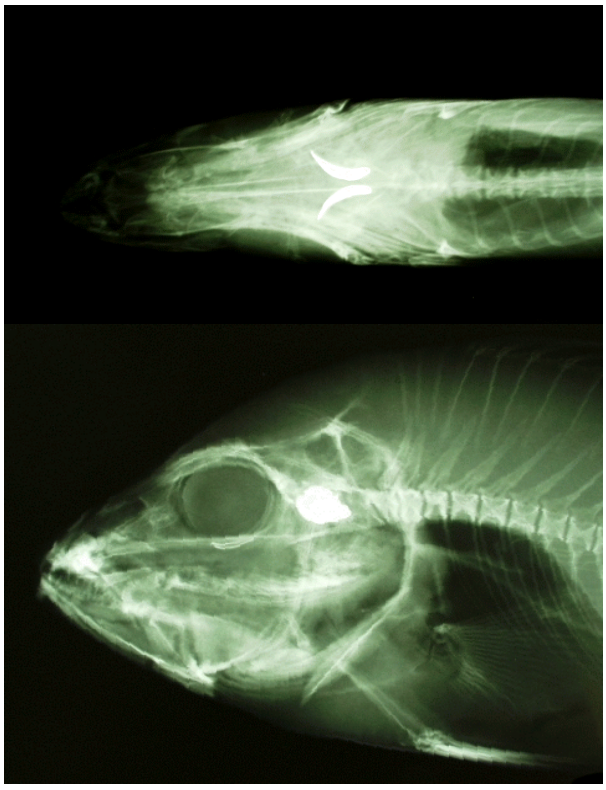


Figure 5.44 Location of sagittal otoliths in red snapper.

Extraction

Red snapper otoliths may break during contact with certain extraction tools. The otic capsule in red snapper is located near the posterior base of the skull behind the gills. The surface of the otic capsule is convex and easily discernible once the gills have been removed or scraped back. The capsule surface is fairly thin, can appear transparent, and is relatively easy to chisel away.

Bottom Method

The method of otolith extraction through the gill cavity is preferred when sampling a commercial catch intended for market as it minimizes visible damage to the fish.

1. Pull open the opercle to expose the gills.
2. Pull the gill arches back to expose the otic capsule.
3. Carefully chisel away the otic capsule to expose the sagitta (Figure 5.45).
4. Remove the otolith.
5. Repeat for the other side.

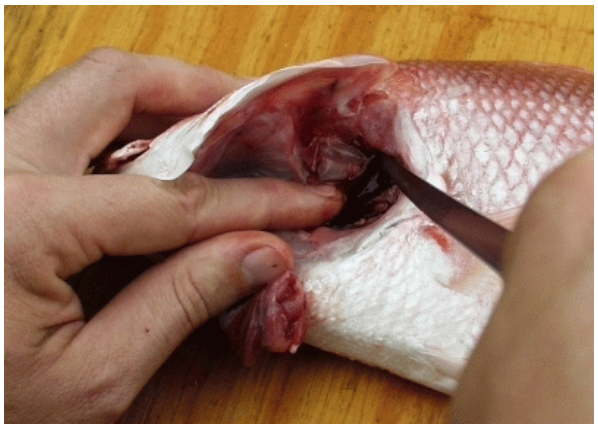


Figure 5.45 Removal of red snapper otolith through the operculum.

Top Methods

Smaller Fish

1. Make a cut from the back of the skull to a point below and behind the eyesocket exposing the brain (Figure 5.46).
2. Remove brain to reveal the otoliths.
3. Remove the sagittal otoliths.

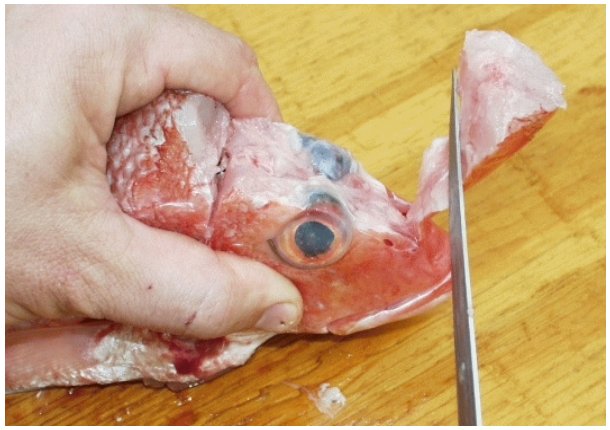


Figure 5.46 Extraction of red snapper otoliths through the top of the neurocranium.

Larger Fish

1. Make a vertical cut in the skull at a point just behind the centerline of the opercle through the otic capsule.
2. Bend the head of the fish forward to reveal the sagittae (Figure 5.47).
3. Remove the sagittal otoliths.

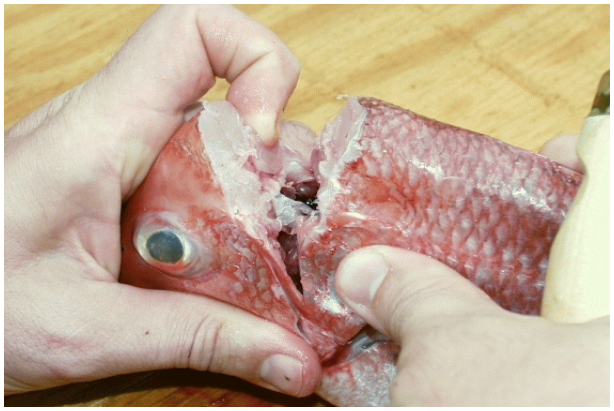


Figure 5.47 Meatsaw technique for extraction of otoliths from red snapper.

Processing

Due to the relatively large size of red snapper otoliths, multiple processing techniques

are acceptable. The technique chosen will likely reflect available equipment. Generally red snapper sections are processed at approximately 0.5 mm. The following techniques have been used throughout the Gulf.

Low Speed Wafering Saw Technique

Embedded Whole Otoliths (Section 3.4.2.1)

1. Embed the otolith with the long axis (anterior-posterior axis) parallel to the long axis of the mold.
2. Locate the core and position block in chuck.
3. Adjust arm weight (50-75 g) and speed (8-10). Make successive 0.5 mm cuts to obtain the core region.
4. Mount the core sections onto slides.

Thin Section Machine

Free-Hand Whole Otolith Sectioning (Section 3.4.3)

1. Firmly grasping both ends of the otolith, make initial cut adjacent to the core.
2. Hand grind additional material until core is visible.
3. Mount otolith half with core on labeled slide.
4. Place slide in chuck and section off remaining material.
5. Place slide into precision grinder arm and adjust caliper to 0.5 mm.

Age Determination

Enumeration of annuli in red snapper otolith sections can be challenging to inexperienced personnel. The problem encountered most often by readers is determining the position of the presumptive first opaque increment nearest the core (Figure 5.48). Due to a protracted spawning season (early May through late September) (Figure 5.49), there is assumed to

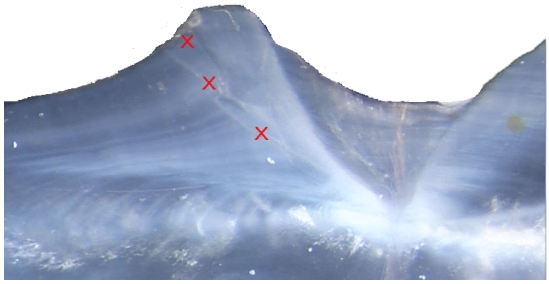


Figure 5.48 Section from the sagittal otolith of an age-3 red snapper showing first annuli as a diffuse opaque zone (reflected light).

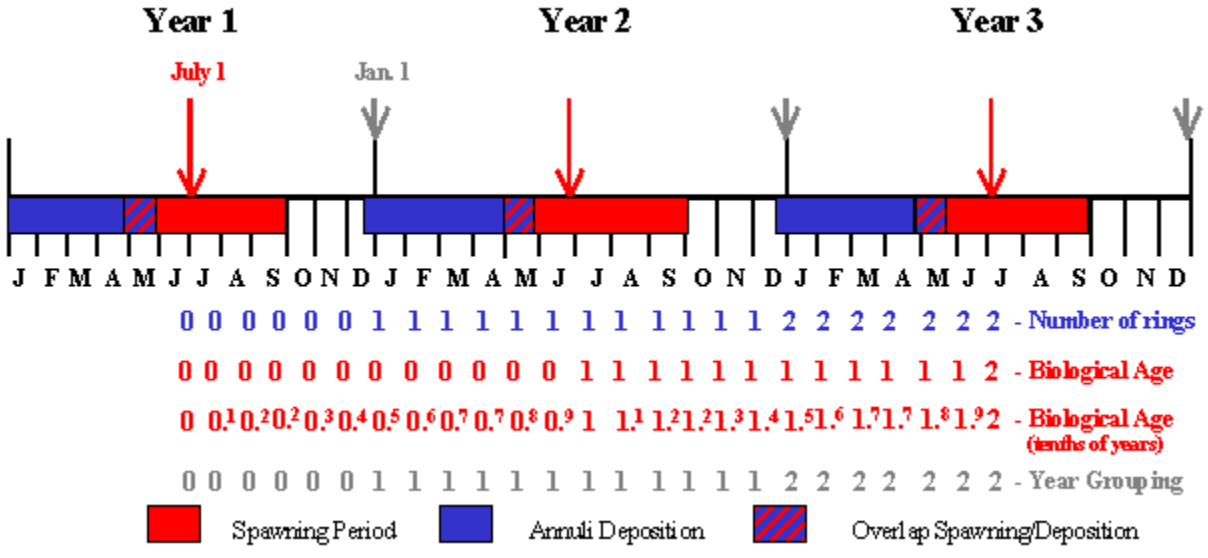


Figure 5.49 Birthdate assignment timeline for red snapper. Age and year group based on biological birthdate (July 1), number of rings, and January 1 to December 31 year.

be considerable variation in the distance from the core to the first opaque increment, which can appear as a diffuse "smudge." The increment may appear adjacent to the core region if the individual was spawned in the fall (Figure 5.50A) or may appear as an annuli outside the core if an individual was spawned in early summer (Figure 5.50B). The longevity of the species also increases the difficulty in obtaining accurate age estimates of older individuals. After age-10, red snapper somatic

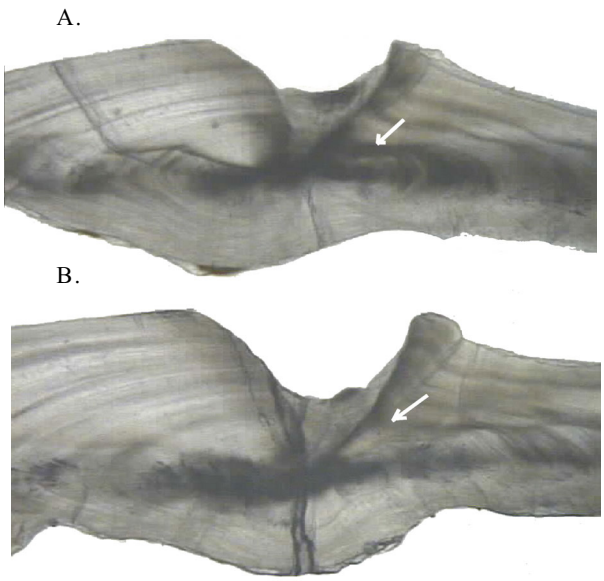


Figure 5.50 Transverse sagittal otolith sections of A) fall spawned and B) summer spawned red snapper (arrows indicate position of 1st increment).

growth slows dramatically and is reflected by a decrease in the accretion rate in the otolith. The opaque rings will appear much closer together with distance from the otolith core (Figure 5.51).

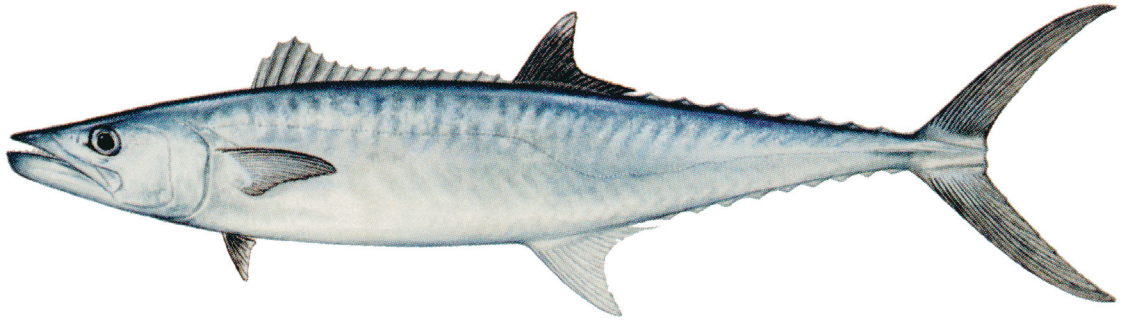
Figure 5.51 Transverse section of sagittal otolith from an age-52 red snapper.

Other Ageing Methods

Bomb radiocarbon is a recent technique used to validate otolith age, which utilizes the increase in oceanic ^{14}C resulting from atmospheric testing of nuclear bombs during the 1950s and 1960s. Otolith section ages were validated through accelerator mass spectrometry analysis of bomb-produced ^{14}C in red snapper otoliths hatched before, during, and after the nuclear testing periods (Baker and Wilson 2001).

Break and burn has not been attempted on this species in the Gulf. Whole otoliths have not been used with any success. Scales have been unsuccessful after the first few years of age.

5.8 King Mackerel *Scomberomoros cavalla*



Highlights

- Otoliths are elongate, laterally compressed.
- Otoliths relatively easy to locate and remove.
- First ring may resemble a diffuse "smudge" in section.
- Whole otoliths can be successfully aged up to age-6.
- Rings in sectioned otoliths are usually distinct in older fish.

Otolith description

King mackerel sagittae are small, elongate, laterally compressed, and have an indented sulcus on the medial side (Figure 5.52). The rostrum and antirostrum are easily distinguishable and extremely fragile. The location of the otolith is illustrated in Figure 5.53.

Figure 5.52 Medial view of king mackerel sagittal otolith.

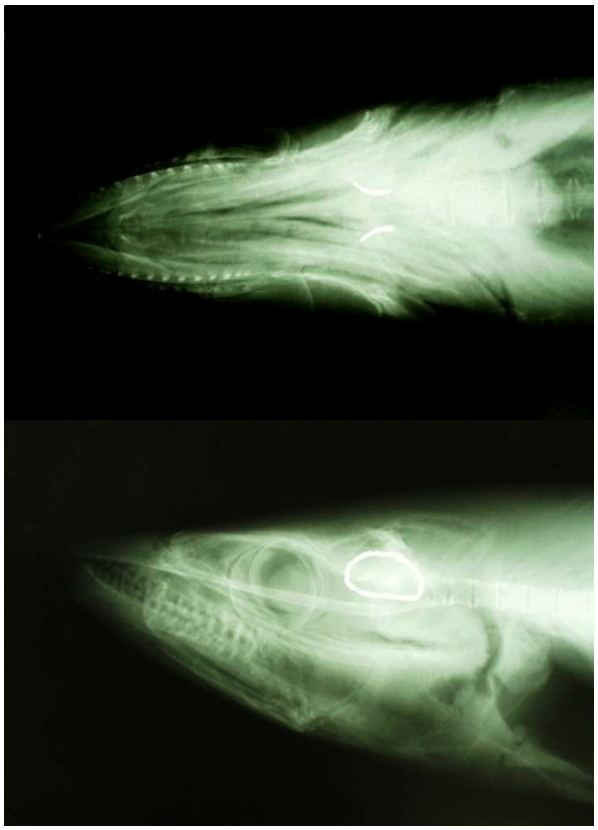


Figure 5.53 Location of the sagittal otoliths in king mackerel.

Extraction

Otolith removal in king mackerel is relatively easy; therefore, any of the techniques illustrated in Section 3.1 can be used. Due to the fishes size, the meatsaw technique is

recommended when the condition of the head is not important. The otic capsule in king mackerel is located near the posterior base of the skull behind the gills. The surface of the otic capsule is convex and easily discernible once the gills have been removed or scraped back. The capsule surface is fairly thin, can appear transparent, and is relatively easy to chisel away.

Bottom Method

The method of otolith extraction through the gill cavity is preferred when sampling a commercial catch intended for market as it minimizes visible damage to the fish.

1. Pull open the opercle to expose the gills.
2. Pull the gill arches back to expose the otic capsule.
3. Carefully chisel away the otic capsule to expose the sagitta (Figure 5.54).
4. Remove the otolith.
5. Repeat for the other side.

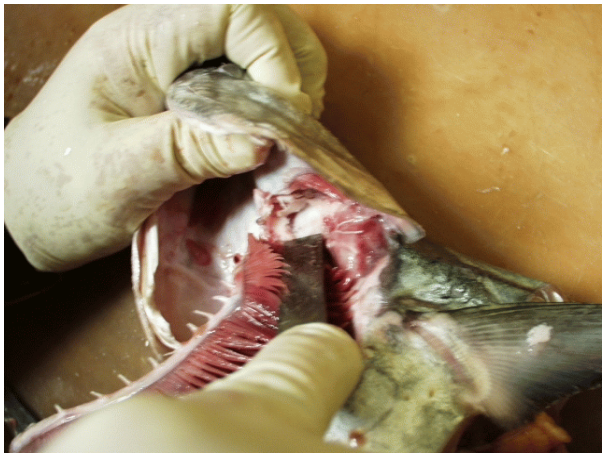


Figure 5.54 Removal of king mackerel otolith through under the operculum.

Top Methods

Smaller Fish

1. Make a cut from the back of the skull to a point below and behind the eyesocket exposing the brain (Figure 5.55).
2. Remove brain to reveal the otoliths.
3. Remove the sagittal otoliths.

Larger Fish

1. Make a vertical cut in the skull at a point just behind the centerline of the opercle through the otic capsule.

Figure 5.55 Extraction of king mackerel otoliths through the top of the neurocranium.

2. Bend the head of the fish forward to reveal the sagittae (Figure 5.56).
3. Remove the sagittal otoliths.

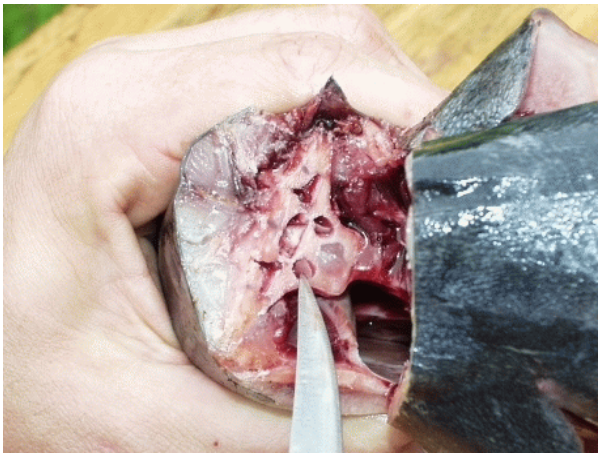


Figure 5.56 Meatsaw technique for extraction of otoliths from king mackerel.

Processing

Sectioning preparation typically consists of

embedding the otoliths in bullet molds (Section 3.3.1.3). In the Gulf, the primary sectioning apparatus used is the low speed saw although the thin sectioning machine has also been used successfully. It should be noted that the NMFS Panama City Laboratory strongly recommends the use of the low speed saw for small otoliths such as the mackerels and suggests a comparison of the results from both types of saw before making a long-term equipment choice. For very young fish the otoliths can be read whole (see age determination below).

Low Speed Wafering Saw Techniques

Embedded Whole Otoliths (Section 3.4.2.1)
NMFS

1. Embed the otolith with the long axis parallel to the long axis of the mold.
2. Locate core and position block in chuck.
3. Adjust arm weight and speed. Make successive 0.5 mm cuts to obtain the core region.
4. Mount the core sections.

Thin Section Machine

Free-Hand Whole Otolith Sectioning (Section 3.4.3)
LSU, AMRD

Note: Only use the grinder on small/fragile otoliths.

1. Firmly grasping the posterior end of the otolith, grind material until adjacent to the core.
2. Mount otolith half with core on labeled slide.
3. Holding slide in hand, grind down remaining material to approximately 1 mm.
4. Place slide into precision grinder arm and adjust caliper to 0.5 mm.

Age Determination

Whole Otoliths (Section 3.5.5)
NMFS

With few exceptions, small king mackerel up to age-4 are much easier to age using whole otoliths. A good rule of thumb is to use whole otoliths to age males <80 cm FL and females <90 cm FL. The following is a brief methodology for ageing king mackerel using whole otoliths.

1. Place otolith, distal or concave side up, in watch-glass with water.
2. Use a dark stage and reflected light (preferably a fiber optic light) to view otolith.
3. Annuli are read on the distal side of the posterior half of the otolith; those in the corner formed by the posterior and ventral edges are often the easiest to identify.
4. Readability can almost always be improved by rotating the watch-glass and adjusting the angle and intensity of the light. Try illuminating the otolith through the side of the watch-glass if you have a fiber optic light.
5. Changing magnification, especially lowering it, will also improve readability on some otoliths.
6. Examine both left and right otoliths if available, as they often vary in readability.

In most cases the distance from the core to the first annulus will be much larger than all subsequent increments, although the increment between the first and second annuli will sometimes be quite large as well (Figure 5.57). If a whole otolith from a small fish seems especially difficult to read, try sectioning it, as occasionally the section will be more readable than the whole otolith, even in younger fish.

Ageing Sections

Annuli in sectioned king mackerel otoliths

Figure 5.57 Whole otolith from an age-2 king mackerel.

are almost always most readable in the dorsal portion, especially along the sulcal groove. With transmitted light and a compound microscope, all annuli except the first appear as fairly narrow dark marks (Figure 5.58). The first annulus is almost always the most difficult to identify, as it is often just a broad, diffuse

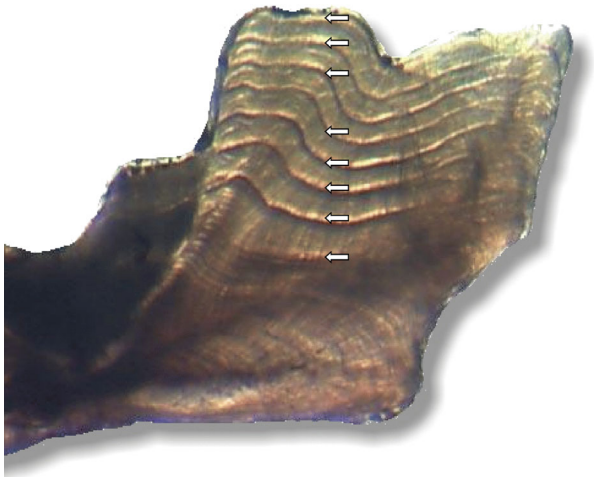


Figure 5.58 Otolith from an age-8 year-old king mackerel sectioned on a low-speed saw.

dark band. This first annulus sometimes is more apparent on the ventral portion of the otolith, even if subsequent annuli are not, so it always pays to examine that area if it is not clear on the dorsal end. One other time when the ventral portion should be examined is when the sectioned fish is very young (i.e., two or three) as sometimes the annuli will be clearer there than on the dorsal portion. A common phenomenon in king mackerel otolith sections is for annuli to appear as doublets or couplets, which can lead to significant overageing problems if one is not careful. Adjusting the focus often helps resolve
this problem. Another characteristic of these sections is that after the second or third annulus, the growth increments are almost always quite uniform in size, with little or no decrease in size with increasing age. Because of this trait, ageing older fish is no more difficult than ageing younger ones and suggests that otolith growth and fish growth seem to become decoupled in king mackerel at a fairly young age. Two techniques which may improve readability are using a polarizing filter and flipping the slide over on the microscope stage (this can make a big difference). If a section is very difficult to read and the fish is close to the minimum size for sectioning, examine the remaining otolith whole if available. Measuring increment distances from the core is somewhat problematic because the axis of growth in the otolith changes after the first ring is formed. Age determination in king mackerel is further complicated by its protracted spawning period (Figure 5.59) – May through October in the northern Gulf (Finucane et al. 1986) with a peak in September (Grimes et al. 1990). Annulus deposition occurs from March to May (Beaumariage 1973, Johnson et al. 1983). The oldest king mackerel aged to date was 26 years old (DeVries and Grimes1997).

Other Ageing Methods

Break and burn is not recommended for this species due to the sagittal otoliths small size. Currently spines and other hard parts have not been attempted for this species, and no information exists on the use of scales for ageing king mackerel.

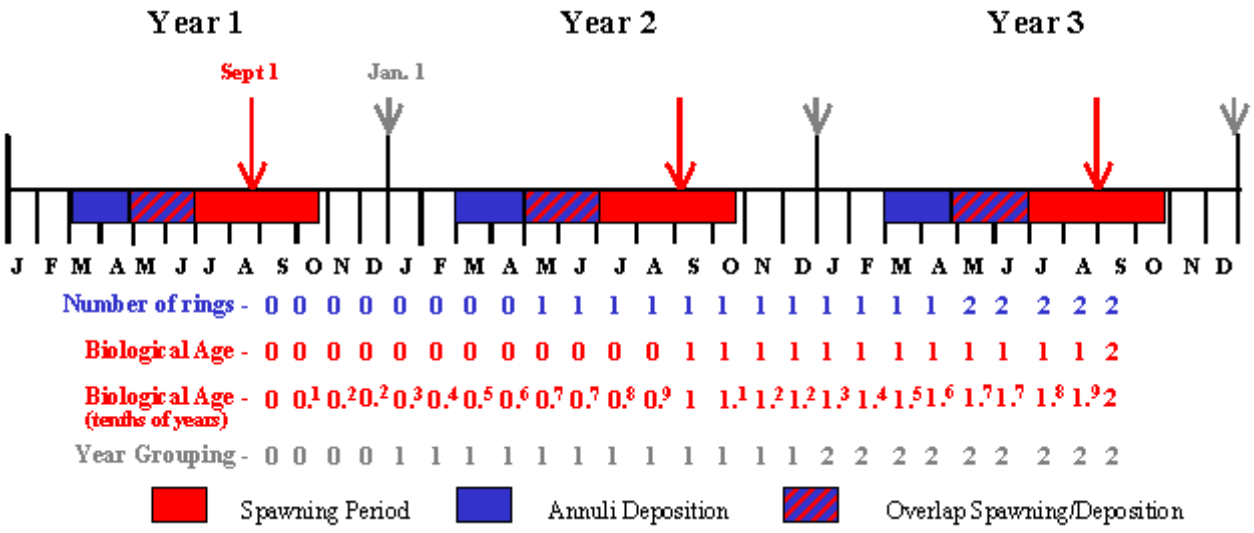
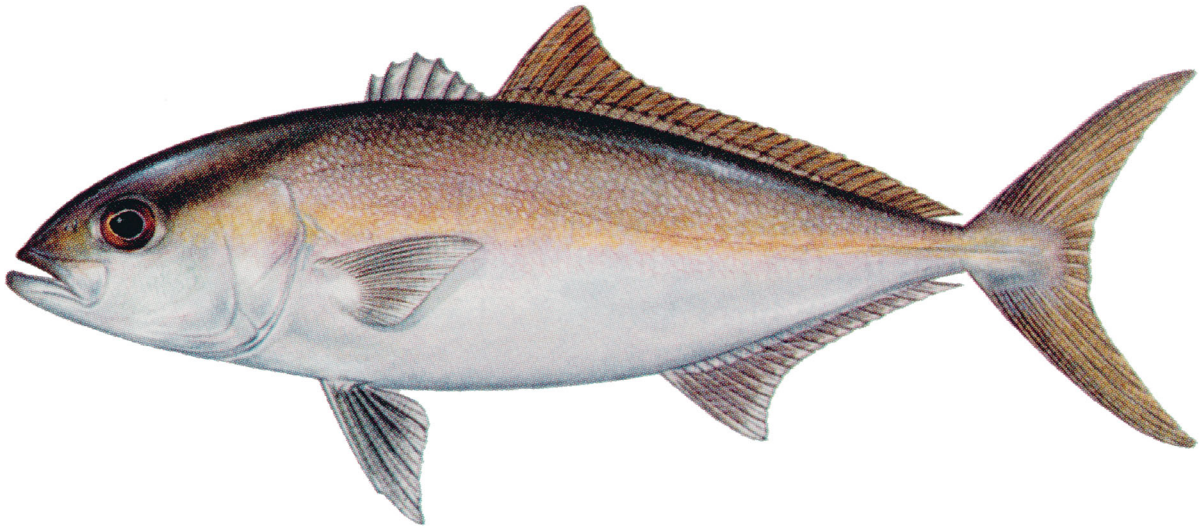


Figure 5.59 Birthdate assignment timeline for king mackerel. Age and year group based on biological birthdate (Sept 1), number of rings, and January 1 to December 31 year.

5.9 Greater Amberjack *Seriola dumerili*



Highlights

- Otoliths small and fragile, easy to break during extraction.
- Typically require embedding to section.
- Rings not always discernable requiring manipulation to read.
- Average life-span reported at 8-12 years but as old as 15.

Otolith Description

Thompson et al. (1999) described greater amberjack sagitae as follows:

> "Greater amberjack sagittae are small, thin, fragile and elongate in the anterior direction and bluntly crenelate at the posterior end. The medial surface is convex and has a deep, prominent sulcus. The anterior portion of the sagitta is curved laterally and the posterior end is relatively flat. The rostrum is longer than the antirostrum, but the difference increases with fish size. Prominent grooves and ridges are present on the lateral side of the sagittae and are nearly absent on the medial side" (Figure 5.60A and B).

The location of the otolith in the neurocranium is illustrated in Figure 5.61.

Figure 5.60 Greater amberjack sagittal otolith, A. medial and B. lateral.

Extraction

Otolith removal in greater amberjack is not easy. The otoliths are small and fragile making it easy to damage them during extraction; however, while any of the techniques illustrated in Section 3.1 can be used, a few tend to be easier than others. The otic capsule in

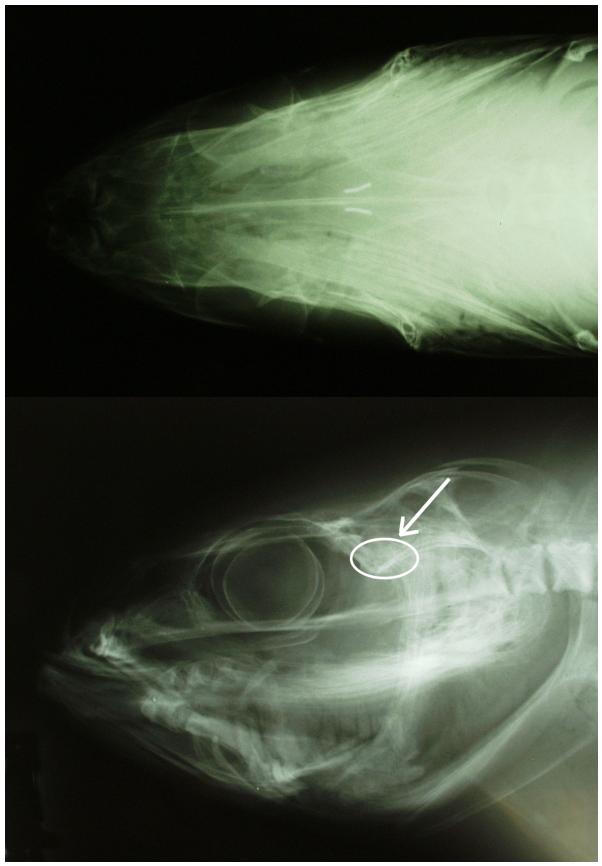


Figure 5.61 Location of the sagittal otoliths in greater amberjack.

greater amberjack is located directly behind and under the brain making it difficult to get into through the gill cavity, although it can be done. The recommended approach is to cut through the head using the meatsaw technique or through the top of the neurocranium.

Bottom Method

The method of otolith extraction through the gill cavity is preferred when sampling a commercial catch intended for market as it minimizes visible damage to the fish, although it is difficult.

1. Pull open the opercle to expose the gills.
2. Pull the gill arches back to expose the otic capsule (Figure 5.62).

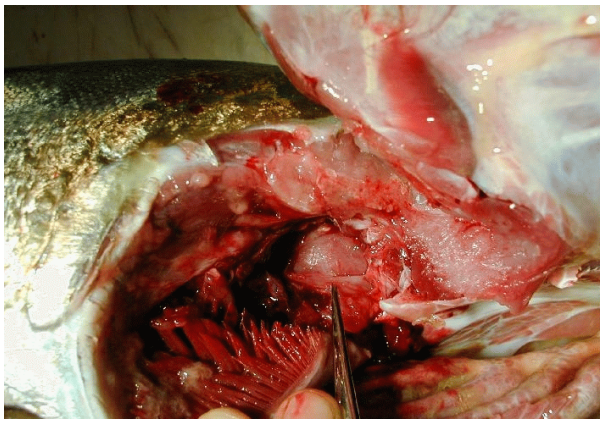


Figure 5.62 Exposure of otic capsule in greater amberjack.

3. Carefully chisel away the otic capsule to expose the sagitta (Figure 5.63).
4. Remove the otolith.
5. Repeat for the other side.

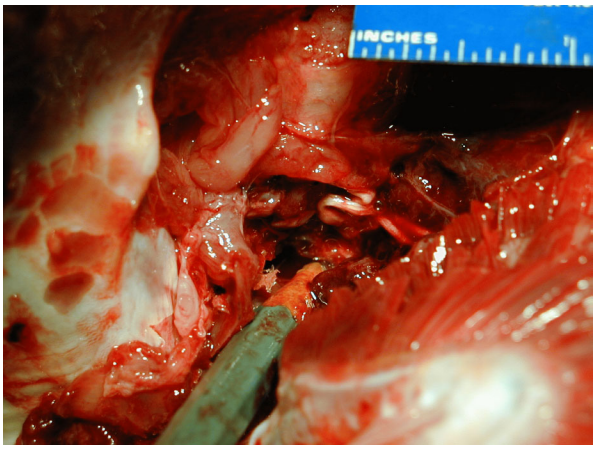


Figure 5.63 Removal of greater amberjack otoliths after chiseling capsule open.

Top Methods

Smaller Fish

1. Make a cut from the back of the skull to a point below and behind the eyesocket exposing the brain (Figure 5.64).
2. Remove brain to reveal the otoliths.
3. Remove the sagittal otoliths.

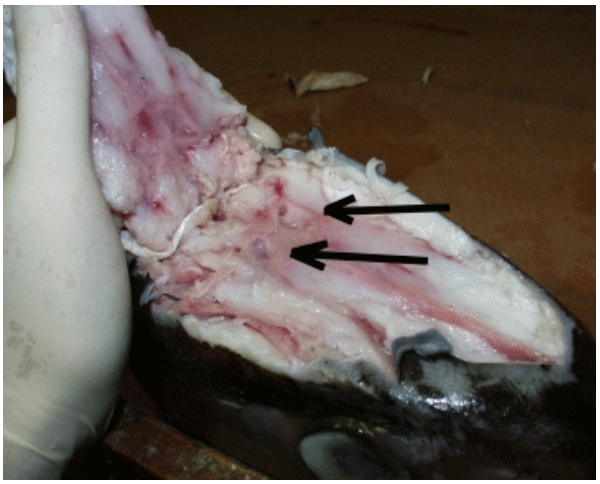


Figure 5.64 Extraction of greater amberjack otoliths through the top of the neurocranium.

Larger Fish

1. Make a vertical cut in the skull at a point at the leading edge of the opercle between the brain and the otic capsule (Figure 5.65).
2. Carefully clean the cut to determine position relative to the otic capsule.
3. Some 'digging' may be required to locate the otic capsule; if necessary, another thin section can be cut to reach the capsules (Figure 5.66).
4. With great care, remove the sagittal otoliths.

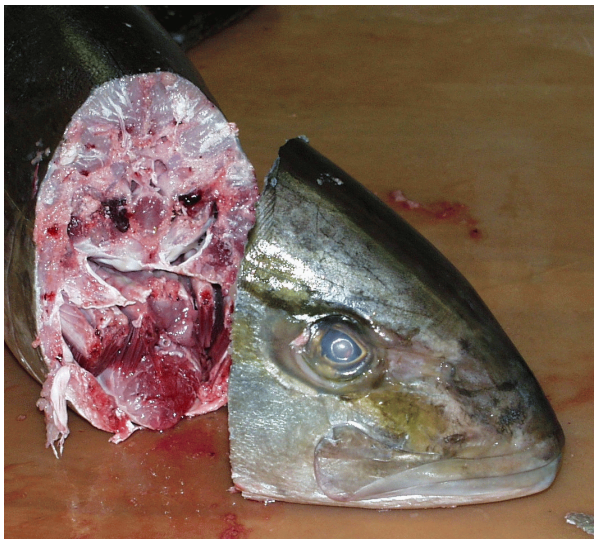


Figure 5.65 Relative location of cut when sectioning greater amberjack head.

Figure 5.66 Otic capsules opened and sagittal otoliths exposed in posterior cross-section of greater amberjack head.

Processing

Sectioning preparation typically consists of embedding the otoliths in bullet molds (Section 3.3.1.3). In the Gulf, the primary saw which has been used is the low speed saw, although the high speed saw could also be used. The thin sectioning machine has been used successfully with this species using the freehand technique.

Low Speed Wafering Saw Techniques

Embedded Whole Otoliths (Section 3.4.2.1) NMFS

1. Embed the otolith with the long axis parallel to the long axis of the mold.
2. Locate core and position block in chuck.
3. Adjust arm weight and speed. Make successive 0.5 mm cuts to obtain the core region.
4. Mount the core sections.

Thin Section Machine

Free-Hand Whole Otolith Sectioning (Section 3.4.3)
AMRD

Note: Only use the grinder on small/fragile otoliths.

1. Firmly grasping the posterior end of the otolith, grind material until adjacent to the core.
2. Mount otolith half with core on labeled slide.
3. Holding slide in hand, grind down remaining material to approximately 1 mm.
4. Place slide into precision grinder arm and adjust caliper to 0.5 mm.

Age Determination

While difficult, greater amberjack can be aged when viewed in thin section. Very little work has been done with this species to validate the annuli, but it is assumed at this time that the rings can be interpreted as annual events (Figure 5.67). Spawning of greater amberjack occurs in the spring with both male and female GSI reaching a maximum in April and May.

Figure 5.67 Otolith section of an age-2 greater amberjack. Black arrows indicate annuli.

Annulus deposition probably occurs just prior to spawning, suggesting that the first annuli should be far from the core and represent the first full year of growth although a smudge near the core does occur (Thompson et al. 1999) (Figure 5.68). In these cases, the first readable annulus is actually deposited between 15 to 21 months.

Like many of the pelagics, the difficulty in ageing greater amberjack is due to the small size of the otolith. If the otolith is broken or damaged during extraction, age determination can be impossible. In addition, otoliths in this species, while not deformed, can lack any evidence of rings at all; some otoliths just cannot be aged. While it is not practical to throw out difficult otoliths, it may be necessary at times for this species.

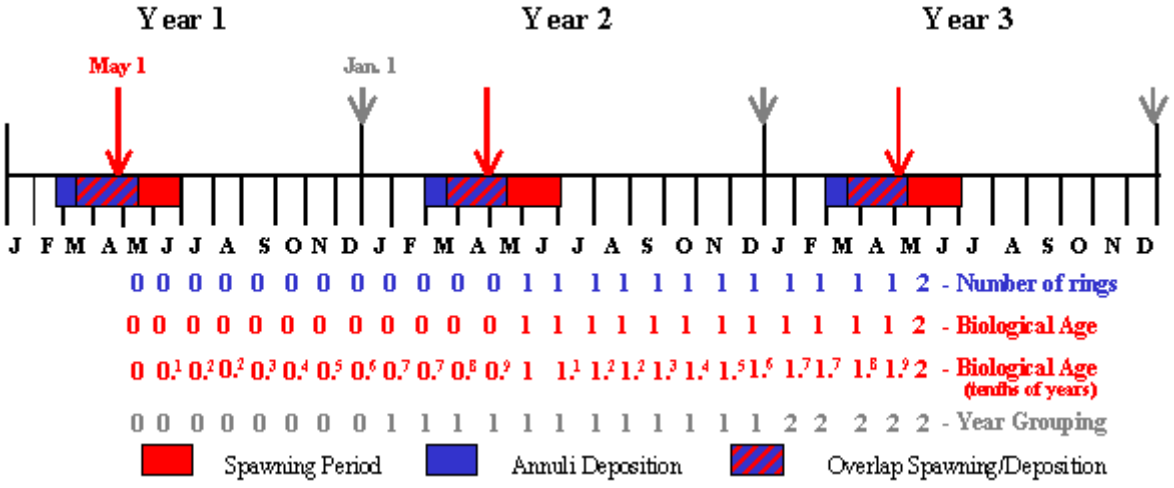


Figure 5.68 Birthdate assignment timeline for greater amberjack. Age and year group based on biological birthdate (May 1), number of rings, and January 1 to December 31 year. A mark (ring or dark smear) can occur close to the core; however, the first true annuli does not occur until the fish is actually a year old.

Other Ageing Techniques

Whole otoliths were not readable due to the lack of translucence even when immersed in clove oil or glycerin (Thompson et al. 1999). Break and burn is probably not practical due to the small size of the sagittal otoliths.

5.10 Spanish Mackerel *Scomberomorus maculatus*

Highlights

- Otoliths are elongate, laterally compressed.
- Otoliths relatively easy to locate and remove.
- First ring may resemble a diffuse "smudge" in section.
- Whole otoliths can be successfully aged up to age-6.
- Rings in sectioned otoliths are usually distinct in older fish.
- Spanish mackerel generally do not live past age-11 on the Atlantic Coast.

Otolith Description

Spanish mackerel otoliths (sagittae) are small, elongate, laterally compressed, and have an indented sulcus on the medial side (Figure 5.69). The rostrum and antirostrum are easily distinguishable and extremely fragile due to their small size and the overall thinness of the entire otolith. The location of the otolith is illustrated in Figure 5.70.

Extraction

Otolith removal in Spanish mackerel is relatively easy; therefore, any of the techniques

Figure 5.69 Medial view of Spanish mackerel sagittal otolith.

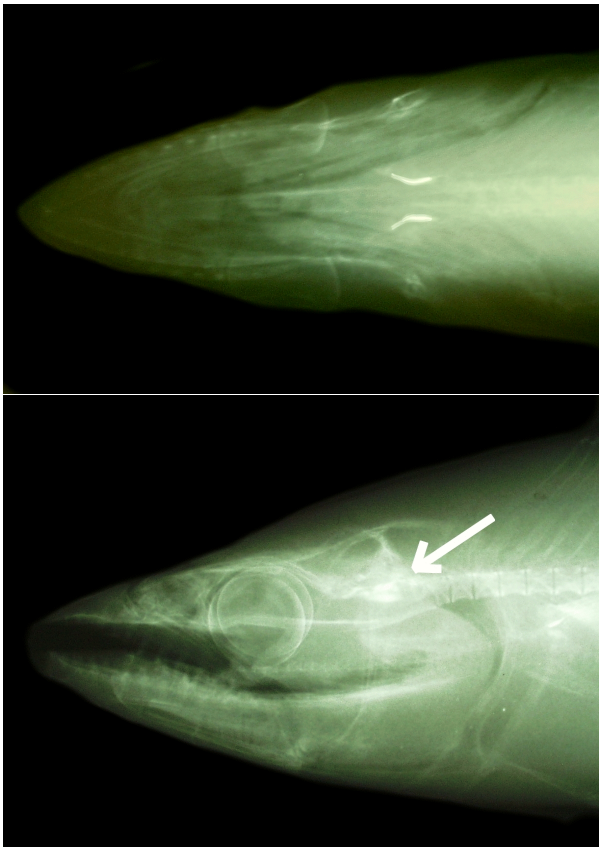


Figure 5.70 Location of the sagittal otoliths in Spanish mackerel.

illustrated in Section 3.1 can be used. Due to their small size, however, the meatsaw technique is not recommended. The otic capsule in Spanish mackerel is located near the posterior base of the skull behind the gills. The surface of the otic capsule is convex and easily discernible once the gills have been removed or scraped back. The capsule surface is fairly thin, can appear transparent, and is relatively easy to chisel away.

Bottom Method

The method of otolith extraction through the gill cavity is preferred when sampling a commercial catch intended for market as it minimizes visible damage to the fish.

1. Pull open the opercle to expose the gills.
2. Pull the gill arches back to expose the otic capsule.
3. Carefully chisel away the otic capsule to expose the sagitta (Figure 5.71).
4. Remove the otolith.
5. Repeat for the other side.

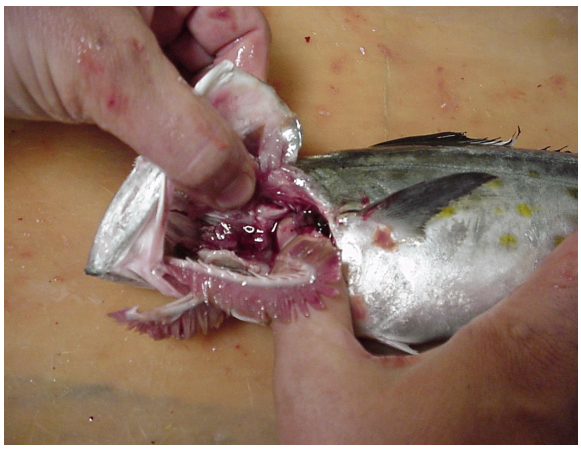


Figure 5.71 Removal of Spanish mackerel otolith through under the operculum.

Top Methods

Smaller Fish

1. Make a cut from the back of the skull to a point below and behind the eyesocket exposing the brain (Figure 5.72).
2. Remove brain to reveal the otoliths.
3. Remove the sagittal otoliths.

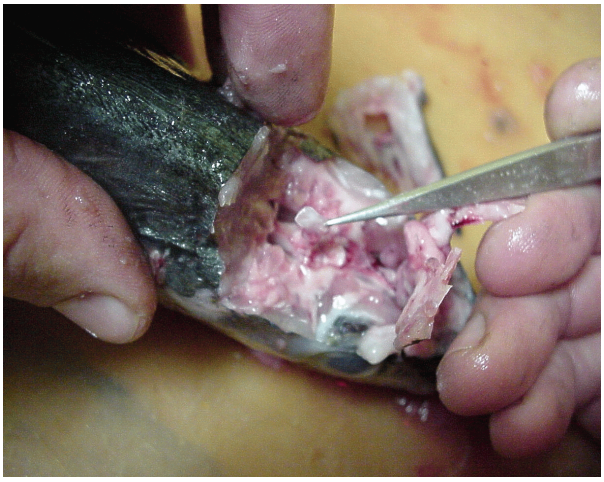


Figure 5.72 Extraction of Spanish mackerel otoliths through the top of the neurocranium.

Processing

Sectioning preparation typically consists of embedding the otoliths in bullet molds (Section 3.3.1.3). In the Gulf, the primary saw which has been used is the low speed saw. For very young Spanish mackerel, otoliths can be read whole (see Age Determination below). The NMFS Panama City Laboratory strongly recommends the use of the low speed saw when sectioning this species to ensure section clarity. It is suggested that a comparison of the results from both saws be made before making a long-term equipment choice.

Low Speed Wafering Saw Techniques

Embedded Whole Otoliths (Section 3.4.2.1) NMFS

1. Embed the otolith with the long axis parallel to the long axis of the mold.
2. Locate core and position block in chuck.
3. Adjust arm weight and speed. Make successive 0.5 mm cuts to obtain the core

region.
4. Mount the core sections.

Thin Section Machine

Free-Hand Whole Otolith Sectioning (Section 3.4.3)
LSU, AMRD

Note: Only use the grinder on small/fragile otoliths.

1. Firmly grasping the posterior end of the otolith, grind material until adjacent to the core.
2. Mount otolith half with core on labeled slide.
3. Holding slide in hand, grind down remaining material to approximately 1 mm.
4. Place slide into precision grinder arm and adjust caliper to 0.5 mm.

Age Determination

Whole Otoliths (Section 3.5.5)
NMFS

With few exceptions, small Spanish mackerel up to age-3 are much easier to age using whole otoliths rather than sections. A good rule of thumb is to use whole otoliths to age males <45 cm FL and females <55 cm FL. It should be noted, however, that specimens as large as 60cm FL have been aged using whole and sectioned otoliths with high levels of agreement (J. Mareska, AMRD, personal communication). The following is a brief methodology for ageing Spanish mackerel using whole otoliths.

1. Place otolith, distal or concave side up, in watch-glass with water.
2. Use a dark stage and reflected light (preferably a fiber optic light) to view otolith.
3. Annuli are read on the distal side of the posterior half of the otolith; those in the corner formed by the posterior and ventral edges are often the easiest to identify.
4. Readability may be improved by rotating the watch-glass and adjusting the angle and intensity of the light. Try illuminating the otolith through the side of the watch-glass if you have a fiber optic light.
5. Changing magnification, especially lowering it, will also improve readability on some otoliths.
6. Examine both left and right otoliths if available, as they often vary in readability.

In most cases the distance from the core to the first annulus will be much larger than all subsequent increments, although the increment between the first and second annuli will sometimes be quite large as well. If a whole otolith from a small fish seems especially difficult to read, try sectioning it. Occasionally the section will be more readable than the whole otolith, even in younger fish.

Ageing Sections

Annuli in sectioned Spanish mackerel otoliths are most readable in the dorsal portion, especially along the sulcus. With transmitted light and a compound microscope, all annuli except the first appear as fairly narrow dark marks. The first annulus is usually the most difficult to identify, as it is often just a broad, diffuse dark band (Figure 5.73). This first annulus sometimes is more apparent on the ventral portion of the otolith, even if subsequent annuli are not, so it always pays to examine that area if it is not clear on the dorsal end. One other time when the ventral portion should be examined is when the sectioned fish is very young (i.e., two or three) as the annuli will be clearer there than on the dorsal portion.

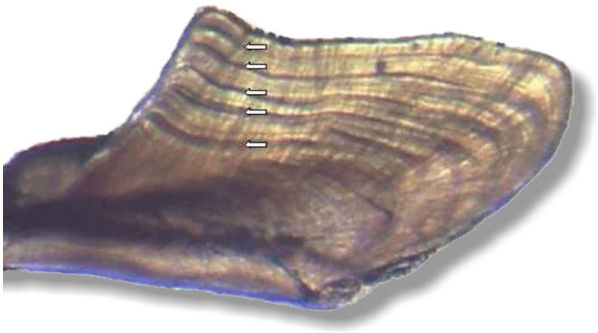


Figure 5.73 Sagittal otolith section from age-5 Spanish mackerel. White arrows indicate annuli.

A common phenomenon in Spanish mackerel otolith sections is for annuli to appear as doublets or couplets, which can lead to significant overageing problems if one is not careful. Adjusting the focus often helps resolve this problem. Another characteristic of these sections is that after the second or third annulus, the growth increments are usually uniform in size, with little or no decrease in size with increasing age. Because of this trait, ageing older fish is no more difficult than ageing younger ones and suggests that otolith growth and fish growth seem to become decoupled in Spanish mackerel at a fairly young age. Two techniques which may improve readability are using a polarizing filter on the light source and flipping the slide over on the microscope stage (this can make a big difference). One other thing to try if the section is very difficult to read and the fish is close to the minimum size for sectioning is to examine the remaining otolith whole if available. Measuring increment distances from the core is somewhat problematic, because the axis of growth in the otolith changes after the first ring is formed.

Age determination in Spanish mackerel is further complicated by its protracted spawning period (Figure 5.74) – typically May through October in the northern Gulf (Powell 1975, Finucane and Collins 1986). Annulus deposition occurs during the spring or early summer (Powell 1975, Fable et al. 1987). The oldest Spanish mackerel aged by the NMFS Panama City Laboratory to date was age-11.

Alternative Techniques

Break and burn is probably not practical due to the small size of the sagittal otoliths, and the use of scales for this species has not yet been determined.

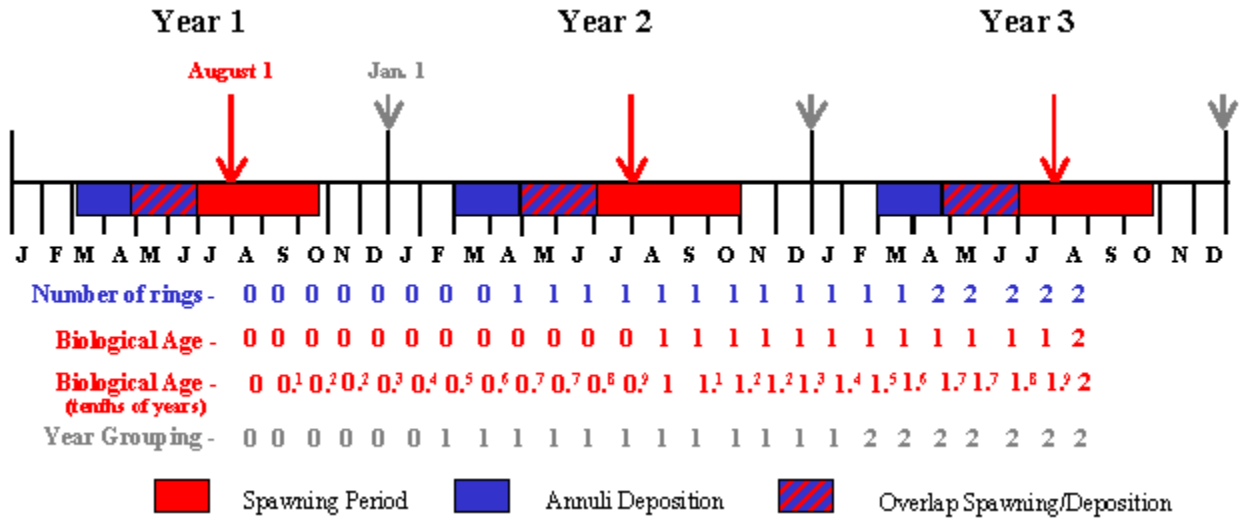
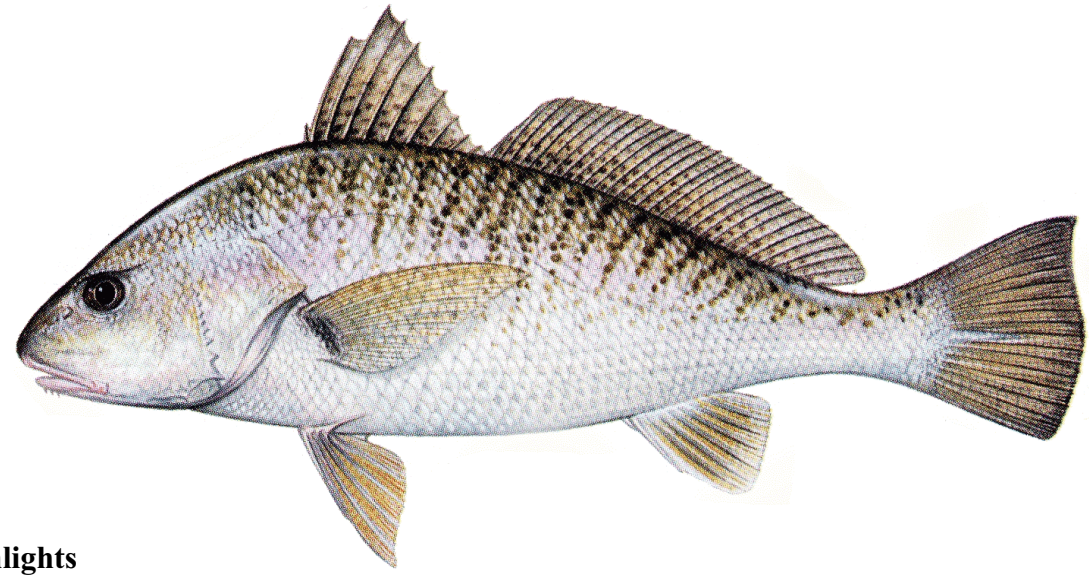


Figure 5.74 Birthdate assignment timeline for Spanish mackerel. Age and year group based on biological birthdate (Aug 1), number of rings, and January 1 to December 31 year.

5.11 Atlantic Croaker *Micropogonias undulatus*



Highlights

- Otoliths relatively easy to locate and extract.
- Multiple sectioning techniques successful.
- Rings easily discernable.
- First distinct opaque ring forms at approximately 1.5 years of age.
- Generally less than ten rings.

Otolith Description

The sagittae in Atlantic croaker are very thick and shield shaped, often with a shelf or flange on the outer surface or on the dorsal margin (Figure 5.75). The ostium of the sulcus is large, pear-shaped, and its expanded part does not reach the anterior margin. The 'J' shaped cauda of the sulcus acousticus is sharply bent, and its dorsal edge extends further into the ostium than its ventral edge. The rostrum and anterostrum are not distinguishable from one another. The core of the otolith usually lies just interior to the surface that faces outward from the midline of the fish. In the antero-posterior axis, the core lies adjacent to the junction of the ostium and cauda regions of the sulcus acousticus. The location of the otolith in the neurocranium is illustrated in Figure 5.76.

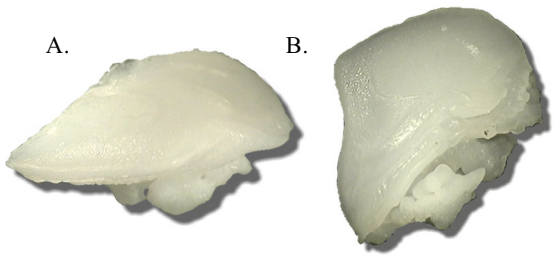


Figure 5.75 Atlantic croaker sagittal otoliths A) medial and B) top view.

Otolith Extraction

Atlantic croaker otoliths can withstand expected impacts from otolith extraction devices without breaking. The otic capsule of Atlantic croaker is somewhat convex making it easy to identify through the gill cavity near the posterior base of the skull above the gills. It is relatively easy to cut away the surface of the exposed otic capsule with a heavy knife. At larger sizes, otoliths can be removed using a

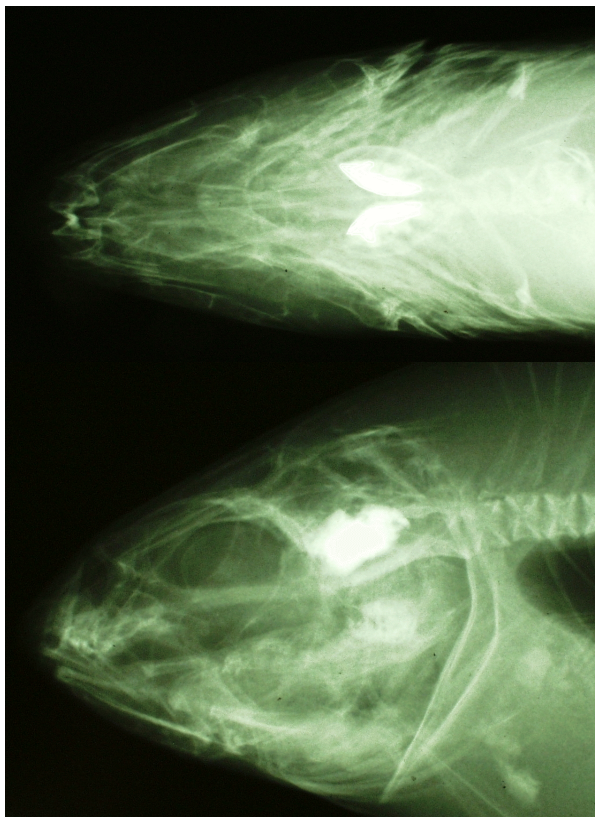


Figure 5.76 Location of Atlantic croaker sagittal otoliths.

hacksaw cut made from the dorsal surface of the head to the otic capsule. Atlantic croaker otoliths are relatively robust across all life stages, but due to the still fragile nature of young otoliths, extraction should be executed with care at smaller sizes.

Top Methods

Smaller Fish

1. Make a cut from the back of the skull to a point below and behind the eye socket exposing the brain (Figure 5.77).
2. Remove brain to reveal the otoliths.
3. Remove the sagittal otoliths.

Larger Fish

1. Make a vertical cut in the skull at a point just behind the centerline of the opercle through the otic capsule (Figure 5.78).
2. Bend the head of the fish forward to reveal the sagittae.
3. Remove the sagittal otoliths.

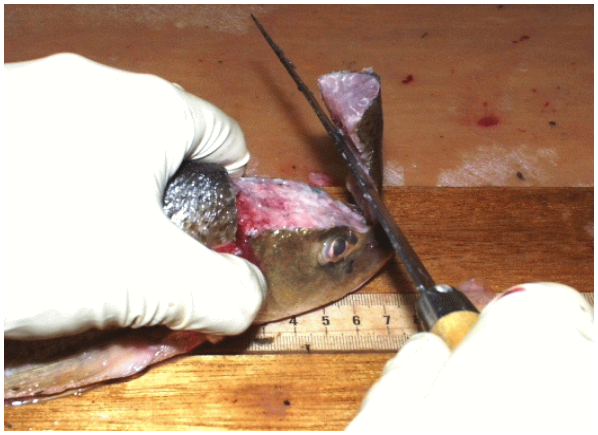


Figure 5.77 Extraction of Atlantic croaker otoliths through the top of the neurocranium.

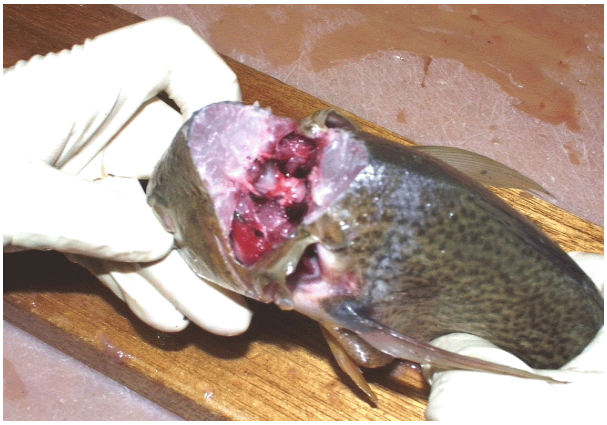


Figure 5.78 Meatsaw technique for extraction of otoliths from Atlantic croaker.

Bottom Method

This method causes minimal visible damage to the fish.

1. Pull open the opercle to expose the gills.
2. Pull the gill arches back to expose the otic capsule (Figure 5.79).
3. Chisel away the otic capsule to expose the sagitta.
4. Remove the otolith.
5. Repeat for the other side.

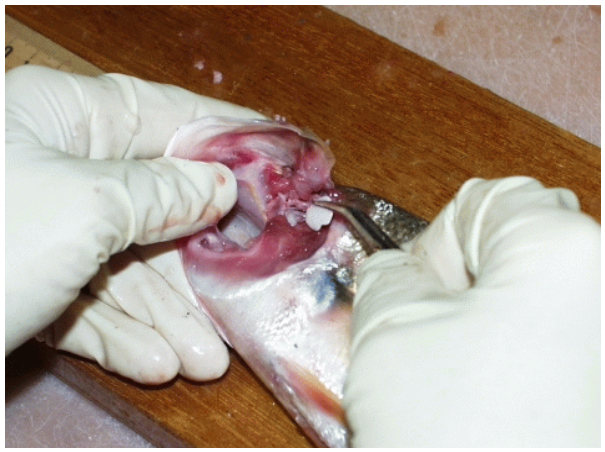


Figure 5.79 Extraction of otoliths from Atlantic croaker through the operculum.

Otolith Processing

Due to the robust nature of the otoliths in this species, multiple techniques are acceptable and usually reflect available equipment. Generally, Atlantic croaker sections are processed at approximately 0.5 mm. The following techniques have been used successfully throughout the Gulf.

Low Speed Wafering Saw Techniques

Embedded Whole Otoliths (Section 3.4.2.1)
LDWF, GCRL, MDMR, FMRI

1. Embed the otolith with the long axis parallel to the long axis of the mold.
2. Locate core and position block in chuck.
3. Adjust arm weight and speed. Make successive 0.5 mm cuts to obtain the core region.
4. Mount the core sections.

Mounted Whole Otoliths (Section 3.4.2.2)
FMRI

1. Mount whole otolith to slide, concave side down with the long axis parallel to the long side of the slide using thermoplastic.
2. Locate core and position slide in chuck.
3. Adjust arm weight (50-75 g) and speed (8-10). Make successive 0.5 mm cuts to obtain the core region.
4. Mount the core sections.

High Speed Wafering Saw Techniques

Embedded Whole Otoliths (Section 3.4.2.1)
TPWD

1. Embed the whole otolith with the long axis parallel to the long axis of the mold.
2. Locate core and position block in chuck.
3. Adjust load (1,000 g) and speed (3,000 rpm). Make successive 0.5 mm cuts to obtain the core region.
4. Mount the core sections.

Thin Section Machine

Free-Hand Whole Otolith Sectioning (Section 3.4.3)
LSU, AMRD

1. Firmly grasping both ends of the otolith, make initial cut adjacent to the core.
2. Hand grind additional material until core is visible.
3. Mount otolith half with core on labeled slide.
4. Place slide in chuck and section off remaining material.
5. Place slide into precision grinder arm and adjust caliper to 0.5 mm.

Age Determination

Transverse otolith sections of Atlantic croaker show very clear, easily identified marks that can be used for aging. Typical sections have an opaque core surrounded by a blurred opaque band, composed of fine opaque and translucent zones (Figure 5.80). This band represents the first annulus. Because of Atlantic croaker's spawning season, the width

of the first annulus varies among individuals. Spawning typically occurs from November through January while annuli deposition occurs from December through May (Figure 5.81). Late-spawned fish have a very narrow band that is almost continuous with the core; early-spawned fish have a wide, well-defined band clearly separated from the core. Because of this variation in width and proximity to the core, the first annulus is sometimes difficult to identify.

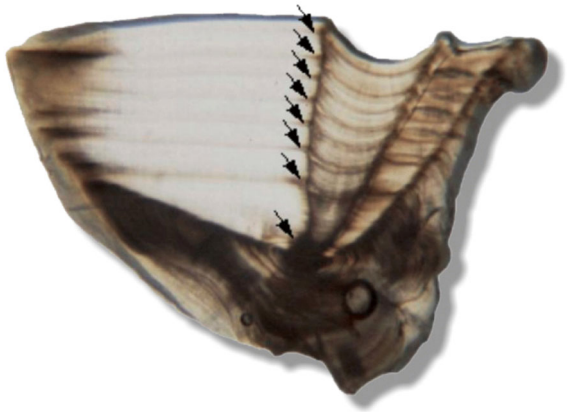


Figure 5.80 Otolith section of an age-8 Atlantic croaker. Black arrows indicate annuli. Note first annulus appears as a blur or smudge.

Subsequent annuli are represented by easily identified, narrow, opaque bands that alternate with wider translucent bands outside the proximal margin of the first annulus.

For regional stock assessment purposes, three minimal parameters are recorded: number of rings, presence or absence of an opaque ring at the margin, and month of capture. Based on these three parameters, cohort and biological ages can be determined.

Other Ageing Methods

Whole otoliths have not been used successfully in the Gulf region. The usefulness of break and burn techniques for Atlantic croaker has not been determined; however, this species may be a good candidate for the technique. Atlantic croaker scales have not been demonstrated to be useful in the Gulf yet.

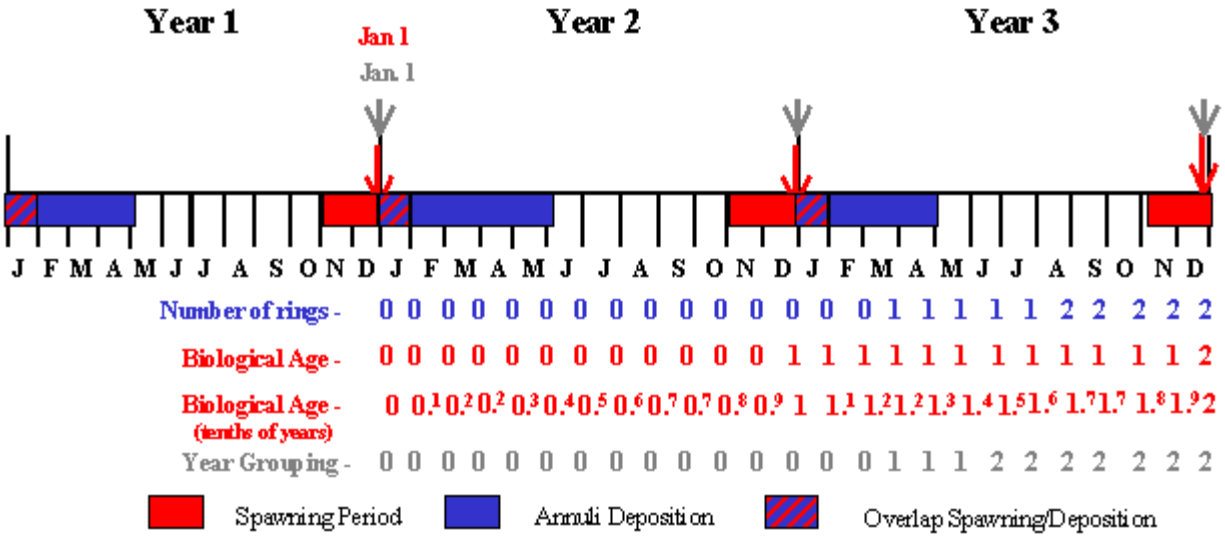


Figure 5.81 Birthdate assignment timeline for Atlantic croaker. Age or year group based on biological birthdate (January 1), number of rings, and January 1 to December 31 year.

5.12 Sheepshead *Archosargus probatocephalus*

Highlights

- Otoliths are ovate, laterally compressed.
- Otoliths relatively easy to locate and extract.
- Otoliths are relatively large, and multiple sectioning techniques can be used.
- Maximum age varies by region ranging from 14 yrs (FL), 20 yrs (LA), to 26 yrs (SC).

Otolith Description

Sheepshead otoliths (sagittae) are relatively large, ovate, laterally compressed, and exhibit an indented sulcus on the proximal surface (Figure 5.82). The rostrum and anterostrum are easily distinguishable. The location of the sagittae in the neurocranium is illustrated in Figure 5.83.

Figure 5.82 Medial and lateral view of sheepshead sagittal otolith.

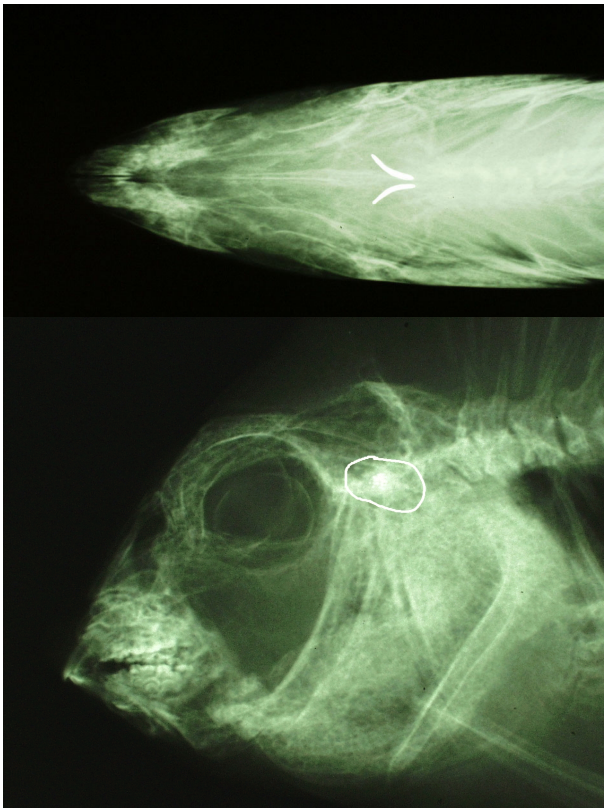


Figure 5.83 Location of sagittal otoliths in sheepshead.

Extraction

Sheepshead otoliths are not terribly fragile, but caution should be taken during extraction as they may break during contact with certain extraction devices. The otic capsule in sheepshead is located near the posterior base of the skull behind the gills. The surface of the otic capsule is convex and easily discernible once the gills have been removed or scraped back. The capsule surface is fairly thin, can appear transparent, and is relatively easy to chisel away.

Bottom Method

The method of otolith extraction through the gill cavity is preferred when sampling a commercial catch intended for market as it minimizes visible damage to the fish.

1. Pull open the opercle to expose the gills.
2. Pull the gill arches back to expose the otic capsule.
3. Carefully chisel away the otic capsule to expose the sagitta (Figure 5.84).
4. Remove the otolith.
5. Repeat for the other side.

Figure 5.84 Removal of sheepshead otolith through under the operculum.

Top Methods

Smaller Fish

1. Make a cut from the back of the skull to a point below and behind the eyesocket exposing the brain (Figure 5.85).
2. Remove brain to reveal the otoliths.
3. Remove the sagittal otoliths.

Figure 5.85 Extraction of sheepshead otoliths through the top of the neurocranium.

Larger Fish

1. Make a vertical cut in the skull at a point just behind the centerline of the opercle through the otic capsule.
2. Bend the head of the fish forward to reveal the sagittae (Figure 5.86).
3. Remove the sagittal otoliths.

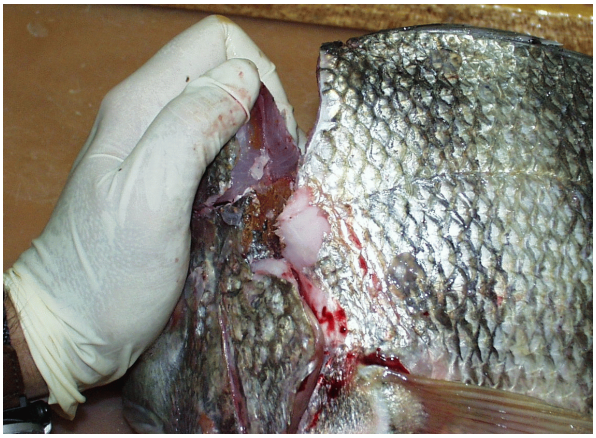


Figure 5.86 Meatsaw technique for extraction of otoliths from sheepshead.

Processing

Due to the relatively large size of sheepshead otoliths, multiple processing techniques are acceptable. The technique chosen will likely reflect available equipment. Generally sheepshead sections are processed at approximately 0.5 mm. The following techniques have been used throughout the Gulf.

Low Speed Wafering Saw Technique

Embedded Whole Otoliths (Section 3.4.2.1) GCRL

1. Embed the otolith with the long axis (anterior-posterior axis) parallel to the long axis of the mold.
2. Locate the core and position block in chuck.
3. Adjust arm weight (50-75 g) and speed (8-10). Make successive 0.5 mm cuts to obtain the core region.
4. Mount the core sections onto slides.

Mounted Whole Otoliths (Section 3.4.2.2) University of Florida

1. Mount whole otolith to slide, concave side down with the long axis parallel to the long side of the slide using thermoplastic.
2. Locate core and position slide in chuck.
3. Adjust arm weight (50-75 g) and speed (8-10). Make successive 0.5 mm cuts to obtain the core region.
4. Mount the core sections.

Thin Section Machine

Free-Hand Whole Otolith Sectioning (Section 3.4.3)

1. Firmly grasping both ends of the otolith, make initial cut adjacent to the core.
2. Hand grind additional material until core is visible.
3. Mount otolith half with core on labeled slide.
4. Place slide in chuck and section off remaining material.
5. Place slide into precision grinder arm and adjust caliper to 0.5 mm.

Age Determination

Enumeration of sheepshead annuli in otolith sections is straightforward with the exception of the first ring (Figure 5.87). The period of annulus formation in the northern Gulf is from

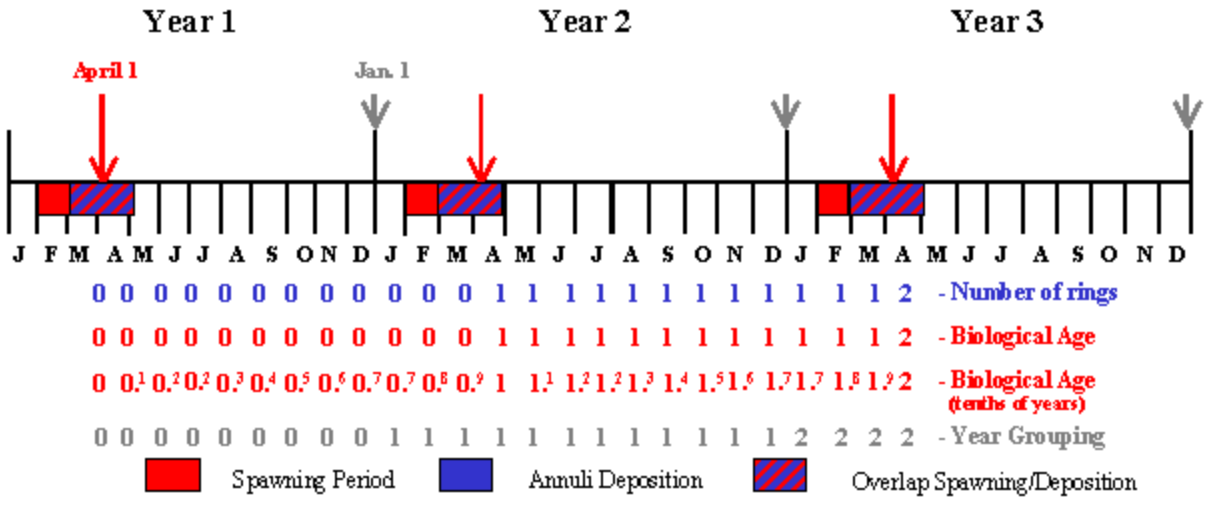


Figure 5.87 Birthdate assignment timeline for sheepshead. Age or year group based on biological birthdate (April 1), number of rings, and January 1 to December 31 year.

March through May, and spawning occurs offshore from February through April with a peak in March and April. The coincidence of ring formation and spawning can lead to dark cores in early spawners and opaque cores in late spawners (Figure 5.88A and B). In general, it is accepted that the core mark is not interpreted as a true annuli.

A.

B.

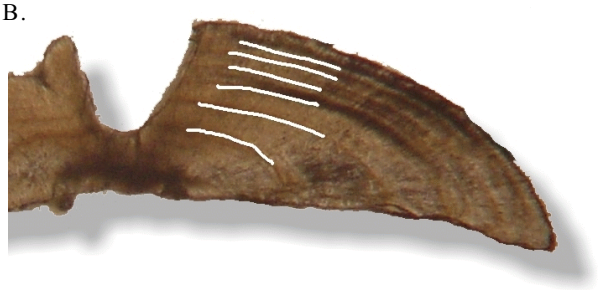


Figure 5.88 Core region of A) early spawned and B) late spawned sheepshead.

Other Ageing Methods

Break and burn has not been attempted on this species in the Gulf. Based on the size of the otolith, this technique may warrant further investigation. The ageing of whole sheepshead otoliths has not been attempted in the Gulf. Scales have been used in the past to age sheepshead, but when compared to otoliths, the use of scales was found to underestimate age by age-3.

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7.0 Glossary of Terms Used in Age and Growth Studies

Sources used to compile this glossary include: Summerfelt and Hall 1987, Secor et al. 1991, Kalish et al. 1995, C.A.R.E. 1997, Old Dominion University/Virginia Marine Resources Commission 2001.

A

accuracy - the closeness of a measure or computed value to its true value.

age - a unit to express the passage of time to capture measured in years, months, days or other units.

age-group (age-class, cohort-age) - a group of fish that have the same assigned age within a given time period (e.g., five-year-old age-group); the term is not synonymous with year-class.

age estimation, age determination - the preferred terms for the process of assigning ages to fish as opposed to the term aging (ageing), which refers to time-related processes such as the alteration of an organism's composition, structure, and function.

ampulla - the enlarged chamber containing a patch of sensory epithelium at one end of each semicircular canal of the inner ear.

annual age - an integer enumeration of age corresponding to year-class.

annual growth zone - all growth on a structure which forms during one year; consisting of an opaque zone or annulus and a translucent zone, generally formed during the winter and summer months, respectively.

annulus (pl. annuli) - a continuous, concentric growth zone that forms once a year, for most fish during a period of slow or no growth (see opaque growth zone, winter growth zone); the optical appearance of these marks depends on the otolith structure and the species.

antirostrum - an anterior projection of the sagitta located dorsal to the sulcus acousticus and rostrum; generally shorter than the rostrum.

aragonite - an inorganic, crystalline polymorph of calcium carbonate that combines with otolin to form the otolith matrix.

asteriscus (pl. asterisci) - one of the three otolith pairs found in the membranous labyrinth of osteichthyan fishes; lies within the lagena of the pars inferior.

B

biological age - the time elapsed from estimated birth to capture expressed in years and fractions of years.

birth date (theoretical) - calendar date that coincides with the mode of spawning activity for a given species.

blind reading - visual assessment of otolith annuli and margin/edge development with no knowledge of fish size and date of capture.

C

calendar age - the age of a fish based on a calendar year rather than to the true date of hatching.

calendar birthdate - January 1; used to maintain year classes when biological birthdate is unknown.

cauda - the posterior, medial-extending section of the sulcus acousticus.

check - a discontinuity (e.g., a stress-induced mark) that forms within the translucent zone, denoting a slowing of growth; checks do not form annually but reflect various environmental or physiological changes; distinguished by the width of the zone relative to annuli, location relative to annuli, and incomplete formation or poor definition.

circuli (circulus; singular) - fine ridges laid in a circular pattern around the focus of a scale.

cohort - group of fish that begins life about the same time and is produced during a relatively discrete spawning event; difficult to apply to fishes that spawn monthly or some other periodicity; does not imply year-class.

cohort age - see annual age.

core - the primordium of the otolith (sometimes used synonymously with focus).

core region - the area or areas surrounding one or more primordium.

corroboration - a measure of the consistency or repeatability of an age determination method when two different readers agree on the number of zones present; not to be confused with validation.

crystallized otolith - an otolith displaying inadequate calcification; age determinations are generally not possible due to missing annuli.

D

daily increment - an increment formed over a 24-hour period; synonymous with daily growth increment and daily ring.

distal edge - the external margin of an otolith cross-section.

distal surface - the external surface of a whole otolith; the surface opposite the sulcus.

E

edge type - synonymous with edge zone; extent of opaque or translucent deposition on the outer margin of the otolith representing the most recent growth.

F

false annulus (pseudoannulus) - sometimes used synonymously with "check" or "check mark;" refers to a zone of slow growth that is not a true annulus; also, a characteristic ring on otoliths that occurs before the first annulus and fairly close to the nucleus.

focus - the hypothetical or real point of origin of an otolith or scale; the starting point of a sectioned or whole otolith where the reader chooses to start a count or use as a reference point for measurement.

I

increment - the region between similar zones on a structure used for age estimation; the term refers to a structure, but it may be qualified to refer to portions of the otolith formed over a specified time interval (e.g., subdaily, daily, annual); an annual increment is made up of an opaque zone and a translucent zone, whereas a daily increment consists of a D-zone and an L-zone.

L

lagena - an organ of non-mammalian vertebrates analogous to the cochlea.

lapillus (pl. lapilli) - one of the three otolith pairs found in the membranous labyrinth of osteichthyan fishes; lies within the utriculus of the pars superior.

M

marginal increment - the region beyond the last identifiable estimation mark at the otolith margin; usually expressed in relative rather than quantitative terms, i.e., as a fraction or proportion of the last complete increment; see edge type.

N

nucleus - central portion of an otolith; used synonymously with core, focus, kernel, or primordium.

O

opaque growth zone - usually synonymous with winter growth zone; a banded region of an otolith

section that interferes with the passage of transmitted light and therefore appears dark relative to adjacent translucent growth zone(s); appears bright under reflected light; usually an area of high concentrations of calcium aragonite relative to otolin; occasionally, opaque zones are formed in areas where the aragonite crystal alignment interferes with light transmission through the otolith section; the opaque and translucent growth zones together form the annual growth zone.

ostium - the anterior section of the sulcus acousticus.

otolin - the organic protein found in the otolith, closely related to conchiolin of some mollusks.

P

precision - the closeness of repeated measurements of the same quantity; in age determination, it relates to the variability between or within readers.

primordium (pl. primordia) - the initial deposition site of organic matrix and calcium carbonate of an otolith; if several primordia are present, they generally fuse to form the otolith core.

proximal edge - the internal margin of an otolith cross-section.

proximal surface - the internal surface of a whole otolith; the surface on which the sulcus is found.

R

radii (radius; singular) - linear extensions of ridges from the focus to the anterior margin of a scale.

reading axis - preferred path along which annuli are counted; see sulcus edge.

ring (band, zone, check) - a descriptive term used in determining the age of a fish from hard parts; does not necessarily designate yearly or annual marks.

rostrum - anterior-most, ventral projection of the sagitta; generally longer than the anterostrum.

S

sacculus - the smaller chamber of the membranous labyrinth of the inner ear.

sagittae (sing. sagitta) - largest of three otolith pairs within the membranous labyrinth of osteichthyan fishes and therefore most often selected for otolith studies; lies within the sacculus of the pars inferior; generally compressed laterally and elliptical in shape with wide variation in appearance among species.

semicircular canal - any of the loop-shaped tubular parts of the labyrinth of the inner ear that together constitute a sensory organ associated with the maintenance of bodily equilibrium,

that consist of an inner membranous canal and a corresponding outer bony canal formed in a group of three in planes nearly at right angles to each other.

split - discontinuity in an annular zone, analogous to a check; causes the annulus to appear as two or more closely spaced winter zones.

subdaily increments - an increment formed over a period of less than 24 hours.

sulcus acusticus/acousticus - commonly called sulcus or sulcus groove; a longitudinal sculptured groove extending down the convex (medial) surface of a sagittal otolith through which an auditory nerve passes; frequently referred to in otolith work because of the clarity of increments near the sulcus in transverse sections of sagittae.

sulcus edge - on an otolith cross-section, the margin adjacent to the sulcus on the internal or proximal surface.

summer growth zone - see translucent growth zone.

T

transition zone - a marked change in the annual growth zone requiring an adjustment to age-reading criteria.

translucent growth zone - the banded regions on an otolith section that allow a greater passage of transmitted light relative to the opaque or winter zones; usually an area of high concentrations of otolin relative to calcium aragonite; represents a period of faster growth; also called summer zone; the term hyaline has been used, but translucent is the preferred term.

U

utriculus - the part of the membranous labyrinth of the inner ear into which the semicircular canals open.

V

validation - the process of proving that otolith rings accurately represent annual growth patterns which can be used to assign an age to a fish; methodologies include tag and recapture, hatchery releases, and chemical or temperature marking of otoliths.

verification - the process of determining ageing precision comparing ages assigned blindly by multiple readers.

W

winter growth zone - see opaque growth zone; represents a period of slower growth.

Y

year class - fish spawned or hatched in a given year.

8.0 Appendices

Appendix 8.1 Suppliers and Supplies for Otolith Processing - Permanent Equipment and Apparatus

Item Description	Purpose	Manufacturer or Supplier	Model or Stock No.	Count per Unit	Current Price
Small hotplate, 120v	Warming slides mounts for thermoplastic adhesive	Barnstead/Thermolyne	HP2305B	1	141
Motorized polishing apparatus	Polishing otolith sections	Crystalite Corp.	C5505070 Crystal Master Plus 6 and Master Lap	1	225
Diamond-tip glass cutter	Cutting glass slide-mounts from microscope slides	Science supply or hardware store	---	1	4
Top-loading balance	Gravimetric measurement of embedding epoxy resin and hardener components	C & H Distributors	76-5990 2000 gram capacity balance and AC adapter	1	112
Low-speed diamond wafering saw	Cutting otolith thin sections	Buehler, Ltd.	Isomet Model 11-1180 Low Speed Saw	1	8,500
Low-speed diamond wafering saw	Cutting otolith thin sections	South Bay Technology	Model 650		3,495
Low-speed diamond wafering saw	Cutting otolith thin sections	Struers	Minitom	1	3,750
High-speed diamond wafering saw	Cutting otolith thin sections	Buehler, Ltd.	Isomet Model 2000 High Speed Metallurgical Saw	1	8,500
Thin section machine	An otolith thin section cut-off saw and grinder incorporated into one unit	Hillquist	Model 1005	1	6,400
Stereo-microscope with transmitted and reflected light sources	Use with or without imaging system to read otolith annuli and edge zones	Wild-Leitz, Olympus, Parco, etc.	---	1	Call vendor for price quote

Item Description	Purpose	Manufacturer or Supplier	Model or Stock No.	Count per Unit	Current Price
Compound microscope	Use with or without imaging system to read otolith annuli and edge zones	Wild-Leitz, Olympus, Parco, etc.	---	1	Call vendor for price quote
Color video image analysis system consisting of a stereo microscope, transmitted-light base, cold light source, fiber optic ringlight, polarizer filter, color video camera and monitor, computer and monitor, frame grabber, Optimas software, and other accessories	Otolith imaging and measurements; photographing otolith sections under the microscope	Meyer Instruments	Contact for regional sales representative and technical support	1	Call for price quote
Optimas color optical image analysis software	Otolith image capture, enhancement, and display; spatial measurement of growth zones	Meyer Instruments Optimas Corp.	Contact for regional sales representative and technical support	1 1	6,500
Low-power dissecting microscope	Judging quality of otolith sections	Wild-Leitz, Olympus, Parco, etc.	---	1	Call vendor for price quote
Incubator oven	Curing embedded otoliths	Fisher Scientific			
Growth zone macro, used with Optimas software	Data manipulation of growth zone measurements	Meyer Instruments	Contact for regional sales representative and technical support		

Appendix 8.2 Suppliers and Supplies for Otolith Processing - Expendables

Item Description	Purpose	Manufacturer or Supplier	Model or Stock No.	Count per Unit	Current Price
Sample envelopes	Field collection and permanent archiving of whole otoliths	Allometrics	VW 56775-039 3 x 5 brown Kraft tin-tie safety fold	250/box	24.50
Sample vials	Field collection of small otoliths	VWR Scientific	20170-610 Micro-centrifuge tubes with caps	500/bag 10 bags/case	189.00
Tissue culture trays, 24 well	Storing/Archiving of whole otoliths and otolith sections	VWR Scientific	29443-952	50/case	97.00
Polyethylene embedding molds, 22 x 30mm	Embedding otoliths for thin sectioning	VWR Scientific Polysciences	15160-270 Peel-Away embedding molds	8 cells/tray 36 trays/case	53.00 50.00-60.00
Flat embedding mold, reusable	Embedding very small otoliths for thin sectioning and whole larval fish for grinding	Ted Pella Polysciences	110 Pelco 20-cavity, 5mm x 15mm x 5mm	1 1	10.00
Histological disposable base molds	Embedding otoliths for thin sectioning	Surgipath Medical Industries		500/case	50.00-60.00
Clear silicon embedding molds	Embedding otoliths for thin sectioning	Electron Microscopy Sciences	70900		8.50
Araldite resin and hardener	Embedding otoliths for thin sectioning	Ciba-Geigy Corp.	Epoxy resin: CY 8702 Araldite-D-US Epoxy hardener: HY 956 EN/US	6 qt. resin and 2 qt. hardener	127.00

Item Description	Purpose	Manufacturer or Supplier	Model or Stock No.	Count per Unit	Current Price
Low viscosity resin (Spurr)	Embedding otoliths for thin sectioning	Ted Pella Electron Microscopy Sciences	18300 14300		
Disposable plastic beakers, 100 ml	Mixing two-part embedding epoxy	VWR Scientific	13915-624 Tri-Pour	100/box	29.00
Latex gloves	Skin protection while mixing and pouring embedding media	Ward's	15 W 1071	100/box	18.00
Magni-Visor	Close-up work: cleaning otoliths, positioning in embedding molds, marking epoxy blocks for sectioning	Ward's	25 W 2101	1	25.00
Metal spatulas	Mixing embedding epoxy	Ward's	15 W 4313	12/pack	23.00
Thin Section Machine Diamond Blade	Cut-off Wheel	Hillquist	8 inch	1	180.00
Thin Section Machine Diamond Cup Wheel	Precision Grinding	Hillquist	8 inch	1	665.00
Diamond wafering blade	Low-speed saw	Buehler, Ltd.	11-4244 Isomet High Concentration Wafering Blade	1	227.00
Diamond wafering blade	High-speed saw	Precision Surfaces International	4 inch 6 inch	1 1	177,300.00
Diamond wafering blade	Low-speed saw	Struers	230CA Diamond cut-off wheel	1	200.00
Diamond wafering blade	Low speed saw	South Bay Technology	DWH4122 Diamond wheel - 4" x .012" x medium/high concentration	1	

Item Description	Purpose	Manufacturer or Supplier	Model or Stock No.	Count per Unit	Current Price
Diamond wafering blade	Low-speed saw	Alro Industrial Supply	Norton Diamond Wheel, Product No. JO-588-678	1	99.00
Diamond wafering blade	Low-speed saw	Diamond Wheel, Inc	ME120928	1	96.00
Glass microscope slides, 1.2 mm thickness	Otolith mounts	Ward's	14 W 3501	72/pack 10 packs/box	85.00
Thermoplastic mounting adhesive	Securing otolith thin sections and whole otoliths on glass slide mounts	Aremco Products	Crystalbond 509	5 sticks/pack	70.00
Thermoplastic mounting adhesive	Securing otolith thin sections and whole otoliths on glass slide mounts	Hugh Courtright & Co.	Lakeside Brand (Quartz) Thermoplastic Cement, Stock No. 70C	12 bars/box	33.45
Loctite mounting adhesive	Securing otolith thin sections and whole otoliths on glass slide mounts	Loctite Corp. Motion Industries	Loctite 349 (It is important to use the 349 adhesive.)	1 1	
Micropolishing compound, .3 micron	Polishing apparatus	Buehler, Ltd.	40-6363-006 Alumina II	6 oz.	16.00
Wet-dry sandpaper, 600 grit	Polishing apparatus	Hardware store	---		
Polishing cloth	Polishing apparatus	Buehler, Ltd.	40-7216 40-7218	10/pack	3,226.00
Pointed scalpels	Etching species and sample number codes on otolith slide mounts	Ward's	14 W 0966	1	6.00
Disposable glass pipettes, 10 ml	Dispensing epoxy into small molds (e.g., those used for larval fish otoliths) during otolith embedding	Ward's	17 W 1308	1	7.00
Blunt-fine tipped scissors	Trimming otolith sections prior to mounting	Ward's	14 W 0940	1	5.00

Item Description	Purpose	Manufacturer or Supplier	Model or Stock No.	Count per Unit	Current Price
Kimwipes	Wiping saw coolant from otolith sections	Lab Safety Supply	14011 Extra Low Lint, Delicate Task, 11" X 17"	140/box	6.00
Teri-Towels	General housekeeping	Ward's	15 W 1024		17.00
Baby oil Mineral oil	Diamond saw blade lubricant and coolant	---	---	---	---
Water soluble saw coolant	Diamond saw blade lubricant and coolant	South Bay Technology	02-02460	1 qt. concentrate	---
Soluble oil	Diamond saw blade lubricant and coolant	Buehler, Ltd.		1 gallon	50.00
Glycerine		Fisher Scientific	G33-4	4 bottles/case	305.33
Plastic slide boxes		Fisher Scientific	03448-5	72 boxes/case	565.25
Plain glass slides		Fisher Scientific	12-550A	10 gross/case	136.81
Frosted glass slides		Fisher Scientific	12-550-43	10 gross/case	175.66
Mounting media		Fisher Scientific	Flo-Texx, Stock No. 143903 Flo-Texx, Stock No. 143904	4 pack 6 pack	
Disposable droppers		Fisher Scientific	6219-0068		
Rubber cement	Securing polishing cloth and wet-dry sandpaper to polishing apparatus	Office supply	---	1	---
Forceps	Handling otolith thin sections and otolith mounts	Science supply companies	---	---	---
Ultraviolet flourescent light bulb (24")	curing of Loctite adhesive	General Electric	F20T12BLB	1	40.00

Appendix 8.3 Contact information for Appendix 8.1 and 8.2

Allometrics, Inc.
PO Box 15825
Baton Rouge, LA 70895
(800) 528-2246
www.allometrics.com

Alro Industrial Supply
12490 49th Street
Clearwater, FL 34622-4310
www.alro.com

Aremco Products, Inc.
PO Box 429
Ossining, NY 10562
(914) 762-0685
www.aremco.com

Barnstead/Thermolyne
PO Box 797
2555 Kerper Boulevard
Dubuque, IA 52004-0797
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Crystalite Corporation
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Diamond Wheel, Inc.
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diamondwheelinc.com/

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Fisher Scientific
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www.hillquist.com

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