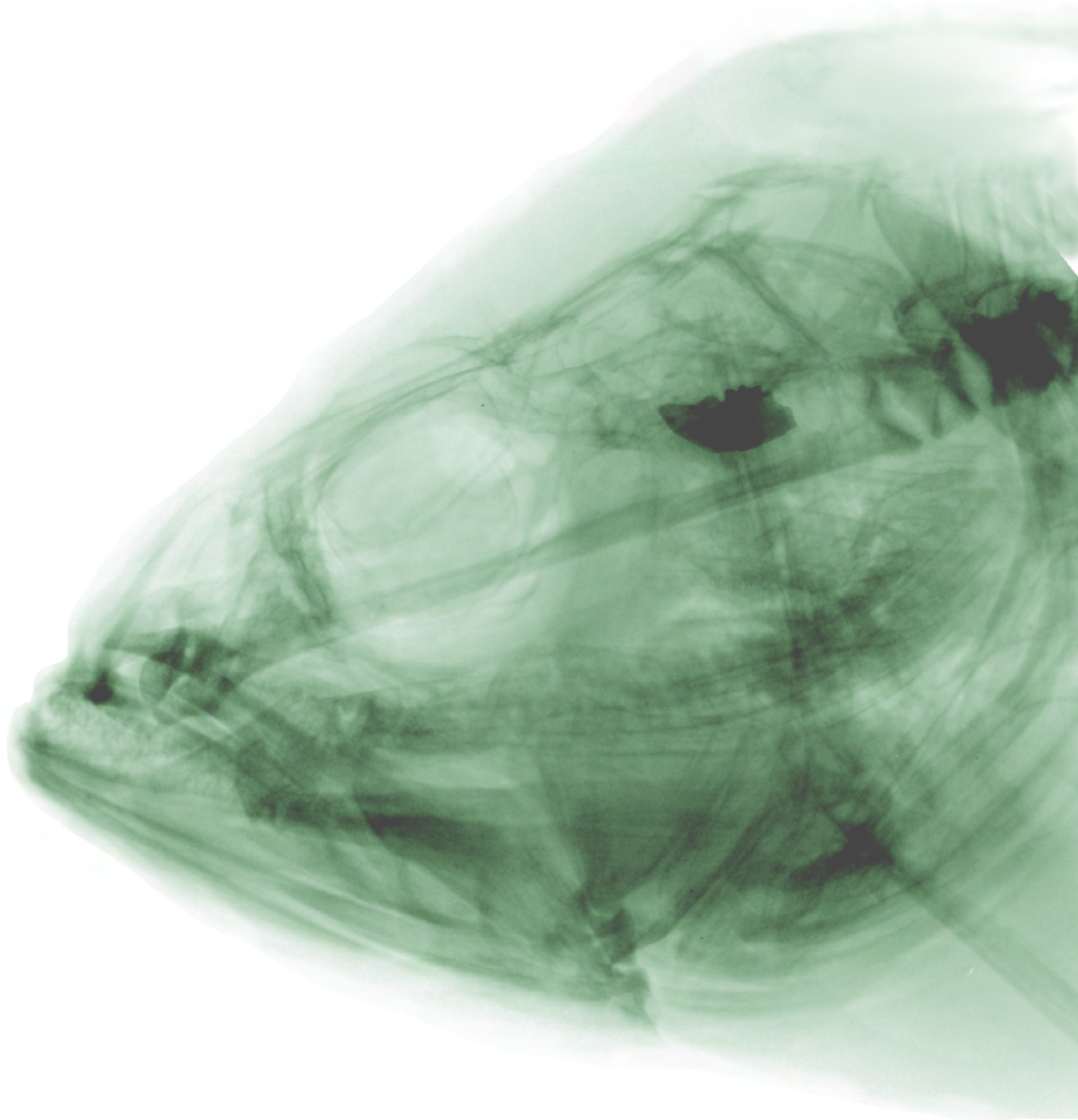


A Practical Handbook for Determining the Ages of Gulf of Mexico and Atlantic Coast Fishes

THIRD EDITION



GSMFC No. 300

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Gulf States Marine Fisheries Commission

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A Practical Handbook for Determining the Ages of Gulf of Mexico and Atlantic Coast Fishes

Third Edition

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Preface

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 by the Gulf's marine agencies to process the 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 Oceanographic and Atmospheric Administration's (NOAA's) National Marine Fisheries Service (NMFS). The original 'otolith manual' was completed in 2003 after two years of effort by the work group. The Second Edition (2009) was a continuation of the standardization effort developed previously by the Gulf States Marine Fisheries Commission's Otolith Work Group and included contributions from 14 state agencies, federal laboratories, and universities. Following the release of the revision, the Atlantic States Marine Fisheries Commission (ASMFC) reached out and requested that Atlantic Coast fishes be included in any future revision since a number of species overlapped with the Gulf. The first meeting for the next edition took place at the NOAA Fisheries Panama City Laboratory on August 19, 2014. The first face-to-face meeting of the combined Atlantic and Gulf of Mexico Ageing Work Group took place November 30-December 3, 2015 at the NOAA Fisheries Woods Hole Laboratory in Massachusetts. Several other joint meetings took place over the next three years which included the NOAA Fisheries Beaufort Laboratory in North Carolina as well. While the draft was mostly complete in 2019, the COVID shutdowns of 2020 provided more time for detailed review and final editing and polishing of the Third Edition.

The Third Edition includes contributions from many more agencies and universities from the Gulf region as well as the Atlantic. A number of techniques and species are described beyond the original scope of the manual. We have tried to provide information on all the various techniques that have proven to be useful or unsuccessful for each of the species covered in Chapter 9.0 and ensured that validation is included wherever possible. It is hoped that the wide variety of species will allow anyone interested in exploring the ageing of fish species new to them or their agency to find common techniques and accounts which will shorten the time required to pursue something that can be a trial and error process.

The most widely used methodologies and approaches are included in each species account but additional methods and details can be found in the appendices (Chapter 12.0). While the techniques in Chapter 12.0 are represented in the literature, the contributors to the current edition of the manual did not feel they were as common and didn't want to suggest expertise on our part.

As always, this manual is available online at the GSMFC website (www.gsmfc.org) and the ASMFC website (www.asmf.org) or through either Commission office.

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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 calcified structures (or hard parts) 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 alternating zones or 'rings' are distinguishable throughout the life of most fishes. This provides a reliable and permanent record of 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 by species. Ages of individual fish are required to estimate growth rate, age-at-recruitment, age-at-migration, 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 requires age data. In the simplest sense, this time frame may involve estimating the number of years a fish spends in a particular life history 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 analyses of exploited fish populations that most agencies responsible for fisheries management have begun to routinely collect and process ageing structures 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 objectives of 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 (NMFS) to process samples from Northwest Atlantic fishes and mollusks. Other reports have targeted the interpretation of daily growth increments (Pannella 1971) 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. 1995a).

Advances in microchemistry with regard to the use of diagnostic isotopes incorporated within the aragonite matrix of the otolith allows scientists to evaluate extant populations and the area or environment they grew in as well as the environmental conditions of long extinct populations. The physical makeup of an otolith encourages incorporation of various chemical markers throughout the life of the organism offering insight into the natal conditions or environment the fish originated from, the movement of the fish at various life history stages, and evidence of long distance migrations into systems with widely different chemical signatures. In addition, ecopathogens and contaminants can be tracked in the otoliths as unique chemical signatures encountered by the animal in their environments. This can be useful in identifying point and non-point source pollution.

These advanced techniques are not detailed in this manual since its focus is on the processing of hard parts for direct, visual interpretation. However, a number of papers describing the techniques have been published (Campana et al. 1995 and 1997, Sinclair et al. 1998, Radtke et al. 1999, Markwitz et al. 2000, Limburg et al. 2007) along with a thorough review of the most common methodologies, their assumptions, and their value as life history tools (Elsdon et al. 2008). This manual provides standard procedures for age determination using annual marks. A number of other techniques may be applied to daily marks but will not specifically be covered in this publication.

The intent of this manual is to be a dynamic resource that changes as species-specific processing nuances are developed and to serve as a training tool for new laboratory personnel. Descriptions of new and changing techniques will be included in future editions and techniques which become outdated or determined to be of less use may be removed as well. Standardization of techniques is a cornerstone of fisheries science, and we believe that this manual will facilitate the adoption and incorporation 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 at state and regional levels.

2.0 Pre-ageing Considerations

Prior to assigning ages to fish, there are a number of preliminary items that should be considered. First and foremost is identifying and understanding the annual marks used to estimate age. Given the variety of structures available for age estimations, it is then important to choose one which will give you precise and hopefully accurate ages. The technique used to interpret those structures should ideally be validated as precise and accurate. After ages have been assigned, it is also important to think about the future use/storage of the structures. All of these topics are outlined in this section.

2.1 Introduction to Annual Growth Zones

The successful application of techniques to detect and interpret age-related marks in the calcified structures of 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.

Enumeration of annular marks for the purpose of assigning age estimates in fish is analogous to the practice of dendrochronology, the ageing of trees using tree ring counts from a cross section of the trunk. 'Annual growth zones' can be summarized as alternating periods of slow and fast growth which are generally seasonal and occur once a year (Figure 2.1). In most fishes, marks resulting from varying growth can be seen in the layers of otoliths, bones, and other calcium-based structures; however, each 'mark' must be evaluated independently to determine if they are produced annually or are due to other biological functions which are not necessarily repeated on a regular basis. These non-annular marks can be the result of habitat or foraging shifts during ontogeny, reproduction, environmental stress, or injury. In Figure 2.1, the repeating marks (Panel A) are considered growth zones and represent slow growth periods (O – Opaque) and fast growth periods (T – Translucent), very similar to tree rings (Panel B). There are a variety of ways to validate annual growth zone formation relevant to all of these structures in fish (see Campana 2001 and Chapter 2.0, Section 2.2).

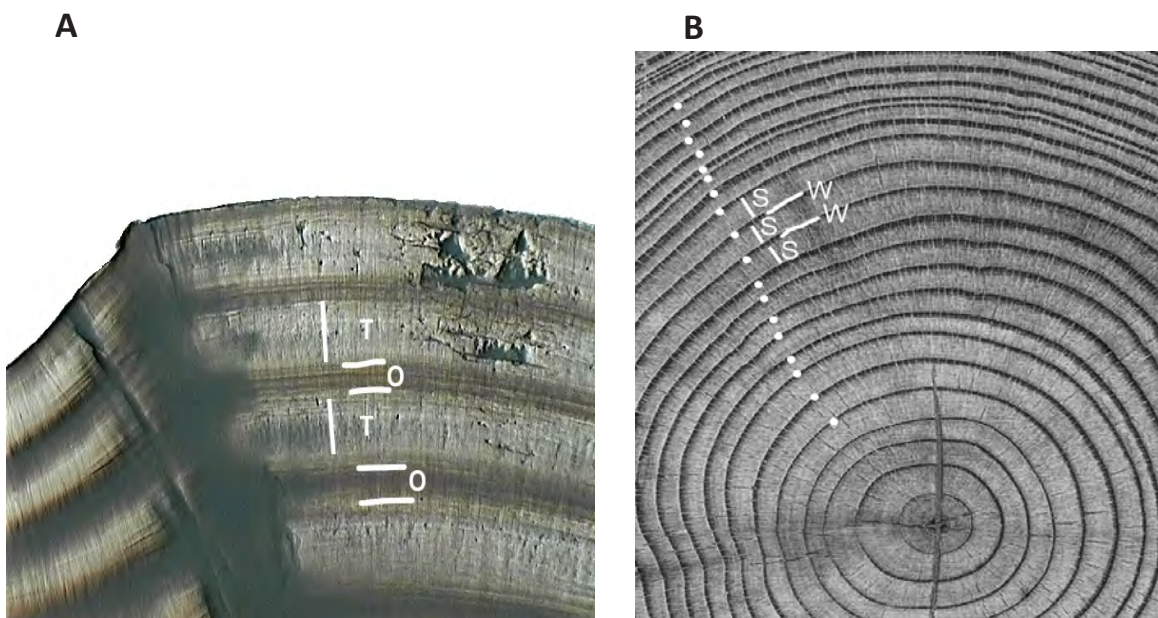


Figure 2.1 Annuli in a black drum otolith section (A) and annual rings in a cross section of a tree trunk (B); O = opaque slow growth, T = translucent fast growth, S = summer fast growth, W = winter slow growth.

In the following sections, we outline the basics of gathering hard part samples for ageing which includes otoliths, scales, opercle bones, fin spines, and fin rays. This is not an exhaustive list of biological samples which can provide information but it does include parts utilized for the work conducted along the Gulf and Atlantic Coasts of the United States. These same basic techniques would work for species originating from anywhere else in the world and can be applied in any laboratory with the same basic equipment.

2.2 Structure Choice

Several factors could determine what hard part should be used to age a species. First and foremost is maximizing the accuracy and precision of the age estimate. In many cases this leads to using otoliths. There are instances, however, where the optimal structure cannot be obtained. For instance, many commercially captured species are sold whole or even alive. Removal of otoliths in this instance is impractical or impossible without purchasing the fish, and as a result, an alternate structure has to be used but might require a sacrifice of accuracy and precision. See individual species accounts in Chapter 9.0 for species-specific guidance.

2.3 Annulus Validation

As a general rule when working with a new species, it should not be assumed that pairs of translucent and opaque zones are annuli. As such, validation of an ageing methodology is a critical, though often overlooked, component. The primary goal of any age validation study is to determine whether age estimates produced are, on average, approximately correct for the species in question (Francis et al. 2010). The caveat “on average” is necessary because even with the most rigorously controlled ageing procedure, one expects to find differences among repeated age estimates for the same fish. The goal is that independent age determinations, although likely variable, will be unbiased (Francis et al. 2010). Therefore, the focus of any age validation, implicitly, is to determine the bias of age determinations, not the precision of individual, independent reads (Francis et al. 2010). There are a multitude of age validation techniques, the most common of which will be discussed below, but for a more comprehensive list, see Campana (2001). Care should be taken when discussing validation to understand what exactly is being validated. The term “age validation” gets used a lot but is rarely accurate. In order to validate age, you must start with known age fish. All other procedures merely verify that annuli are being laid down annually.

2.3.1 Physical/External Marking

Unlike angler-based tag-recapture studies which collect data on movement and migration, growth rates, habitat preference, and post-release survival, tag-recapture for age validation relies on the tagging of known aged fish in order to verify the presence of annual increments in hard structures like otoliths. Releasing fingerling fish with internal anchor tags, dart tags, or coded wire tags into the wild, allows researchers to compare the time at liberty for the fish with the age data acquired upon its recapture and, in most cases, death. In some cases, the fish can be sampled using non-lethal means such as clipping fin rays and pulling scales and the animals can be released alive for additional recaptures in the future.

External tagging and long-term maintenance of known aged fish can be done in a laboratory or hatchery setting as well rather than using chemical marking. However, captive rearing does not always reflect natural conditions or growth patterns that might be observed in the wild. For that reason, captive experiments are only occasionally used for annuli validation when no other information is available.

Tagging wild fish of unknown age and removing a non-lethal ageing structure can be used akin to the chemical marking below (Chapter 2.0, Section 2.2.2). At the time of recapture a second structure can be

taken and compared to the first. This works well with scales and fin rays or spines. This method provides verification of annual growth zone formation but not of actual age.

2.3.2 Chemical Marking

The best method of annuli validation involves exposing a fish to oxytetracycline (OTC), calcein, alizarin complexone (ALC), or some other chemical that incorporates a mark on the otolith (and/or other hard parts) through a physiological process. The chemical combines with calcium in the various structures such as otoliths and spines and produces a mark that will fluoresce under UV light (Figure 2.2). An unknown age fish can be ‘tagged’ by injection with the chemical and the duration until the time of death can be determined directly on the calcium structure. Through release and recapture of this marked fish over time, one has a direct method for validating that one opaque ring is deposited on an annual basis. However, the potential for recapture can be low in open marine system, and chemical marks can fade over time, making this method less practical. In addition, there are legal restrictions in many places regarding releasing fish which have been chemically tagged using OTC (an antibiotic) back into the environment. As an alternative, a marked individual can be held in captivity for an extended length of time for validation. However, the timing of annuli formation of a fish held in captivity may not reflect natural conditions in the wild and should be interpreted with caution. **Note:** The USFDA should be consulted before using any chemical marking technique for validation work if one intends to release fish into the wild.

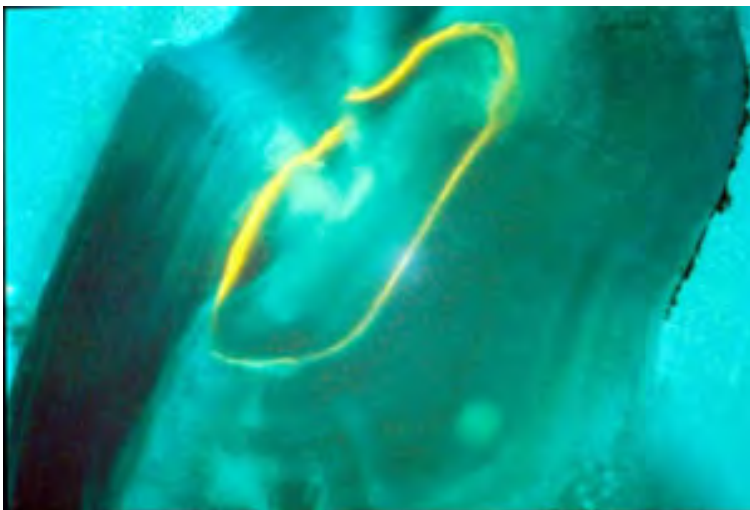


Figure 2.2 OTC marked Red Drum otolith under fluorescent light.

2.3.3 Marginal Increment Analysis (MIA)

Annual formation of the opaque zone is commonly validated by marginal increment analysis (MIA). The examination of the edge of a hard part for multiple fish captured over a time continuum (typically monthly) reveals the timing of formation of the last opaque zone. For example, if an opaque zone is found at the edge of the structure only during one time period per year, it is inferred that the process is a yearly event (see Campana 2001 for review of otolith MIA; Figure 2.3). Many times, these data are presented as the monthly mean distance from the proximal edge of the last visible opaque zone to the margin of the otolith (Figure 2.4). Lowest monthly values

of margin increments observed during a calendar year reveal the timing of opaque zone formation and, if the minimum value is observed only once per year, it is inferred that the process is an annual event (Figure 2.4). Although slightly less informative for MIA, standard margin codes can be used to describe the growth at the margin relative to the previous year's growth. See Chapter 8.0 for a full description of margin codes.

2.3.4 Radiocarbon ¹⁴C

Bomb radiocarbon (¹⁴C) dating is the most recently developed technique used in age validation studies, and has quickly become accepted as one of the most accurate methods for age validation of long-lived fishes. During the 1950s and 1960s, surface ¹⁴C activity doubled in the world's oceans due to extensive nuclear arms testing. Subsequently, testing dramatically declined after this era. These elevated levels of ¹⁴C were incorporated into the calcium carbonate structures of bivalves, corals, fish, and other organisms

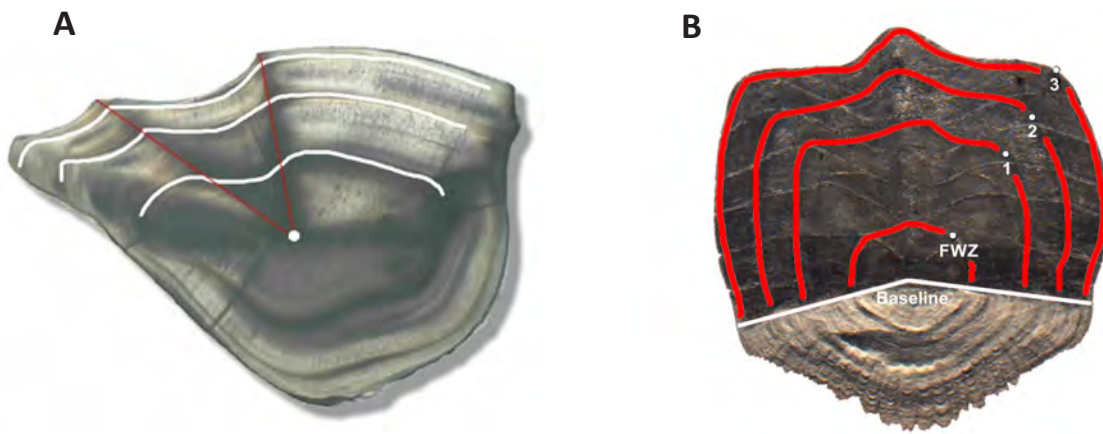


Figure 2.3 Examples of the growth zones in an A) otolith section and a B) scale inferred as ‘annual’ marks. The relative distances and condition of the ‘edge’ are examined for validation in marginal increment analysis (MIA).

growing during the 1950s and 1960s, creating a time-specific chemical marker in the structure. The first use of ^{14}C age validation was on growth rings present in hermatypic corals (Druffel 1989). Researchers found ^{14}C levels within the annual growth zones proportional to those found in the water column. In a pivotal paper, Kalish (1993) identified the utility of these increased levels in validating the age in fish. Through use of an accelerated mass spectrometer, Kalish (1993) was able to validate the ages of a New Zealand fish by comparing the ^{14}C level chronology found within the otolith’s core to a coral standard ^{14}C chronology from nearby waters.

Two pieces of information are necessary to use ^{14}C to validate an aging method: 1) a test data set that includes estimated ages and the associated radiocarbon values for the current study and 2) an accepted reference data set that contains ages and ^{14}C values for another species (Francis et al. 2010).

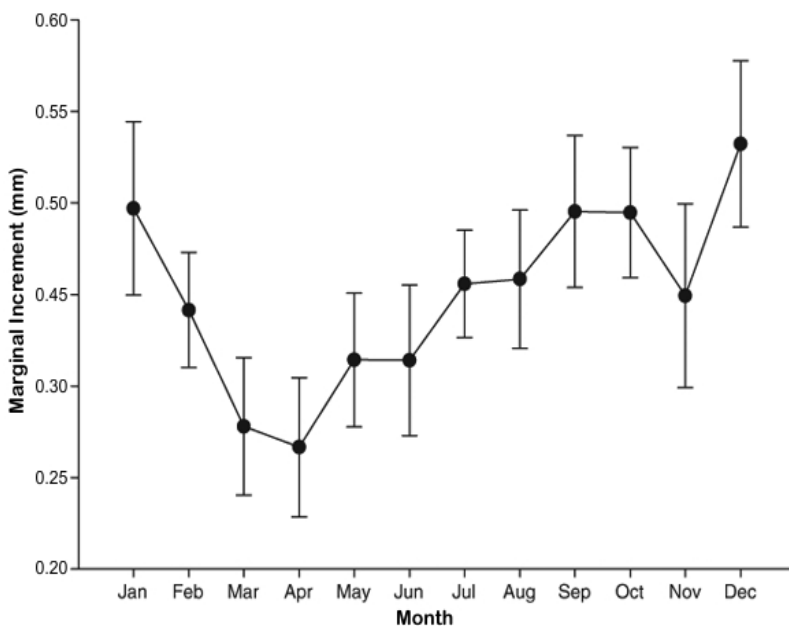


Figure 2.4 Example of marginal increment analysis (MIA) using distance to outer margin from the last completed annual growth zone. The average distance from the margin is the smallest around the period of annuli formation which is around March and April in the example.

An often non-explicit assumption of age validation using ^{14}C is that the test and reference species occupy the same, or similar, environments with respect to ^{14}C availability, so that the carbon incorporated into the carbonate structures of the two species in the same year will contain the same proportion of ^{14}C (Francis et al. 2010). However, as more ^{14}C age validation studies have become published, mainly on deep-water species, a time-lag or phase shift has become apparent when comparing them to commonly used published reference chronologies, formed from what are typically species found at much shallower depths. This phase shift has been proposed to result from differences in oceanic mixing rates causing a delay in the rise of ^{14}C with increasing depths and evidence supporting this can be

found throughout the literature (Horn et al. 2010, Grammar et al. 2015, Campana et al. 2016). Prior to performing a ^{14}C age validation study, a researcher must take into account potential impacts of hydrographical differences between the reference and test chronology.

The benefits of performing a ^{14}C age validation study are two-fold because it can validate a potential 'lowest maximum' age and annual increment formation. A lowest maximum can be validated by analyzing specimens born prior to the onset of ^{14}C , generally accepted as 1958, which should contain no detectable amounts of ^{14}C . One caveat of validating a lowest maximum age is that it only allows a maximum age to be validated to the year of ^{14}C onset. This means that even though a fish may have an estimated age of 30 years old and a capture year of 1985, a researcher can only say with certainty the fish is 27 years old; hence the term lowest maximum age. When selecting specimens to validate annual increment formation, it is best to select specimens that have birth years that fall within the period of rapid increase, typically 1958 to 1970. Historically, ^{14}C chronology comparisons were analyzed qualitatively by examining for phase shifts between the curves, with good phase agreement suggesting ages have been validated. However, there are three quantitative methods available:

1. Campana et al. (2008) proposed a method to estimate year of ^{14}C onset; comparison of the estimated values allows for identification of a potential phase shift.
2. Hamel et al. (2008) proposed the coupled-functions model which provides estimates of year of ^{14}C onset and the temporal midpoint of ^{14}C increase. The results are compared as in the Campana et al. (2008) study.
3. A final method was proposed by Francis et al. (2010) which allows for the estimation of a 95% confidence interval for aging bias between a test and reference chronology. Using this method, a strong validation produces a narrow confidence interval containing zero and a weak validation produces a wide interval.

While ^{14}C age validation is accepted as one of the best methods for age validation, it does come at a cost, with sample analysis costing anywhere from \$200 to \$850 depending on the amount of samples being processed and the type of agency performing the study. Despite these high costs, it requires one sample to validate a lowest maximum age and as little as 8-10 specimens to validate annual increment formation.

2.3.5 Otolith Microchemistry

Microchemical analysis of otoliths is a technique that has been used to aid in validating age estimation techniques in multiple species. Strontium has been at the forefront of otolith microchemical analyses, used to discriminate salinity and temperature differences for movement (Steer et al. 2009, Tzeng et al. 1999) as well as inferring age and growth (Radtke and Targett 1984, Gauldie et al. 1995, Sherwood et al. 2012).

Otolith mineralization, that is inversely proportional to Sr:Ca, is controlled by protein matrix formation which in turn is associated with metabolic rate, temperature, and growth rate (Campana 1999). This suggests that the strontium available in the endolymph surrounding the otoliths will be incorporated as a function of growth and supports the findings of increased Sr:Ca ratio during periods of low growth (typically in the winter) (Radtke and Targett 1984, Tzeng et al. 1999, Gauldie et al. 1995, Sherwood et al. 2012). These studies have successfully correlated Sr:Ca sinusoidal periodicity to annuli formation on otoliths, corroborating age data.

By measuring Sr:Ca ratios with Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICPMS), seasonal changes along an otolith growth axis can be identified with high precision due to sampling at very small increments (microns). This method provides an accurate way to measure Sr:Ca along an otolith

growth axis, assisting in the visual identification of annuli in difficult to age species. Gauldie et al. (1995) were able to better distinguish between annuli and 'checks' in otoliths of orange roughy (*Hoplostethus atlanticus*) by looking at Sr:Ca ratios and Sherwood et al. (2012) demonstrated this technique in identifying the first annulus of Monkfish (*Lophius americanus*). Due to the high cost, this method should be used to corroborate current aging methods and to help discern annuli in difficult to age species rather than a stand alone validation method.

2.4 Archiving and Long-Term Storage

Archiving of ageing structures may be useful for a number of reasons. First, archiving allows researchers to go back to the original material (un-sectioned otolith, raw scales etc.) should a sample be lost or need confirmation. Second, additional opportunities may arise for other researchers in need of historic material (daily growth, microchemistry, genetic material etc.). In most cases, structures should be stored clean (majority of tissue removed) and dry. Best practice is to have a storage location that is climate controlled, where moisture and pests can be kept away.

Whole otoliths, opercles, scales, fin-spines, and fin-rays can all be stored in paper envelopes once they are clean and dry. When left and right samples are provided, they should be stored together if possible. Small and/or fragile structures can be stored in small vials. These envelopes or vials can be placed in larger storage boxes. Boxes will preferably be made of a material that will be impervious to moisture and pests. Stackable plastic boxes with snap-on lids are an economical option. Boxes that have built in rows are helpful; however, rows can be added using cardboard or some other stiff material. Rubber bands should not be used to hold together envelope bundles, as they degrade and break in a very short time period. If there is a desire to bundle groups of envelopes together, binder clips, paper clips or sandwich size plastic bags can be used. Care should be taken with fragile structures to ensure they are not damaged by not packing the box so tightly as to squeeze and subsequently break them. Small vials can be grouped (100-200 vials) in labeled plastic bags prior to storage in boxes. More vials can be placed in the bags but the time to retrieve specific individual samples increases dramatically. Proper labeling and organization of archived samples is critical so they can be easily found and identified in the future. Structures that have been sectioned and mounted to slides can be stored in most slide boxes although newer drawer-type storage exists similar to card catalog files drawers used in libraries. **Note:** Storage of specimens in formalin will degrade otoliths by reacting with the protein matrix and should be avoided.

2.4.1 Concerns with Long-Term Physical Storage

Space for long-term storage is the biggest issue with archiving. Most of the biological materials require some sort of climate control to prevent deterioration of the samples. While some facilities have the luxury of on-site storage, most necessitate off-site storage locations. Slide storage boxes for mounted sections generate a similar concern, although most slide boxes are stackable. In addition, the ability to 'move' samples from storage to avoid damage or loss from tropical storms/hurricanes is a problem near coastlines. Finally, the ageing data forms which are typically paper require storage as well. While many of the agencies' labs have transitioned to electronic reporting by samplers, historical forms still need to be maintained posing similar space and climate control issues.

Flo-Texx® and Cytoseal® seem to hold up over the long-term, as they have not been found to degrade, crack, or change color over time. Loctite should be stored in a climate-controlled space because heat will soften the material, and slides stored horizontally will have the sections slide off the slide and stick to the surrounding material (slide box, aluminum sheet, etc.). Loctite® will also crack and turn brittle over time (Figure 2.5); however it can be ground off to reveal the section underneath.

Lack of climate control can result in humidity issues with mold and mildew of envelopes. There can also be issues with bugs degrading tissues over time (scales, etc.). In some cases, even when something like cryo-vials are used to store small structures, without labeling the vials themselves, loss of samples can result should the envelope deteriorate.

2.4.2 Benefits of Long-term Physical Storage

Structures that are organized, catalogued, and stored in a climate controlled environment provide a valuable resource. They potentially offer a historical value, providing access to specimens that are potentially older, possibly before fishing pressure existed (virgin fishery), or before regulations were established, as well as, a source of environmental event information (climate change) that can impact fish growth and annuli formation. Archived structures offer the ability to re-age specimens at a later date, especially with improving technology and explore drift by readers over time. They provide the ability for conducting microchemistry studies and offer the potential to conduct genetic studies (see Chapter 2.0, Section 2.2 and Chapter 12.0, Section 12.2.4). Finally, stored structures have an educational value, providing materials for training new personnel in specific ageing techniques.

2.4.3 Digital Storage

Considering the issue many laboratories have with long-term storage space, some labs have begun digitizing their structures, cataloging the images for use in the future, and discarding the physical samples after a given period of time (five years for example). The images can be stored and organized electronically in multiple locations and separate hard drives for backup. Care must be taken to ensure the structure is photographed in a way in which it can be used for future age estimations. Prior to discarding any samples, it is best to check with other potentially interested agencies/universities to see if there is any need for the samples.

2.4.3.1 Benefits of Digital Storage

Digital storage can be combined with physical storage. In the event that there is a question regarding an archived sample, digital images can be retrieved quickly and easily without the need to physically find the structure. If further investigation is needed the physical structure can then be located. Digital images can be stored easily in multiple locations in the event of a catastrophe (computer crashes, severe weather events, fire etc.). In some species where otoliths are read whole prior to being sectioned, it can be advantageous to save images of the whole otoliths that can be compared with the sections later.

Digital storage allows labs with limited storage capabilities a chance to maintain vital age records electronically. For these images to be beneficial in future use, they must be high resolution, on plane, and fully in focus.

2.4.3.2 Concerns with Digital Storage

While digital images can be used for future age estimations, many readers would prefer to be able to manipulate the physical structure rather than look at a 2D image. The photographer takes an image that



Figure 2.5 Otolith section that has been covered with Loctite® and has cracked over time.

is representative of the age estimation that they made. The reader/photographer may save an image that focuses more or less on parts of a structure that another reader might not. This can lead to inadvertently biased ages by a future reader.

Digital storage does not enable future studies to look at different aspects of the structures/tissues such as daily growth, chemical analysis, genetic tests, etc. High resolution images also require a large amount of electronic storage space and will need to be backed up in the event of computer failure.

3.0 Otoliths

3.1 Introduction (Function, history, pros and cons)

In general, teleosts 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 ampulla, a fluid filled chamber for sensing inertia (Figure 3.1A 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 ampullae 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 lapillus otolith, which is used predominantly to detect gravitational force and sound (Popper and Lu 2000).

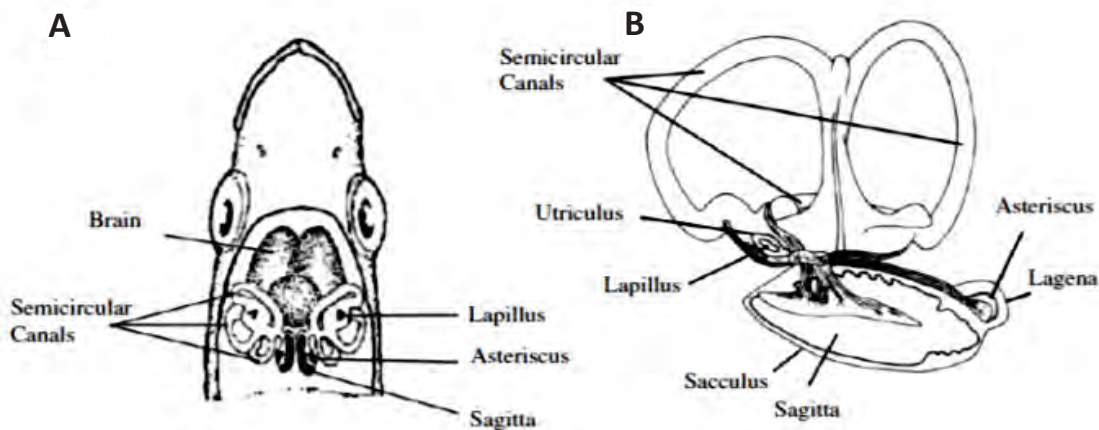


Figure 3.1. A) Location of the otolith pairs within a generalized fish (*modified from Secor et al. 1991*) and B) medial view of the inner ear (*modified from Moyle and Cech 1988*).

The ventral portion of the teleost inner ear includes the sacculus and lagena that each 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 that have a higher density than the fluid-filled chambers they occupy. As sound waves are intercepted, the otoliths move independently of the surrounding chamber, causing mechanical stimulation of the hair cells (Figure 3.2). This process results in an auditory signal allowing the fish to 'hear.'

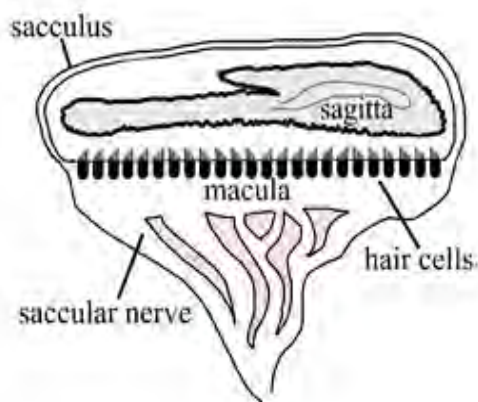


Figure 3.2 Generalized structure and components of the sacculus.

The sagittae are typically the largest otoliths in most fishes and are therefore the most often used for ageing. Lapilli can be used for daily rings and a number of the catfishes have a larger lapillus which is more helpful for ageing. Each species group may have differences that would necessitate alternative structure to the sagittae, but for the purpose of this manual, the sagitta will be primarily used to age the majority of the species. Please note, however, that some researchers recommend the use of other otolith pairs (Secor et al. 1991).

The sagittae lie within the saccula and are attached to a noncellular, otolithic membrane. Along the medial surface of the otolith lies a gelatinous pad known as the macula and

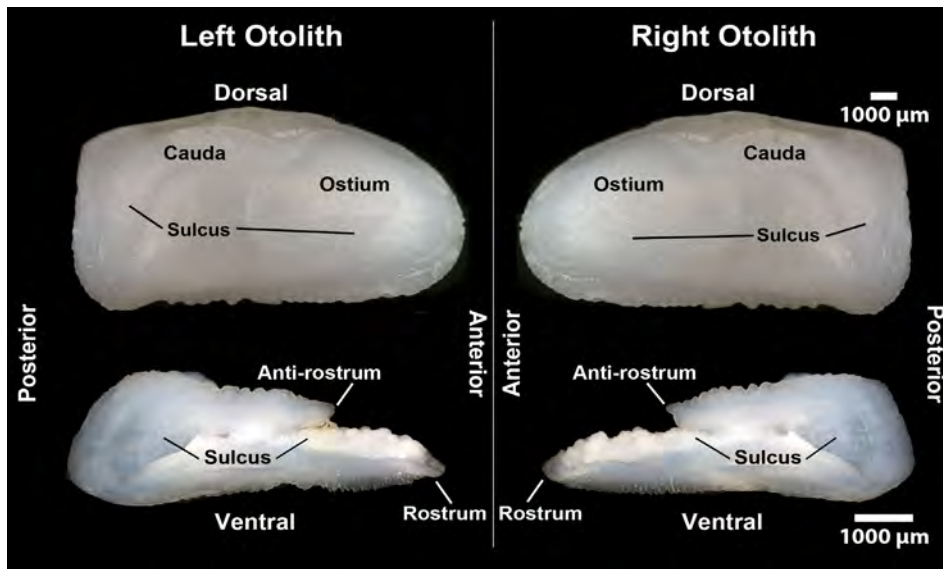


Figure 3.3. Medial surface of the left and right sagittal otoliths from a Red Drum (top) and King Mackerel (bottom).

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 anterior end of the otolith and the sulcus acousticus that forms a furrow (sulcal groove) along the medial surface of the otolith (Figure 3.3). 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 medial surface of the sagitta.

Otoliths are crystalline in nature and are built up around a primordium/core region 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

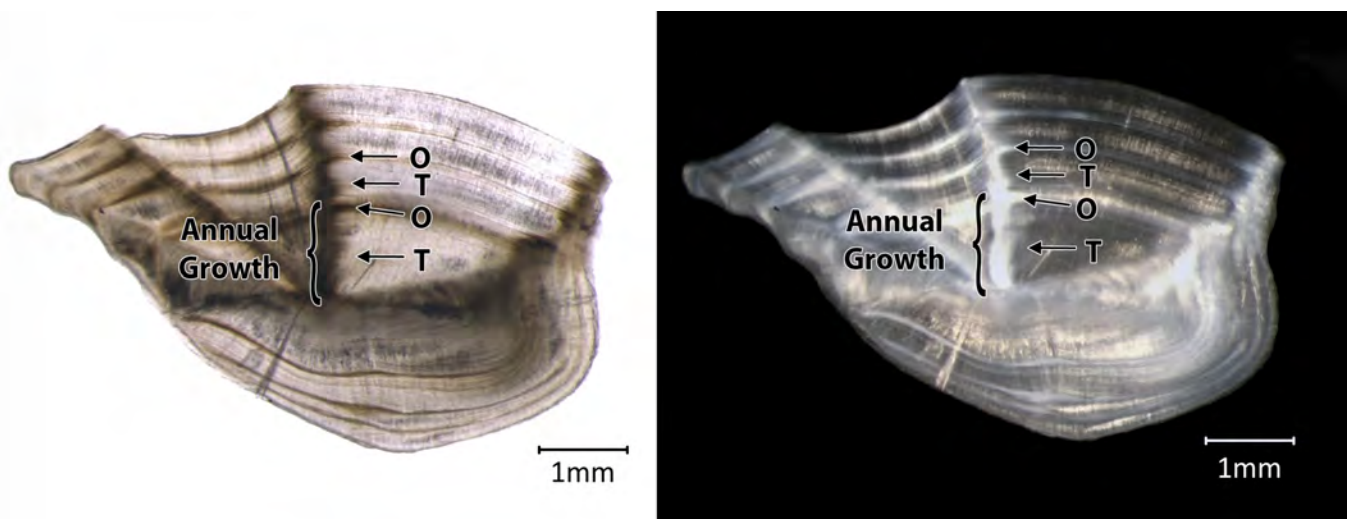


Figure 3.4. Close-up of alternating opaque (O) and translucent (T) zones in a sectioned spotted seatrout sagittal otolith under transmitted (left) and reflected (right) light.

densely spaced during periods of faster growth during warmer months make up the translucent rings (Figure 3.4). An annual zone consists of one opaque and one translucent ring. When the formation of successive opaque and translucent rings occurs on an annual basis, they are collectively referred to as annual growth zones, and the opaque rings are frequently called the annuli (singular: annulus).

When the alternating rings of an otolith cross section are viewed under magnification, the opaque rings lying along a 'reading' or 'counting' axis are conventionally the ones tallied for age estimates. The counting axis is generally described by a hypothetical line on one side or the other of the sulcus, extending from the core to the outer edge of the otolith (Figure 3.5).

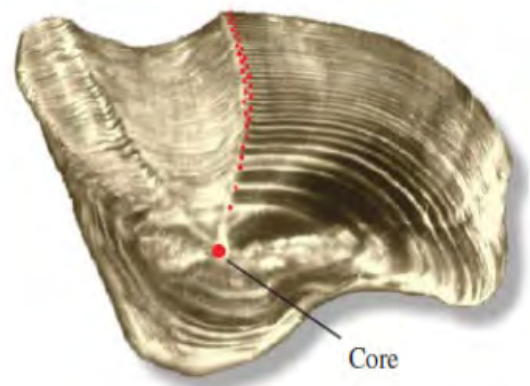


Figure 3.5. Transverse section of a Black Drum sagittal otolith including location of the core and rings along the sulcus. Red dots denote the annuli along the counting axis.

Sagittal and lapilli otoliths have been used to estimate daily growth during the first year of life and specific intervals later in the fish's life. The astericii are

not typically used for daily growth, because they are formed later in life than the other two pairs of otoliths that are present in the fish at hatching/birth. Daily growth is beyond the scope of this manual however, but is described well in Pannella 1971 and 1974, Brothers et al. 1976, Brothers 1984, Campana and Neilson 1985, Radtke 1989, and Wenner et al. 1990.

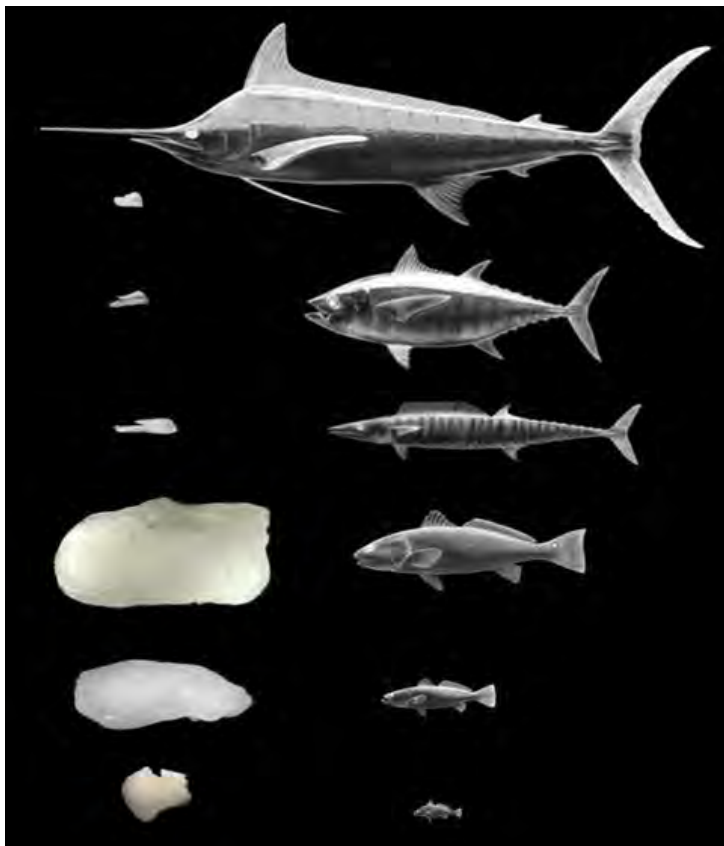


Figure 3.6 Relative otolith size and body size of several species of Gulf of Mexico fishes (not to scale). From top to bottom: Blue Marlin (*Makaira nigricans*), Yellowfin Tuna (*Thunnus albacares*), Wahoo (*Acanthocybium solandri*), Red Drum (*Sciaenops ocellatus*), Spotted Seatrout (*Cynoscion nebulosus*), Atlantic Croaker (*Micropogonias undulatus*).

Otolith morphology differs by species. 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). The relative size of the otolith also varies widely, but is somewhat based on the needs of the particular species. Pelagic fish which live offshore in clear water tend to have very small otoliths and large eyes, relying more on vision than the sensory information derived from the 'inner ear.' In contrast, the nearshore species, which live in much more turbid water, have larger, thicker otoliths since they require more auditory information when sight is limited. Figure 3.6 provides the

relative size of a few pelagic species and their otoliths compared to three species of the drum family, which inhabit the nearshore environments.

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, to fishery scientists, are as biological and ecological information storage units 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). When comparing otoliths to other fish hard parts, such as vertebrae, scales, fin rays, and spines, otoliths often provide more accurate age estimates due to their continuous accretion and limited resorption, whereas other hard parts tend to underestimate age.

3.2 Preparing Otoliths for Ageing

3.2.1 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. Otoliths should be removed (post-mortem) after these data are recorded since the otolith removal process will often physically alter the fish, making some of these features impossible to accurately assess.

Sagittal otoliths (the otoliths most commonly used for ageing) lie inside the otic capsule located toward the posterior end of the ventral surface of the skull (Figure 3.1A). 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. Some of the more common techniques are described here, as well as in each species account in Chapter 9.0 of this manual.

In the first method (Figure 3.7), useful for any fish when the external appearance of the whole 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 (Figure 3.7A). 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. Under this muscular tissue and lateral to the midline is the outer wall of the otic capsule (Figure 3.7B). Its location and shape varies by species and is described in greater detail in Chapter 9.0.

Using a stout knife or chisel (depending on the thickness of the capsule wall), layers of the otic capsule wall are removed until the sagitta and its surrounding membrane are fully exposed (Figure 3.8A and B). Using appropriately sized forceps, the sagitta are gently removed (Figure 3.8C). Both sagittae can often be extracted through the single opening in the otic capsule. If not, the process can simply be repeated on the opposite side. If the external appearance of the fish is not



Figure 3.7 Otolith removal through the gill arches under the operculum; ventral view.

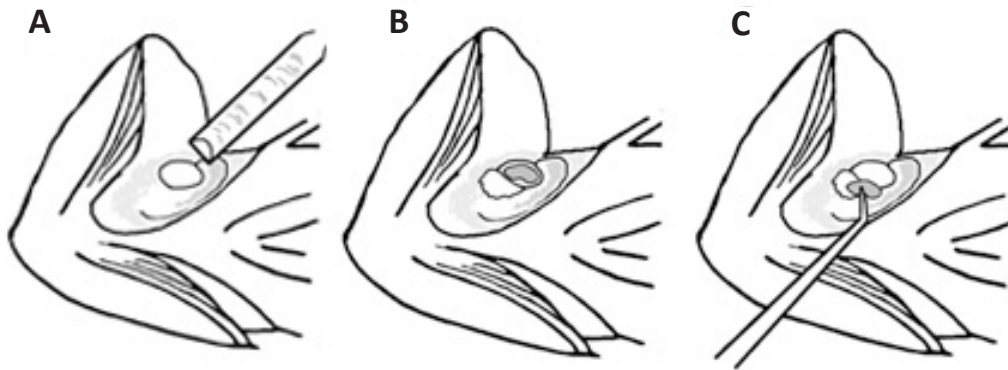


Figure 3.8 Removal of the otolith by exposing the otic capsule through the gill cavity using a sharp chisel. A) Gill cover flared with gills removed exposing otic capsule. Utilization of a chisel or other sharp object to scrape or shave off capsule surface. B) Open otic capsule with otolith exposed. C) Otolith removal.

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.

A second 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 3.9A). 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 pried open, exposing the split otic capsule, the otoliths are removed using forceps (Figure 3.9B). **Note:** It is important to make the cut down the center of the head to prevent damaging the otoliths.

The third 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 3.10 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 to make a transverse cut (Figure 3.10 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 (Figure 3.10). The otoliths are then removed using forceps.

The 'Score and Break' technique is also useful on small to medium-sized fish that do not need to be kept in saleable condition, or on filleted carcasses. The otic capsule is exposed by removing the gills (Figure 3.11A). The otic capsule is scored with a knife, then broken open by pressing

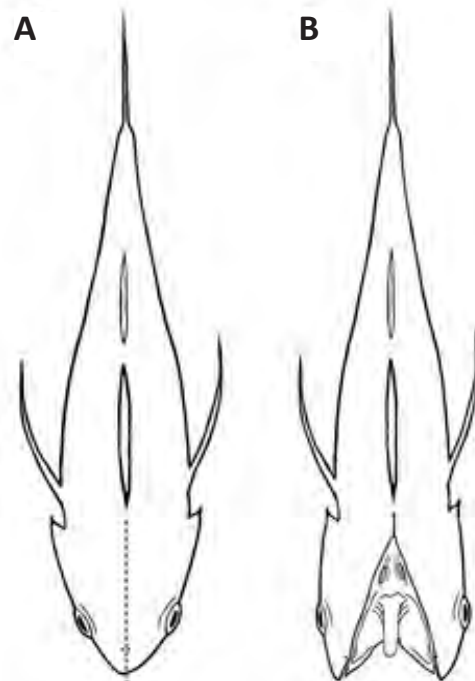


Figure 3.9 The butterfly method. A) Split the head laterally through the center line and B) expose the otoliths above and behind the buccal cavity.

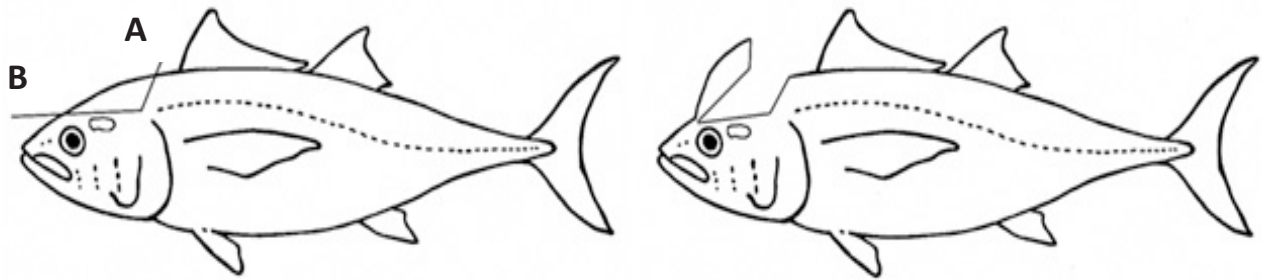


Figure 3.10 Cutting planes A and B for excision of the sagittal otolith through the upper neurocranium.

down on the anterior portion of the fish head (Figure 3.11B). Breaking the otic capsule can be facilitated by placing the dorsal surface of the skull over a board or pipe that is aligned with the score, holding the body of the fish firmly and pressing down forcefully on the skull. When the otic capsule breaks, the otoliths are exposed and can be easily removed (Figure 3.11C)

3.2.2 Cleaning Otoliths

Otoliths have been traditionally used for ageing fish; however, analysis of otolith 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 distilled or purified water and allowed to air-dry before storage. Otoliths for solution-based inductively coupled plasma mass spectrometry (ICP-MS) or laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) analysis should be removed by a technician wearing gloves using non-metal instruments, such as acid-washed glass probes or ceramic-tipped forceps, and cleaned of proteins using a couple drops of ultrapure hydrogen peroxide followed by triple rinsing with purified water to flush the surface. But, recognizing that in many cases this is not possible or otoliths will be sectioned using metal blades, sectioned otoliths for LA-ICP-MS analysis can be ground with a fine-grained sandpaper to remove the contaminated surface and then sonicated. 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.

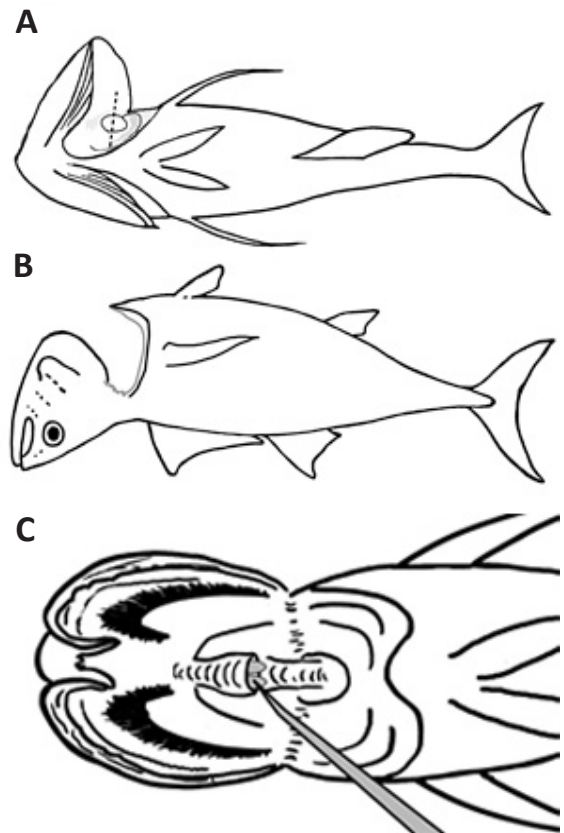


Figure 3.11 A) Expose the otic capsule through the operculum and score it with a sharp tool. B) Press the head down and away from the body by the opercula. C) Remove otoliths.

3.2.3 Evaluating Otolith Condition

In order to be useful for age determination purposes, otoliths need to have formed correctly. There are a few instances where this does not happen. These malformed otoliths need to be very carefully considered and/or excluded from further analyses.

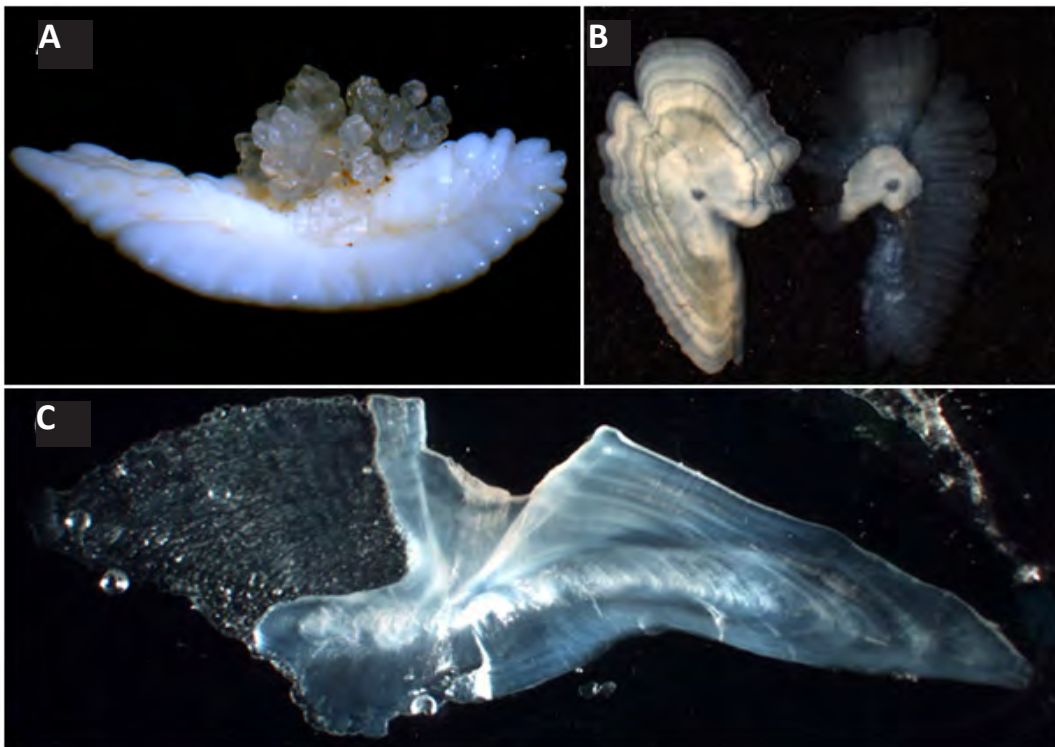


Figure 3.12 A) An Atlantic Cod otolith showing significant malformation due to crystallization. B) A pair of American Shad otoliths. One of normal formation and the other almost completely crystallized. C) A partially crystallized Red Snapper otolith cross section.

Typical otolith formation is the aragonite form of calcium carbonate precipitated on a protein matrix. Otoliths can at times form with either the vaterite or calcite forms of calcium carbonate instead. These otoliths are referred to as crystallized (Figure 3.12A). Otoliths can be partially or completely crystallized (Figures 3.12B and C). The crystallized portion of these otoliths does not show the same growth rings as the aragonite portions of the otoliths and should therefore not be used. As the crystalline structure of these otoliths can be difficult to see under some lighting situations, extreme care should be taken when trying to interpret a partially crystallized otolith.

Some otolith malformations may not be evident until post processing. For example, otoliths can have a shift of growth axis which is not evident until a cross section is taken (Figure 3.13). If the otolith shifts in relation to the canal it is in, the axis of growth can shift, leading to difficulty interpreting annual growth zones immediately following the shift.

Occasionally otoliths are seen that exhibit color other than the typical white (Figure 3.14). The color does not seem to negatively impact the age determination of these otoliths.

3.2.4 Examining Whole Unsectioned Otoliths

Examination of a whole otolith using transmitted light can often reveal marks expressed on the surface (Figure 3.15). This technique has predominantly been used for otoliths taken from larval and small fish, but also has been used successfully to age older Gag (*Mycteroperca microlepis*; McErlean 1963) and Red Grouper (*Epinephelus morio*; Johnson and Collins 1994). 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 annuli counted on whole otoliths correspond with the ‘correct’ number

of annuli observed in sections. Annuli counted on whole otoliths for Striped Mullet (*Mugil cephalus*) in Mississippi were consistently one fewer than the counts from sections of the same otoliths (J. Warren personal communication). Inconsistencies have also been observed when comparing whole and sectioned Southern Flounder (*Paralichthys lethostigma*) otoliths (A. Fischer personal communication). As a rule, whole otoliths should be stored dry. Small or fragile otoliths should be protected in a hard rigid container such as a small glass scintillation vial or a plastic microcentrifuge vial. Larger otoliths may be stored whole using a variety of containers.

3.2.5 Sectioning Preparation for Otoliths

Before sectioning, whole otoliths should be examined because some can be read in certain situations and sizes. When deciding to section, the techniques presented here will depend on individual laboratory preferences, budgets, available equipment, and otolith morphology. Three methods of preparation for sectioning are generally used: embedding whole otoliths in an epoxy resin, mounting whole otoliths to glass slides, and free hand cutting of whole otoliths followed by mounting on slides for sectioning.

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. If one otolith is preferred over the other, the species specific chapter will address it.

3.2.5.1 Marking the Core (Focus/Nucleus)

It is helpful to mark the core or some frame of reference prior to sectioning to ensure a perpendicular section. With an ultra-fine point pen or pencil, place a mark over the core of the otolith (Figure 3.16).

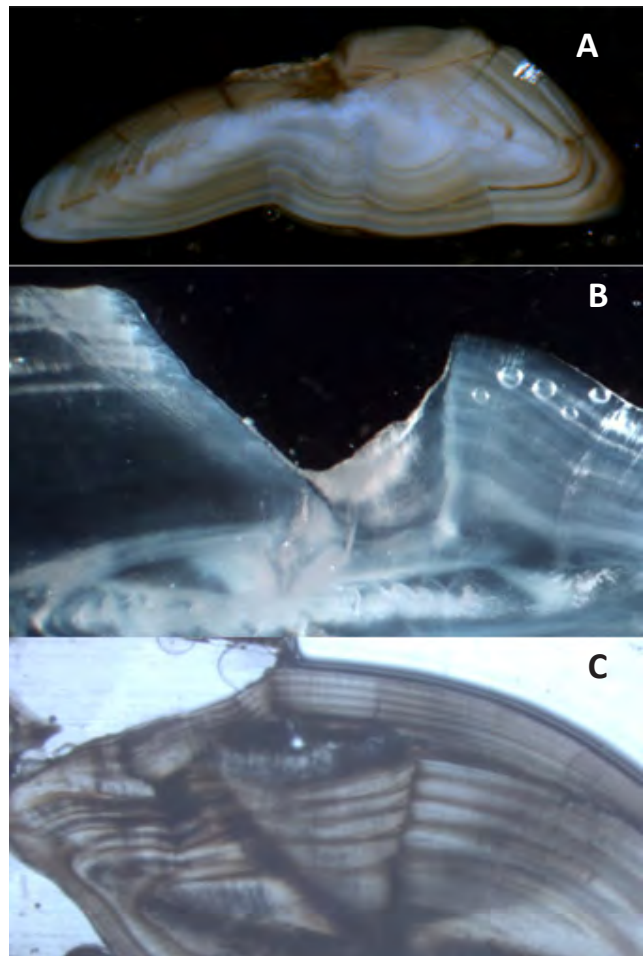


Figure 3.13 A) Atlantic Cod otolith section exhibiting a minor shift in growth axis. B) Red Snapper otolith section with a shift near the core. C) Red Drum otolith with shifted growth axis.

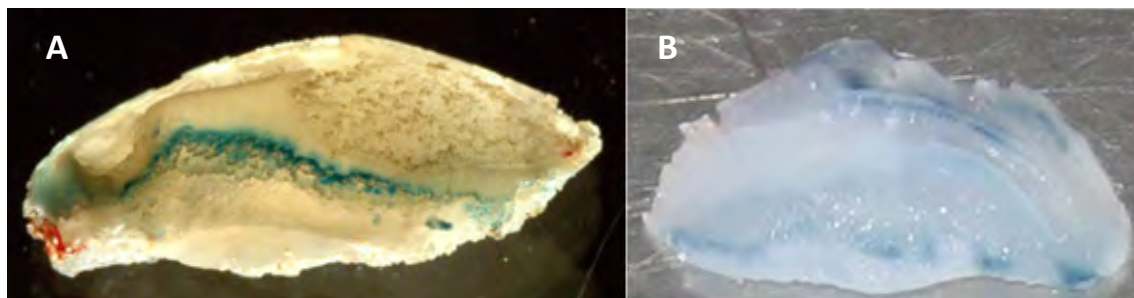


Figure 3.14 Two whole Spotted Seatrout otoliths showing traces of A) green and B) blue coloring.

Otoliths can be marked before or after embedding. On one side of the mark, a reference line can be drawn in the transverse plane of the otolith or an embedding block to assist in aligning the blade for sectioning. Depending on the size of the otolith, marking may need to take place under a dissecting scope. 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.



Figure 3.15 Ventral posterior edge of a stained whole sagittal otolith from an age-5 King Mackerel.

3.2.5.2 Embedding Otoliths

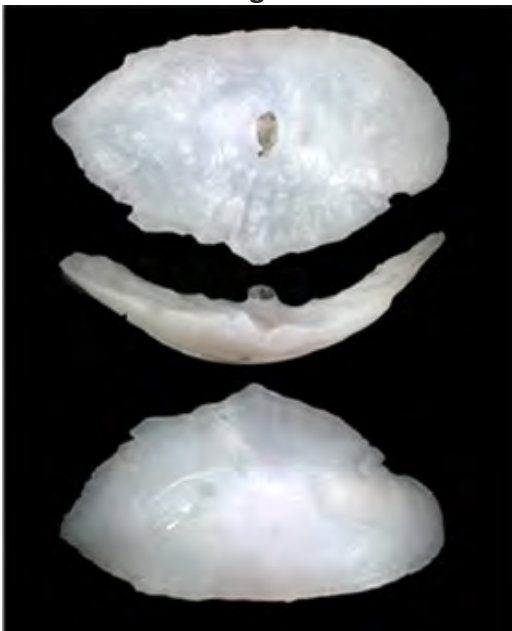


Figure 3.16 Well marked core on a Red Snapper otolith prior to embedding or sectioning.

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 materials safety data sheets (MSDS) available. Several embedding media are available and are widely used throughout the various marine agencies. The most common, two-part epoxy resin, will be generally discussed for embedding large and small otoliths (Figure 3.17).

A wide variety of options are available as mold trays for embedding otoliths. These include specific use products designed for scientific purposes as well as simple, household/cooking products that work equally well. Silicon is generally being used in most labs today because it doesn't have the problems which can occur with plastic based trays and molds. In addition, some labs spray/coat their silicon molds before each use to ease the removal of the cured epoxy resins.

Typically a number of commercially available, two-part epoxy resins can be used to embed otoliths in molds. **Note:** *While most of the two-part epoxies are non-carcinogenic, check the MSDS on any materials and use accordingly.* Resin and hardener should be measured by weight in separate containers and combined in a disposable plastic beaker. The combined resin and hardener should be mixed thoroughly but not 'whipped' as bubbles may form in the resin and disrupt the readability of the otolith.

In the single-pour method, if the mold does not have its own label, a permanent ink marking pen can be used to

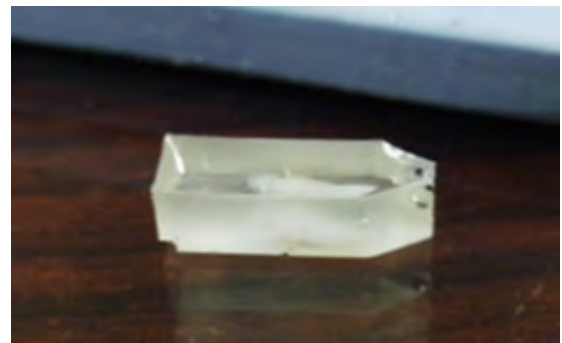


Figure 3.17 Small otolith embedded in a block of resin or embedding media that has been removed from the flexible, reusable bullet mold.

label the inside of each mold with the unique otolith identification number or a small paper label may be included in the block (Figure 3.18). The mold should then be filled with epoxy and the corresponding otolith. A probe should be used to manipulate the otolith into a proper position in the mold after epoxy is added. The otolith should then be gently rolled from side to side to release trapped air bubbles.

In the two-pour method, a small amount of epoxy 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. Once labeled, the otolith is placed in the mold, on the false bottom, and covered with a second pour of epoxy. Bullet molds are recommended for small, fragile otoliths (Figure 3.19) and a two-step pour can be used. These bullet molds have labels that transfer from the mold directly onto the embedding material, so no internal labeling is necessary; however, the individual mold number needs to be recorded on the slide or cardstock.

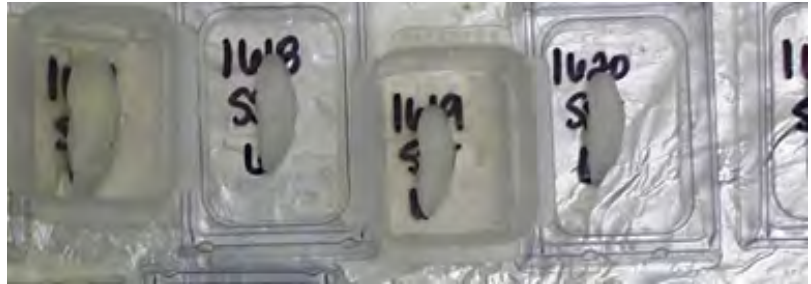


Figure 3.18 Embedding molds labeled with identification information.

The epoxy should be allowed to cure based on the manufacturer's guidelines. 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 should transfer 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.

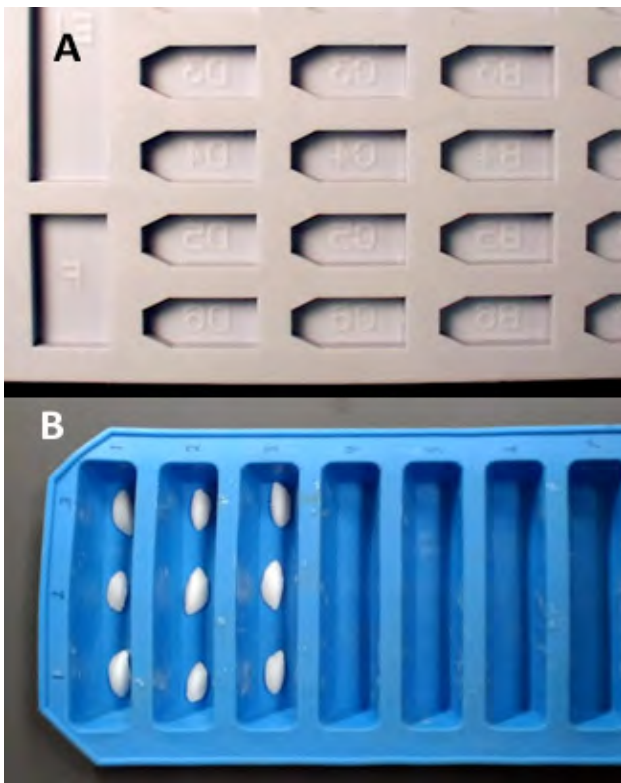


Figure 3.19 A) Typical silicon bullet mold used for embedding small and/or fragile otoliths and B) water bottle ice cube mold for larger otoliths.

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, or hand sanding if desired.

3.2.5.3 Mounting Whole Otoliths on Slides

The following technique works well for both fragile and robust otoliths, but fragile otoliths should be embedded first to prevent breakage. Otoliths to be sectioned should be clean and dry. Whole otoliths are adhered to a slide using thermoplastic cement. To begin, place the slide on a hot plate set at medium to high heat. Apply a small amount of thermoplastic directly onto the slide and allow it to melt. Keep in mind, the slide will have to fit into the saw chuck so it is necessary to leave adequate space at one end of the slide. Remove the slide from the hot plate and be prepared to 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.

While the thermoplastic is still soft, place the posterior end of the otolith into the pile of thermoplastic on the slide and pack 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 the slide 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 which the sections will be cut (Figure 3.20). 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. An alternative process used by some to adhere the ends of the otolith is to use two-part epoxy or even hot-melt glue applied with a glue gun. 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 core of the otolith. As an alternative to glass slides, otoliths can be mounted/glued to heavy cardstock and clipped onto the chuck with minor modifications for multi-blade or high volume sectioning (See Chapter 3.0, Section 3.2.6.2.3 Multi-blade Sectioning).



Figure 3.20 Otolith mounted to a glass slide using thermoplastic on each end. The central portion of the otolith must remain clear of adhesive.

3.2.6 Sectioning Techniques

Otoliths and other hard parts are generally sectioned using rock and gem cutting (lapidary and metallurgical) saws. Three main saw types are currently used by the various ageing labs: the high and low speed wafering saws, and the thin sectioning machine. With the wafering saws, thin circular saw blades coated with diamond particles are passed through the otolith or sample in serial cuts to achieve thin sections, which allow the transmittance of light. When using the wafering saws, it is practical to cut three or four sections from the otolith to ensure a section containing the otolith core is obtained. Depending on the species, size of the sample, weight, and saw speed, it can take anywhere from five seconds to several minutes to cut through a sample. The thin sectioning machine relies on a larger, single blade to make an initial cut and then the remaining half of the otolith or resin block is adhered to a slide and ground on a second portion of the machine to a single thin section ready to read (Chapter 3.0, Section 3.2.6.3). Most of the saw manufacturers provide repair services, technical support, and can recommend appropriate-sized chucks for a variety of cutting techniques. Also note that all labs have different equipment and may do things slightly different. This manual is just a general outline for saw use.

3.2.6.1 Breaking

Although this method does not create a thin section as the following methods do, it does produce a cross section of the otolith for examination of growth zones. With this method, the sagittal otolith is literally broken in half (dorsal-ventrally) through its nucleus (core) (Figure 3.21). This can be facilitated in thicker otoliths by scoring the otolith through the core using a diamond-point marker. After breaking, the exposed surface is typically heated over an alcohol flame to enhance the contrast between the organic and inorganic components of the matrix (Christensen 1964). This additional process is enhancement called burning that can be found in Chapter 7.0. 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. In the southeast, this method has been successfully used on White Grunt (*Haemulon plumieri*)



Figure 3.21 Sagittal otolith scored and broken through the core.

(D. Murie personal communication) and Red Porgy (*Pagrus pagrus*) (Devries 2006). This method has been used extensively for production ageing for stock assessments in the northwest (e.g., Alaska Fisheries Science Center - Chapter 12.0, Section 12.7). The surface of the broken otolith is usually not a perfectly flat surface, however, and it may be more difficult to measure annuli if necessary for back-calculation.

3.2.6.2 Wafering Saws (High and Low Speed) (Buehler®, Southbay®, Allied Tech®, MTI®)

Wafering saws can reduce the processing time for sectioning high volumes of samples and result in sections that need little to no polishing and can be read clearly after applying a liquid cover slip or mounting solution. These saws can also be equipped with multiple blades which can further decrease sectioning times as well as enable the user to section samples at smaller thicknesses (Chapter 3.0, Section 3.2.6.2.3). Both saws are relatively simple to operate. Blades for these models can be expensive, so care should be taken to reduce blade breakage and corrosion. Problems with electrolysis or corrosion between the aluminum blade flanges and the copper-coated saw blades have been encountered, but do not appear to impact saw operation or blade life. These problems can be avoided with proper daily cleaning/drying and regular saw maintenance.

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High speed saws are capable of sectioning larger samples much faster. Advantages with these types of saws include faster sectioning times, digital displays of section thickness and blade speed, as well as the adaptability for multi-blade sectioning (Figure 3.22). Speeds range from 150 to 900 rpms but are generally run around 300 rpms. Sectioning times through a resin-embedded otolith on the high speed saw will vary based on block size, but usually take anywhere from 5 to 20 seconds. On certain models of saw, cutting speed, load, and chuck position are controlled by pressure pads and settings for all three are displayed digitally and will need to be adjusted for each species being processed. One downside to the high speed wafering saw is that the saw has a safety switch which prevents blade or pump operation when the cover is open. In addition, sectioning at higher speeds can increase blade breakage, especially if the block is not completely secure in the chuck which is why most of the high speed saws are still used at lower speeds.

Low speed saws with their small size and reduced cost allows for simultaneous operation of multiple saws to achieve a high sectioning production rate (Figure 3.23). The low speed saws have a maximum speed of 300 rpm and generally use 3-inch, diamond wafering blades. The cross feed micrometer can be adjusted by a dial and moves the chuck arm across the blade.



Figure 3.22 High speed wafering saw (cover opened).

3.2.6.2.1 Embedded Otoliths

The resin block containing the otolith is placed in the chuck of a wafering saw equipped with a diamond blade. 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. 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 3.24). For otoliths embedded in small bullet molds, it may be necessary to first mount the block onto a slide using thermoplastic or other adhesive and then align the slide in the chuck. Failure to tighten the block in the chuck appropriately may result in sample destruction and a ruined blade. Do not start the saw while the specimen is resting on the blade as it could damage the sample and/or the blade. Gently lower the sample onto the turning blade to begin sectioning. Sectioning begins just posterior to the otolith core, and sequential sections are made approaching the core region 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 3.25). Sections are examined under a dissecting microscope or magnifying glass to identify that the otolith core was captured. If the core was missed, another attempt at sectioning can be made. They are then affixed to a labeled glass slide (Chapter 7.0, Section 7.1.4). Depending on the type, size, or fragility of the otolith and embedding medium used, the saw speed can be 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.



Figure 3.24 Embedded otolith mounted in low speed saw with resin block with otolith oriented against a blade at 90°.



Figure 3.23 Low speed wafering saw

reached within two to three cuts.

3.2.6.2.2 Mounted Otoliths

When sectioning whole mounted otoliths using a low speed wafering saw, check the recommended arm weight and blade speed for that species (some specifics are provided in Chapter 9.0). This may require some trial and error with new species. Secure the slide with the adhered otolith in the chuck, but do not over tighten as the slide can break (Figure 3.26A). Check the angle of the sample to ensure that the blade will section the otolith in the transverse plane. Line up the blade based on the core. Gently lower the otolith onto the turning blade to begin sectioning. Depending on the species, size of the otolith, weight, and saw speed, it can take anywhere from 30 seconds to several minutes to cut through the otolith. Thickness of the samples can be altered depending on the species (see Chapter 9.0 for specific recommendations). When the blade 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. It is practical to cut three or four sections from the otolith to ensure the core was captured (Figure 3.26B).



Figure 3.25 Alignment of the block to the blade is made by adjusting the micrometer.

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 water and allow them to dry. Examine the sections under a magnifying glass or low-power microscope to ensure that a good core section has been obtained (Figure 3.27).

Sectioning whole mounted otoliths (glass slide or cardstock) with the high speed saw can be difficult, but with reduced speeds of 300 rpms or less is possible. Sectioning techniques are the same as the low speed saw except high speed saws do not have an easily swinging specimen basket to catch the fallen section. Sectioning fish spines can be done with great success on a high speed saw using plastic cardstock and hot glue. The glue keeps the sections attached to the cardstock so they do not fall into the coolant reservoir.

3.2.6.2.3 Multi-blade Sectioning

A number of laboratories have begun utilizing multiple blades with spacers on low speed and high speed saws to obtain simultaneously cut sections from a single pass.

This technique works very well for both fragile and robust otoliths. As noted previously, fragile otoliths should still be embedded before sectioning to prevent breakage. The main advantage of this multi-blade technique is that it results in three or four sections which should contain the core or at least be very close to the core in one-third to one-quarter of the total processing time. Two blades can be used with a single spacer to achieve a single section as well. It should be noted that when sectioning smaller otoliths with this method, the core should be oriented between the two blades.

However, any warp or bend in the blades can result in varying thicknesses of the multiple sections so one must be careful with the blades and always use the same blades together as a set.

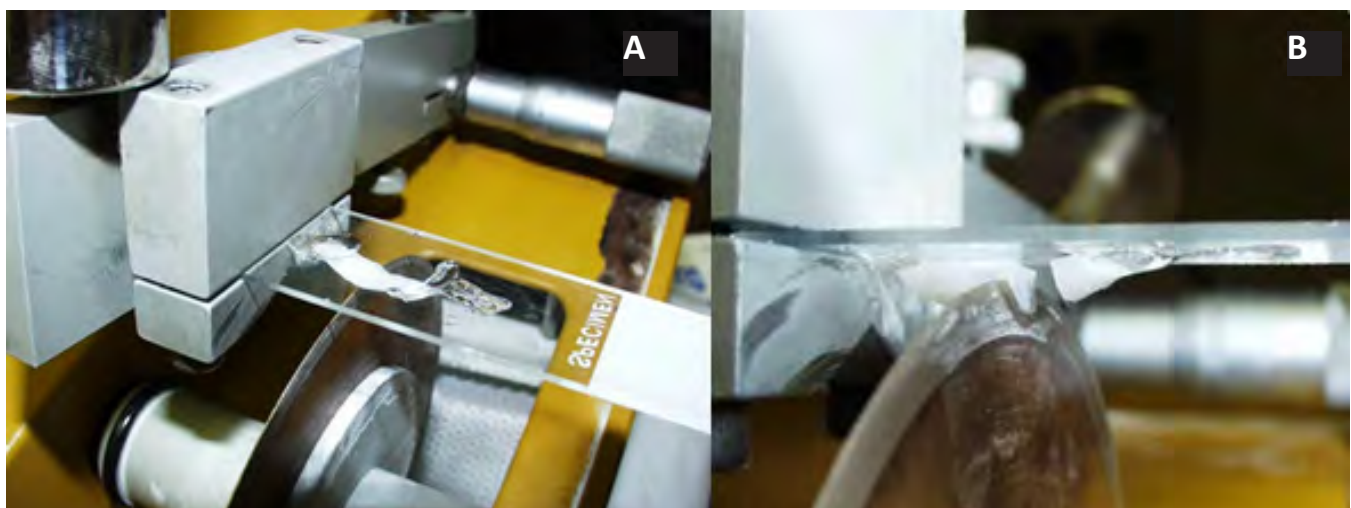


Figure 3.26 A) Mounted otolith aligned in chuck for first transverse cut and B) subsequent serial cuts.



Figure 3.27 Three otolith sections prepared for the final mount and ring enumeration.

Commonly, otoliths embedded in epoxy blocks can be mounted directly to the saw chuck for sectioning. To section unembedded specimens, a blank slide or small piece of tag paper or cardstock cut to the size of a standard slide with hot-melt glue or thermoplastic can be used (Figure 3.28). Paper slides are held to a sacrificial chuck by a small binder clip (Figure 3.29).

The chuck is typically a plate that is about 1 cm thick and can be made of aluminum, plastic, or any other material that can maintain its shape and stability after being cut numerous times. These chucks can range in size and shape. Custom aluminum chucks are common for otoliths mounted to paper or cardstock. Uniformity

is helpful when processing many samples. Regardless of mounting technique, the specimen is slowly lowered onto the spinning blades of the saw with the blades running through the water bath for lubrication.

Thin, transverse sections can be cut with a low or high speed saw. Generally, three to four blades, each separated by a spacer, and are used to yield two to three transverse sections (Figure 3.30). Different spacers can be used to achieve desired thicknesses.

The multiple blade sectioning technique can be used with either 3" or 4" blades, depending on saw type and processing needs. The 3" blades are much thinner, and subsequently grind through less otolith material while cutting. This makes the 3" blades ideal for cutting smaller embedded otoliths, where a precision cut is optimal. The 4" blades are thicker and grind through more otolith material; these are



Figure 3.28 Hot-melt glue applied to small strip of cardstock ready to receive whole otolith for sectioning using the multi-blade sectioning technique.



Figure 3.29 Cardstock with whole otolith attached to chuck with binder clip for sectioning using the multi-blade sectioning technique.

generally used on larger, more robust otoliths. Regardless of blade size, the sections are then recovered from the cardstock or basket, dried, and affixed to a final, labeled slide using a mounting medium (Chapter 7.0, Section 7.1.4). Spacers may be difficult to locate for purchase but can be made easily in the lab by cutting the center sections out of old blades or plastic sheeting of a thicker nature. Spacers may also be 3D printed using standard ABS plastic filaments (Figure 3.31). The spacer allows the blades to run simultaneously and cut consistent sections that often do not require additional sanding.

3.2.6.3 Thin Sectioning Machine (Hilquist®)

The thin sectioning machine is primarily used to section unembedded, whole otoliths. The procedure borrows petrographic techniques from geology and reduces

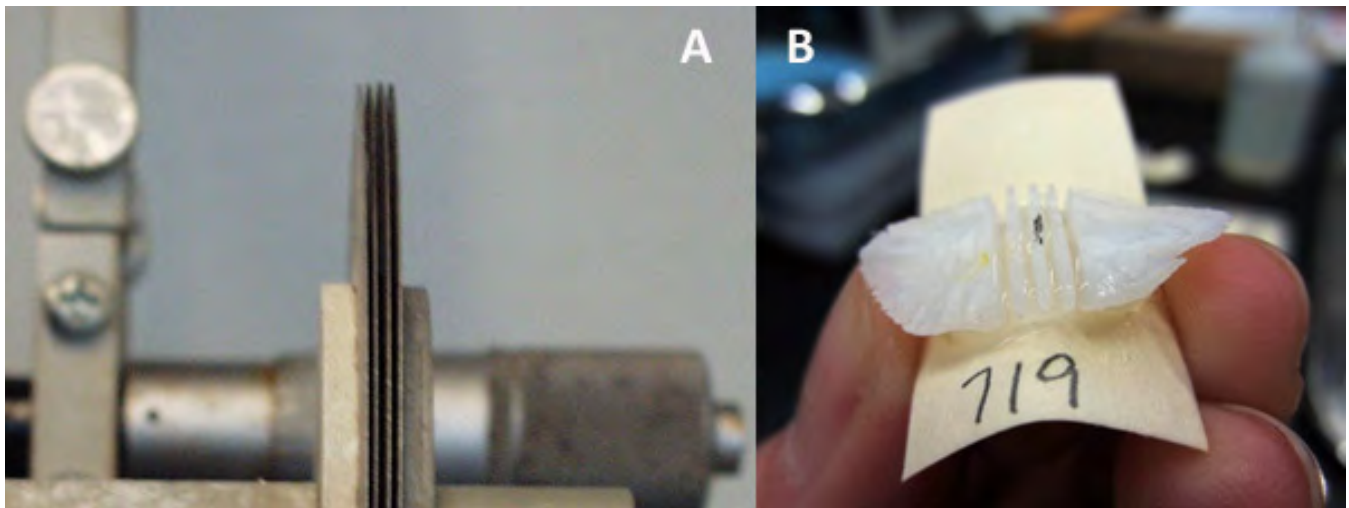


Figure 3.30 A) Low speed saw set up with four blades. B) A single pass through a mounted whole otolith produces three individual thin sections.

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 thin sectioning machine can be used to create ‘frosted’ slides by grinding one end of a less expensive, clear blank slide on the machine’s lap arm pad. **Note:** The sectioning process is quite loud so ear plugs or other hearing protection is strongly recommended.

The water-cooled, thin sectioning machine is equipped with two individual tools, a cut-off saw, and a precision grinder (Figure 3.32). The saw is equipped with a 20 cm diamond blade while 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.

The following is a method for the rapid processing of large otoliths first described by Cowan et al. (1995) with some minor modifications. Otoliths are hand held and cut along the transverse plane near the core using the cut-off saw before mounting onto slides (Figure 3.33). 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. Care must be taken to keep the sulcul groove perpendicular to the blade to ensure a proper cut. 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.

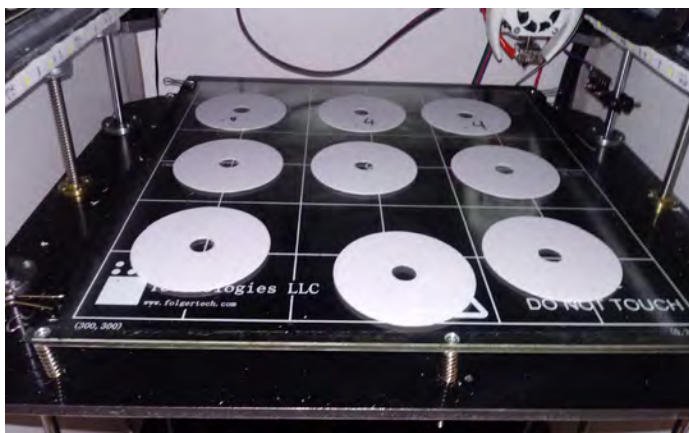


Figure 3.31 Spacers for multiple blade sectioning set up 3D printed with ABS plastic. Thicknesses can be adjusted for a wide variety of applications. Three spacers are required for each four blade setup.

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. Care must be taken to keep the sulcul groove perpendicular to the blade to ensure a proper cut. 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.

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



Figure 3.32 Thin section machine containing a high-concentration-diamond, continuous-rim blade cut-off saw (left) and a precision grinder (right).

identification numbers written under each using a water-proof marker (Figure 3.34A).

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 to get as close to the core as possible. 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 3.34B). Once the slides are dry, the otolith sections on each slide may be covered with a few drops of mounting medium which may eliminate the need for polishing although additional polishing can occur prior to covering the

final section. 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 along the transverse plane keeping the sulcul groove perpendicular to the saw blade near the core. Again, it is imperative to get as close to the core 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



Figure 3.33 Hand cutting an otolith on the high speed thin sectioning saw.



Figure 3.34 A) Otolith halves mounted on microscope slides with Loctite which is cured under ultraviolet (UV). B) Final polishing of otolith sections using grinding arm.

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.

3.2.7 Common Mistakes in Sectioning

Section is 'Off Plane'

When the otolith is cut at an angle, it can cause annuli to blur or appear indistinct (Figure 3.35). Tilting the slide under the scope will sometimes help distinguish annuli when reading imperfect sections. To avoid this problem, the otolith should be kept perpendicular to the cutting blade/grinding wheel and then embedded in epoxy in a flat position w/o tilting (otoliths can easily be manipulated in uncured resin immediately after pouring the molds). Finally, when hand cutting otoliths, the otolith should be held at a 90° angle to the blade without tilting in any direction. In some species, it is important to make sure your cut is perpendicular to the sulcus, which may not be exactly in line with the long axis of the otolith.

Off-core Sections (Tornado or Lost First)

Sectioning anterior or posterior to the core can produce sections in which the sulcus groove has a 'tornado like' appearance or the first annuli is blurred or lost (Figure 3.36A). To avoid this problem, mark the core well and cut multiple sections so you can choose the best.

Thin or Thick Sections

Sections cut too thin will produce faint annuli that are hard to see (Figure 3.36B), while ones cut too thick produce wide, dark bands that can be hard to distinguish from each other, as well as dark outer edges through which the margin is hard to distinguish (Figure 3.36C). To avoid this problem, adjust the thickness of the sections according to the species accounts and recommendations from similar species.

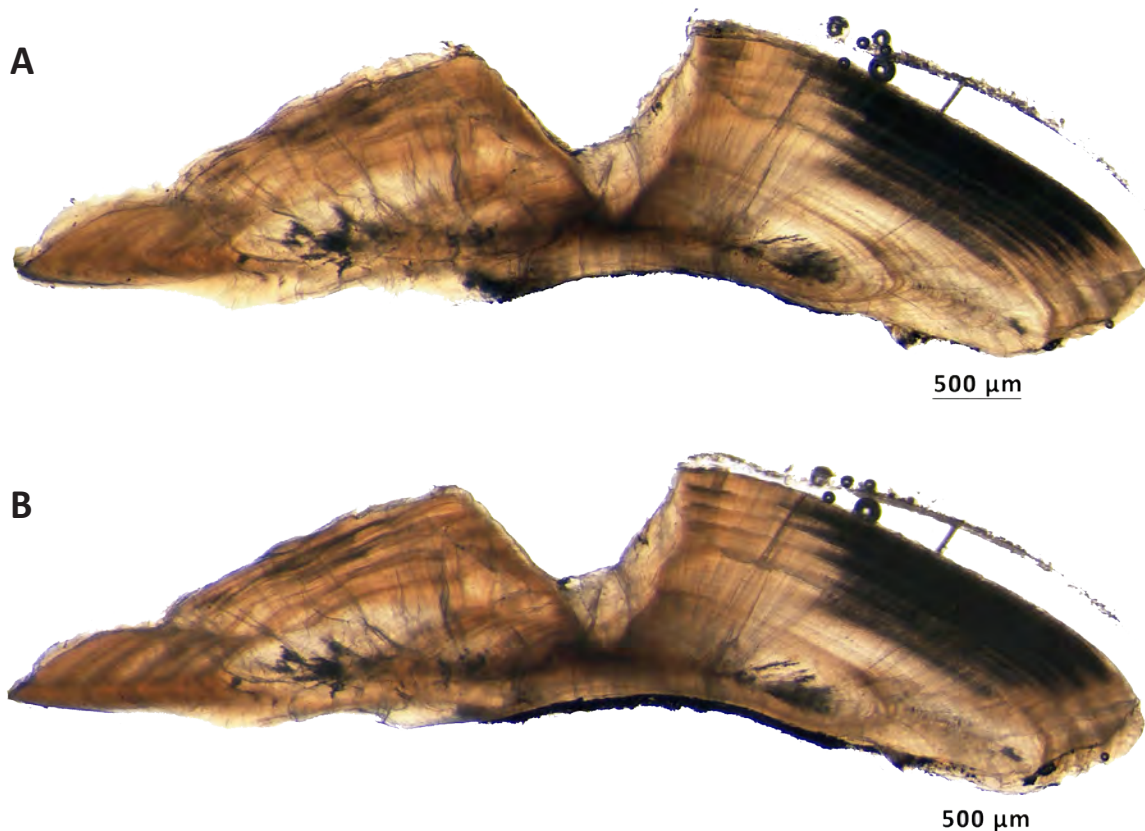


Figure 3.35 Example of an age-6 year Gray Snapper otolith that was improperly aligned for sectioning. A) is the off-plane cut and B) is the same section tilted to correct for the plane.

Variable Section Thickness

A warped blade can cause sections to be thicker on one end vs the other (Figure 3.36D). To avoid this problem, do not force the blades through the material, change blades on a regular basis, and make sure resin is properly cured prior to sectioning. Soft or sticky resin can heat up or drag the blades until they no longer cut true. Improper maintenance of saws can result in warped blades as well if the drive shaft or other components are out of alignment.

Bubbles in the Section

Bubbles in epoxy and adhesive materials make ageing difficult at times and can easily be removed from the molds or slides before curing (Figure 3.36E). To avoid this problem, stir mixture thoroughly but not aggressively and poke/remove bubbles from mold prior to cure using a probe. Bubbles can form in liquid coverslip as gasses escape from the section. Check sections before the coverslip has had time to cure and remove. Using too little coverslip can also leave areas exposed which are just as hard to age through as bubbles.

Scored or Burned Sections

Some blades produce sections with a rough appearance making them difficult to read leaving saw marks or scratches (Figure 3.36F). To avoid the problem, dress blades weekly and consider switching to a finer grain blade. Cutting fluid may need to be changed more often, typically based on number of cuts, not number of days. Additives can be added to cutting lubricant if problems continue.

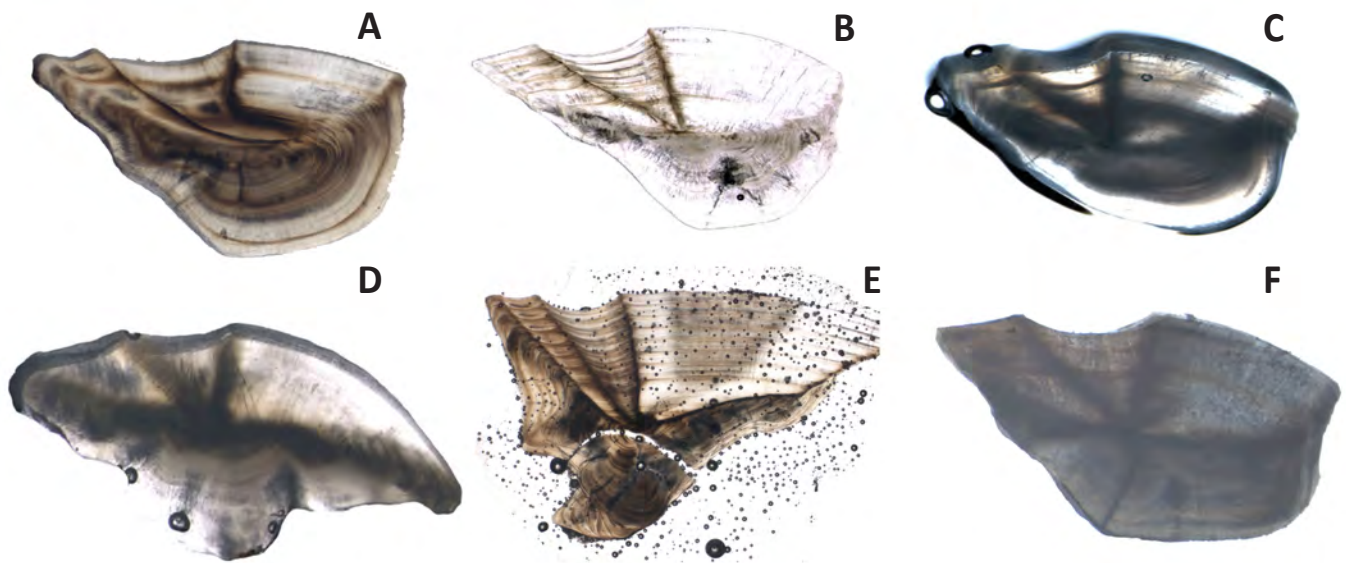


Figure 3.36 A) Tornado otolith section. Otolith sectioned B) too thin and C) too thick. D) Wedge otolith section from improper blade maintenance or warping. E) Bubbles trapped in liquid coverslip and not removed before hardening. F) Otolith section with severe saw marks and generally unreadable in current state.

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4.0 Scales

4.1 Introduction (Function, history, pros and cons)

Fish scales are derived from the dermis and come in multiple types. The three main types of scales found in modern fishes are placoid (found in elasmobranchs), ganoid (found in gars), and elasmoid (found in teleosts). For the purpose of age determination, focus will be on elasmoid scales. Elasmoid scales come in two forms, cycloid and ctenoid. Cycloid scales are smooth edged and found in soft-rayed actinopterygian fishes. Ctenoid scales have small teeth, generally on the posterior edge of the scale, and are found in more derived teleosts. Both forms of elasmoid scales are formed by a collagen layer that is covered by a thin layer of lamellar bone (Barton and Bond 2007). Elasmoid scales overlap each other to provide protection with minimal impact on flexibility.

Scales grow concentrically around their focus or origin. The anterior portion of the scale is embedded within the dermis of the fish while the posterior portion is exposed. Ctenoid scales have cteni on the posterior portion and radii extending from the focus to the anterior portion (Figure 4.1). Many clupeids have transverse grooves that run dorsal to ventral across the scale. The concentric rings formed in the bony layer as the scales grow are called circuli. Scale growth stops during cold periods and causes breakages in these circuli. The circuli start new concentric rings when growth resumes and the resulting pattern can be interpreted as an annulus.

Scales have long been used for ageing fish and are one of the first hard parts in which the well-defined rings were assumed to be annuli. 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

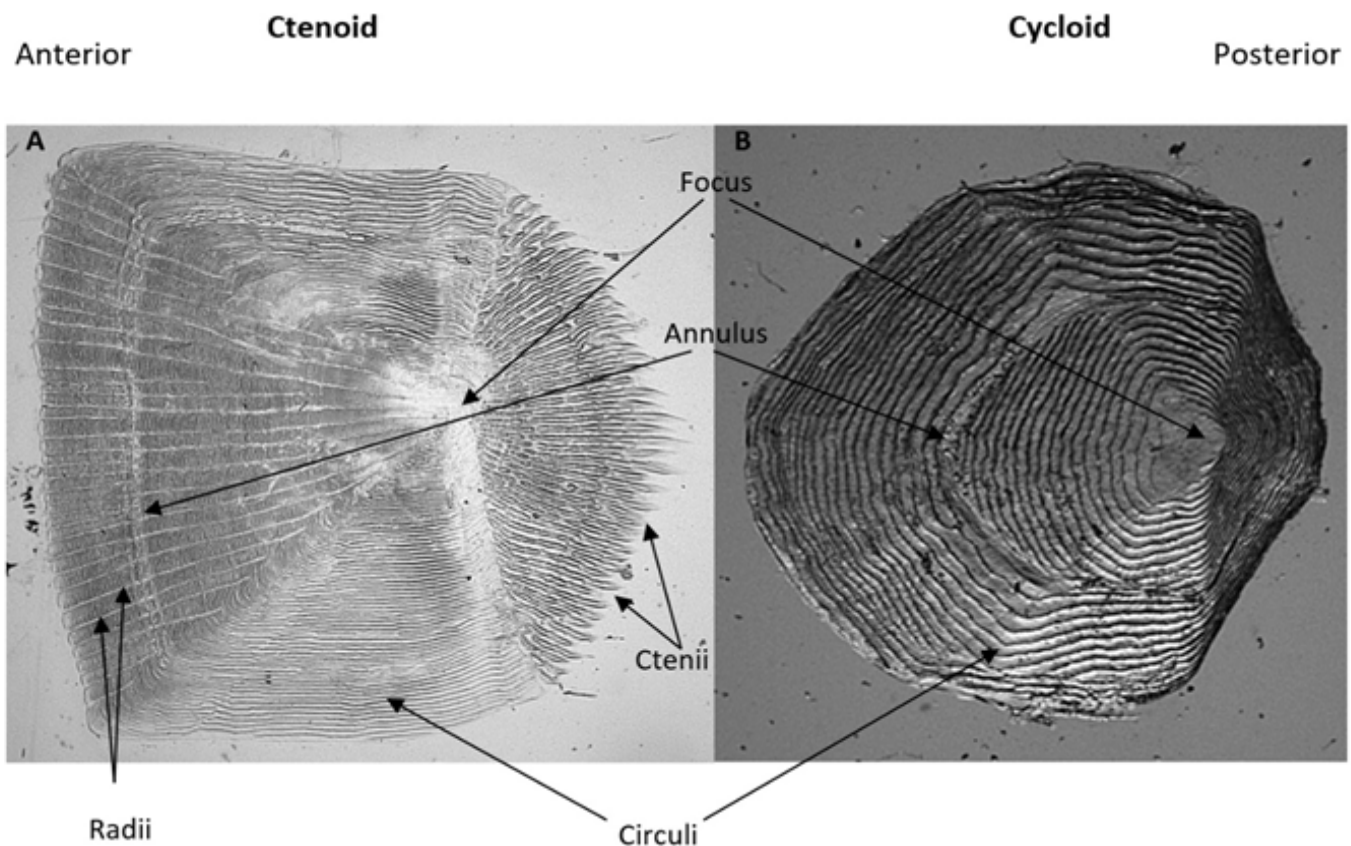


Figure 4.1 Labeled scales. A) Ctenoid scale from a Striped Bass (*Morone saxatilis*). B) Cycloid scale from a Rainbow Smelt (*Osmerus mordax*).

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.

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. 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. In addition, scales can be read 'raw', mounted between glass, or as impressions of their three dimensional structure pressed into acetate.

The biggest disadvantage to using scales is that as external structures they are more vulnerable to environmental changes and damage which can result in a number of false annuli or check marks on these structures. In addition, scale regeneration can occur following injury or trauma to a fish which can result in sampling of poor quality scales which no longer can be used to age. Furthermore, fish can re-absorb calcium from their scales during times of stress which can lead to degradation of the edge and previously laid down growth.

Validation of annuli is essential because scales are not useful for ageing all 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; Secor et al. 1995; Liao et al. 2013). The opposite issue can occur in younger fish where scales may overestimate age due to check marks. 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 (Chapter 2.0, Section 2.2.1).

In summary, scales may not be appropriate for ageing all 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. 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.

4.2 Scale Removal

Scales are often removed from the middle of the body, below the dorsal fin, 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. Given that scales can be differently shaped depending on the body location, it is also important to be consistent in the scale removal location within a species. See individual species accounts in Chapter 9.0 for the preferred collection location in several species.

Scales can be collected by scraping with a knife or other semi sharp object from posterior to anterior on the side of the fish (Figure 4.2). Alternatively, forceps can be used to remove specific scales. When removing scales from live fish, 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. When working with dead fish, it is helpful to wipe away as much mucus coating prior

to collecting scales as possible. This will make cleaning the scales easier later. Because some scales are unsuitable for ageing, it is recommended that one collects six to ten scales per fish (number varies by species). Once removed, scales can be placed in a small envelope to dry. Small and delicate scales should be placed between two pieces (or one folded piece) of paper prior to inserting into the envelope. This will facilitate removal later without destroying the envelope.



Figure 4.2 Scale removal above the lateral line from a Striped Bass.

4.3 Handling, Cleaning, and Preparing for Ageing

In many cases, no further processing is necessary. Raw scales can be examined directly, although some additional effort to clean, mount (either dry or wet), or make an acetate impression of the scale can enhance the details of it for viewing and interpretation. Enhancement techniques are described in much greater detail by Dery (1983).

4.3.1 Evaluating Scale Condition

Scales should undergo a preliminary examination before going through the steps to clean and mount them. Many scales are unsuitable for ageing for various reasons and should be avoided. Scales that have been lost throughout the life of the fish will re-grow quickly to the relative current size of the fish. These re-grown or regenerated scales do not possess the same annual markings as the lost scale and should not be used for age determination. Future growth will, however, follow the same pattern as the rest of the scales. Scales can be damaged while being removed from the fish or can be misshapen (Figure 4.3A). This tends to be the case when taken from the wrong area of the fish. In rare circumstances, scales can be semi-dislodged but continue to grow (Figure 4.3B). This will lead to the appearance of the center being twisted in relation to the rest of the scale. Counting annuli on a shifted scale is unreliable and should be avoided.



Figure 4.3 A) Three unacceptable Striped Bass scales from the same fish. The one on the left is regenerated, the middle one is torn around the edge, and the one on the right was taken from the wrong place on the fish and is misshapen. B) A Rainbow Smelt scale with the growth axis shifted (right).

4.3.2 Cleaning Scales

Wiping scales clean when initially collected can save time later. Several methods have been used to clean scales. In the simplest method a small brush, such as a toothbrush, and a cleaner (e.g., a mild soap solution, alcohol, or diluted bleach) are applied to the scale to remove the dried mucus. Time spent soaking in the cleaner varies by species and cleanliness of the scales. This can be a time-consuming process for dirty scales and requires great manual dexterity for small scales.

A second method for cleaning scales involves soaking in an ultrasonic bath. The advantage of this is that, once out of the bath, the scales can be easily wiped clean. This method is outlined quite well by Whaley (1991). The scales are placed into small vials containing a solution of 5% pancreatin. This enzyme helps to break down the mucus coat on the scales. The vials are floated in an ultrasonic bath (jewelry cleaners work as do lab grade ultrasonic baths). A 10 minute soak time works well for most species but results may vary. The pancreatin is then drained from scales which are then rinsed in clean water and wiped dry. The pancreatin solution can be used multiple times but should be changed when its effectiveness diminishes. Care should be taken not to leave scales soaking in pancreatin solution overnight as it can cause damage.

4.3.3 Mounting to Slides

Some scales can curl and shrink as they dry. One way to prevent this and allow for later reading or repeat readings is to mount the scales between blank microscope slides (Figure 4.4). Clean and dry scales are laid out on a glass microscope slide, leaving room at one end for a label. To facilitate easier reading later, scales should be put down all in the same orientation. A second glass slide is then placed on top of the scales and, using a small piece of tape at either end, the two slides are affixed to each other. The top slide is then labeled accordingly. For small and/or thin scales, a glass cover slip can be used instead of the top slide. Scales can be numbered to help with data sharing and QA/QC when being reviewed by multiple readers and/or labs.

4.3.4 Scale Impressions

Making impressions is a more laborious technique, but the time and cost can often be justified by providing several advantages over raw scales.

1. Impressions can enhance the details of scales with delicate features.
2. 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.
3. Larger scales may be too thick to be transparent enough for direct viewing while impressions can be viewed using transmitted or reflected light.
4. The texture on the back of some scales can inhibit a clear view of the circuli.
5. Multiple scale impressions on a single slide can be easier to handle than many small, loose scales in an envelope, so the best scales can be easily selected for reading.
6. Impressions can be archived indefinitely.



Figure 4.4 Alewife Herring (*Alosa pseudoharengus*) scales mounted between two glass slides.

4.3.4.1 Jewelers Press

The sculptured side of a fish scale can be imprinted on laminated plastic by using pressure, such as with a roller press. When using a roller press, clean and dry scales are typically placed on a blank slide in order and a single acetate slide (0.010" thick) is laid over them. The sandwiched scales are carefully placed into the rollers of the press (Figure 4.5A). As the machine passes them through, the scales are pressed into the acetate and the result is a negative relief of the scales' surface features. The acetate is removed from the slide and the scales, which may stick to the acetate, are returned to their archive envelope for storage. The acetate slide is labeled and can now be read (Figure 4.5B).

4.3.4.2 Heat Press (Carver® Laboratory Press)

Another option for making scale impressions is the use of a heated press such as a Carver® Laboratory Press (Figure 4.6A). Although a heat press can make up to 12 slides at a time, this process can be more time consuming than a roller press. With the correct settings, a heat press will produce better quality impressions than a roller press, thereby potentially speeding up the ageing process and making up for some of the extra time. Clean, dry scales are placed dull (rough) side down on top of a sheet of acetate (clear acetate 0.6-1.0 mm thickness) which is then sandwiched between two mirrored stainless steel plates. The acetate can be pre-cut into 6" x 6" square pieces. Alternatively the acetate may be cut smaller prior to pressing to facilitate more even pressure. Trying various configurations with the press available is advisable to achieve the best results. If using larger pieces of acetate, one of the mirrored steel plates can be etched to reflect the final size of the slides in order to facilitate easier and more precise scale placement prior to pressing. Using sandpaper to roughen the edge of the acetate can be helpful to enable labeling, as writing on smooth acetate can be difficult. The amount of heat, pressure, and time used varies between labs, species, and scale size depending on the preference of the lab. It is important to ensure that scales are dry prior to pressing. Large, thick scales can hold water inside and, when exposed to the heat, will expand and cause fractures, making age determination much more difficult. Once the allotted time has elapsed, the sample can be removed from the heat press and allowed to cool for several minutes. The top plate can be removed followed by the sheet of acetate. The scales can then be removed from the acetate and returned to the original scale envelope. The slides are then labeled and separated using sharp scissors or other acetate cutting device. The slide is now ready for reading (Figure 4.6B). The completed slides can be stored in a slide box or returned to the original sample envelope.

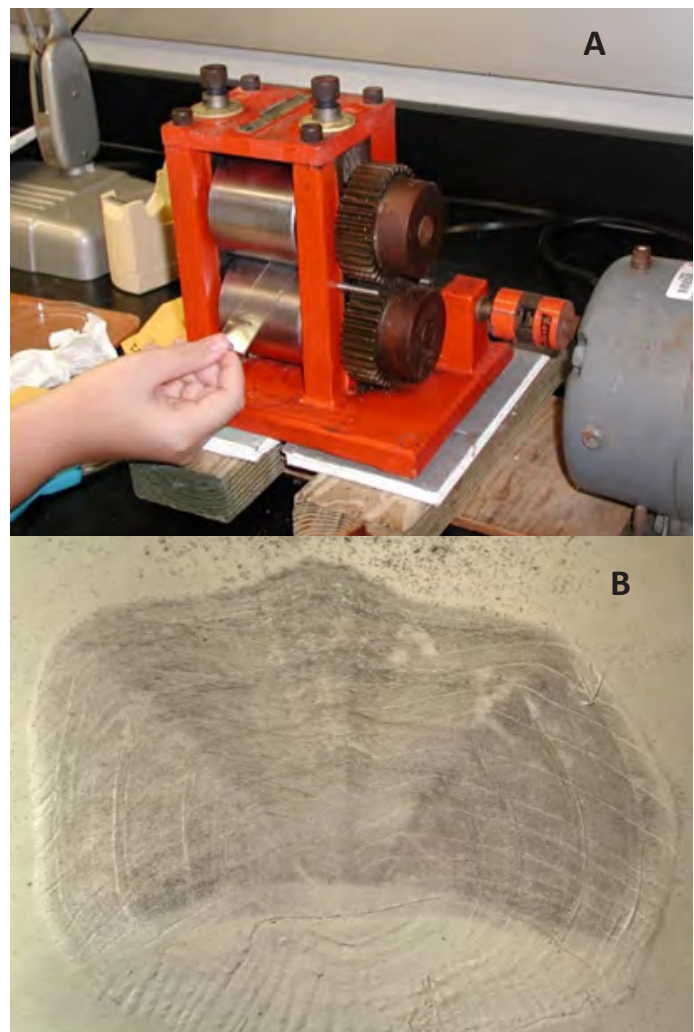


Figure 4.5 A) Jewelers press or roller press used to imprint scales on cellulose acetate. B) An acetate impression of an *Alosa* sp. scale produced through a roller press.

4.4 Storage

Scale samples can be archived as outlined in Chapter 2.0, Section 2.4. Care should be taken, however, in selecting a storage container for mounted scales as they can become loose between the slides and fall out over time as they dry out.

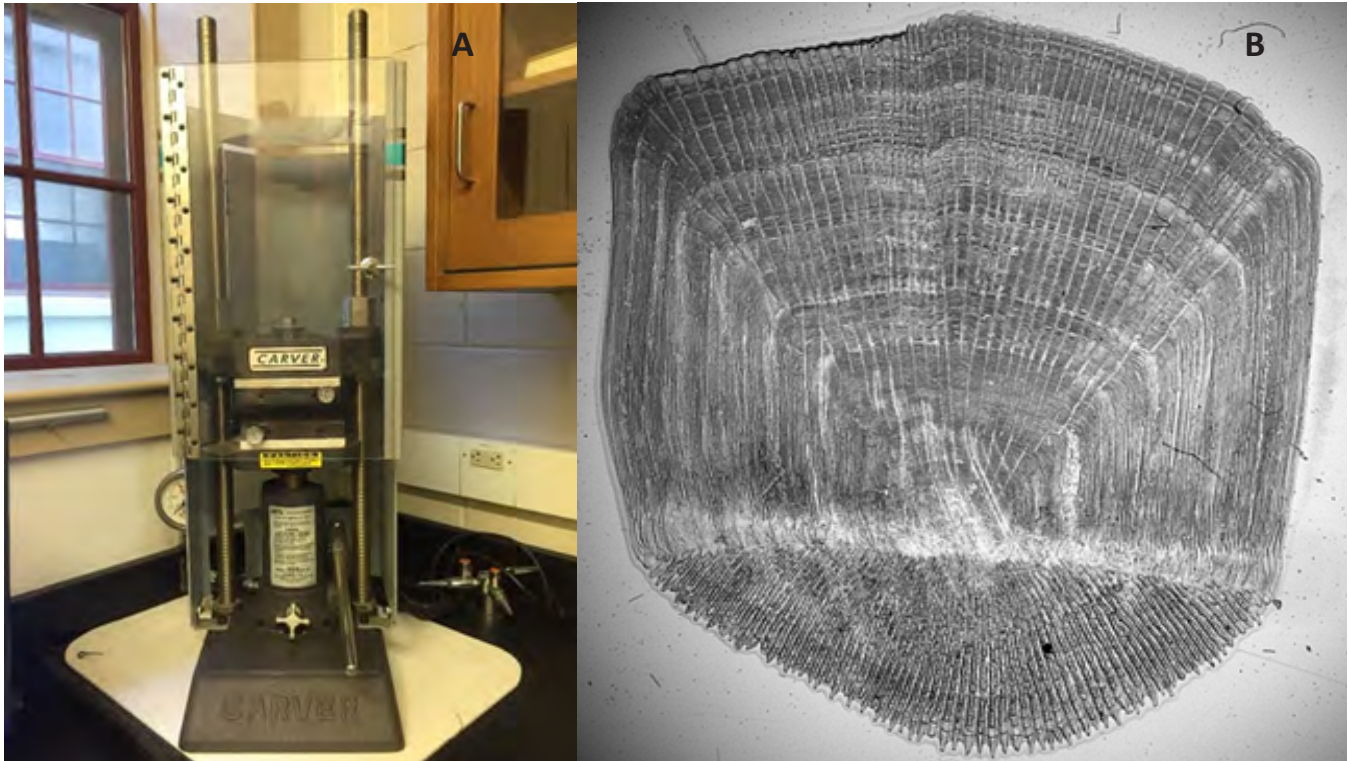


Figure 4.6 A) The Carver® laboratory heat press for generating scale impressions. B) An acetate impression of a Striped Bass scale produced on a heated press.

5.0 Opercles

5.1 Introduction (Function, history, pros and cons)

The operculum of teleost fishes serves two main functions – protecting the gills and alternately serving as a water vent and check-valve during respiration (White 2002). Four bones typically comprise the opercular series (White 2002, Lagler et al. 1962). The most anterior bone is the typically j-shaped preopercle. Ventral to the preopercle is the interopercle. The opercle is the largest bone and lies posterior to the preopercle. Ventral to the opercle is the subopercle. These bones can be discerned in a *Tautog* (*Tautoga ontis*) skull in Figure 5.1. Ageing is typically performed using the opercle.

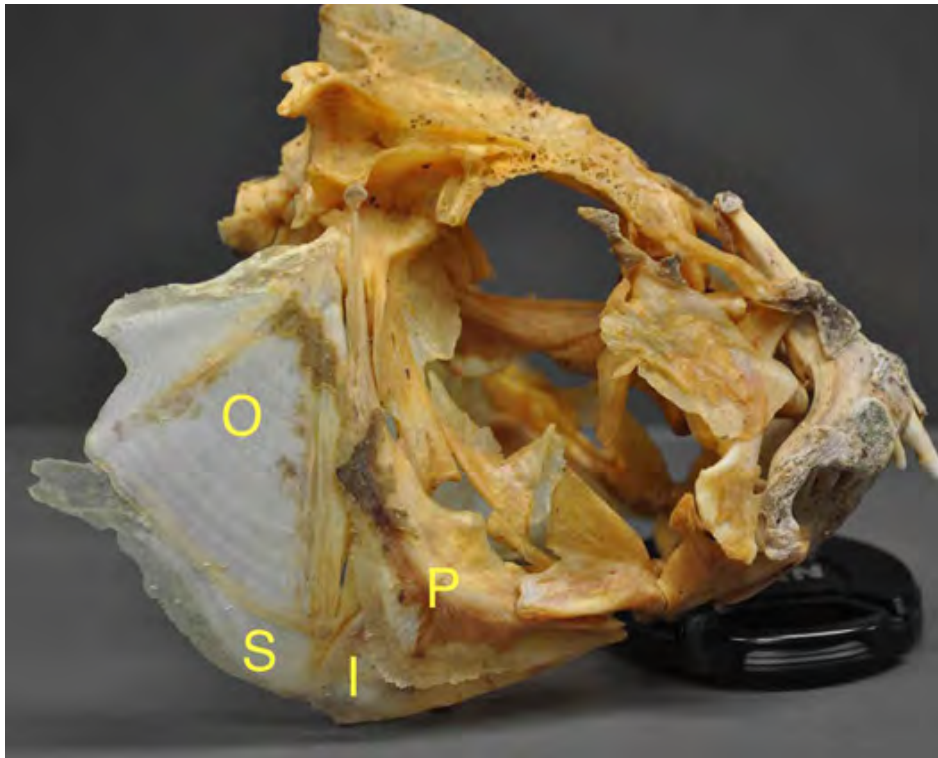


Figure 5.1 *Tautog* skull with labeled preopercle (P), interopercle (I), opercle (O), and subopercle (S) (Skull by Elzey).

A comprehensive review of literature describing the use of bones other than otoliths for ageing by Menon (1950) indicated that opercles have been considered for ageing since at least 1904 and used for ageing since at least 1910. Opercles have been most commonly used for ageing various fresh water fish. Le Cren (1947) used opercles to examine the age and growth of Eurasian Perch (*Perca fluviatilis*) and is quite often cited in other works that involve ageing using opercles. He noted that opercles had several useful characteristics including easy removal and preparation, a size that made them easy to handle and store, and usually being large enough that the annual zones could be seen with the naked eye. He also noted that, at least for the perch he examined, opercles were generally easier to read than scales. Bardach (1955) similarly used opercles to age North American Yellow Perch (*Perca flavescens*). McConnell (1952) used opercles to examine age and growth in Carp (*Cyprinus carpio*) and observed that the opercle method was superior to other methods for examining age and growth and that there was much less variation of the opercle-body length relationship than of the scale-body length ratio. Campbell and Babaluk (1979) performed comparative ageing in Walleye (*Stizostedion vitreum vitreum*) using scales, fin spines, vertebrae, pelvic fin rays, pectoral fin rays, opercles, branchiostegal rays, and otoliths. They found that opercles were among the easiest to delineate annuli, provide age determinations comparable

to otoliths, and were one of the easiest bony parts to remove and prepare. They also validated the annual growth increments in walleye using tetracycline dye (Babaluk and Campbell 1987). Although useful for age determination in some species, the opercles are not a good choice for all species. Elzey et al. (2015) found that the ages derived from opercles of American Shad (*Alosa sapidissima*) were of low precision and biased as compared to other ageing structures.

Typically, opercles are roughly triangular in shape (Figure 5.2). The dorsal and anterior edges intersect at the articular apex, a thickened structure that contains the cup of a ball and socket joint that serves as the hinge point for the opercle. The articular apex corresponds to the center of bone growth and thus serves as the origin for radial measurements (Le Cren 1947, McConnell 1952, Bardach 1955, Cooper 1967, Hostetter and Monroe 1993). The outer surface of the opercle is convex and the inner surface concave, with both surfaces coming together to form a thin, delicate edge along the margin. This thin ventral margin is where new growth is most apparent.

Le Cren (1947) opined, and others (Bardach 1955, McConnell 1952) concurred that in opercles, the broad translucent zones correspond to rapid growth which gradually fades into narrow opaque zones that correspond to slow growth. The arrowheads in Figure 5.2 mark the transition from the translucent zones to the opaque zones in a Tautog opercle.

5.2 Preparing Opercles for Ageing

5.2.1 Opercle Removal

When removing opercles, care must be taken not to damage or cut through the articular apex (center of growth), the anterior or dorsal margins, or the ventral margin (the most prominent area of new growth; Figure 5.2).

General Method

1. Gently pry up the operculum and make a posterior to anterior cut along the dorsal edge of the operculum (Figure 5.3A). As you proceed with the cut, continue to lift the operculum, angling the knife edge slightly away from the operculum and medially toward the body of the fish. This avoids accidentally cutting into to the dorsal surface of the operculum. Discontinue cutting when you begin to encounter resistance (bone). **Do not sever the bone at the apex as this is an important landmark in ageing.**
2. Flex the operculum anteriorly along the intersection of the opercle and preopercle until you feel the articular joint dislocates (Figure 5.3A). Using a knife to pry the joint apart while flexing the operculum helps in larger fish. When this happens, the whole operculum will snap forward.

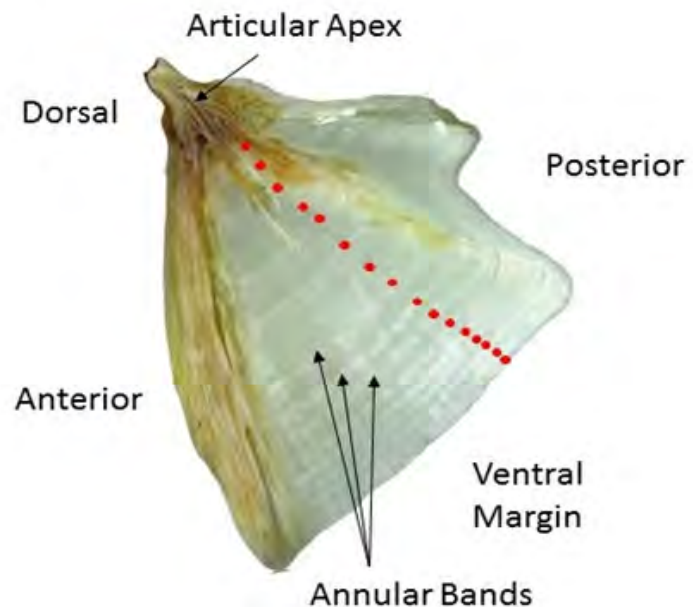


Figure 5.2 Tautog (*Tautoga onitis*) opercle cleaned and ready for ageing.

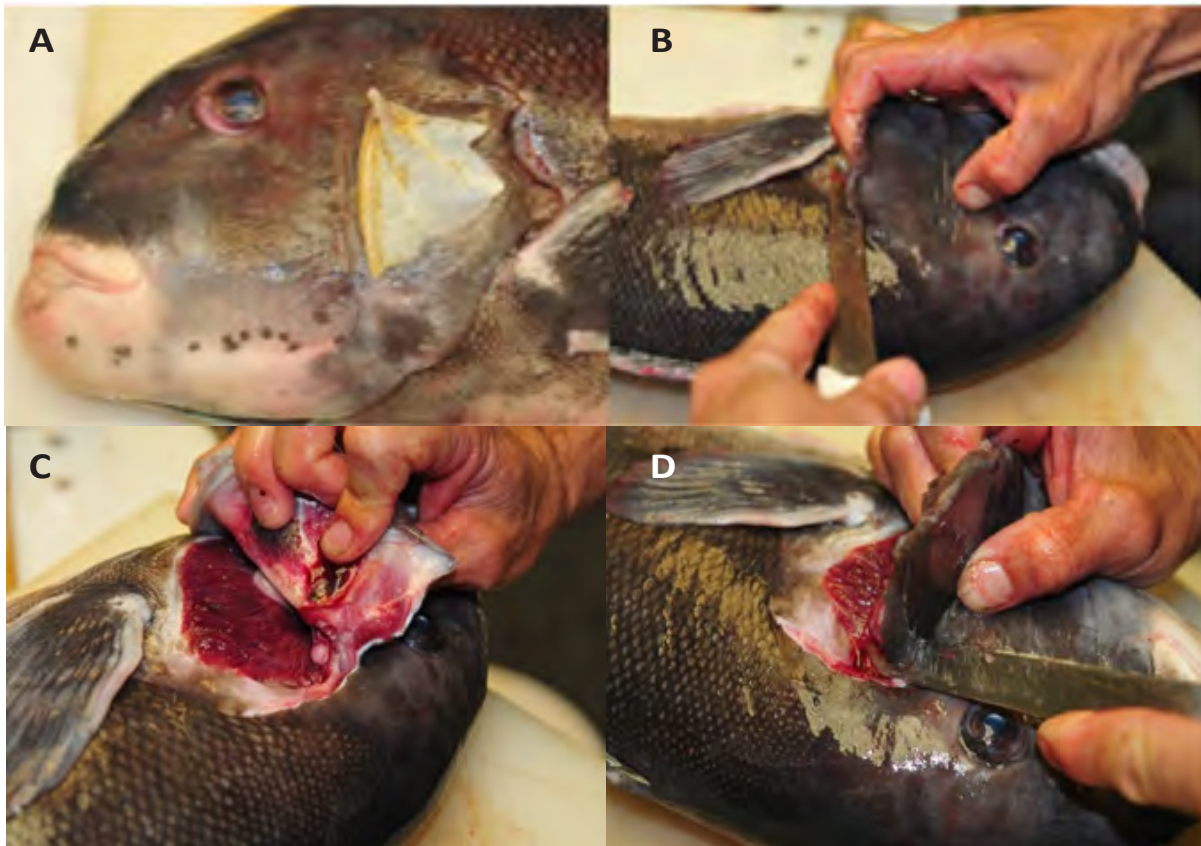


Figure 5.3. A) Location and orientation of a Tautog opercle and B) beginning dissection of the dorsal edge of the operculum. C) Dislocated articular joint in Tautog showing the opercle's ball and socket. D) Redirecting the cut in a ventral direction.

Observation of the ball and socket of the dislocated articular joint should be possible (Figure 5.4B).

3. Cut the opercle away from the fish by cutting ventrally along the crease between the preopercle and opercle (Figure 5.4B).
4. The cut should continue until the entire opercle has been cut from the connecting tissue (Figure 5.5). Frequently the subopercle will still be attached. This is fine as it will be removed during the cleaning process described below.

It should be noted that this is a general method and several labs have small variations to the above mentioned procedures depending on what they are most comfortable and familiar with. Methods may also slightly vary by species. The most important thing is to not damage the opercle during removal. A fast technique used in tautog, which results in very little attached tissue, is illustrated by ODU's Center for Quantitative Fisheries Ecology (<https://www.odu.edu/content/dam/odu/offices/center-for-quantitative-fisheries/videos/Tautog%20operc.mp4>).

5.2.2 Opercle Cleaning

Once the opercle is free from the fish, the cleaning process is relatively simple.

1. Boil the opercle in water for 1-3 minutes to loosen the soft tissue that is adhered to the bone.
2. Remove large pieces of tissue with forceps, and the rest with water and a small soft bristle brush. Care must be taken as stiff bristles will leave brush marks or streaks. On the concave side of the



Figure 5.5. Concluding the removal of the Tautog opercle separation.

opercle near the apex, there are often one or more small blind holes that need to be cleared of tissue.

3. Rinse the opercle with clean water.
4. Wipe the opercle dry with a paper towel.
5. Allow the opercle to air dry for at least 24 hours and store in labeled paper coin envelopes (Figure 5.6). Damp opercles placed in envelopes will dry as long as the envelopes are allowed adequate air flow.

5.3 Evaluating Opercle Condition

There are several instances where an opercle may not be useful for age determination. Opercles may be damaged during removal or during the life of the fish. Opercles are also occasionally malformed (Figures 5.7A-D). Caution should be used when determining age based on damaged or malformed opercles.

5.4 Long-Term Storage and Archiving

Dry opercles are stored in envelopes with the appropriate sample information. The envelopes should be stored in rigid boxes to minimize the potential for physical damage. The boxes should be stored in a secure and dry location. Very large opercles or opercles from oily fish may continue to leach oils for an extended period of time. Paper envelopes will wick the excess oil away. Opercles that are not cleaned and dried well prior to storing in air tight containers may degrade due to bacterial and or fungal growth.



Figure 5.6. Clean Tautog opercle ready for reading.

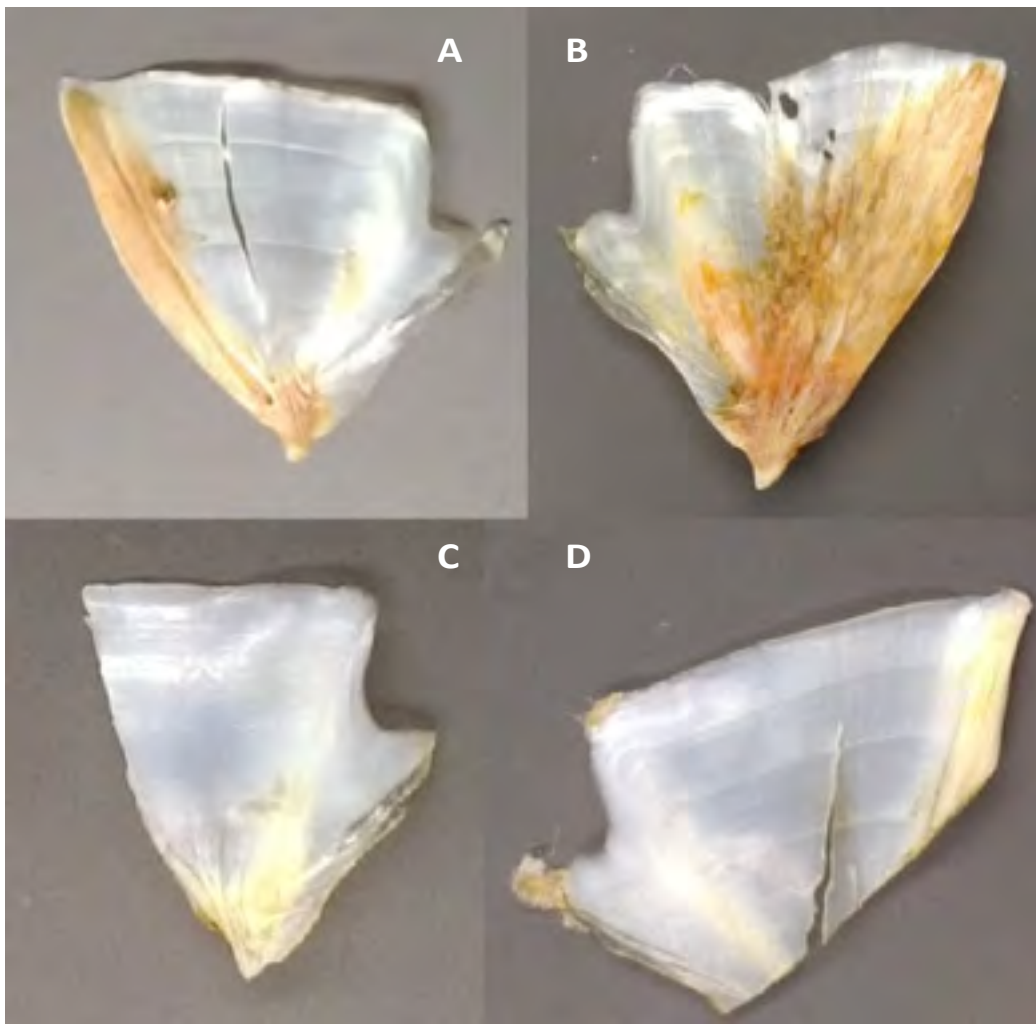


Figure 5.7 Examples of tautog opercles in various conditions. A) Minor crack in opercle that does not affect age determination. B) Major growth deformation that renders the opercle unusable. C) Poorly removed opercle with the anterior portion cut off. This opercle may or may not be useable. D) Poorly removed opercle with the articular apex cut off, rendering it unusable.

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6.0 Spines and Fin Rays

6.1 Introduction (Function, history, pros and cons)

Spines and fin rays have been used to age diverse groups of fishes since at least the 1960s, primarily focused on fishes from cold temperate regions where the annual growth zones in the ageing structures (whether otoliths, scales, or spines and fin rays) are relatively wide and distinct (Table 6.1). More recently, spines and fin rays have been used to successfully age fishes from warm temperate waters of Florida, such as Gag and Goliath Groupers (Debicella 2005, Murie et al. 2009), and from equatorial waters of French Guiana (Artero et al. 2015). Spines are the preferred ageing method for some pelagic billfishes whose otoliths have been deemed unreliable (Kopf et al. 2010), and also have been used for non-lethal ageing of reef fish in closed areas of the Florida Keys (FWC Unpublished Data). In contrast to scales, spine and fin ray methods of ageing can be used for fish living into their late teens and twenties. In addition, some fish groups, such as the sturgeons, can be aged over 100 years using their fused fin rays (e.g., Lake Sturgeon *Acipenser fulvescens* aged to 152 years of age, Anderson 1954) (Table 6.1).

Table 6.1. Examples of fish species that have been aged using spines (S) or fin rays (R), along with their maximum observed ages.

| Common and Species Name | Structure | Maximum Age (Yrs) | Reference |
|---|-----------|-------------------|----------------------------|
| Albacore Tuna (<i>Thunus alalunga</i>) | R | 12 | Beamish 1981 |
| Arctic Grayling (<i>Thymallis arcticus</i>) | R | 11 | Sikstrom 1983 |
| Black Marlin (<i>Makaira indica</i>) | S | 13 | Speare 2003 |
| Blue Throat Wrasse (<i>Notolabrus tetricus</i>) | S | 12 | Metcalf and Swearer 2005 |
| Common Carp (<i>Cyprinus carpio</i>) | S | 18 | Yates et al. 2016 |
| Gag Grouper (<i>Mycteroperca microlepis</i>) | R | 17 | Debicella 2005 |
| Goliath Grouper (<i>Epinephelus itajara</i>) | R | 18 | Murie et al. 2009 |
| Gray Triggerfish (<i>Balistes caprisucus</i>) | S | 14 | Allman et al. 2017 |
| Lake Sturgeon (<i>Acipenser fulvescens</i>) | Fused R | 152 | Anderson 1954 |
| Lingcod (<i>Ophiodon elongatus</i>) | R | 21 | Beamish and Chilton 1977 |
| Sockeye Salmon (<i>Oncorhynchus nerka</i>) | R | 4 | Bilton and Jenkinson 1969 |
| Tautog (<i>Tautoga onitis</i>) | S | 20 | Elzey and Trull 2016 |
| Walleye Pollock (<i>Theragra chalcogramma</i>) | R | 9 | Beamish 1981 |
| White Sturgeon (<i>Acipenser transmontanus</i>) | Fused R | 104 | Rien and Beamesderfer 1994 |
| White Sucker (<i>Catostomus commersoni</i>) | R | 14 | Beamish and Harvey 1969 |

Using spines and fin rays for age and growth studies offers certain advantages over otoliths and other hard parts. In particular, it is a non-lethal method as spines and fin rays can usually be removed without sacrificing the fish. This may be an important consideration when studying fish that are an endangered or threatened species, or living within an area that is closed to fishing. Physical damage to live fish that will subsequently be released, or mutilation of fish being port sampled (which could reduce the market value of the fish), can be minimized with experience. Additionally, using spines or fin rays may be the only choice of an ageing structure for a fish landed with its head removed and its scales damaged. In many cases spines and fin rays can also be removed more easily, and therefore faster, than otoliths. This can be a significant consideration if sampling time is limited, such as when port sampling or sampling fish at sea.

Although spines and fin rays can be useful in the estimation of age-and-growth in fish, there are two major disadvantages that must be considered and investigated on a species-specific basis, including:

- The core of the spine or fin ray can undergo resorption and become vascularized, thus obscuring or eliminating the first few annuli in older fish, which results in an underestimation of age (Figure 6.1). This problem is more prevalent in spines because they are formed as a single fused element of bone with a central lumen that may be filled with vascular tissue or be hollow due to resorption of the spine nucleus (Penha et al. 2004). Although occlusion or resorption processes can occur in fin rays (Beamish and Chilton 1982, McFarlane and King 2001), each fin ray is comprised of two parallel fin ray elements (lepidotrichia) and the vascular tissue of the fin ray lies between the lepidotrichia and is thus offset from the core of the fin ray (which itself does not occupy a central position in the fin ray).
- Annuli may accumulate close together on the edge of the fin ray or spine in older fish, making it difficult to distinguish and count each individual annulus (Beamish 1981, Cass and Beamish 1983). The age at which this accumulation occurs is species-specific, however, and may not occur within the range of age determination required for management. For example, Lingcod are routinely and reliably aged up to about 20 years of age before the accumulation of annuli on the edge interferes with accurate age estimates, although there are very few Lingcod greater than 10 years taken in the fishery (McFarlane and King 2001).

Checks, or false annuli, appear similar to annuli but are associated with 'marks' that are often incomplete and irregular, and frequently found only in one region of the structure. Although they may be prominent, checks 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. Since spines and fin rays record the physical growth in the body of the fish, they are particularly prone to depositing checks due to environmental conditions, reproductive events, and feeding.

For both spines and fin rays, successfully determining the age requires that the structure is sectioned near the base in a precise transverse plane, although the exact location of the section depends on the species. Sectioning too far up the structure results in the first annulus, in particular, being missed or difficult to interpret.

As with any ageing structure, it is necessary to validate the use of spines and fin rays to confirm that observed marks are, in fact, produced annually (see Chapter 2.0, Section 2.3). Such a validation can be done in a tag-recapture study in which the same fish can be aged for twice when it is tagged and recaptured, respectively, using its spines and fin rays. This allows the method to be validated using tag and release studies. Similar to otoliths, the method can also be validated using recaptured oxytetracycline (OTC) tagged fish, as has been shown for Lingcod *Ophiodon elongatus* (McFarlane and King 2001).

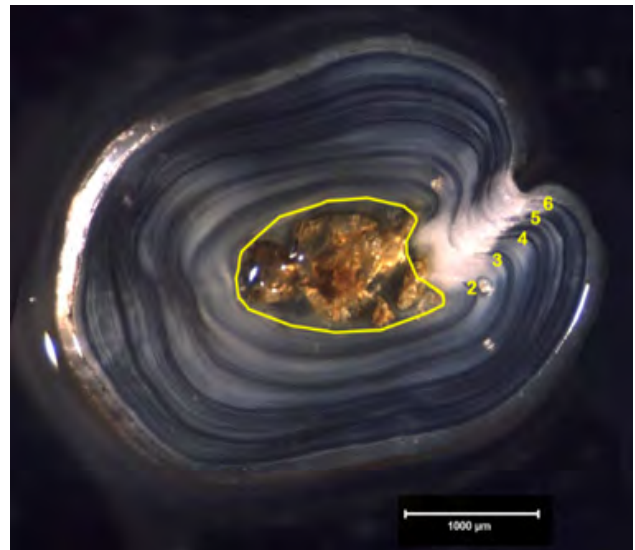


Figure 6.1. Resorption and deterioration of the first annulus (indicated by the yellow line) of the second dorsal spine of a Red Grouper (*Epinephelus morio*).

6.2 Spines

Fin spines are formed as a single fused element of bone with a central lumen that is connected to the inner support system (pterygiophores) within the body cavity of a fish. Spines are rigid and unsegmented (Figure 6.2); they articulate from the base (condyle) for defense or to orient the fish in the water column. The central lumen of the spine may be filled with vascular tissue, or it may be resorbed and hollow; in either case the earliest growth rings in older individuals can become obscured.

Spines that are used for age determination are typically removed from the dorsal fin (Speare 2003, Metcalf and Swearer 2005, Brusher and Schull 2009, Kopf et al. 2010, Lombardi et al. 2015), but anal and pelvic spines have also been successfully used for ageing (Speare 2003, Elzey and Trull 2016, Pons et al. 2016). When ageing a species for the first time using spines, it is preferable to take samples of all the fins and compare readability among them before choosing one alone.

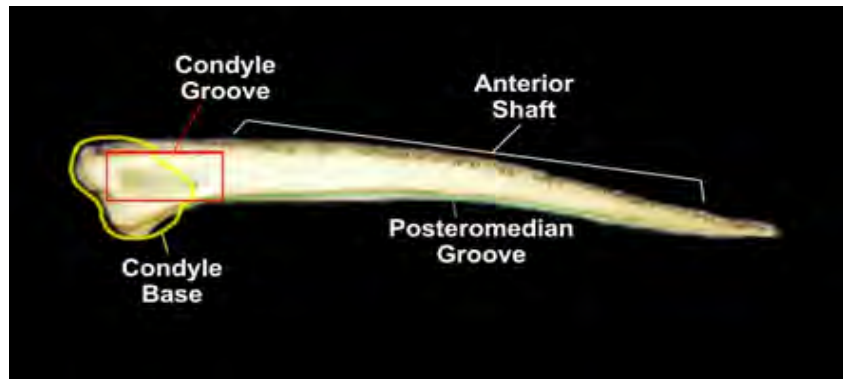


Figure 6.2 Generalized dorsal fin spine showing the various regions of the structure.

6.2.1 Spine Removal

The methods for removing spines for ageing purposes revolve around getting the spine cut as close to the body as possible. In fish that are to be kept alive or in marketable condition, this will involve cutting the membrane between the desired spine and the subsequent spines, then the spine can be cut as close to the body as possible with a pair of wire cutters, strong scissors, fingernail clippers, knife etc. In fish that are already deceased and do not need to be kept in pristine condition, a knife or scalpel can be used to cut the entire spine including the condyle from the fish. Typically the longest spine along the fin is used for ageing, and in many cases, this is the third spine (Figure 6.3). The first and second spines are generally not used for ageing because they are the smallest spines of the fin. The first or second spines may also have encountered physical damage at some point and would be deemed unreliable for ageing.

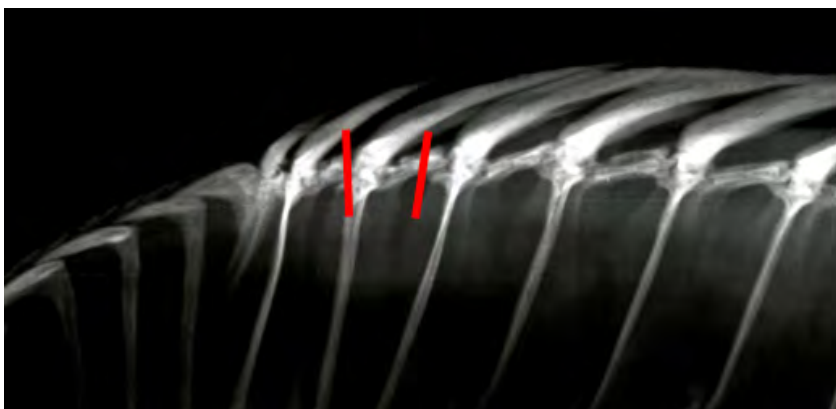


Figure 6.3 Radiograph of dorsal fin spines and condyles. In most species, the third dorsal spine (usually the longest) is selected for ageing. Red lines indicate location of cuts to remove spine and condyle.

A notable exception to this common rule is the Gray Triggerfish *Balistes capriscus*, in which the first dorsal fin spine (the trigger) is the largest spine, and is thus used for ageing.

6.2.2 Spine Preparation

Regardless of the processing technique, a few standard storage and cleaning protocols are recommended for spines. Cleaning is not a requirement for some spines, but it is recommended to ensure that the external surface of the section is free of tissue for ageing. Most often, spines are stored in a freezer in order

to prevent rot and fouling on both the exterior and interior portions of the structure. Frozen spines are removed from the freezer and cleaned just prior to sectioning. Spines can be stored unfrozen in an envelope; however, care must be taken to ensure the spine is completely dry prior to storage, otherwise mold and rot could ruin the sample.

Spines should be cleaned prior to sectioning so that all tissue is removed from the exterior of the spine; the easiest method to do so is by boiling the spine for a short time period. The amount of time spent boiling can vary depending on the size of the spine, the species of fish, the storage status (frozen, fresh, or dried) and the amount of tissue on the spine. Boiling times can range anywhere from 20 seconds to a few minutes depending on the factors listed above. If the sample does not contain the base of the spine, the boiling time will be significantly reduced. Along with boiling water, a soft bristle brush (i.e., toothbrush) can be used to aid in tissue removal.

The condyle (base) of the spine is not needed for ageing, so it can be removed by cutting with scissors, diagonal pliers, rotary tool or, if available, a high speed saw. Removing the condyle of the spine serves a dual purpose because it allows the spine to lay flat on a surface for sectioning, thus ensuring a perfectly transverse cut. However, care must be taken to only remove the condyle of the spine during this process; if the cut to remove the condyle is made too far distal, the earliest growth zones will likely be removed, and the fish will be underaged (Figure 6.4).

6.2.3 Sectioning Spines

The thickness of the transverse section must be adjusted to assure that annuli are visible, though this is often species-specific. If the spine was not previously cleaned, 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; however, care must be taken not to damage the outer edge of the section while performing this cleaning, as important information might be lost when removing this tissue.

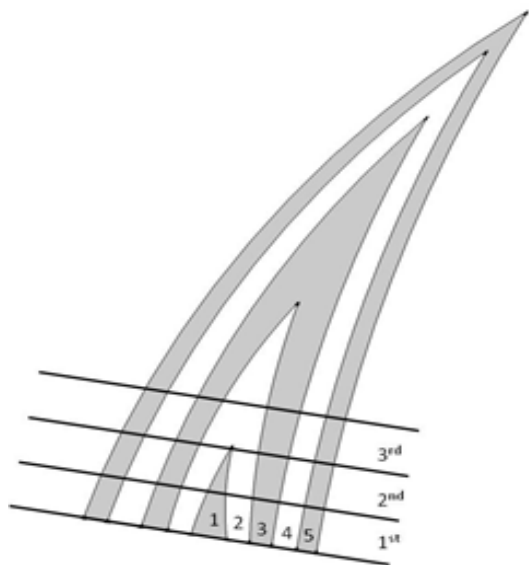


Figure 6.4 Diagram of the growth of a spine showing five annual growth zones and three sectioning areas. Note how the first annulus is missed in the 3rd section where the cut is too far distal.

In general, 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 cut that is made too far distal (Figure 6.4, 3rd cut; Figure 6.5C) will result in a loss of the earliest growth zones. A basal cut will result in sections that are abnormally shaped, and incorporate the convolutions of the condyle of the spine (Figure 6.5A and 6.6)

6.2.3.1 Low Speed Wafering Saw

Most of the methods described in Chapter 3.0, Section 3.2.6 can be applied to sectioning spines; however, the most successful spine processing technique has been using a multi-blade setup, and most states have applied a form of this technique for their spines. If the spine has the base attached, the base on the flattest side of the spine needs to be trimmed, so that the spine will lay flat, ensuring a perpendicular transverse cut. A mounting technique using cardstock and hot glue is used, as described in Chapter 3.0, Sections 3.2.5.2 and 3.2.6.2.3 (Figure 6.7).

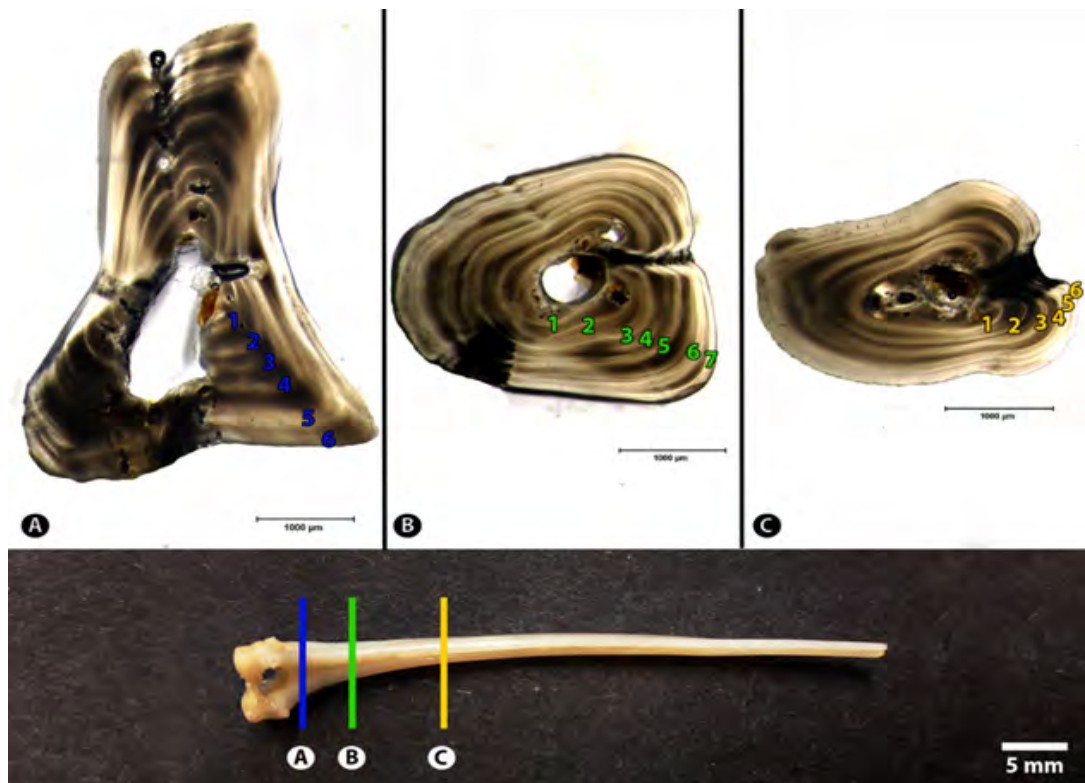


Figure 6.5 Cutting regions and resultant sections from a Red Grouper *Epinephelus morio* spine. Cut lines along the whole spine correspond to the labeled sections. A) A basal cut of the spine, which incorporates the convolutions of the spine, and is the most vascularized. B) The ideal region for a cut, and is the only section that incorporates all seven annuli. This section can be successfully obtained from a spine that is sampled whole (as in the picture), or one that is sampled non-lethally, as long as the spine is clipped off the fish as close to the muscle of the back as possible. C) A distal cut of the spine, where the true first annulus is no longer apparent, and the outer annuli have begun to compact. **Note:** These regions are specific to this species, and should be used only as a general spine sectioning guideline.

The mounted spine is placed on a chuck assembly on the low speed saw (Figure 6.8) and lowered onto the spinning blades. If necessary, multiple binder clips can be used to stabilize the card (and attached spine) during processing. The spine needs to be perfectly perpendicular to the blades to ensure that the sections are on-plane. The cardstock can be adjusted to ensure a proper cut and, if needed, a perpendicular line

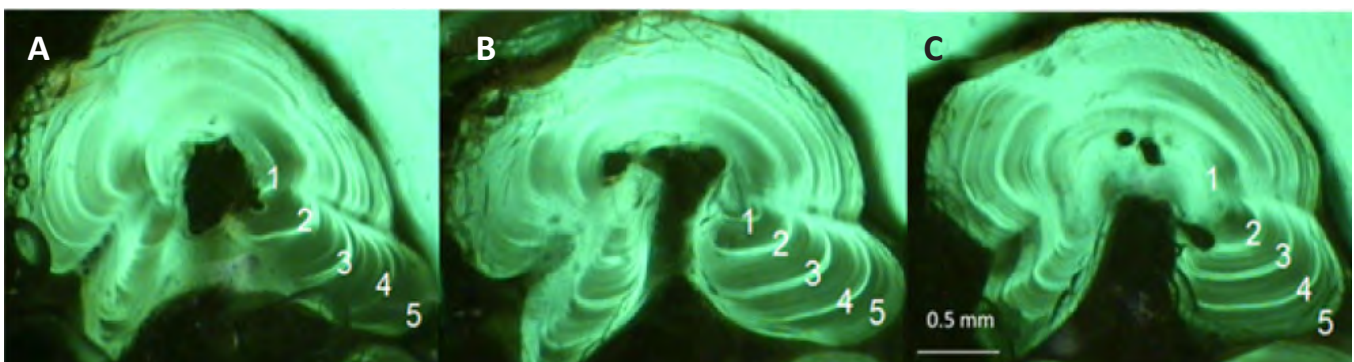


Figure 6.6. Annuli visible in basal versus distal sections of fin ray from an individual Gag *Mycteroperca microlepis*: A) basal section from just above pterygiophore on an age-5 fish; B) section from a fin ray clipped level with the back of the Gag; and C) section from about 1 cm distal on fin ray of the same fish, where first annulus is present but more difficult to identify.

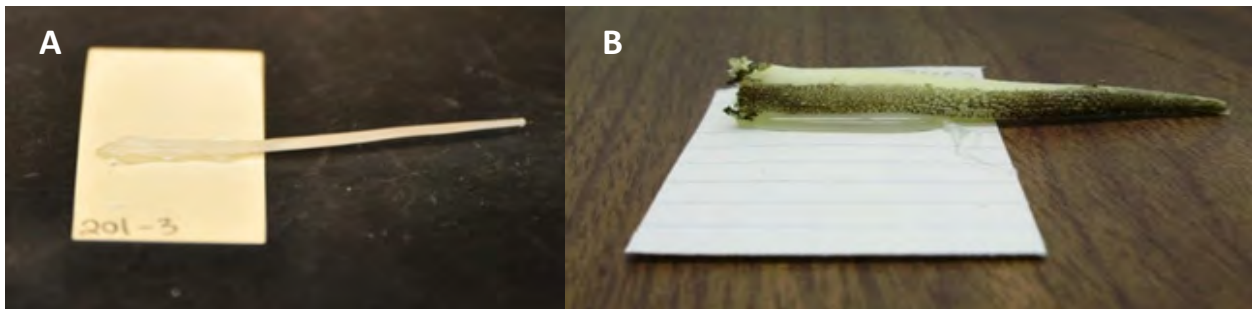


Figure 6.7 A) Red Snapper *Lutjanus campechanus* and B) Gray Triggerfish *Balistes capricus*) spines mounted on cardstock using hot glue. Note that bases of spines are trimmed so that the spines lay flat on the cardstock surface.

can be drawn on the cardstock to aid in alignment. The processing is complete when the blades have cut completely through the spine and are starting to cut through the glue. Smaller spines can also be embedded in epoxy, as described in Chapter 3.0, Section 3.2.5. Trimming the length of the spine can help it fit in a smaller mold, thereby saving epoxy. Final spine sections are mounted onto the slide using a liquid coverslip.

6.3 Fin Rays

Fin rays are comprised of two parallel elements (lepidotrichia) and are segmented, allowing them to have flexibility (Figure 6.9). Unlike a fin spine, a fin ray does not have a central lumen completely surrounded by bone. Rather, the vascular tissue lies between the lepidotrichia and is offset from the core (lies in a medial groove). There is a pterygiophore, or knuckle of cartilage or bone, at the base of each fin ray pair that articulates with the dorsal skeletal elements, or the pelvic/pectoral girdles.

Fin rays used for age determination are typically removed from the dorsal or pectoral fin. A modification of the method of Beamish and Chilton (1977, 1982) has been used successfully with dorsal fin rays to estimate ages for Gag Grouper up to age-17 (Debicella 2005) and Goliath Grouper to 18 years (Murie et al. 2009). For Greater Amberjack, however, pectoral fin rays provide clearer annuli compared to dorsal fin rays (Murie personal observation), similar to Pacific Cod (Beamish and Chilton 1982). When ageing

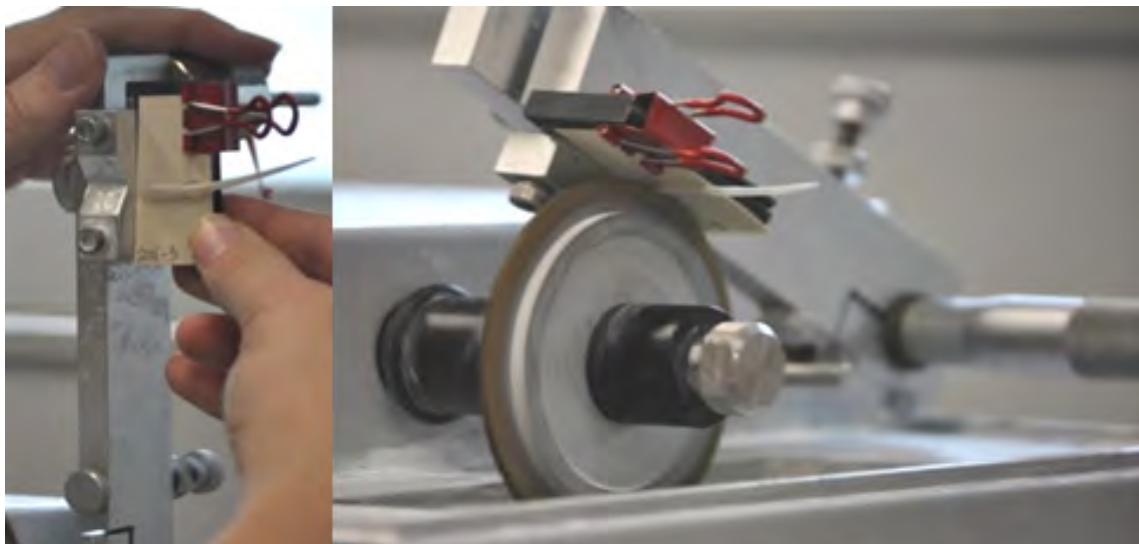


Figure 6.8 Dorsal fin spine mounted on low speed saw. A binder clip is used to keep the cardstock in place on the saw arm.

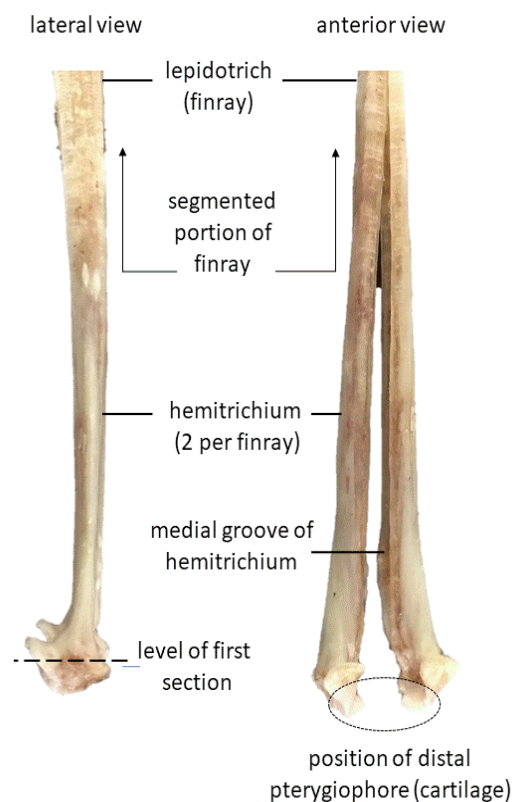


Figure 6.9 Generalized fin ray showing the various regions of the structure.

processing, fin rays should first be thawed and trimmed of as much excess tissue as possible. Unlike spines, fin rays cannot be boiled because excessive heat may cause the segments to fracture or fall apart (i.e., fin rays are segmented structures). Instead, the proximal part of the fin rays may be submerged in simmering water for 20-30 seconds at a time and carefully cleaned after each submergence so that the tissue on the outer surface and between each fin ray is removed. After cleaning, fin rays should be placed with the cut surface exposed to the air and with the fin rays lying parallel to one another to air dry completely (usually two to five days) (Figure 6.10). It is important to arrange the fin rays parallel to one another as they dry so that they remain aligned prior to sectioning.

Once dried, most fin rays need to be embedded or coated with epoxy to strengthen their structure for sectioning, as fin rays are usually flexible to some degree. In addition, the epoxy will help to keep the cross-sections together when the fin rays are sectioned. The fin rays may be embedded or coated using a two-part epoxy resin that cures relatively quickly (Figure 6.11); the use of a mold is not necessary. To epoxy the fin rays, the dried set of rays (usually two to four fin rays) are placed on a piece of waxed paper (to which the epoxy does not stick to when cured) and the epoxy is applied over the

a species for the first time using fin rays, it is preferable to take samples of all the fins that can be sampled non-lethally and compare readability among them before choosing one alone.

6.3.1 Fin Ray Removal

Soft fin rays must be removed as close to the surface of the body as possible to make sure that all annuli (especially the first) are present in the base of the fin ray (Figure 6.10). On dead fish, the rays can be removed down to their base (knuckles), which extends into the muscle of the fish. As with spines, the fin membranes between the fin rays are cut down to the back of the fish and then the fin rays are cut off as close to the back of the fish as possible, usually using heavy duty lab scissors or pruning shears. Usually two to four fin rays are removed from the second dorsal fin of most fishes. The fourth through seventh fin rays typically work well as they are slightly larger than the first few fin rays, which are usually smaller and structurally different in most fishes.

6.3.2 Fin Ray Preparation

Once collected, it is easiest to keep fin rays frozen until processed, but they can be air-dried if necessary as long as they are not exposed to humid conditions (i.e., they will grow mold), or damaging organisms (i.e., insects). When



Figure 6.10 Dorsal fin rays from a Red Grouper *Epinephalus morio* arranged for drying in a coin envelope. Note the parallel placement of the fin rays.



Figure 6.11 Embedded dorsal fin rays from a Goliath Grouper *Epinephelus itajara* (rays are partially obscured by cured resin). Sections have been removed from the basal portion (left side) of fin ray block.

proximal ends of the dried fin rays. Care must be taken to ensure that the epoxy encapsulates the entire fin ray base (i.e. the region that will be sectioned). In addition, the epoxy should fill the space between the two elements of each fin ray, and between the fin rays, to provide structural support while sectioning.

6.3.3 Sectioning Fin Rays

After curing for at least 48 hours to allow the epoxy to harden, the fin rays can be processed on a variable speed sectioning saw. Processing occurs sequentially from the base of the fin rays out toward their tips (distally). Sectioning should start immediately at the plane of the knuckles of the fin rays (or as close as possible) and progress sequentially up the ray so as to not miss sectioning the first annulus (Figure 6.12). Sectioning should occur until the first annulus can no longer be distinguished. For small fin rays,

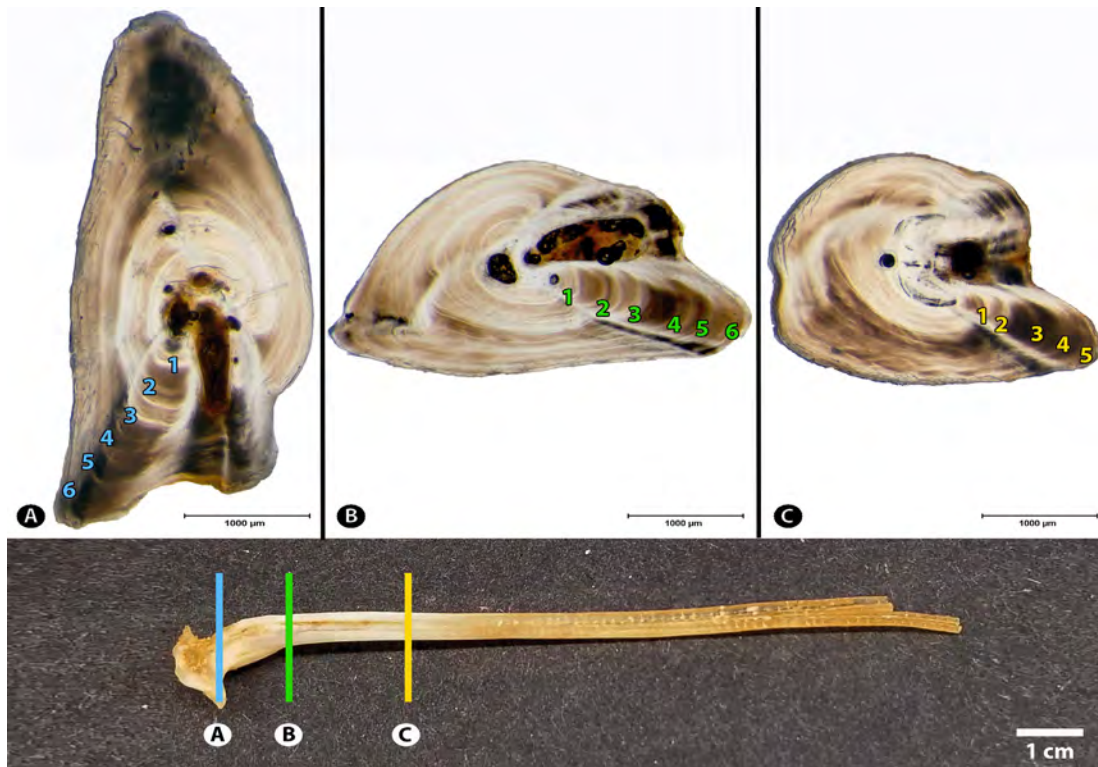


Figure 6.12 Cutting regions and resultant sections from a Goliath Grouper fin ray. Cut lines along the whole fin ray correspond to the labeled sections. A) A basal cut of the fin ray, which incorporates the convolutions of the fin ray, and is the most vascularized. B) The ideal region for a cut, and is the only section that incorporates all six annuli. C) A distal cut of the fin ray, where the true first annulus is no longer apparent, and the outer annuli have begun to compact. **Note:** These regions are specific to this species, and should be used only as a general fin ray sectioning guideline.

this typically occurs within the first five or six serial sections, but for larger fish, this can take up to eight sections depending on the species. Once the level of sectioning needed to capture the first annulus, while simultaneously decreasing the amount of vascularization present, is known for the fish species specifically, then the number of sections taken can be reduced to target that specific fin ray area. Taking multiple sections of the fin rays may be time consuming but is worthwhile because of the individual variability in their structure; it also allows for multiple views of multiple fin rays, which can aid in the ageing process (see Chapter 8.0, Section 8.2.4).

Section thickness may vary between species and the optimal thickness should be determined through a process of trial and error prior to sectioning all the fin rays. The optimal section thickness can range anywhere from 0.7 mm to 1.4 mm or more, and it is important to define an appropriate thickness on a species by species basis. Ideal sections should have thick, clear annuli, but allow adequate transmitted light to penetrate through the translucent zones while using a microscope. Final sections are permanently mounted on a labeled slide using a liquid cover slip (i.e., Flotexx®) (Figure 6.13).

6.4 Troubleshooting Bad Sections

The most common problem encountered when mounting spine and fin ray sections to glass slides using Flotexx® (or other mounting media) is the occurrence of an air bubble over a critical area needed for ageing (e.g., the core). This can generally be avoided by checking for air bubbles under a dissecting scope after applying the Flotexx® and dragging the air bubbles off to the side of the slide using any long and fine-pointed tool (insect pin, toothpick, forceps, etc.).

When the structures are not sectioned perfectly perpendicular to the long axis, the resulting sections will not be perfect cross-sections but instead will be slightly oblique. For spines and fin rays this can cause problems when viewing them under transmitted light because the bottom of the section may prevent the light from transmitting directly up through the translucent part of the annulus. Although it is best to take care that the spine and fin rays are sectioned perpendicular to their axis in the first place, oblique sections can still be read by tilting the slide until the top and bottom of each section line up and thereby allow the transmitted light to pass through the entire structure without obstruction. Ideally, the translucent annuli in the spine and fin rays should act as “light pipes” and so adjusting the tilt or overall position of the sectioned spine and fin rays relative to the light source can improve the clarity of the annuli.

6.5 Long Term Storage

Once spines and fin rays are processed and mounted to a slide, they can be stored and archived according to the same protocol as otolith slides. Typically once a readable section is removed from the structure, the remainder is discarded, since the optimum area for ageing has been removed. If there is a desire to retain a spine or fin ray for future analysis, they can be stored in an envelope, or if space permits, in a plastic vial. If the spines or fin rays have been cleaned and dried, then they can be stored in labelled manila coin envelopes but must be protected from humidity and/or bug infestations, as they can serve as a growing media and attractant for mold and pests.

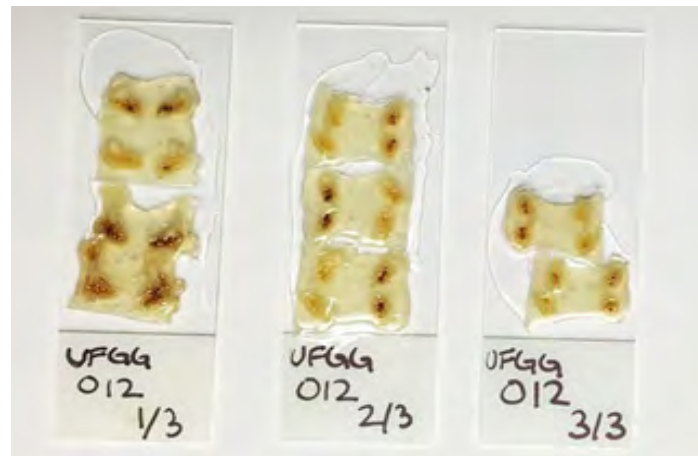


Figure 6.13 Sections of dorsal fin rays from a Goliath Grouper mounted to final slides.

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7.0 Structure Enhancement

When reading structures for age estimation, it is often necessary to perform techniques which will enhance the structure, thereby enabling better visualization of the growth zones. Physical techniques to enhance the readability of ageing samples include polishing, etching, staining, clearing, and baking. Other enhancement techniques such as alternative lighting types, filters, polarizers, and light sources may improve readability without directly affecting the structure. The resolution on most samples can be improved by using one or more of these techniques; however, a bit of trial and error must occur first. The species-specific sections of the manual (Chapter 9.0) will highlight enhancement techniques that have been used successfully.

7.1 Physical Enhancement

7.1.1 Polishing

Polishing involves using various grades of abrasive papers and polishing compounds to smooth the surface to be read. Large sectioned otoliths, embedded or not, can be polished with 400-800 grit wet-dry sand paper while larval and juvenile embedded otolith sections are typically polished with 1000-1500 grit. Oliveria (personal communication) polishes with various lapping films estimated between 1,800 to 8,000 grit (3-9 μm). Spine, fin ray, and vertebrae sections can also be polished. Electric polishers, gem polishers, buffing wheels, and hand polishing have all been used to remove saw marks and other surface imperfections. Care must be taken though not to polish away too much of the material which can eliminate ageing structures.

7.1.2 Etching

Etching is a technique used to enhance otolith microstructure, especially daily growth zones on younger fish. This technique is also employed when otoliths contain growth zones 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 resulting in three-dimensional relief on the surface which increases readability of the structure (Pannella 1980). The chemical is most often an acid solution applied to a thin otolith section that will partially dissolve the calcified zones and leave deposits of insoluble matrix proteins which will take stain if desired. Three solutions used for etching by Davies et al. (1988) include immersion in 0.1 M disodium salt EDTA (ethylenediaminetetraacetic acid) for 15 to 20 minutes, immersion in 1% HCl (hydrochloric acid) solution for 20 to 30 seconds, or immersion in 2% Histolab[®] RDO (a commercial etching solution comprising a mixture of HCl and EDTA) for five minutes. Etching may be followed by staining. Both EDTA and stain may be applied by placing a drop on the surface of the otolith or by placing the end of the slide in a histological staining jar. Etched otoliths may require some sort of wetting solution or cover to eliminate light refraction (Figure 7.1). **Note:** Etching too aggressively may result in the loss of edges.

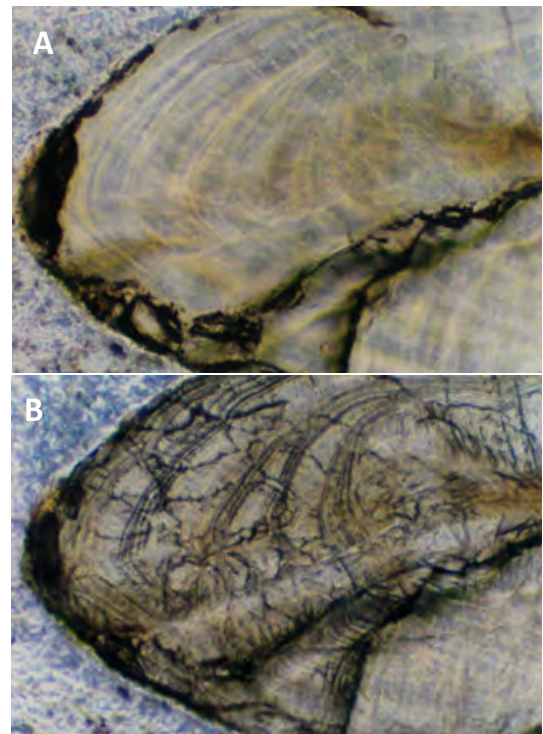


Figure 7.1. American Eel (*Anguilla rostrata*) sagittal otolith section before (A) and after (B) treatment with 5% EDTA.

For some applications, etched otolith sections may be viewed directly under a Scanning Electron Microscope (SEM) (Figures 7.2) or a replica of the etched surface can be examined using an acetate peel. However the majority of otolith studies focus on species that do not require the use of etching for analysis.

7.1.3 Physical Clearing

The physical clearing of an otolith refers to the process of submersing, or soaking a whole or sectioned otolith in a fluid medium that facilitates the passage of light through the specimen.

Soaking the sample in either clove oil, cedar oil, mineral oil, or glycerin will allow the perfusion of the clearing medium into the otolith microstructure. The soaking media effectively saturates the protein between the calcium carbonate crystals, resulting in better definition between translucent and opaque zones. Clearing usually affects the translucent growth zones first. The duration of soaking is critical in achieving good contrast; however, once applied, the effect can continue and eventually render a section unreadable. These clearing techniques can be permanent. Therefore, caution must be exercised when attempting this technique as time of soaking is dependent upon objective, species, and the otolith size.



Figure 7.2 Cross section of Moray Eel (*Gymnothorax* sp.) leptocephalus otolith to show growth rings using SEM at a magnification of 4,930X.

7.1.4 Mounting

Mounting or covering a sectioned sample can help reduce light refraction from imperfections, making annuli easier to identify (Figure 7.3). This can be achieved with a variety of products, such as Flo-Texx®, Loctite 349Impruv® Light Cure Adhesive (UV activated), Cytoseal®, or thermoplastic.

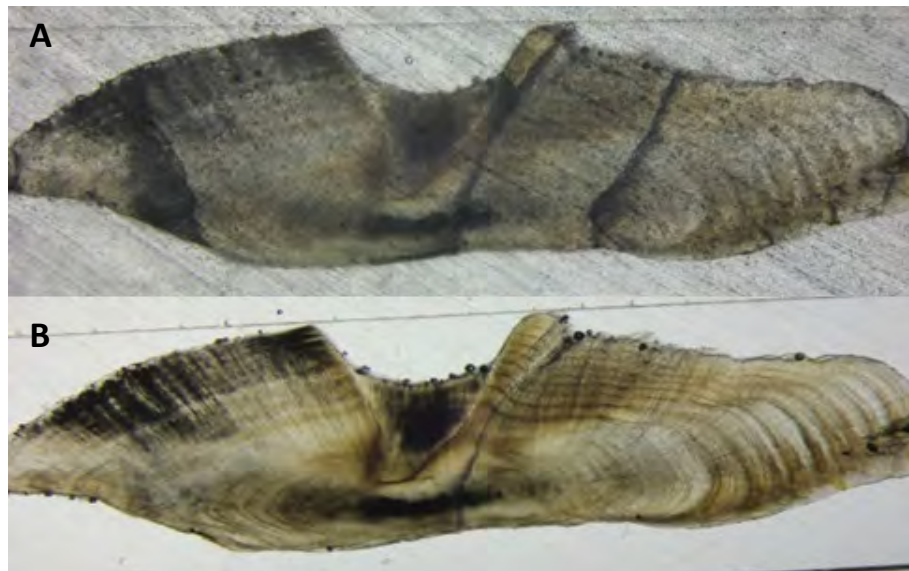


Figure 7.3 Two views of the same otolith section A) with no covering and B) with a liquid coverslip.

Mounting in this way will not only make it easier to read, but once properly cured, will protect the sample. For best results, it is important to avoid any bubbles in the mounting medium both above and below the sample. Care should be taken to ensure otolith, spines, and fin ray sections are completely dry before the application of a mounting medium.

7.1.5 Staining

Similar to the application of 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 sample (Figure 7.4). Dyes for this purpose generally act in one of two ways: 1) differential diffusion (uneven staining) of the protein and calcium matrix or 2) reaction solely with the calcium carbonate portions of the otolith (Gauldie et al. 1998). Histological stains are most effective, and commonly used stains include Alizarin Red, Aniline Blue, Crystal Violet, and Toluidine Blue; the darker colors prove to be most effective (Richter and McDermott 1990). It is recommended that otolith 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

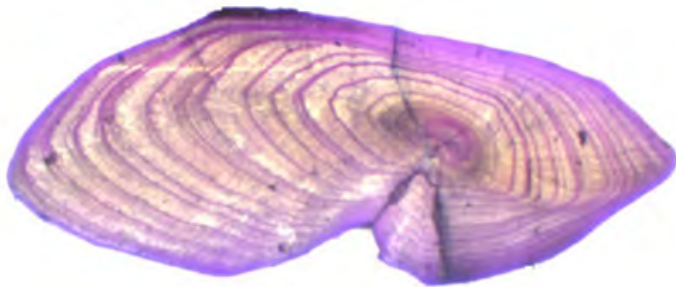


Figure 7.4 Transverse section of an EDTA etched and toluidine blue stained sagittal otolith from an American Eel.

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 techniques such as acid etching (acidification of the stain), thin sectioning, and use of transmitted light (Albrechtsen 1968, Bouain and Siau 1988, Richter and McDermott 1990, Gauldie et al. 1998). Staining is often successful when used to aid in interpretation of otoliths that exhibit indistinct growth zones or annuli.

7.1.6 Burning and Baking

'Burning' an otolith that has been sectioned by breaking (Chapter 3.0, Section 3.2.6.1) differentially burns the organic matrices within the annuli of the otolith, with the protein dense zones darkening faster than the rest of the otolith. The broken surface of one-half of the otolith is then held at an angle and moved 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. 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 7.5: Demonstration by Alaska Fisheries Science Center Chapter 12.0, Section 12.7).

Long-term storage of burnt otoliths does not appear to result in the fading of bands (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.



Figure 7.5 A broken and burnt otolith placed on a plasticine block to be examined under a microscope.

'Baking' otoliths is an alternative to 'burning' over an alcohol flame (Robillard et al. 2009). Ovens, ranging from toaster ovens to muffle furnaces, can be used to darken the proteins in an otolith. Otoliths are placed on a heat-proof surface (ceramic well trays work great for muffle furnaces) and placed into the oven for several seconds to several minutes depending on the species and temperature of the oven. The otoliths are removed when they reach a 'caramel' color. Alternatively, when ceramic trays are fully heated in a 400°C oven, otoliths can simply be placed on the tray and the latent heat will toast them in a few seconds while you watch, eliminating the chance of over baking. Otoliths can be baked prior to embedding and sectioning or broken, sectioned and baked

after. However, caution must be used when handling baked sections as they can become quite brittle.

7.2 Visual Enhancement

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.

7.2.1 Tilting and Flipping Samples

The nature and shape of whole otoliths will require some moving (tilting and flipping) of the sample to ensure visualization of all the zones. When reading otoliths whole or sectioned, physically moving the sample on the microscope stage will enable the reader to observe separate zones that, when observed from above may blend together in to one resulting in under-estimation of the sample's age. Sections are not always cut exactly perpendicular to the margin resulting in a blurring of the annuli and margins through the section (Figure 7.6). If the microscope slide is slowly tilted by hand or flipped over and viewed from the other side, the front and back planes of the section are realigned, creating a perpendicular margin relative to the viewer. The perceived opaque zone caused by an oblique view of the margin will disappear as the sharp edge of the section is presented. This same method can be used when reading sectioned

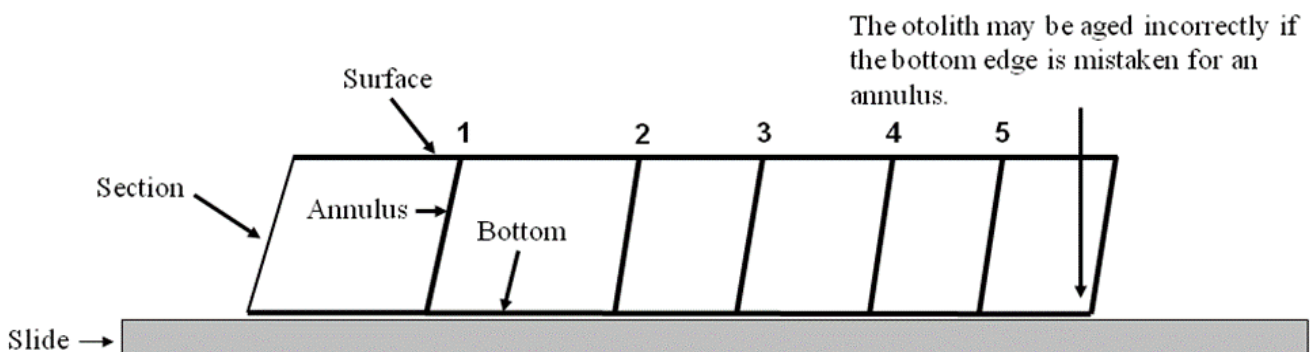


Figure 7.6 An illustration of a mounted section from a fish with five annuli. Because the otolith was sectioned slightly off-axis or off-plane, the section appears to have six annuli. This mistaken opaque zone is the bottom edge of the section visible through the thin section.

spines and rays as well. It is important to achieve alignment of the zones to avoid under-estimations, especially in slow-growing deep water species.

7.2.2 Submersion (Temporary Covering)

Temporarily submerging a sample in water or other fluid to change the light refraction rather than change the structure of the sample is an easy and inexpensive way to improve readability. Whole otoliths can be read while immersed which greatly reduces the glare from the surface of the sample. Immersing a sectioned sample can reduce the appearance of saw marks and other surface imperfections. This method also includes wetting a sample previously affixed to a slide with water, oil, or other fluid. **Note:** Prolonged exposure to clove oil, cedar oil, or glycerin will result in reduced readability and should be used with caution.

7.2.3 Lighting Options

The appearance of structures used to age fish will vary under different illumination methods. Transmitted light (light from below passed upward through the sample) and reflected light (light from above) will produce opposite contrasts in the observed zone patterns. Transmitted light is the most commonly used method for reading sectioned samples and scales while reflected light is used mainly for reading otoliths whole.

Different light types such as LED, incandescent, and halogen are available but it will depend on what each lab has at its disposal. Fiber optic lighting is convenient due to the ability to easily direct a focused beam from any angle which will aid in the reduction of glare. The ability to focus or diffuse the light source can be important as well to eliminate glare when reading samples.

The wave length/color of the light may impact the way a sample looks as well. Many LED light sources tend to give a bright blue tint to samples which does not regularly affect the ability to estimate the age. The warmer lighting associated with incandescent bulbs tends to allow visualization of texture and structure on scales better than LED or halogen lighting.

7.2.4 Filters

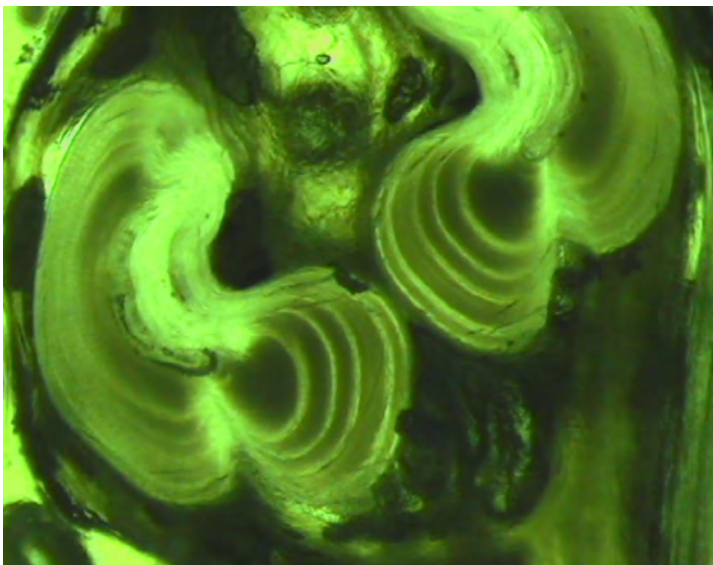


Figure 7.7 Cross-section of the dorsal fin rays from an age-6 White Grunt (*Haemulon plumieri*) viewed with a green filter (540 nm narrow-band).

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. Each microscope filter is used for a different purpose and all are typically placed in the light path, either over the illuminator or in a filter slot that lies in the light path. Polarization is commonly used to enhance ring identification. Color filters have also been used with moderate success for particular species (Figure 7.7).

7.2.5 Digital Analysis

Although binocular dissecting microscopes provide a clear view of most structures, many labs have found it advantageous to use more advanced image analysis systems. 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 or measuring to the margin for MIA. Image analysis systems have also been used to rapidly enumerate measurements used to back-calculate the length at annuli development and automatically determine number of annuli on the otolith. Image analysis is also beneficial in that two or more scientists can discuss the features of a sample without looking into a microscope. This allows for quick resolution of differences between readers within labs as well as between labs.

8.0 Age Determination

8.1 Getting Started

This chapter is designed to give the reader guidance in age interpretations using hard parts. Throughout Chapter 8.0, an example data sheet (Figure 8.1) is provided to track the procedure as the structure is read and an age determined for a fish with a predetermined birthdate. A section of a common sciaenid otolith is used as an example here because its features are typically clear and obvious (Figure 8.1). Other species' otoliths and other hard parts can be more difficult to interpret and details are covered in the species-specific accounts in Chapter 9.0. The only data that should be available to the reader during ageing is the sample ID of the fish to ensure blind reading, assuming no age information can be inferred from the unique sample ID. Blind reading is the practice of counting annuli directly from the hard part without knowledge of length or capture date that a reader can use to infer age, as these data can potentially bias, consciously or subconsciously, the reader's estimate. If age information can be inferred from the sample ID, a different reader should generate and assign random numbers to each sample and only provide the random numbers in the data sheet in place of sample ID. Before ageing is begun, it should be determined if any data in addition to the data in the example data sheet is needed by the end user. If ages are being recorded into a data file that already contains other fish information, be sure to hide the columns containing everything but fish ID prior to beginning ageing.

| Fish ID | Read Date | Reader | # Annuli | Margin Code | Readability Code | Comments | Capture Date | Age Group | Biological Age |
|---------|-----------|--------|----------|-------------|------------------|----------|--------------|-----------|----------------|
| MS00001 | | | | | | | | | |
| MS00002 | | | | | | | | | |
| MS00003 | | | | | | | | | |



Figure 8.1. Example blank datasheet and common sciaenid otolith section prior to assignment of annuli, margin code, readability code, or age.

The first items to record are the date the sample was read and the reader's name (Figure 8.2). This information will become useful for evaluating ageing error and for adjustments to age estimates, if necessary, in the future.

8.2 Annuli Enumeration

A basic understanding of hard structure development through successive periods of growth zone

formation is necessary to interpret the information contained in the structure. Hard parts contain annual growth zones (i.e., annulus), each made up of subzones representing periods of slow growth and periods of fast growth (Chapter 2, Section 2.1). Generally, an annulus consists of one slow growth subzone and one fast growth subzone. However, this varies across species, and when more than two subzones occur in an annulus it will be noted in Chapter 9.0. The slow growth zone is usually narrow relative to the fast growth zone.

| Fish ID | Read Date | Reader | # Annuli | Margin Code | Readability Code | Comments | Capture Date | Age Group | Biological Age |
|---------|------------|-----------|----------|-------------|------------------|----------|--------------|-----------|----------------|
| MS00001 | 12/12/2016 | Joe Smith | | | | | | | |
| MS00002 | | | | | | | | | |
| MS00003 | | | | | | | | | |

Figure 8.2. Example data sheet with read date and reader name.

8.2.1 Otolith Annuli Interpretation

Otoliths contain annuli made up of a translucent zone and an opaque zone, which appear differently depending on the type of light used during viewing (Chapter 3, Section 3.1). The translucent zone is usually wider than the opaque zone and represents a period of faster growth. The opaque zones are generally counted to represent the number of annuli and, in practice, the terms opaque zone and annuli are used interchangeably. Counting opaque zones in an otolith may seem straightforward, but for some species, separate opaque zones are not distinct. Two specific problems can be encountered: 1) identifying the location of the first opaque zone near or within the core, and 2) identifying an opaque zone beginning formation very near or on the outer edge, or margin, of the otolith. If the timing of opaque zone formation is concurrent with or immediately following spawning, the first opaque zone may be hidden within the core region. If time of capture is concurrent with zone formation, a distinct zone may or may not be observed at the otolith's margin (Chapter 8, Section 8.3). When zones are not particularly clear, techniques can be used to help discern zones (Chapter 7.0) and are discussed separately within each species account when they apply (Chapter 9.0).

8.2.1.1 Sectioned Otolith Annuli Enumeration

Annuli in sectioned otoliths can be viewed under compound or dissecting microscopes using either transmitted or reflected light. The thickness of the section as well as the otolith properties will determine the best lighting option for each species. Annulus enumeration in sectioned otoliths is typically made along the edge of the sulcus from the center of the core to the otolith margin (Figure 8.3). Some species will require an alternate plane on which to enumerate annuli. These species-specific differences will be noted in Section 9.0. The number of annuli (opaque zones in most species) are counted and recorded (Figure 8.3).

8.2.1.2 Whole Otolith Annuli Enumeration

Whole otoliths can be read using a dissecting microscope with either reflected or transmitted light. When using reflected light, placing the otolith on a black background increases contrast considerably. Submerging the otolith in one of a variety of fluids can also reduce glare for easier viewing (see Chapter 7, Sections 7.1 and 7.2 for further enhancement techniques). The sulcus of the otolith can distort the view of the annuli so enumeration is typically performed along the distal surface. Annuli are enumerated from

| Fish ID | Read Date | Reader | # Annuli | Margin Code | Readability Code | Comments | Capture Date | Age Group | Biological Age |
|---------|------------|-----------|----------|-------------|------------------|----------|--------------|-----------|----------------|
| MS00001 | 12/12/2016 | Joe Smith | 2 | | | | | | |
| MS00002 | | | | | | | | | |
| MS00003 | | | | | | | | | |

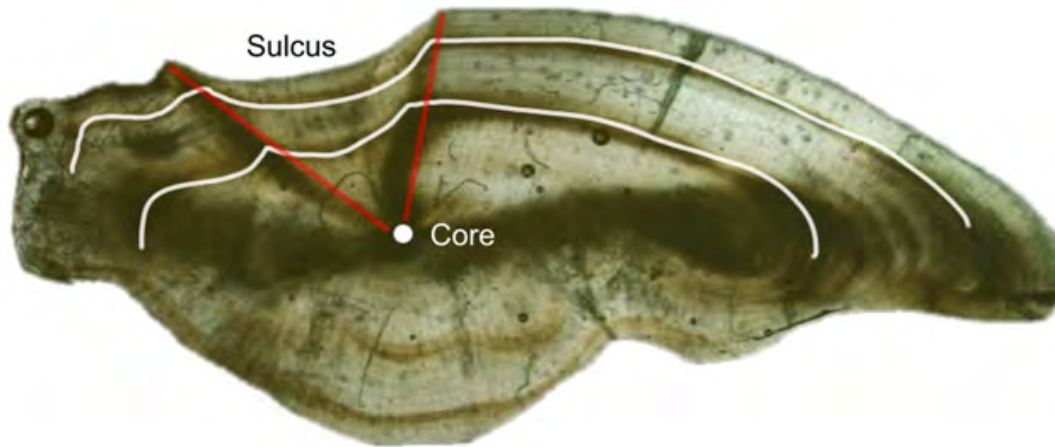


Figure 8.3. Example data sheet with the number of annuli counted on the sciaenid otolith section.

the core to the edge of the otolith. The specific counting plane will vary based on species but is typically along an axis with the most growth. As fish grow older, the otoliths tend to get thicker and therefore can inhibit visualization of the first annulus. Furthermore, growth at the margins may slow considerably, causing difficulty enumerating annuli near the edge. As with all techniques, validation studies should be conducted for all representative ages.

8.2.2 Scale Annuli Enumeration

Scales or scale impressions are typically read with transmitted light and can be viewed with a light microscope, a microfiche reader, or a microprojector (Figure 8.4). Annual growth zones in scales typically consist of a fast growth zone which is characterized by concentric and continuous growth of circuli, and a zone where growth slows or stops (Figure 8.5). This zone where growth has stopped (or nearly stopped) is characterized by circuli which are not continuous (broken) and often tightly packed together. When fast growth resumes, the circuli once again grow continuous and concentric. Depending on the species, these transitions between slow and fast zones may appear as dark (closely spaced broken circuli) or light (empty space between broken circuli and resumption of continuous circuli) lines. Several life history circumstances may lead to broken circuli that are not associated with the slow growth zone. These broken circuli can usually be distinguished from the slow growth zone because they are not continuous around the anterior portion of the scale. In a true slow growth zone the circuli breakages continue across the transition from the anterior to the posterior portion of the scale.

Annuli enumeration typically starts from the center of growth (focus) and continues along an axis either to the lateral edge or to the most anterior edge of the scale. Annual growth zone measurements are typically made along a straight line toward the center of the anterior margin. Scale annuli enumeration

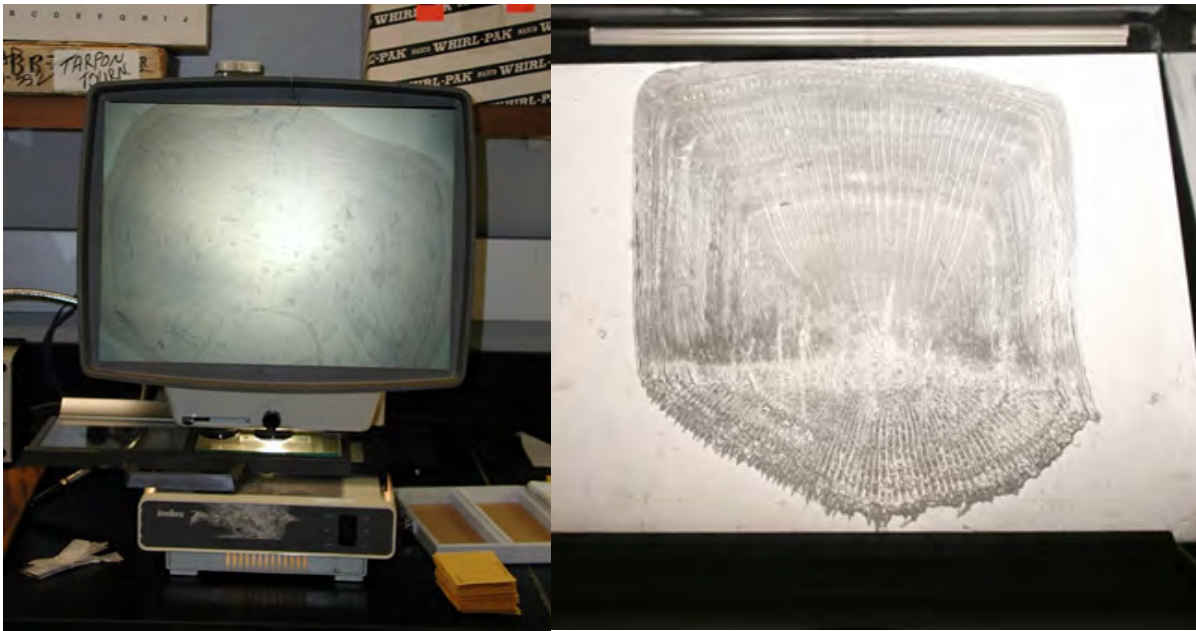


Figure 8.4. Scale impression being read on a microfiche.

becomes more difficult in older fish because as growth slows, the number of circuli laid down during the fast growth zone decreases making breakages harder to identify.

8.2.3 Opercle Bone Annuli Enumeration

Opercles are read using either transmitted light (window, overhead light, microprojector) or reflected light (ambient), with and/or without magnification. Reflected light is most helpful when the opercle is placed on a dark surface. While magnification reveals more detail, a more gestalt view afforded without magnification often presents a clearer pattern of annual growth. Magnification using a microprojector is best for discerning subtle annuli closest to the articular apex (annuli 1 and 2; Figure 8.6). A combination of both methods is helpful with difficult opercles.

Notice that in Figure 8.6, each opaque zone appears to be preceded by a translucent zone. This zone is useful in helping to distinguish between annuli and check marks. Check marks can also be distinguished from annuli because they frequently are not continuous onto the margins of the operculum. The first annulus or two can be difficult to distinguish as the operculum gets thicker. Depending on the species, the distance from the articular apex to the first visible annulus can help inform the reader as to the possibility of missing annuli.

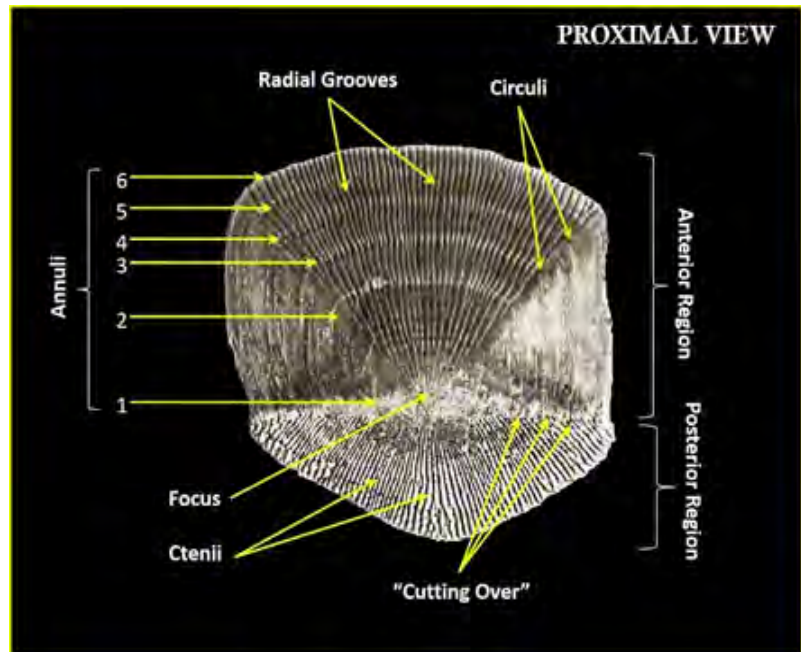


Figure 8.5. An acetate impression of a scale from an age-6 Striped Bass (*Morone saxatilis*) depicting annuli, cutting over, and other features.

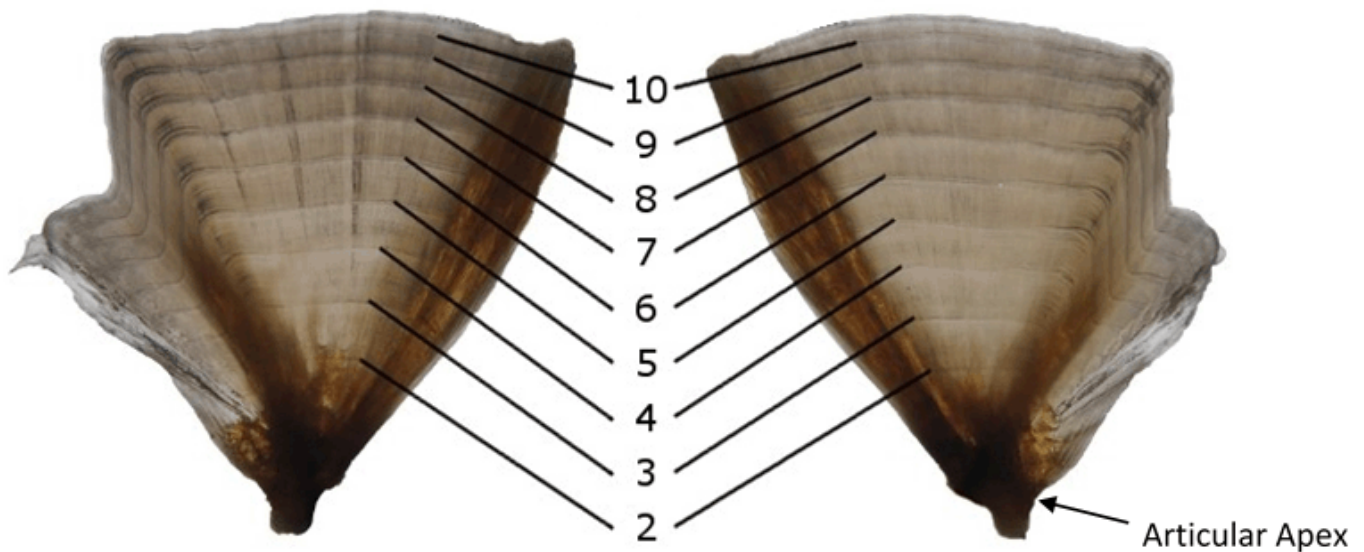


Figure 8.6. Annuli on a particularly well-formed age-10 *Tautoga onitis* operculum. The first annulus is not visible and the bones are judged to be showing nine annuli.

8.2.4 Spine Annuli Enumeration

Spines are viewed with either transmitted or reflected light. Stereomicroscopes work for larger faster growing spines but compound microscopes may be necessary for slower growing smaller spines. In most species, annuli appear as a thin translucent zone and a thick opaque zone. The thin translucent zones are counted starting from the core and moving distally (Figure 8.7). The core of some spines become vascularized eliminating the first (or more) annulus. Crowding of annuli near the edge may be present in old specimens, making enumeration difficult. Species-specific accounts (Chapter 9.0) will address these issues where necessary.

8.2.5 Fin Ray Annuli Enumeration

Fin ray 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. In most species, annuli appear as a thin translucent zone and a thick opaque zone. The thin translucent zones are counted starting from the core and moving distally (Figure 8.8). Crowding of annuli near the edge may be present in old specimens, making enumeration difficult. Species-specific accounts (Chapter 9.0) will address this issue where necessary.

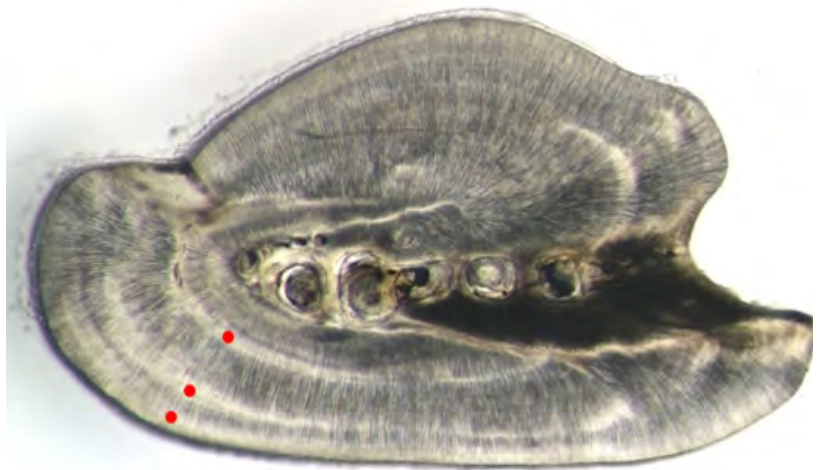


Figure 8.7. Cross section of an age-3 Red Snapper (*Lutjanus campechanus*) dorsal spine. The red dots represent the counted annuli.

8.3 Margin Codes

Another necessary step when assigning ages to fish entails describing the relative stage of annual growth on

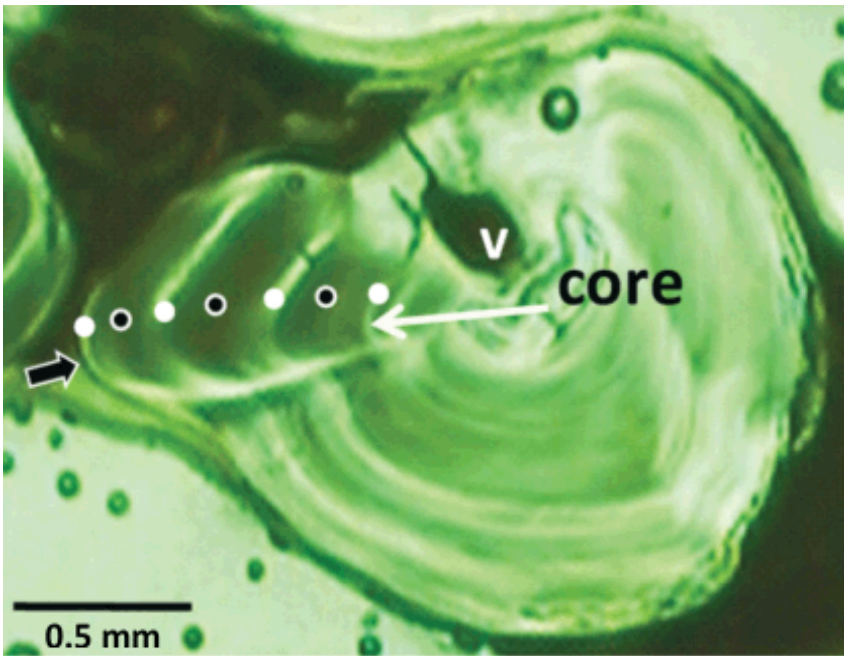


Figure 8.8 Cross-section of a dorsal fin ray from an age-4 Goliath Grouper (*Epinephelus itajara*) showing opaque (black dots) and translucent zones (white dots). Black arrow indicates the start of a new opaque zone at the edge, the white arrow shows the best axis for annuli enumeration, and 'v' indicates vascular tissue (Fig. 1A from Murie et al. 2009).

the margin of a structure. A coding system has been developed to standardize a description of the amount of growth that has occurred on the margin of the structure since the most recent, fully formed annulus (Table 8.1). These codes can be assigned to all hardparts used in ageing for transparency to aid in resolution of reader differences within and between agencies and labs. The inclusion of standard margin codes will assist those using the data to determine why differences may occur in year class/cohort designations.

A code of 1 is assigned if the annual growth zone on the margin is fully formed simultaneous to capture (i.e. opaque zone formed at edge with no growth after). A code of 2 is assigned if the annual growth zone on the margin is less than one third formed (i.e. growth outside the last complete opaque zone is equal to less than 1/3 of the expected growth for that year). A code of 3 is assigned if the annual growth zone on the margin is between one third and two thirds formed; and a 4 is assigned if the annual growth zone on the margin is more than two thirds formed, but not fully formed (i.e., code 1). For the common sciaenid otolith section example, the developing annual growth zone on the margin shows more than two thirds growth relative to the most recent fully formed annulus and is assigned a code of 4. The margin code is recorded in the appropriate column (Figure 8.9).

The determination of which 'third' of the developing annual growth zone has been completed is somewhat subjective; however, the presence/absence of a completed annulus on the margin simultaneous to capture is relatively straightforward. The relative interval distance of subsequent annuli changes as the fish ages, owing to the geometry of the structure and the varying rate of growth, becoming progressively narrower as the fish ages (Figure 8.9). The distances observed in the most recent fully formed annulus closest to the margin are those used to judge the proportion of completion of the annual growth zone on the margin. Multiple codes can be observed in different fish captured at the same time because the

Table 8.1. Coding system for describing the amount of annual growth that has occurred on the margin of the structure compared to the most recent, fully formed annulus.

| | |
|---------|---|
| Code 1. | annual growth zone on margin fully formed |
| Code 2. | annual growth zone on margin less than 1/3 formed |
| Code 3. | annual growth zone on margin 1/3 to 2/3 formed |
| Code 4. | annual growth zone on margin more than 2/3 formed, but not fully formed |

| Fish ID | Read Date | Reader | # Annuli | Margin Code | Readability Code | Comments | Capture Date | Age Group | Biological Age |
|---------|------------|-----------|----------|-------------|------------------|----------|--------------|-----------|----------------|
| MS00001 | 12/12/2016 | Joe Smith | 2 | 4 | | | | | |
| MS00002 | | | | | | | | | |
| MS00003 | | | | | | | | | |

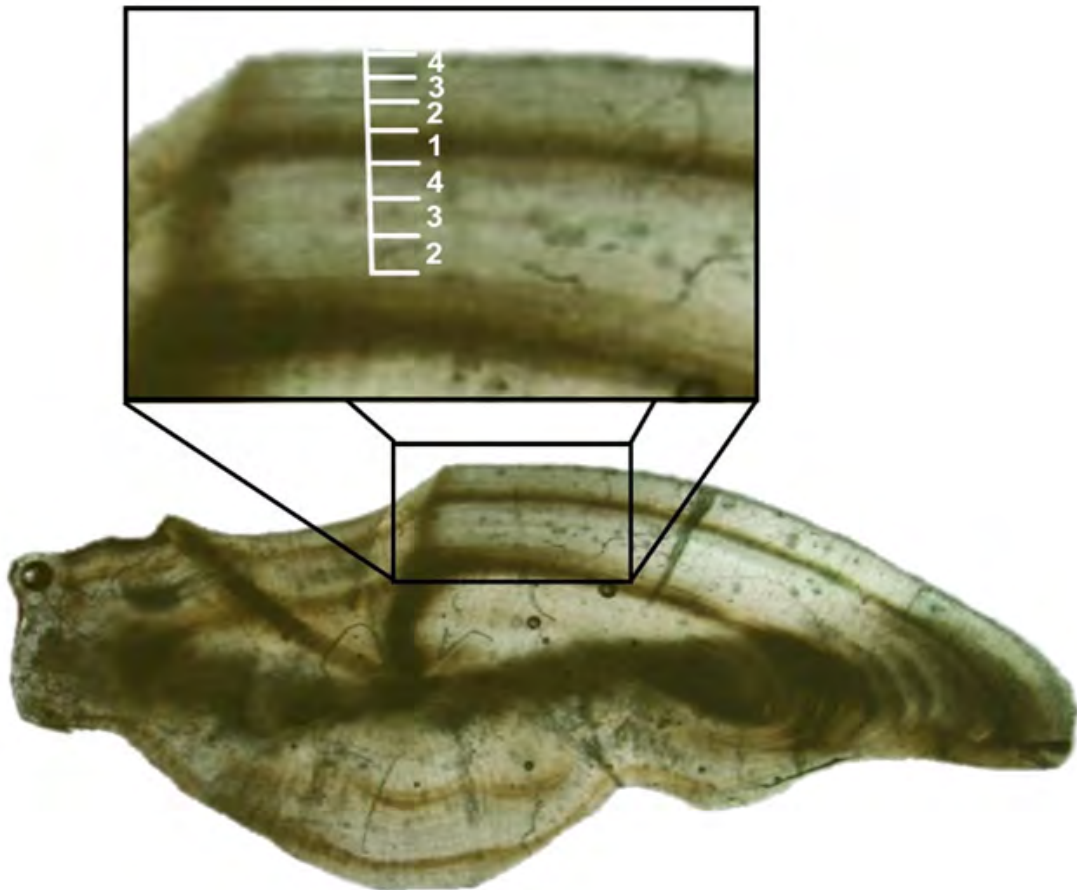


Figure 8.9. Example data sheet with the margin code from the sciaenid otolith section recorded.

timing and duration of annulus formation can be protracted over several months. The margin coding system should also be helpful when working with scales, opercles, and potentially spines and rays. Figure 8.10 provides an example of how the codes might look when judging scale margins.

When viewing cross sections of structures, the reader may incorrectly interpret a fully formed annulus on the margin if the section was not cut absolutely perpendicular to the growth axis (see Chapter 7.0, Figure 7.6). While off-angle sections are a very common and routine occurrence, not all new readers are aware of the phenomenon.

8.4 Readability Code

The reader's confidence in an annuli count, often impacted by the quality of the structure, is also an important variable to consider; how confident is the reader that an annuli count from a sample is repeatable with multiple reads? Confidence code, or readability code, systems are utilized by a number of labs to

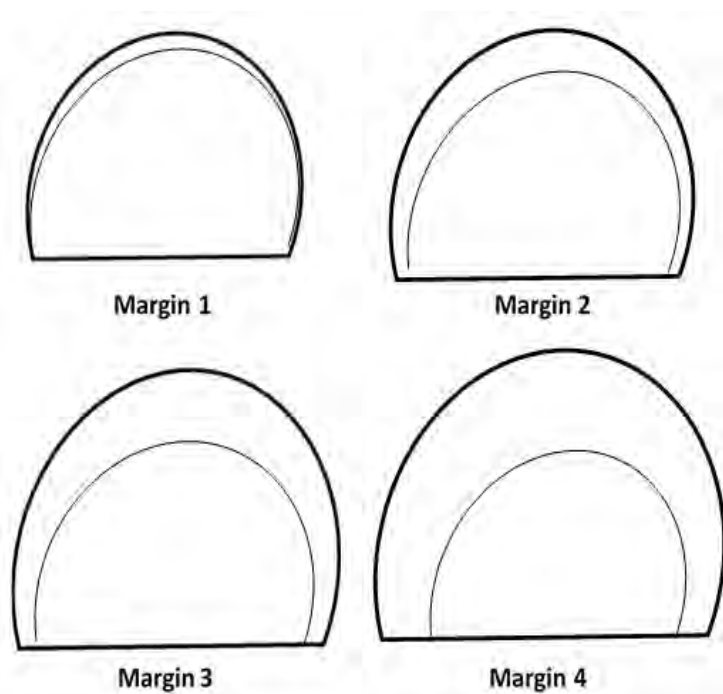


Figure 8.10 Margin codes applied to annual growth on scales.

potentially exclude bad samples from future data analysis (Table 8.2). Bad samples could be a result of poor processing, cloudiness, deformities, or damage. Readability codes are primarily used to indicate unreadable samples and are used internally to the lab or agency. These codes are subjective, but do provide some baseline information on how confident the reader is about the age data derived from the hard parts.

In addition to a readability code, it is best practice to record comments in the data sheet about anomalies or other unusual information that should be considered with the age estimate (Figure 8.11).

8.5 Assignment of Age

The analysis has now provided an annuli count and a margin code. Both of these parameters have been obtained by physically viewing the structure, understanding/recognizing what

the annuli are, counting the annuli, observing the margin and determining a margin code, and recording these data. Once these data are recorded, the capture date can be viewed. For the common sciaenid otolith example, the capture date of November 8, 2016 is provided from a database (Figure 8.12).

Table 8.2 Standard readability codes used to describe the reader's confidence in the repeatability of annuli counts for the same structure.

| Readability Code | Description and analysis consequence |
|---------------------------|---|
| A- Unreadable | Omit sample from analysis. |
| B- Very difficult to read | Age estimate differences between readers are expected to be >2 year for young, and >4 yrs for old fish (>10 yrs). Agreement on age may be difficult to reach, in which case sample should be classified as A and omitted from the analysis. |
| C- Fair readability | Age estimates between readers should be within 2 years in young, and within 4 years in old fish (>10 yrs). Agreement after second reading is expected after some discussion. |
| D- Good readability | Age estimates between readers should be within 1 year for young, to 2 years in old fish (>10 years). Agreement after second reading is expected without much discussion. |
| E- Excellent readability | Age estimates between readers should be the same. |

| Fish ID | Read Date | Reader | # Annuli | Margin Code | Readability Code | Comments | Capture Date | Age Group | Biological Age |
|---------|------------|-----------|----------|-------------|------------------|---------------------------|--------------|-----------|----------------|
| MS00001 | 12/12/2016 | Joe Smith | 2 | 4 | E | Use for example in manual | | | |
| MS00002 | | | | | | | | | |
| MS00003 | | | | | | | | | |

Figure 8.11 Example data sheet with readability code assigned to the sciaenid otolith section and comments about the sample recorded.

Two additional pieces of information, peak timing of annulus formation and birthdate, are necessary to estimate two final age parameters, age group and biological age. These two age parameters have different purposes that will be described below, but once one is estimated, the other can be derived. Timing of annulus formation is usually determined with marginal increment analysis (see Chapter 2.0, Section 2.3.3). An average birthdate can be estimated from fecundity data or from peak densities of larval/post larval fish (Figure 8.13A).

All of this makes assigning an age to a fish more than just using the number of observed annuli as the age of the fish. The necessary information for age assignment can be tied together and visualized with a timeline tracking the age of a hypothetical fish. Timelines also illustrate how fish spawned together are kept together through time in groups for tracking in stock assessments. The timeline for a fish in the Mid-Atlantic (Figure 8.14) is used as an example to assign an age to the common sciaenid otolith section. The timeline shows the accepted birthdate for a newly-hatched fish (August 1) which is assumed to occur at the peak of the June through September spawning period. The timeline then tracks the fish for two subsequent calendar years and shows how the annuli count increases as the fish completes annulus formation at the peak (March 1) of the February through March annulus formation period. All calendar years subsequent to the pictured timeline would show the same annuli formation process as the second and third years and the annuli count would increase accordingly.

Species that cover a broad geographical range, particularly with high latitudinal variation, may experience age processes differently within the range. In these cases, regional timelines should be agreed to and used for fish from a defined region. For example, fish in the South Atlantic and Gulf of Mexico spawn earlier, on average, than their Mid-Atlantic counterparts and, therefore, a slightly earlier birthdate (July 1) is assumed for fish believed to be spawned in these regions (Figure 8.15). Annulus formation occurs

| Fish ID | Read Date | Reader | # Annuli | Margin Code | Readability Code | Comments | Capture Date | Age Group | Biological Age |
|---------|------------|-----------|----------|-------------|------------------|---------------------------|--------------|-----------|----------------|
| MS00001 | 12/12/2016 | Joe Smith | 2 | 4 | E | Use for example in manual | 11/8/2016 | | |
| MS00002 | | | | | | | | | |
| MS00003 | | | | | | | | | |

Figure 8.12 Example data sheet with capture date of the fish that the sciaenid otolith section was extracted from for age determination.

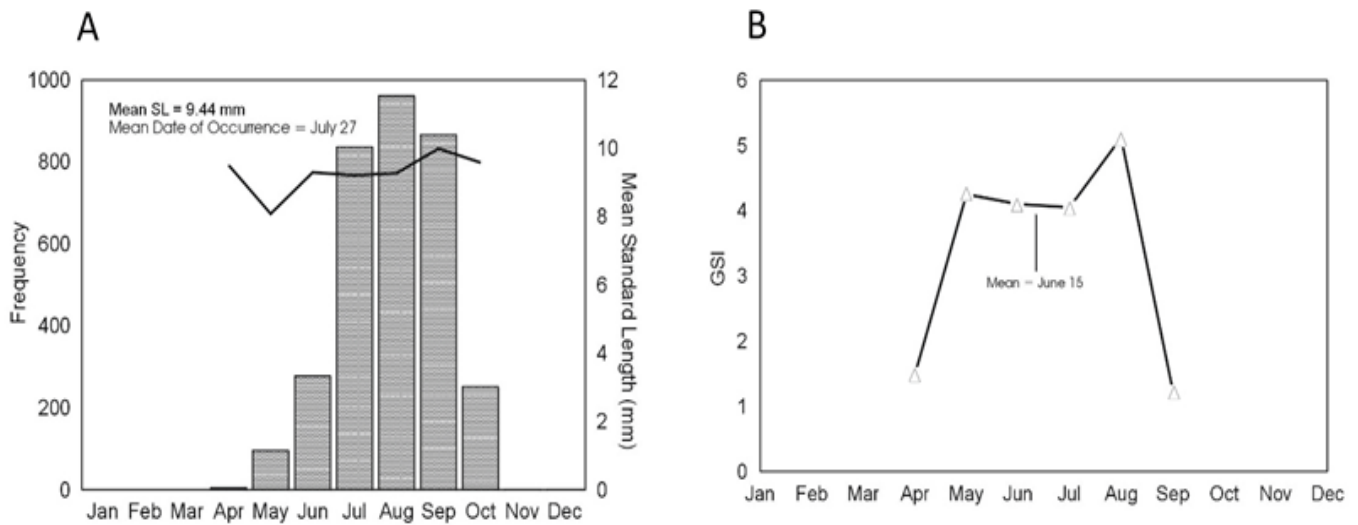


Figure 8.13 Birthdate determination using A) seasonal postlarval fish size and frequency data and B) seasonal Gonadal Somatic Index (GSI) for male or female fish (GCRL unpublished data).

slightly later, on average, in the South Atlantic and Gulf of Mexico, peaking April 1. Species-specific and region-specific, where applicable, timelines are included in Chapter 9.0.

NOTE: *If age data is being provided for a stock assessment, confirmation should be made with the assessment biologists as to exactly what data they require (e.g., just final age determinations, just annuli counts and margin codes, all of the above).*

8.5.1 Assigning Age Groups

Stock assessments utilize catch and population data grouped into age classes, usually recorded as integers, representing unique year classes of fish spawned in the same year. This grouping is needed to keep all fish spawned during the same year together as they are tracked through time when analyzing the population age structure. While each year's offspring are often considered a single cohort, there can be multiple cohorts within the same year class as well. A good example of this is the bimodal spawning in fish; two spawning peaks within one calendar year result in a spring cohort and late summer cohort. Therefore, 'age group' is used here rather than cohort. The term 'calendar age' is often used interchangeably with age group. Fish are assigned to the age-0 age group the year they are spawned and advance to the next

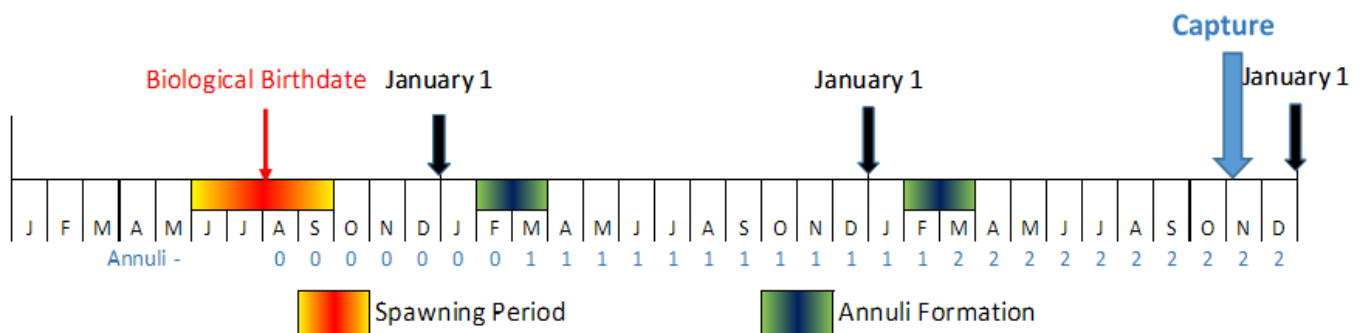


Figure 8.14 Age tracking timeline for a fish spawned in the Mid-Atlantic. The assumed birthdate is August 1, the peak (red) of the June through September spawning season, and annuli form February through March, peaking March 1 (blue). The timeline tracks the number of annuli each month as a fish ages over its birth year and two subsequent calendar years.

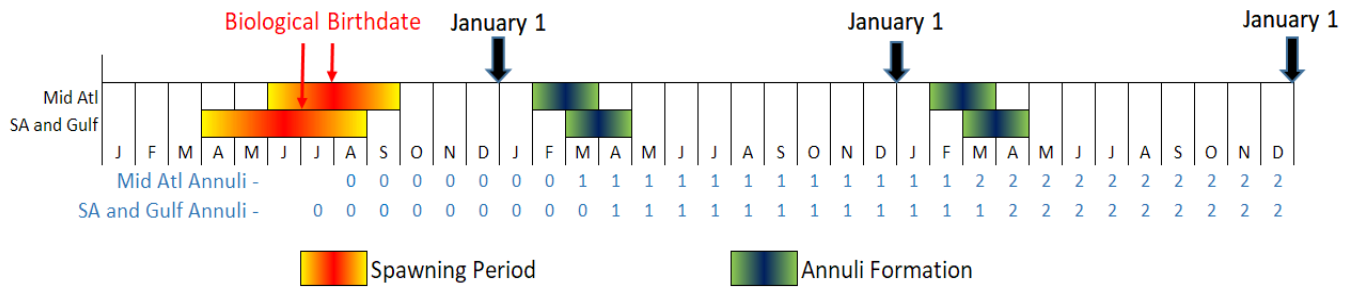


Figure 8.15 Age tracking timeline for fish spawned in the Mid-Atlantic and South Atlantic/Gulf of Mexico regions. The timeline tracks the number of annuli each month as a fish ages over its birth year and two subsequent calendar years.

age group on the first day of each subsequent year. For example, Mid-Atlantic fish tracked over calendar year are assigned to the age-0 age group when spawned, advance to the age-1 age group on January 1 of the second calendar year, advance to the age-2 age group on January 1 of the third calendar year, etc. (Figure 8.16). In any given year there will be unique year classes in each age group that make up the age structure of the population. For example, a population in 2010 will consist of the 2010 year class of fish born in 2010 as the age-0 age group, the 2009 year class of fish born in 2009 as the age-1 age group, the 2008 year class of fish born in 2008 as the age-2 age group, in other words,

$$\text{year-class} = \text{capture year} - \text{age-class}.$$

Using the timing of annulus formation, annuli counts can be converted to age group by combining the margin code and month of capture. If an ageing structure possesses a wide margin (i.e., margin code 3 or 4) and the fish is captured between the first day of the year and annulus formation, its age group is the number of annuli +1. This is based on the assumption that the fish has experienced considerable growth since the most recent fully formed annulus and, therefore, that annulus was formed during the previous year and annulus formation on the margin would have occurred during the year when captured if it had not been captured. Most other fish, regardless of month of capture, are assigned an age group equal to the number of annuli. If an ageing structure has no growth on the margin or a narrow margin (i.e.,

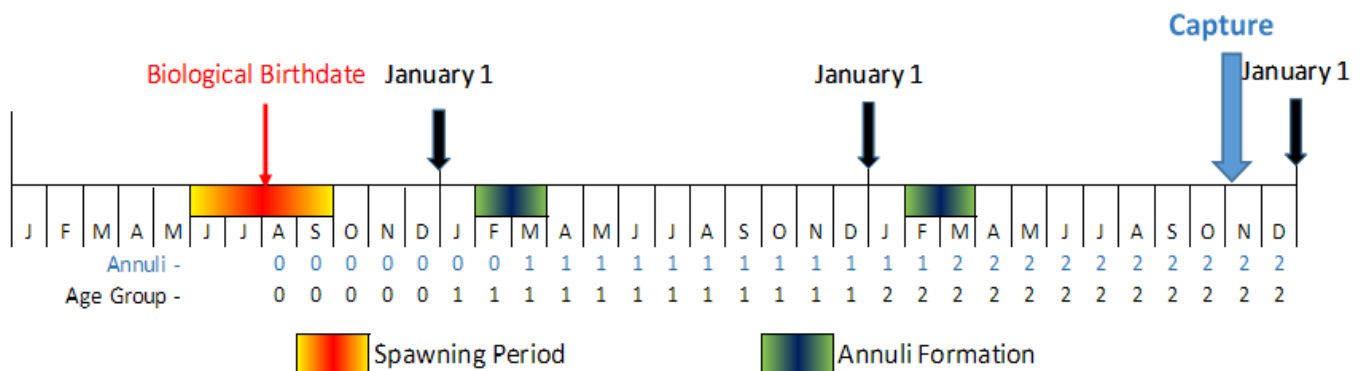


Figure 8.16 Age tracking timeline for fish spawned in the Mid-Atlantic. The assumed birthdate is August 1, the peak (red) of the June through September spawning season, and annuli form February through March, peaking March 1 (blue). The timeline tracks the number of annuli and age group assignment each month as a fish ages over its birth year and two subsequent calendar years.

margin code 1 or 2) and the fish is captured between the first day of the year and annulus formation, it is assumed that the annulus for the year of capture is the most recent fully formed annulus and this annulus formed earlier than typically seen. If a fish is captured after the annulus formation period, it is assumed that the most recent fully formed annulus was formed during the year of capture and any growth on the margin has occurred since. For the sciaenid otolith example, a capture date after the annulus formation period (November 8), a margin code of 4, and annuli count of 2, results in assignment to the age group 2 (Figure 8.17). It is assumed that the growth on the margin has occurred since the annulus formation period of the capture year. However, differences between species in growth rates and annulus deposition timing can introduce variability in the interpretation of margin codes and how they effect age group. When atypical margin codes are seen, careful notes should be taken so that end users can evaluate how to use those data.

8.5.2 Assigning Biological Age

Biological age is defined as the time elapsed between an assumed birthdate and date of capture and is expressed in some unit of time (e.g., days, months, years; Figure 8.18). A biological age estimate and additional biological data are used for relating life history characteristics to ages. For example, an age estimate and a known length of the fish provides a basis for describing growth. Other life history characteristics often related to age include weight, migration patterns, sex transition for hermaphroditic species, maturity, and fecundity. Having age determined with the greatest resolution would, in most cases, yield the most accurate and precise estimates of relationships between age and life history characteristics, and therefore, ages on a finer time scale than year (i.e., integer ages) are used. Because annuli formation and birthdate may not coincide, the number of annuli observed on a structure is not necessarily equal to

| Fish ID | Read Date | Reader | # Annuli | Margin Code | Readability Code | Comments | Capture Date | Age Group | Biological Age |
|---------|------------|-----------|----------|-------------|------------------|---------------------------|--------------|-----------|----------------|
| MS00001 | 12/12/2016 | Joe Smith | 2 | 4 | E | Use for example in manual | 11/8/2016 | 2 | |
| MS00002 | | | | | | | | | |
| MS00003 | | | | | | | | | |



Figure 8.17 Example data sheet with age group estimate for the sciaenid otolith section with two annuli and a margin code of 4.

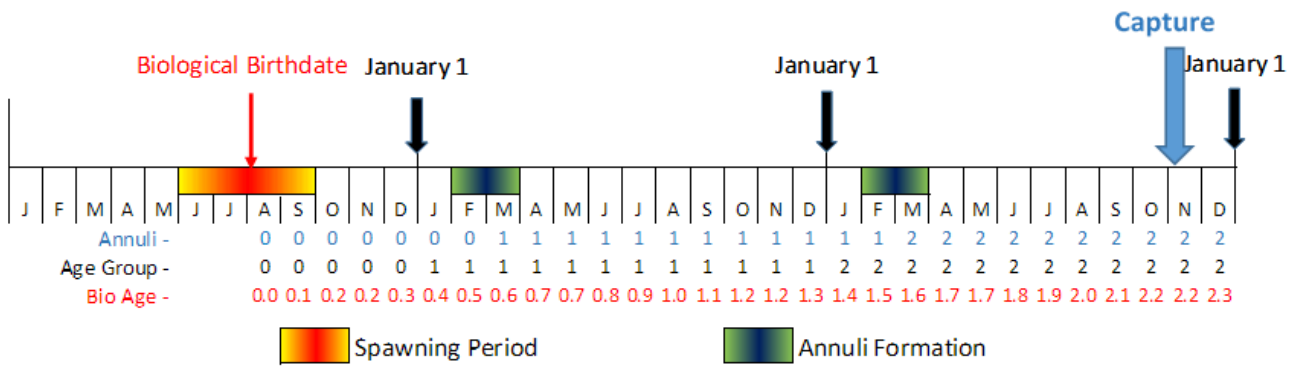


Figure 8.18 Age tracking timeline for fish spawned in the Mid-Atlantic. The assumed birthdate is August 1, the peak (red) of the June through September spawning season, and annuli form February through March, peaking March 1 (blue). The timeline tracks the number of annuli, age group assignment, and biological age each month as a fish ages over its birth year and two subsequent calendar years.

the fish’s biological age. In reality, the biological age of a fish and the number of annuli coincide at only one point in time during each year (August 1 in Figure 8.18). During all other times, the biological age of the fish is the number of annuli, plus or minus the proportion of a year elapsed since its closest birthday. The biological age, also known as a fractional age, represents the combination of month of capture, number of annuli, and the accepted birthdate estimate.

Once age group has been determined, biological age can be derived with the following equation:

$$biological\ age = age\ group + \frac{Month\ of\ Capture - Month\ of\ Birth}{12}$$

The sciaenid otolith example shows a fish that finished forming its second annulus in March, has an August birthday, and is captured in November. The biological age is calculated as 2.25 and recorded in the data sheet (Figure 8.18).

$$2.25 = 2 + \frac{11 - 8}{12}$$

Depending on the intended end use of age data, both age group and biological age may not be necessary to calculate. The main purpose of describing age group and biological age here is to highlight the difference between the two ‘ages’ illustrated in Figure 8.19. Because biological birthdate and the beginning of the calendar year often do not match, fish are shifted into age groups at the beginning of a year that are greater than their biological age.

| Fish ID | Read Date | Reader | # Annuli | Margin Code | Readability Code | Comments | Capture Date | Age Group | Biological Age |
|---------|------------|-----------|----------|-------------|------------------|---------------------------|--------------|-----------|----------------|
| MS00001 | 12/12/2016 | Joe Smith | 2 | 4 | E | Use for example in manual | 11/8/2016 | 2 | 2.25 |
| MS00002 | | | | | | | | | |
| MS00003 | | | | | | | | | |

Figure 8.19 Example data sheet with biological age estimate for the sciaenid otolith section.

8.5.3 “Smudge” Near the Core

One of the more common situations with fish that are spawned over the winter and may have an annulus formed in the spring is the potential appearance of a dark zone near the core or what some people refer to as a ‘smudge’ (Figure 8.20). When spawning occurs just prior to typical annulus formation, juvenile fish only a few months old may develop a mark which is an annual mark but may or may not be in every fish from the cohort. Some later spawned individuals may be too small to develop a mark that is distinguishable from the core. A few examples are Red Drum, Atlantic Croaker, Southern Flounder, and Striped Mullet (specifics for each are covered in Chapter 9.0). Due to variation in presence of a smudge, the problem is whether to count this mark or not, understanding that counting it means the fish may not be close to a biological age of 1 but not counting will potentially make the fish older than a biological age of 1 when the first annulus is counted. Additionally, if the annuli count is used to determine year class, counting the mark would return the fish to the year it was spawned, but only if it forms a smudge. For example, if a fish was born in November 2012 and caught in November 2013 with a smudge, subtracting one from 2013 would give you the birth year or year class of 2012. If the smudge is not counted, the birth year or year class would be advanced to 2013. Red Drum are actually 13-15 months of age when the annulus forms. If the species being aged does have a smudge, it is imperative to communicate with other readers and the user of the age data to develop a protocol addressing the smudge.

8.6 Ageing Error

Final age estimates determined using the process described in this section are just that, estimates. Therefore, ageing error will occur. Ageing error is characterized by two components: bias due to inaccurate age determination and imprecision due to variability of multiple age determinations. A set of age estimates may be accurate and precise, biased and precise, accurate and imprecise, or biased and imprecise (Figure 8.21). Because age estimates can be biased while being precise or vice versa, bias artificially inflates precision (Campana 2001), and imprecision can confound detection of bias (McBride 2015), it is necessary to evaluate both of these ageing error components. Causes of ageing error include incorrect interpretation of marks and margins on the structure and how they relate to age, preparation of structures for ageing, quality of the structure (i.e., environmental effects, oddities, etc.), and reader inexperience. Ageing error can propagate error into population dynamics model estimates, potentially resulting in mismanagement of resources. For example, ageing error will 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 (Liao et al. 2013), or when trying to compare

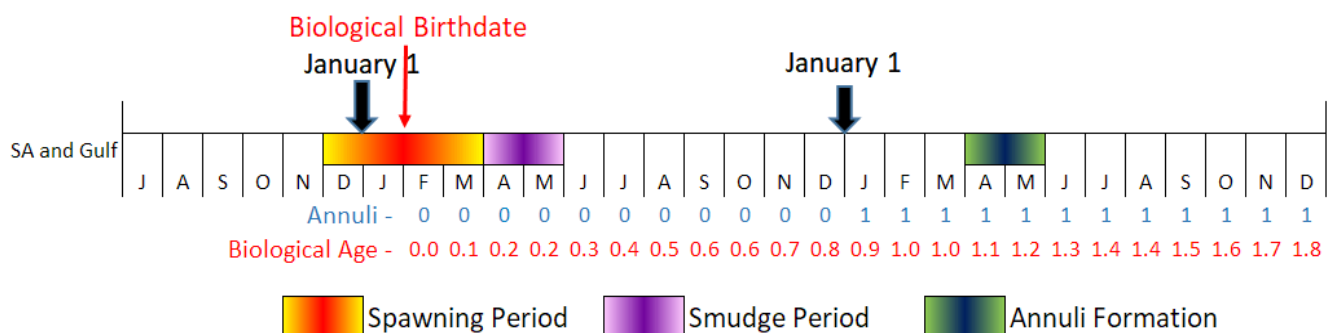


Figure 8.20 Timeline of a species that has a ‘smudge’ (purple) formed near the core. The first annulus which is counted (green-blue) actually occurs when the fish is between 1.3 and 1.4 years of age. Because of the extended spawning period and regional variation, not all the fish in the cohort (i.e., from one complete spawning season, yellow-orange) will form a smudge, therefore, counting will move fish into multiple year classes.

year class strength with environmental indices (Beamish and McFarlane 1995).

8.6.1 Accuracy

The accuracy of an age determination method may be known (validation, Chapter 2.0, Section 2.3), but the accuracy of a particular age estimate following an age validation study is seldom known (Beamish and McFarlane 1995). Validation addresses process error and is the first step to ensure that age estimates are not biased from the true age, but, in practice, there can still be differences in interpretation of annuli (i.e., observation error) over a portion or the full age range commonly aged leading to bias.

If there are multiple age estimates from the same sample that differ, at least one estimate is biased from the true age and it is possible that all estimates are biased from the true age. For example, one reader using otoliths (Figure 8.22B; Reader 2) may misinterpret and not count the first annulus leading to age estimates consistently, negatively biased one year from the true ages. Another reader using scales (Figure 8.22C; Reader 1) may have difficulty interpreting annuli across the age range leading to an inconsistent bias from the true age. These are examples of systematic bias over a range of ages that can be identified with tests of symmetry (Bowker 1948, Evans and Hoenig 1998). In some cases, there may only be one or a few ages that are consistently estimated biased from the true age. Graphical methods such as age-bias plots or Bland-Altman plots have proven effective means for detecting bias, particularly for bias over a subset of the age range that may not be detected by tests of symmetry (Campana 2001, McBride 2015). If there is a set of samples with known ages, these methods can be used to identify bias between true ages and age estimates by readers. In the more likely case when known ages are not available, one set of estimates is often assumed unbiased from the true ages (Figure 8.22A and B, Reader 1) and the methods can be used to test for bias in estimates from other readers. If there is bias detected, readers will need to correct the assumed biased readers' age determinations (either with resolved interpretation differences and training prior to high volume ageing or through calibrations in ageing error matrices for past age estimates).

8.6.2 Precision

Commonly used measures of precision are Average Percent Error (APE, Beamish and Fournier 1981) and percent Coefficient of Variation (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 annuli counts or final assigned ages. Final assigned ages tend to yield lower precision because it is not uncommon for readers to have subtle differences in their interpretation of the margin. Increasingly, measures of precision are being incorporated directly into stock assessment models in order to statistically account for ageing error (Richards et al. 1992, Beamish and McFarlane 1995, Crone and Sampson 1998).

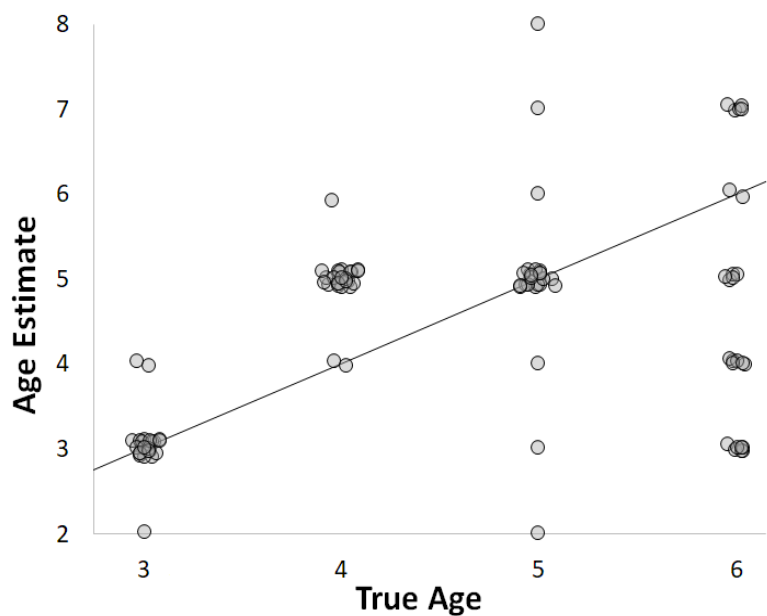


Figure 8.21 Examples of age estimates (circles) that are relatively accurate and precise (true age 3), inaccurate and precise (true age 4), accurate and imprecise (true age 5), and inaccurate and imprecise (true age 6).

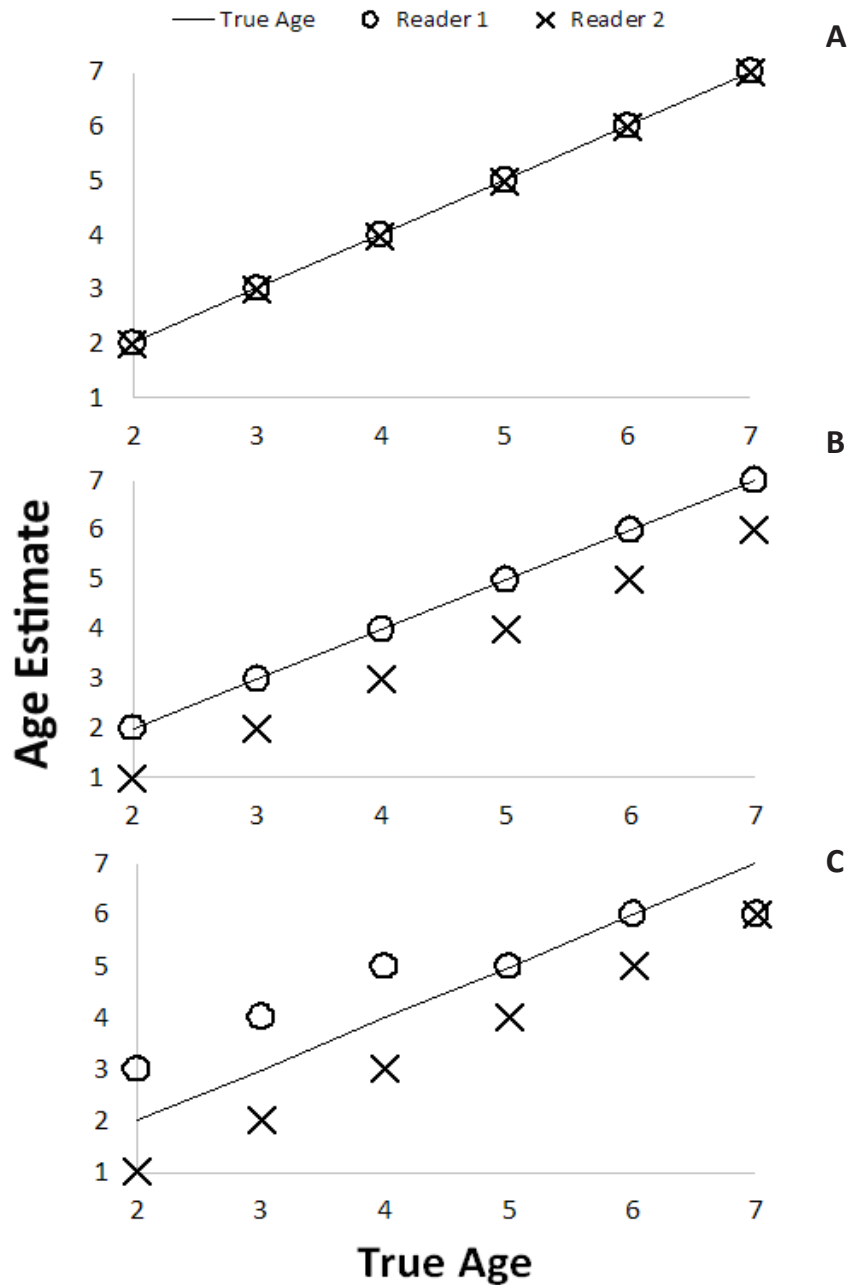


Figure 8.22 Age estimates from two readers that are systematically biased from the true age for A) neither reader, B) one reader (Reader 2), C) and both readers.

As with bias due to observation error, precision is commonly improved by resolving interpretation differences among readers and gaining experience within readers.

However, some level of imprecision is always inherent and it is good practice to establish benchmarks for acceptable imprecision of age estimates depending on the species.

8.7 Quality Control and Quality Assurance

Assuming for a given species that validation of annuli periodicity has been accomplished, initial age and growth characterization is complete, and there is consensus on interpretation of ageing structures as

evaluated with initial bias and precision testing, ageing programs can move into the high volume phase whereby large numbers of samples are aged at regular intervals.

Over time and when multiple labs or readers provide age estimates to a common end user, additional opportunities for error occur. There can be gradual 'drifts' in interpretation of age structures over time, even within readers, that result in gradual changes in ageing error (Campana 2001). Quality control monitoring through paired reading of age samples becomes a very important component of high volume ageing (Boehlert and Yoklavich 1984, Morison et al. 1998).

Ideally, for each sample read within a lab, a second age estimate should be made by another, independent reader. This is commonly referred to as 'verification.' Consensus is achieved by revisiting estimate disparities between readers or by a third party. If a consensus cannot be achieved, it should be noted in the comments column of the data sheet and the source of the final estimate (e.g., most senior reader, mode estimate across readers) should be identified. To evaluate ageing error of recent estimates, readers should apply this method to a subset of recently aged samples. If available resources preclude multiple reads of every sample, an individual reader should reread a subset of all samples. Methods for evaluating bias and precision should be applied to the original and new estimates for the subsample to estimate ageing error for the age data set. Examinations of bias and precision should be recorded and updated annually (Kimura and Lyons 1991).

Similar quality control monitoring should be implemented among all labs providing age estimates for a common use by regularly exchanging sets of samples to be read by all labs. Methods for evaluating bias and precision should be applied to the estimates from different labs to provide estimates of ageing error and determine if current levels of ageing error meet predetermined benchmarks. If levels of ageing error do not meet predetermined benchmarks, exchange participants should revisit estimate disparities to resolve interpretation differences.

Regular evaluation of ageing error and communicating ageing error to the end users of age estimates should increase the acceptance of the science by managers and industry.

8.7.1 Reference Collection

A reference collection is a set of prepared ageing structures for which known or consensus-derived ages are recorded. The idea is to incorporate prepared ageing structures (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 development. If year-specific differences are suspected, including samples from several years should be considered. See Chapters 2.0 - 6.0 for best practices on long term storage of samples to be used in a reference collection.

The use of reference collections serves many of the same purposes as annual quality control and has potential advantages. The dominant uses 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 annual quality control approaches 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.

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 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.

High volume 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 subset should be aged until a sufficient level of precision is achieved and reader bias is minimized (Morison et al. 1998).

9.0 Species-Specific Processing Details

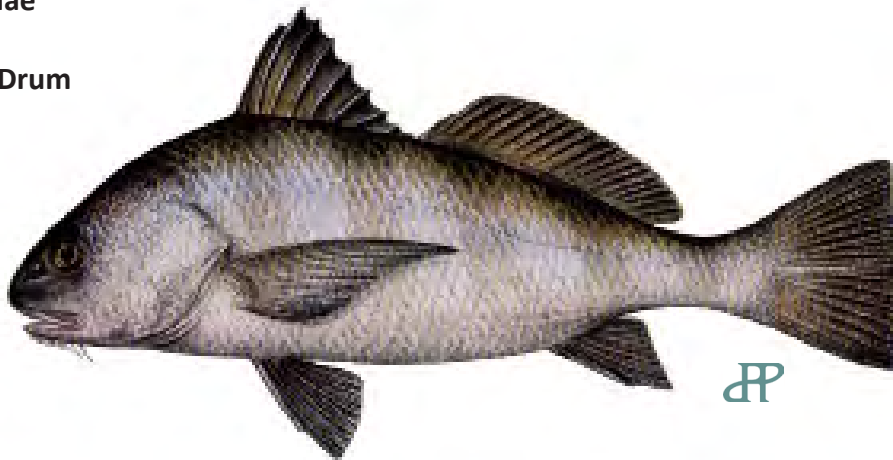
The following accounts provide broad technique details which can be applied to all the species included in the account. In some cases, the account is only for a single species (Cobia, Tripletail, etc.) and others are groups of similar species or that fall into a family (Drums – large, Drums – small, Groupers, Snappers, etc). Those details are intended to be applied across multiple ‘like’ species. Where there are specific differences in hard part removal or processing, they will be noted. Finally, at the end of each account, a timeline or series of species-specific timelines are included that identify the periods of spawning and annulus deposition for each species. The timelines will also include ranges for those periods if they vary by region (Gulf vs Atlantic, South Atlantic vs Mid Atlantic, etc.).

There are a number of species that, while managed by the various state agencies, have little biological data collection associated with them. This may be due to a lack of complete understanding of the ageing process, lack of validation of growth and marks on hard parts, and a lack of funding for species deemed low priority by legislators and funding agencies or a combination. A complete list of those fish for which some data are collected but not aged at this time is included in Chapter 12.0, Section 12.6 by individual agency or institution. Also included in Chapter 12.0, Section 12.5 is the complete list of all the species that each agency or institution is considered to have expertise and may be contacted for further information on any species not included in the manual.

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9.1 Sciaenidae

9.1.1 Large Drum



Black Drum *Pogonias cromis*



Red Drum *Sciaenops ocellatus*

Highlights

- Sagittal otoliths large and relatively easy to locate and extract.
- Multiple otolith sectioning techniques successful.
- Annuli easily discernible.
- First annulus forms at approximately a biological age of 1 in Black Drum.
- First annulus forms at approximately a biological age of 1.5 in Red Drum and there is typically a 'smudge' formed near the otolith core at approximately a biological age of 0.5.
- Maximum observed biological age for Black Drum is 67 (VMRC) for the Atlantic stock and 55+ for the Gulf stock.
- Maximum observed biological age for Red Drum is 62 for the northern Atlantic stock (NC-NJ), 41 for the southern Atlantic stock (FL-SC), and 40 for the Gulf stock.
- Otoliths are the preferred ageing structures, though scales appear reliable age structures for both Black Drum and Red Drum up to age 4.

Otolith Descriptions

Black Drum have a robust otolith that is semi-circular in juvenile fish and becomes somewhat rectangular in mature fish (Figure 9.1.1.1). The otolith has 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 9.1.1.2A.

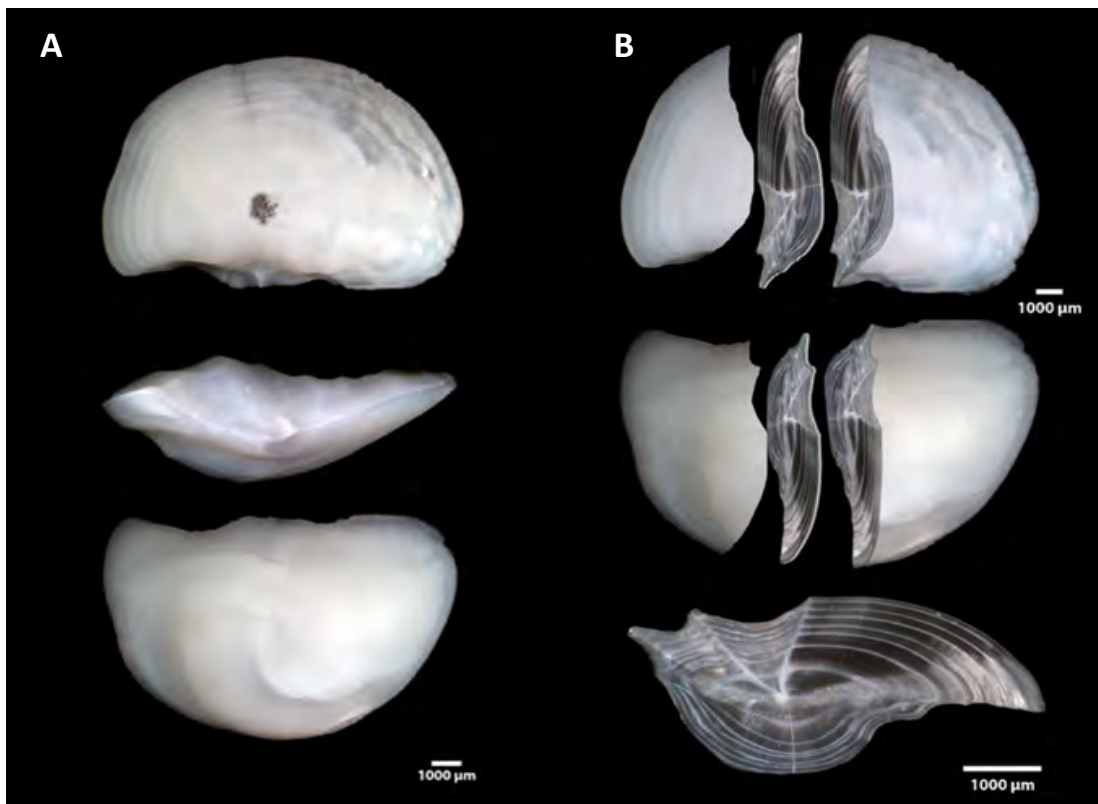


Figure 9.1.1.1 A) Whole otoliths of Black Drum proximal view with core marked (top), dorsal view (middle), and distal view (bottom) and B) Black Drum otolith sectioned with thin-section located and rotated showing location of cut through the core.

Red Drum have large, stout sagittae that are thick enough to be opaque. 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. 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 9.1.1.2B.

Otolith Extraction

Otoliths in these large species of drum are strong enough to withstand expected impacts from otolith extraction devices without breaking. Several different techniques are effective; some may be easier than others on different sized fish (Chapter 3.0, Section 3.2.1). The ventral surface of the otic capsule of Black and Red Drum is somewhat convex, making it easy to identify through the gill cavity near the posterior base of the skull above the gills (bottom method). 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° angle to the back of the ocular socket to open the cranial cavity and expose the sagittae (top method). In larger fish, 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.

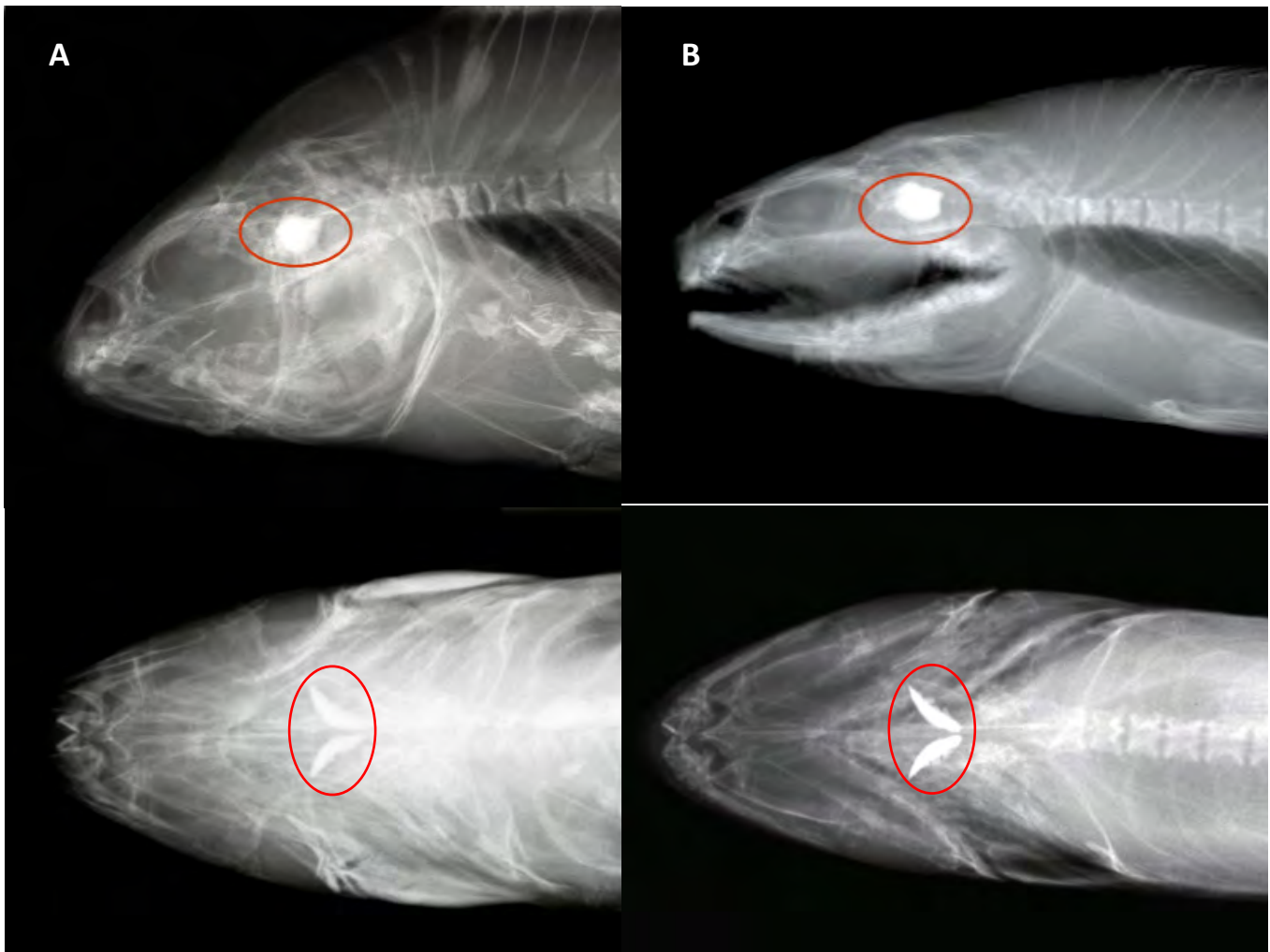


Figure 9.1.1.2 Location of sagittae in the neurocranium of A) Black Drum and B) Red Drum in lateral (top) and dorsal-ventral (bottom) views.

Otolith Processing

Due to the robust nature of large drum otoliths, nearly all techniques outlined in Chapter 3.0, Section 3.2.5 are acceptable for these species. Generally, Black and Red Drum sections are processed at approximately 0.5 mm. The technique chosen will likely reflect the available equipment; however, the following techniques have been used successfully for these species: high speed wafering saw (embedded or whole), low speed wafering saw (embedded or whole) and thin sectioning machine (Hilquist).

Age Determination

Black Drum

It is relatively easy to age Black Drum since opaque zones are normally very distinct even in older fish (Figure 9.1.1.3). Black Drum spawn in the spring (Thomas 1971, Wang and Kernehan 1979, Bobko 1991, Wells 1994, Murphy and Taylor 1989, Fitzhugh and Beckman 1987) at approximately the time of opaque zone formation; therefore, the first distinct opaque zone is deposited when the fish is about one year old (Figure 9.1.1.4).

Murphy and Taylor (1989) indirectly validated the timing of annulus formation using MIA and Murphy et al. (1998) directly validated formation using tag-recapture and determined that annulus formation

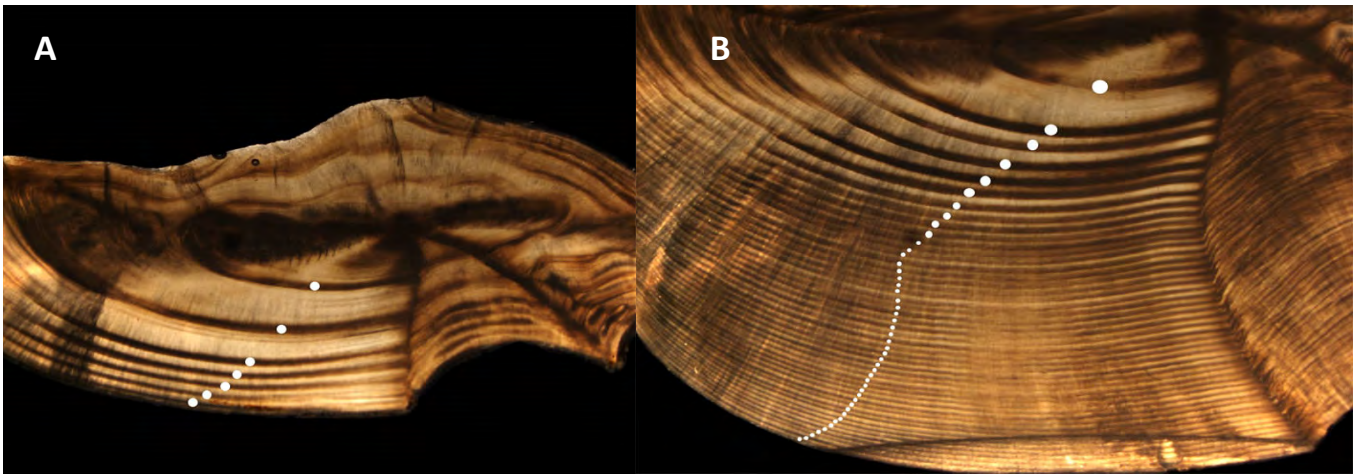


Figure 9.1.1.3 A). Sagittae crosssection from age-7 Black Drum and B). an age-47 Black Drum. Annuli are marked with white dots.

primarily occurs from April to June in Florida waters. Beckman (1989) used MIA to indirectly validate annulus formation from March through May in Louisiana waters. The Old Dominion University Center for Quantitative Fisheries Ecology uses May through June for annulus formation in Virginia (CQFE 2015). The accepted birthdate for this species in the Gulf and South Atlantic is March 1 (Figure 9.1.1.4). The accepted birthdate for this species in the Mid-Atlantic is May 1 (Figure 9.1.1.4).

Red Drum

It is relatively easy to age Red Drum since opaque zones are normally very distinct. Red Drum otolith sections call for special attention in the process of identifying the first annulus. Because Red Drum spawn in the late fall (Murphy and Taylor 1990, Ross et al. 1995, Lowerre-Barbieri et al. 2008), an opaque zone, referred to as a smudge, forms near or in the core region (Figure 9.1.1.5); this smudge is not counted as the first annulus (ASMFC 2008). The accepted first annulus is considered to be the opaque zone that forms during the second winter the fish encounters, when the biological age is 1.2-1.7 years.

Spawning and annulus formation in Red Drum occurs during the same general timeframe along the Gulf and Atlantic Coasts (Music and Pafford 1984, Beckman et al. 1988, Murphy and Taylor 1990, Pafford et al. 1990, Ross et al. 1995), with some minor regional differences (Figure 9.1.1.6). Beckman et al. (1988) used MIA to indirectly validate annulus formation from November through May in northern Gulf of Mexico

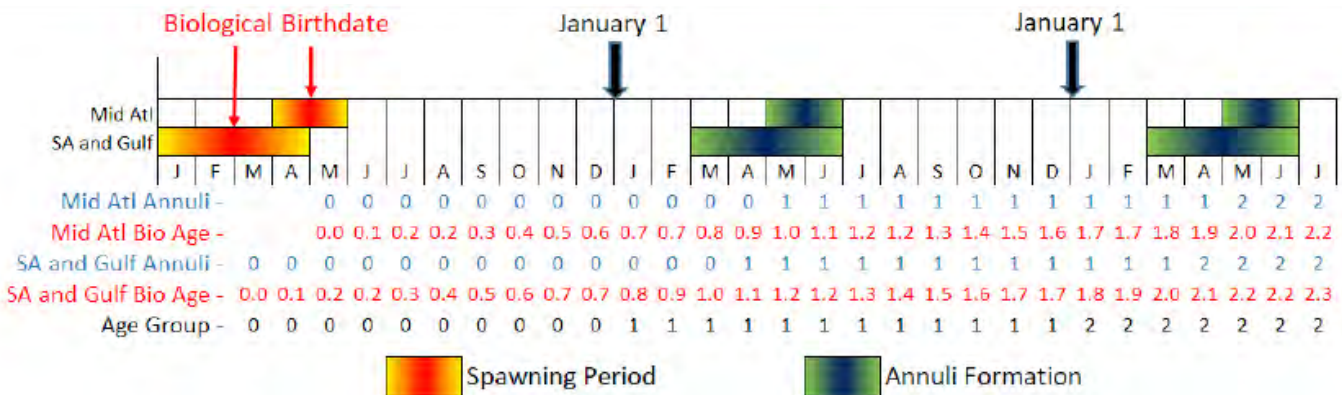


Figure 9.1.1.4 Timeline showing spawning period and annulus deposition ranges for Black Drum in the Mid-Atlantic and the South Atlantic/Gulf.

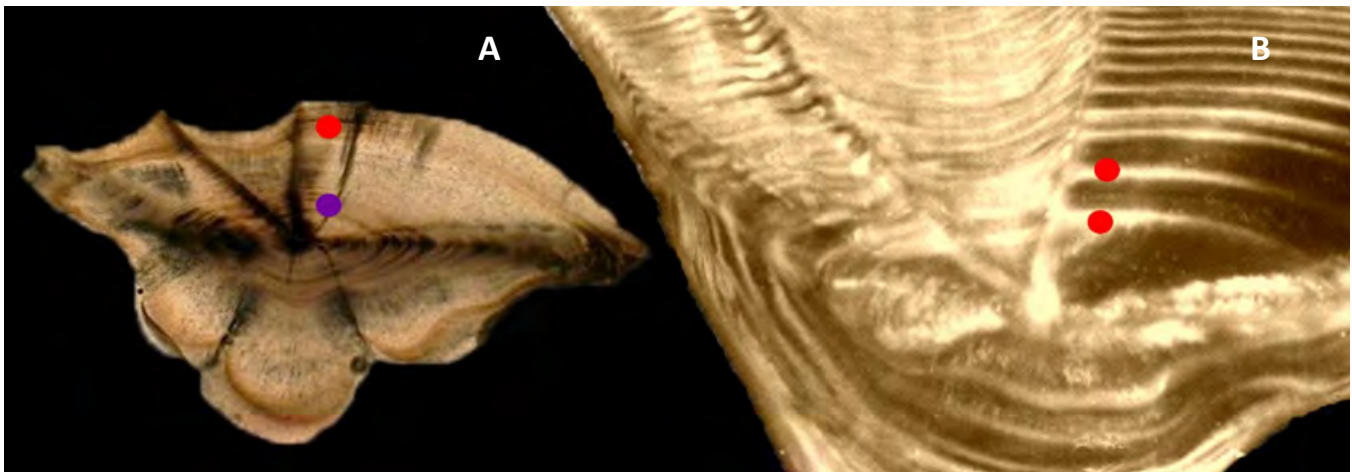


Figure 9.1.1.5 Sectioned otoliths from A). an age-1 Red Drum with smudge in core (purple dot) and first annulus (red dot) and B). an age-32 Red Drum with no smudge and first and second annuli marked with red dots.

waters, with a peak in February. Beckman (1989) validated annulus formation directly in Louisiana using OTC marking of fish. Murphy and Taylor (1990) also used MIA to indirectly validate annulus formation from December through March for fish along Florida’s Gulf Coast. Murphy and Taylor (1991) validated annulus formation using chemical marking and determined formation in Florida waters occurred during the winter. Ross et al. (1995) used MIA to determine that timing of annulus formation in North Carolina primarily occurs from March to May, depending on age. The accepted biological birthdate for Red Drum on the Atlantic Coast is October 1 (ASMFC 2008).

Other Ageing Methods

Black Drum

Black Drum scales have been validated for fish up to age-4 by Matlock et al. (1993) and Richards (1973) aged Black Drum scales up to age-10. Whole Black Drum otoliths have not been used successfully.

Red Drum

Scales have been demonstrated to be useful for ageing Red Drum up to age-4. Ages for older specimens were not reliable due to regeneration and/or reabsorption (Pearson 1929, Simmons and Breuer 1962, Wakeman and Ramsey 1985, Matlock et al. 1987, SCDNR unpublished data).

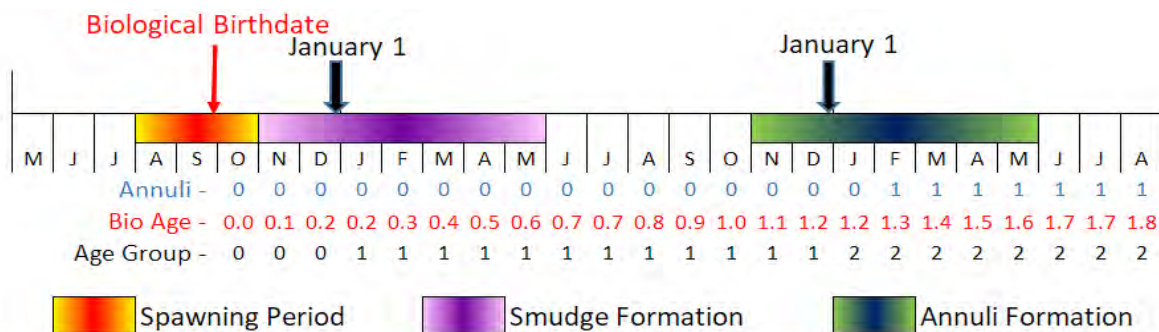


Figure 9.1.1.6. Timeline showing spawning period and annulus deposition ranges for Red Drum in the Atlantic and Gulf.

Bumgardner (1991) examined several structures from Red Drum, including dorsal and anal spines, that had been previously marked using OTC. In cross-section, spines did not provide annual marks as expected.

Research Needs

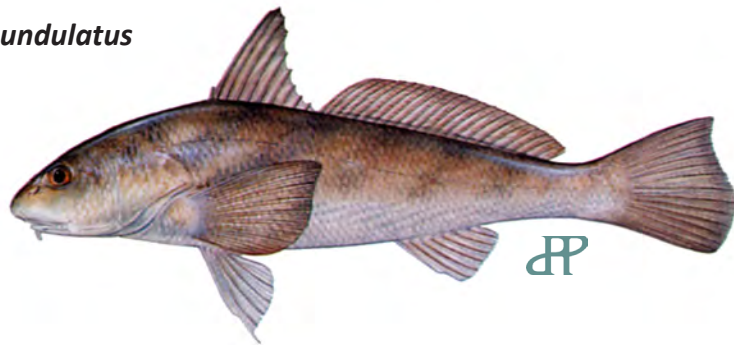
There is very little ageing information needed for Black or Red Drum in the Gulf of Mexico and the South Atlantic.

9.1 Sciaenidae

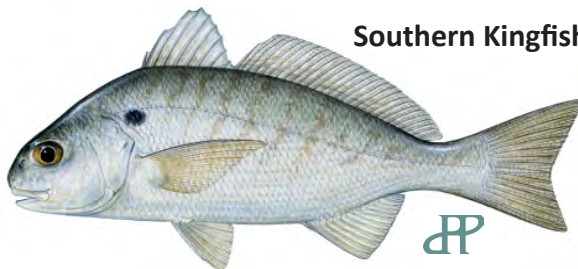
9.1.2 Small Drum



Atlantic Croaker *Micropogonias undulatus*



Southern Kingfish *Menticirrhus americanus*



Spot *Leiostomus xanthurus*

Highlights

- Sagittal otoliths are most commonly used ageing structure.
- Sagittal otoliths relatively easy to locate and extract.
- Multiple otolith sectioning techniques successful.
- Annuli easily discernible.
- Atlantic Croaker first annulus forms from December to May at approximately a biological age of 1.5 and there is typically a 'smudge' formed near the otolith core at approximately a biological age of 0.5.
- Atlantic Croaker generally have less than ten annuli. Maximum observed biological age for Atlantic Croaker is 17 for the Atlantic stock. In the Gulf, the max is eight (Barger 1985).
- Southern Kingfish form the first annulus from April to May at approximately a biological age of 1.
- Southern Kingfish generally have less than five annuli.
- Spot first annulus forms from May to June at approximately a biological age of 1.5.
- Spot generally have less than six annuli with the most common being age-1.

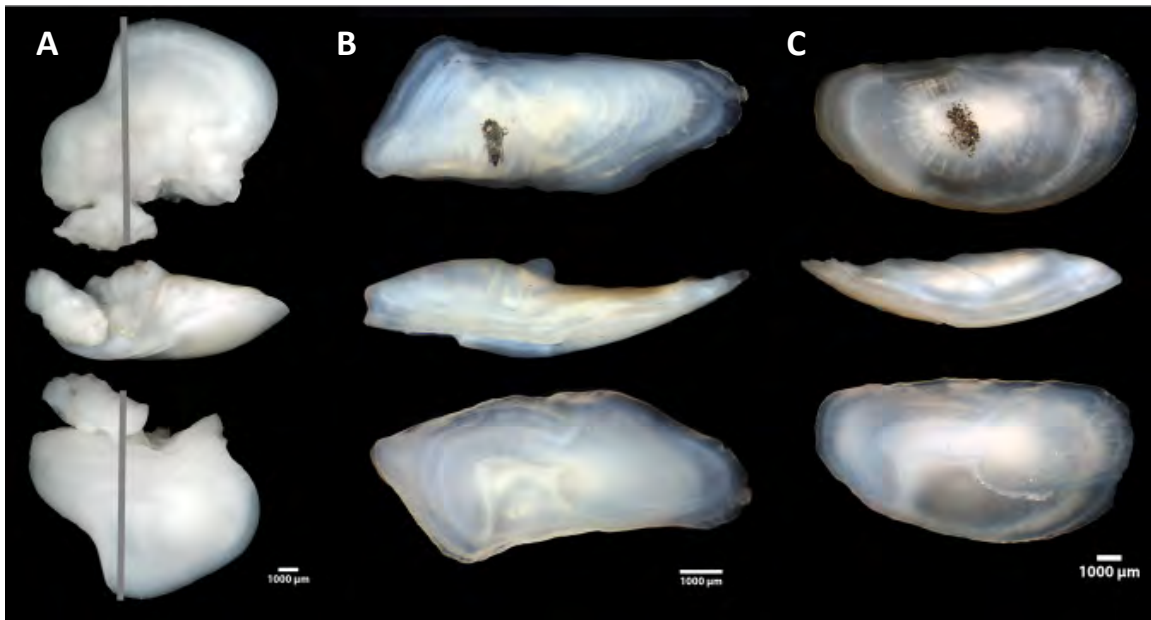


Figure 9.1.2.1 Whole otoliths in proximal view with core marked (top), dorsal view (middle), and distal view (bottom) of A) Atlantic Croaker, B) Southern Kingfish, and C) Spot.

Otolith Description

As in most drum species, 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 9.1.2.1). 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 anteroposterior axis, the core lies adjacent to the junction of the ostium and cauda regions of the sulcus acousticus.

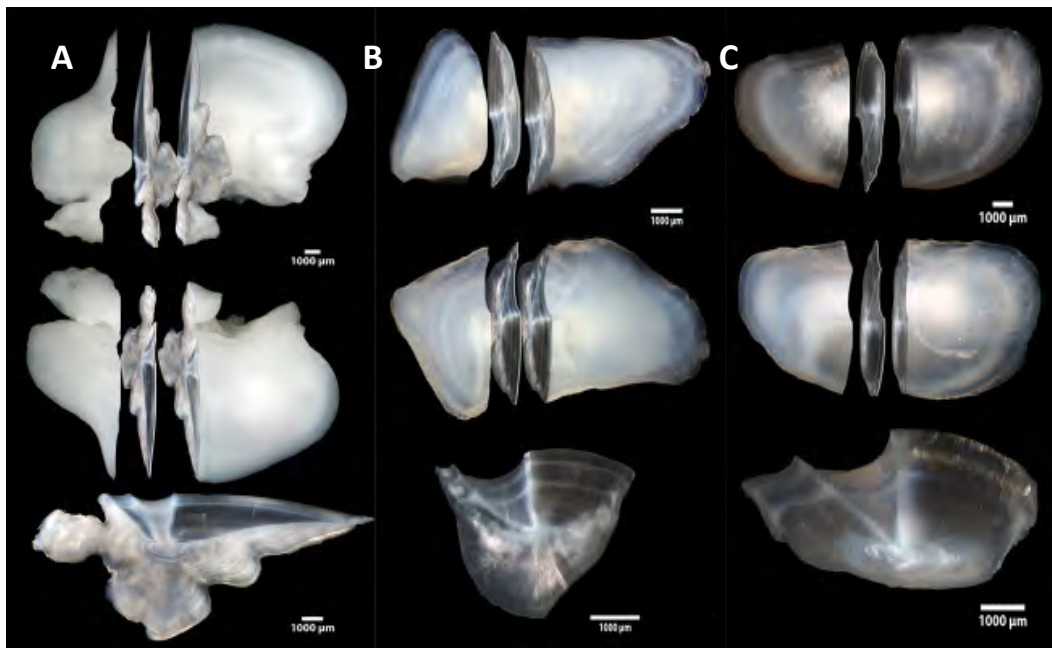


Figure 9.1.2.2 A) Atlantic Croaker, B) Southern Kingfish, and C) Spot otoliths sectioned with thin-section located and rotated showing location of cut through the core.

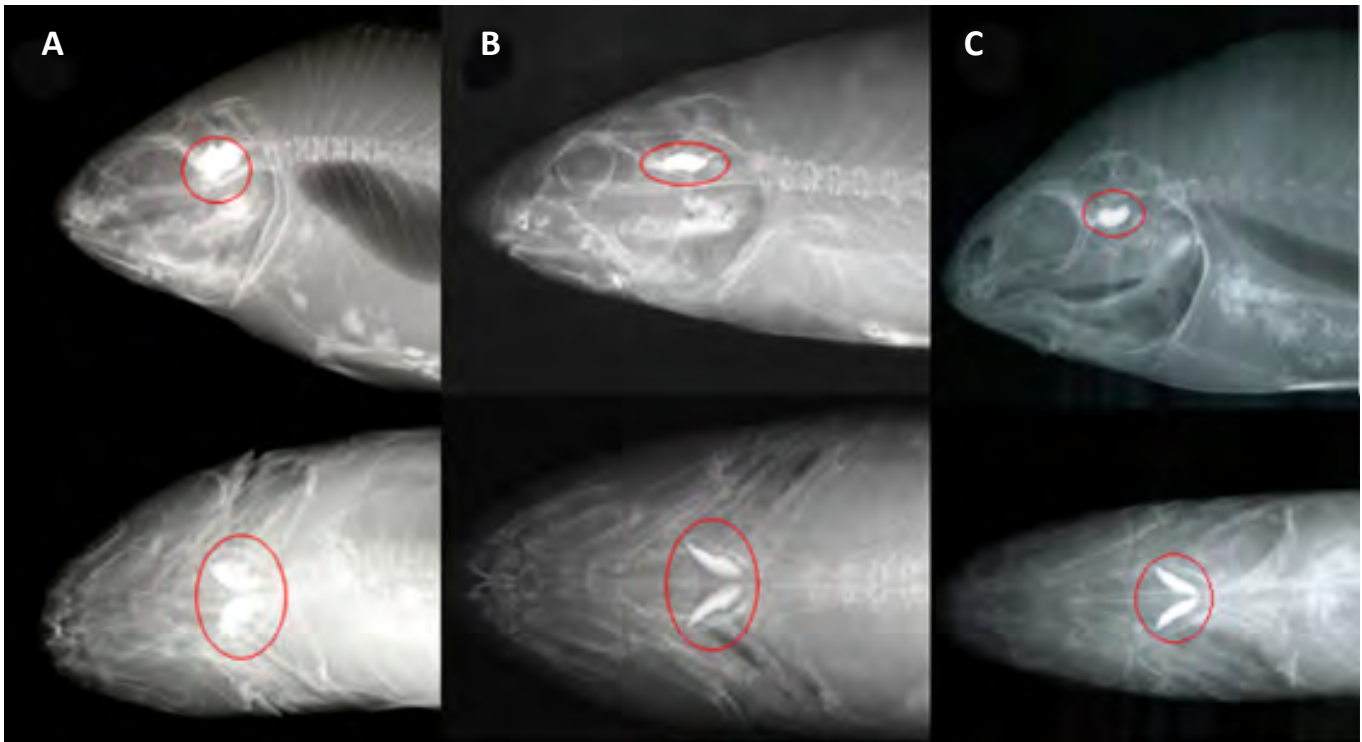


Figure 9.1.2.3 Location (red circle) of sagittae in the neurocranium of A) Atlantic Croaker, B) Southern Kingfish and C) Spot in lateral (top) and dorsal-ventral (bottom) views.

Southern Kingfish sagittae are more like those from *Cynoscion*, elliptical and narrow with an elongate sulcus acousticus (Figure 9.1.2.1). The ostium is ovoid and the cauda is long and bent with a short distal end having a tadpole shape. Spot otoliths are elliptical with an ovoid ostium and a “J” shaped cauda, giving it a tadpole shape (Figure 9.1.2.1).

Sectioning occurs at the core in each of these species (Figure 9.1.2.2). The location of otoliths in the neurocranium of each species is illustrated in Figure 9.1.2.3.

Otolith Extraction

Small drum otoliths can withstand expected impacts from otolith extraction devices without breaking therefore most of the extraction techniques laid out in Chapter 3.0, Section 3.2.1 will work. The otic capsule 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. In larger fish, otoliths can be removed using a cut made from the dorsal surface of the head to the otic capsule. Small drum 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.

Otolith Processing

Due to the robust nature of the otoliths in this species group, multiple techniques described in Chapter 3.0, Section 3.2.5 are acceptable and usually reflect available equipment. Generally, otolith sections are processed at approximately 0.5 mm. The following techniques have been used successfully throughout the Gulf and Atlantic: high speed wafering saw (embedded or whole), low speed wafering saw (embedded or whole) and thin sectioning machine (Hilquist).

Atlantic Croaker can usually be cut without embedding depending on the equipment that will be used. However, Southern Kingfish and Spot are typically embedded in epoxy due to the small size of the otoliths.

Age Determination

Atlantic Croaker

Transverse otolith sections of Atlantic Croaker show very clear, easily identified annuli that can be used for ageing. Typical sections have an opaque core surrounded by a blurred opaque band, composed of fine opaque and translucent zones (Figure 9.1.2.4). This band represents the “smudge”. Due to Atlantic Croaker’s protracted spawning season, the width of the smudge varies among individual fish. Late-spawned fish have a very narrow smudge that is almost continuous with the core whereas early-spawned fish have a wide, well-defined smudge clearly separated from the core. Because of this variation in width and proximity to the core, the smudge is sometimes difficult to identify. The smudge should not be counted as an annulus, but its presence or absence should be recorded (ASMFC 2008). This could potentially result in fish as old as 15 months in the 0 age group (Figure 9.1.2.5), but has been agreed to as the best solution to track cohorts of Atlantic Croaker for stock assessment and management purposes.

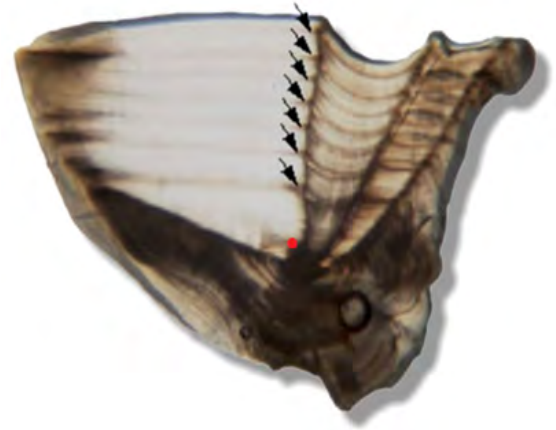


Figure 9.1.2.4 Atlantic Croaker otolith section with 7 annuli marked with black arrows and a red dot near the core marking the “smudge”.

Spawning typically occurs from July through December (Barbieri et al. 1994) with a peak in October (Holt et al. 1985, Barbieri et.al. 1994); therefore the accepted birthdate for this species is October 1 for the north and mid-Atlantic. Spawning occurs from September to April for the South Atlantic and Gulf of Mexico (Barbieri et al. 1994). Annulus formation has been validated for the South Atlantic using MIA and occurs from April through May for both the north and mid-Atlantic and the South Atlantic (Barbieri et al. 1994) and Gulf of Mexico (Hare and Able 2007).

Southern Kingfish

Spawning has been reported anytime from March to October (McDowell and Robillard 2013, Miller and

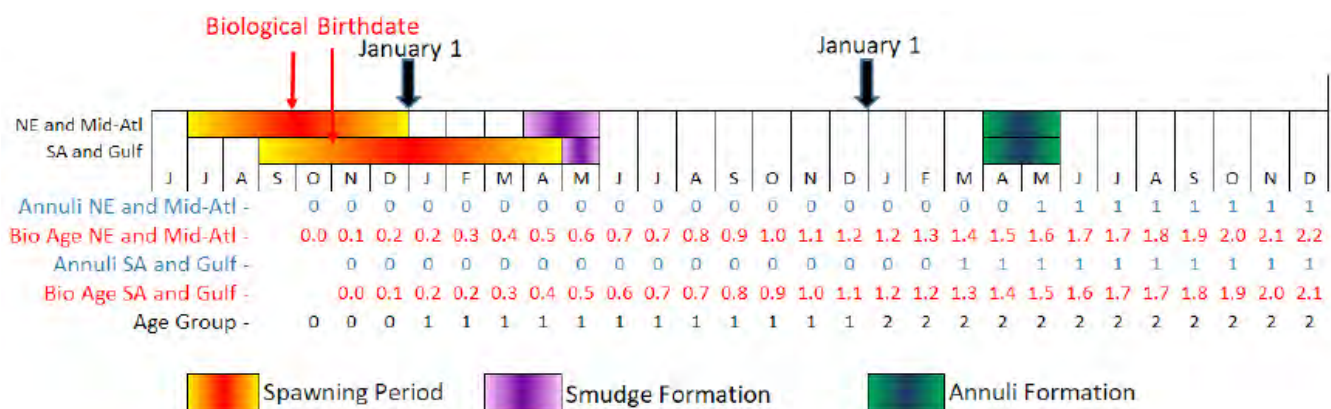


Figure 9.1.2.5 Spawning periodicity and age assignment timeline for Atlantic Croaker from New England to the Gulf of Mexico.

Jorgensen 1969, Smith and Wenner 1985), with a peak in April along the Atlantic Coast (Figure 9.1.2.6). Along the Texas Coast, Southern Kingfish spawning occurred in the early spring (January - April) and fall (August – November) according to Harding and Chittenden (1987). Clardy et al. (2014) confirmed a six month spawning period in the northern Gulf from April-September.

McDowell and Robillard (2013) determined that a single annulus was formed each year between April and May using MIA, suggesting that Southern Kingfish are truly age-1 when they form their first annulus and the distance from the core should be relatively wide. Any occurrence of a ‘smudge’ near the focus should be ignored when assigning ages in Southern Kingfish (Figure 9.1.2.7). In the northern Gulf, Clardy et al. (2014) confirmed that annulus formation in Southern Kingfish coincides with the peak of the spawning season during April–May.

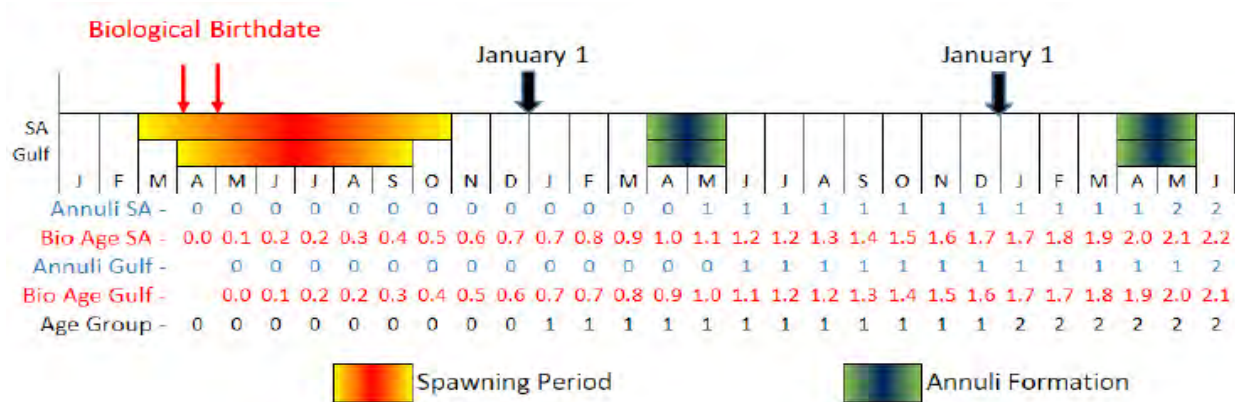


Figure 9.1.2.6 Spawning periodicity and age assignment timeline for Southern Kingfish from the South Atlantic to the Gulf of Mexico.

Spot

There is very little published information on ageing Spot. Spawning occurs from November to February off the continental shelf of the Atlantic Coast (Lewis and Judy 1983, Flores-Coto and Warlen 1993), similar to the October through March period in Georgia (Music 1974) (Figure 9.1.2.8). Peak spawning activity occurs from December to January in North Carolina (Warlen and Chester 1985) and November/December in Georgia (Music 1974). In the Gulf, Spot spawn from October to March (Parker 1971). Piner and Jones (2004) indirectly validated Spot annulus formation using MIA and found it occurs in the Chesapeake from April to July (Figure 9.1.2.8).

Other Ageing Methods

Whole otoliths have not been used successfully for Atlantic Croaker, Southern Kingfish, or Spot. Barger and Johnson (1980) examined Atlantic Croaker vertebrae, scales, and otoliths, but found greatest agreement in otoliths. Both scales and vertebrae provided ‘marks’ but otoliths were the most reliable. Although most of the historic work on Southern Kingfish is based on scales (Smith and Wenner 1985) which were read to age-6, it is generally agreed that scales tend to be less precise than otoliths (Campana and Nielson 1985). Piner and Jones (2004) compared Spot scales, pectoral fin rays, dorsal spines, and otoliths and found otolith

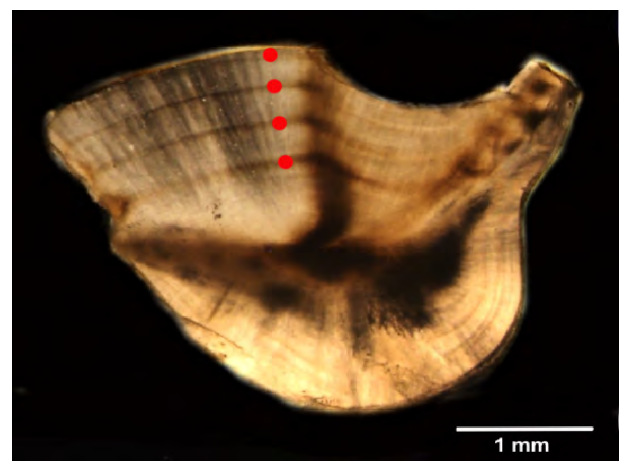


Figure 9.1.2.7 Southern Kingfish otolith section with 4 annuli marked with red dots.

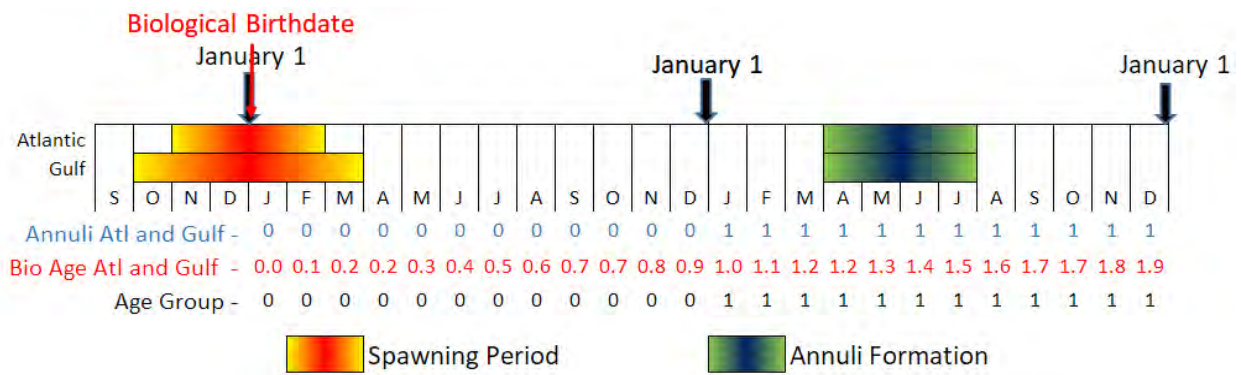


Figure 9.1.2.8 Spawning periodicity and age assignment timeline for Spot across the Atlantic Coast to the Gulf of Mexico.

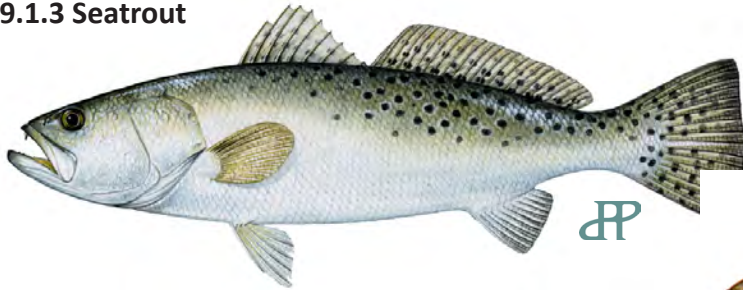
ages were more precise both within and between readers. Likewise, Barger and Johnson (1980) found the greatest agreement in otoliths over scales.

Research Needs

There is very little information available for Southern Kingfish and Spot in the Gulf of Mexico. Most of the validation work originates from the South Atlantic and the Chesapeake; therefore, additional validation needs to be completed in the other regions.

9.1 Sciaenidae

9.1.3 Seatrout



Spotted Seatrout *Cynoscion nebulosus*



Sand Seatrout *Cynoscion arenarius*



Silver Seatrout *Cynoscion nothus*



Weakfish *Cynoscion regalis*

Highlights

- *Cynoscion* otoliths are relatively large and easy to locate and extract.
- Multiple sectioning techniques successful.
- Rings easily discernible.
- Distance from the core to the first opaque ring is variable.
- Spotted Seatrout first ring formation occurs before year 1 and can live up to 10 years (VMRC unpublished data) but are generally less than 5 years old.
- Sand Seatrout form the first ring around 1 year of age with a maximum age of 5 years old (Nemeth et al. 2006).
- Silver Seatrout form their first ring at around 1 year of age with a maximum age of 1.5 years old (DeVries and Chittenden 1982).
- Weakfish form a distinct ring approximately 1 year of age with a maximum age of 17 but are most commonly 6 years old or younger (Lowerre-Barbieri et al 1995).

Otolith Description

Members of the genus *Cynoscion* have relatively large, elliptical, narrow sagittae that are opaque at most sizes (Figure 9.1.3.1). The dorsal margin is smooth and convex, whereas the ventral margin is slightly concave and crenelated (Chao 2002). The posterior portion of the sagittae is wider laterally and generally thicker than the anterior portion.

The sulcus acousticus is elongate with an ovoid ostium and has a long, bent cauda with a short distal end giving it a tadpole shape. The marginal groove is distinct, and the rostrum and anterostrum are not

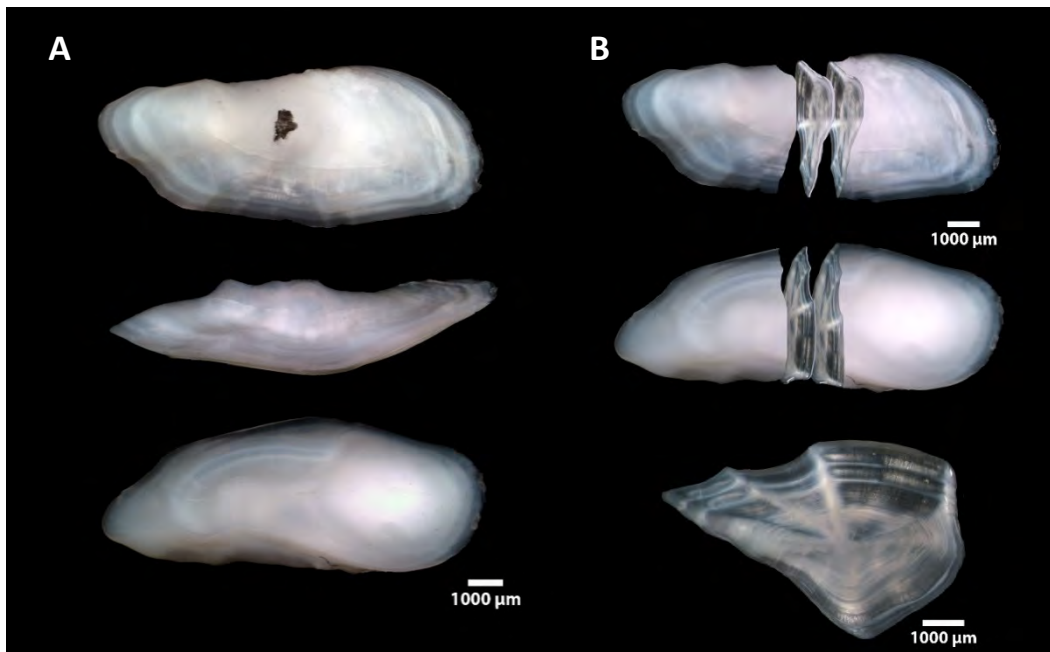


Figure 9.1.3.1 A) Whole otoliths of Spotted Seatrout proximal view with core marked (top), dorsal view (middle), and distal view (bottom) and B) Spotted Seatrout otolith sectioned with thin-section located and rotated showing location of cut through the core.

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 9.1.3.2.

Otolith Extraction

Cynoscion otoliths are strong enough to withstand expected impacts from otolith extraction devices without breaking. They are easy to identify through the gill cavity due to the strongly convex surface of the otic capsule, which is located near the posterior base of the skull. Most of the otolith removal techniques in Chapter 3.0, Section 3.2.1 are effective for these fish; some may be easier than others based on the size and depending on if the fish must be kept in a marketable condition. A video by ODU shows otolith removal from Weakfish (Chapter 12.0, Section 12.7).

Otolith Processing

Due to the robust nature of the otoliths in this genus, multiple techniques described in Chapter 3.0, Section 3.2.5 are acceptable and usually reflect available equipment. Generally, *Cynoscion* otolith sections are cut to approximately 0.5 mm. Any of the saws and techniques included in Chapter 3.0, Section 3.2.6 will work for this species group. The otoliths can be embedded or cut whole, without embedding.

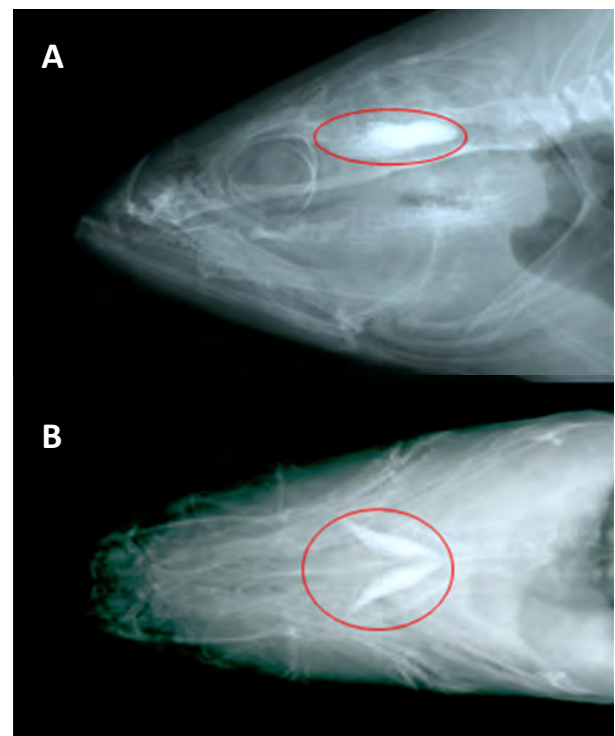


Figure 9.1.3.2 Radiographs showing location of sagittae in *Cynoscion* spp cranium in A) lateral and B) dorsal/ventral views.

Age Determination

Spotted Seatrout

Ageing Spotted Seatrout otoliths is straightforward even though the location of the first annuli can vary widely in its distance from the core. Spotted Seatrout spawn from April to October in the Gulf of Mexico (McMichael and Peters 1989), from April to August with a peak in May from Georgia to the Carolinas (Mahood 1974), and from April through August in the Chesapeake Bay (Ihde 2000). Due to the protracted spawning season there may be a corresponding variation in age (months) at first opaque zone formation, which occurs from March to April in the Gulf of Mexico (Manceina et al 1987), from late February through mid-April in Georgia (Music and Pafford 1984), and from March to April in the Chesapeake Bay (Ihde and Chittenden 2002) (Figure 9.1.3.3).

Maceina et al. (1987) validated annual marks on the otoliths of Spotted Seatrout from Texas waters between ages 1-4 using MIA as did Murphy and Taylor (1994) for Spotted Seatrout from Florida. Ihde and Chittenden (2003) validated ages 1-5 for Spotted Seatrout in Chesapeake Bay.

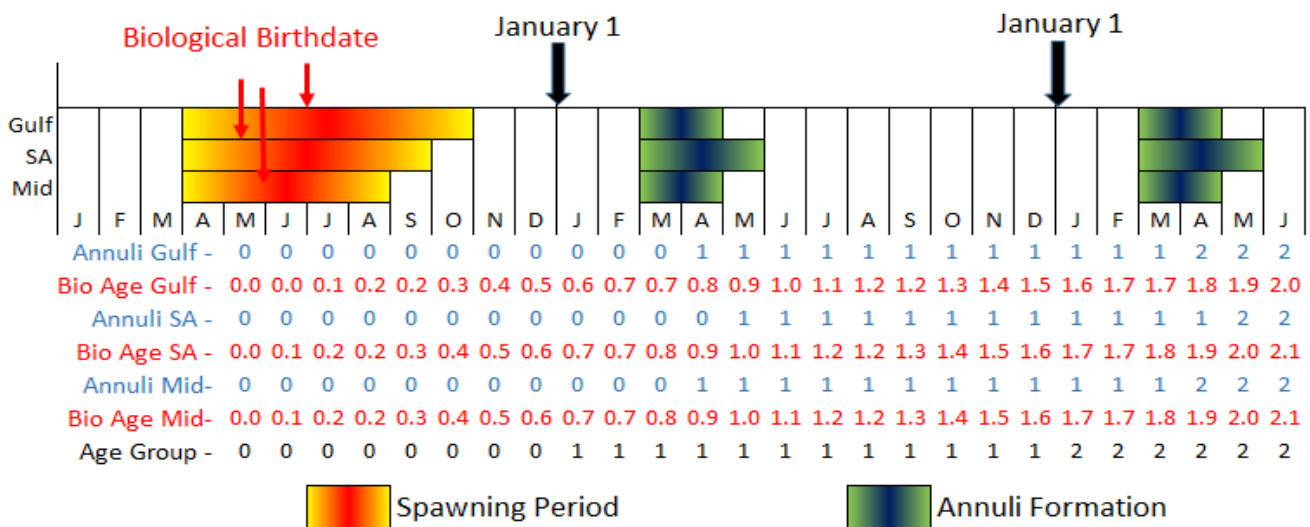


Figure 9.1.3.3 Spawning periodicity and age assignment timeline for Spotted Seatrout from the South Atlantic and the Gulf of Mexico.

Weakfish

Weakfish have a large range from Nova Scotia to Cape Canaveral, Florida, but are most abundant from North Carolina to Long Island (Murdy et al. 1997). Lowerre-Barbieri et al. (1996) determined Weakfish spawned in Chesapeake Bay from May to August with an early and late peak similar to other *Cynoscion*. Mahood (1974) determined Weakfish spawning in Georgia begins in March and continues into August, with the peak from March-May. Weakfish in the northern range spawn from May to early June (Shepherd and Grimes 1984) (Figure 9.1.3.4).

Annulus formation on Weakfish scales was validated by Welsh and Breder (1924), Massmann (1963), and Wilk (1979) using MIA. Music and Pafford (1984) determined annulus formation occurred in Florida from late March through June on Weakfish scales and slightly earlier on their otoliths. They noted that there was 95.9% agreement between scales and otoliths (Music and Pafford 1984). Shepherd and Grimes (1983) also used scales to determine annulus formation in the northern range occurs from April to June. Lowerre-Barbieri et al. (1994) indirectly validated annulus formation from otoliths along the Chesapeake

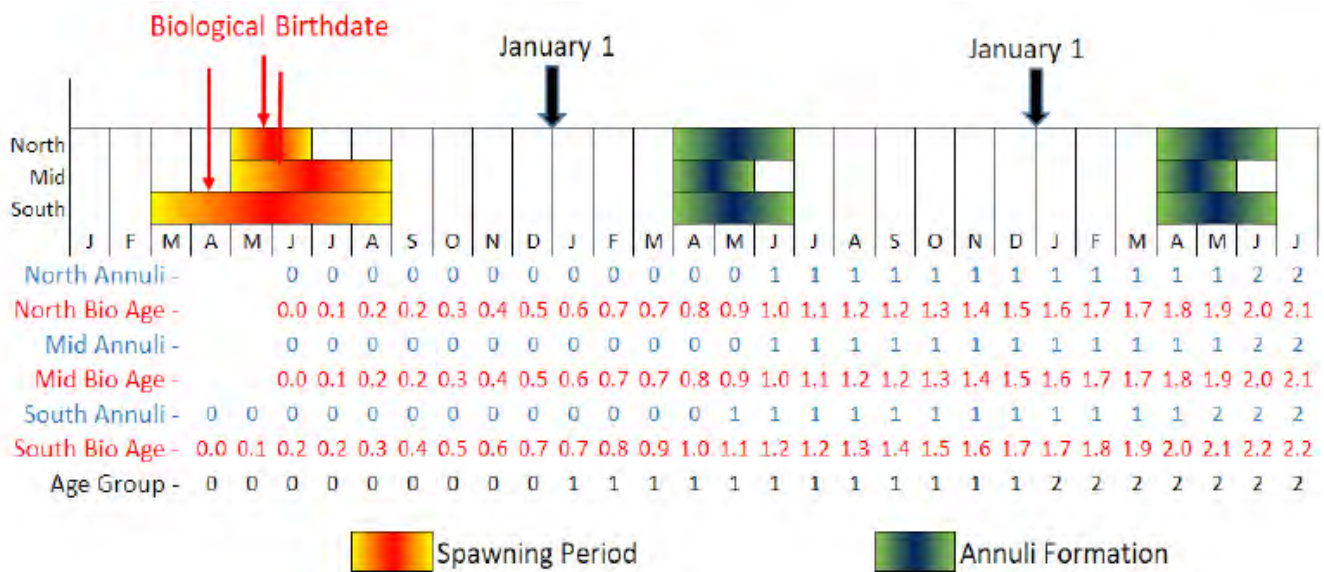


Figure 9.1.3.4 Spawning periodicity and age assignment timeline for Weakfish from New England to the South Atlantic.

Bay using MIA for ages 1-5 and determined annulus formation occurs from April to May. Like many other *Cynoscion*, Weakfish annuli are easy to read in otoliths (Figure 9.1.3.5).

Sand and Silver Seatrout

Sand and Silver Seatrout are not generally considered commercially important but are a primary component of shrimp trawl bycatch and commercial discards. Recreational anglers do not typically target either species but will retain them if they have any size (VanderKooy 2011). Despite their relatively high abundance in most coastal areas, very little has been pursued related to age-and-growth of these species.

Sand Seatrout (*Cynoscion arenarius*) spawn primarily from March to September, exhibiting two distinct spawning peaks, a spring peak from March-April and a late summer peak in August/September (Figure 9.1.3.6A). Sand Seatrout have been aged to age-4 in the northern Gulf (AMRD unpublished data) and

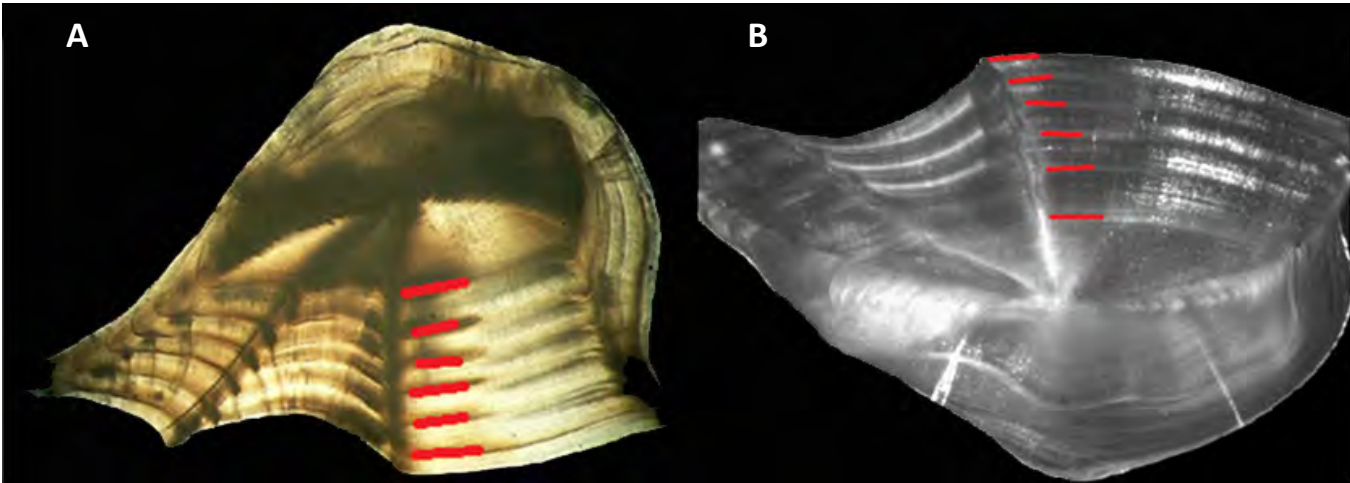


Figure 9.1.3.5 A) Reading plane and annuli location (red lines) on a sectioned otolith from an age-6 Weakfish using transmitted light. B) Reading plane and annuli location (red lines) on a sectioned otolith from a Gulf of Mexico age-6 Spotted Seatrout using reflected light.

age-5 along the Florida Gulf Coast (Nemeth et al. 2006). Nemeth et al. (2006) indirectly validated annulus formation in Sand Seatrout using MIA and determined that formation occurred along the Florida Gulf Coast in January through March.

Silver Seatrout (*C. nothus*) spawn in Texas waters from early May through September or late October (Figure 9.1.3.6B), with the greatest or more successful spawning occurring during the late summer (DeVries and Chittenden 1982). The late summer spawn shows a tendency for two sub-peaks, one in August and one in September. Mahood (1974) never found spawning individuals inshore and determined that spawning by Silver Seatrout off Georgia occurred in the spring through late fall in offshore waters. DeVries and Chittenden (1982) estimated annulus formation for Silver Seatrout using MIA on scales to be from April to June in Texas waters.

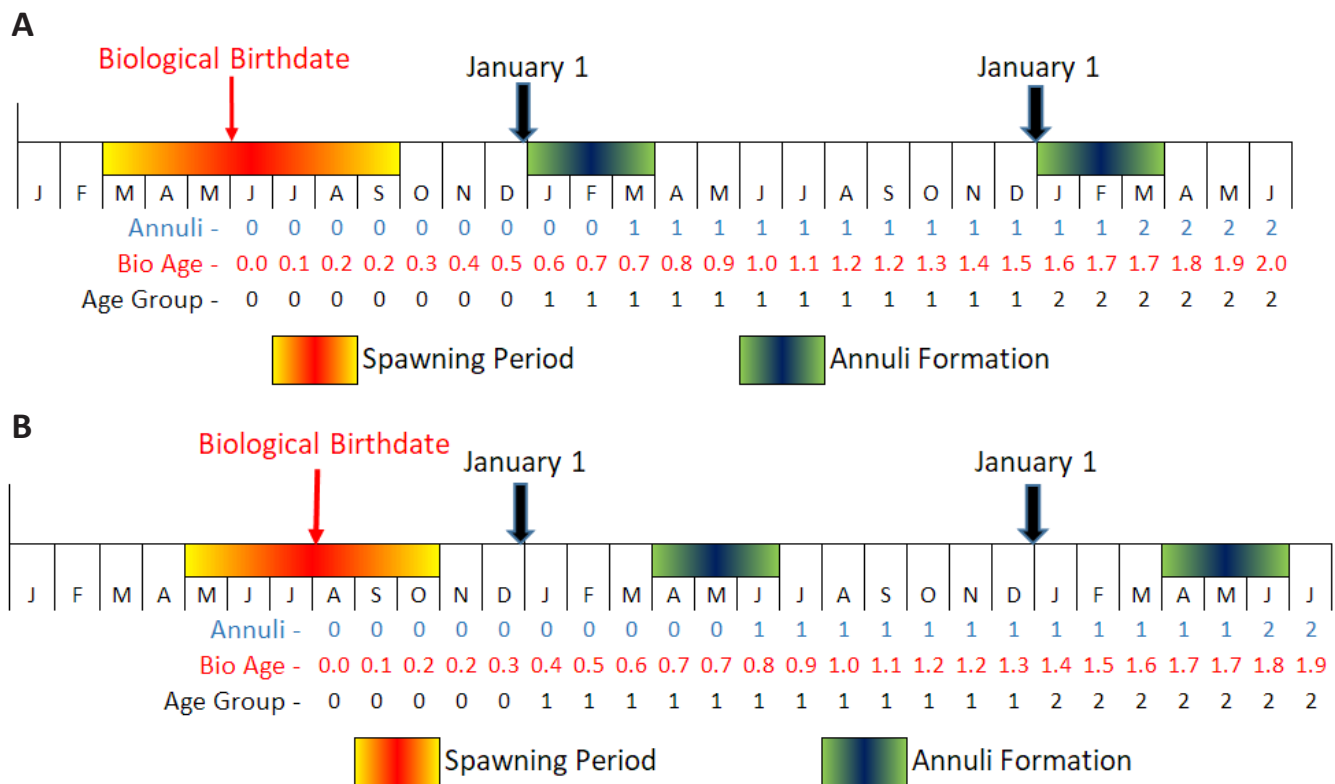


Figure 9.1.3.6 Spawning periodicity and age assignment timeline for A) Sand Seatrout along the Atlantic Coast of Florida and the Gulf of Mexico and B) Silver Seatrout from Georgia to the Gulf of Mexico.

Other Ageing Methods

Due to the thickness, whole *Cynoscion* otoliths have not been used successfully for age determination. Scales in Spotted Seatrout have been demonstrated to be useful in the first few years only along the South Atlantic and Gulf. After age-4 annuli in scales become less consistent, resorption can occur at the core, and false annuli can occur due to spawning checks. Brown (1981) examined scales from Spotted Seatrout in Chesapeake Bay and aged fish to age-15. Ihde and Chittenden (2002) did a comparison of Spotted Seatrout pectoral fin rays, dorsal fin spines, scales, and whole and sectioned otoliths and concluded that sectioned otoliths are the most reliable ageing structure (Figure 9.1.3.7).

Scale-based ages of Weakfish were historically used in stock assessments prior to the 1990s. However, scale and otolith comparisons in the 1990s suggested that otolith ages are more reliable than scale ages,

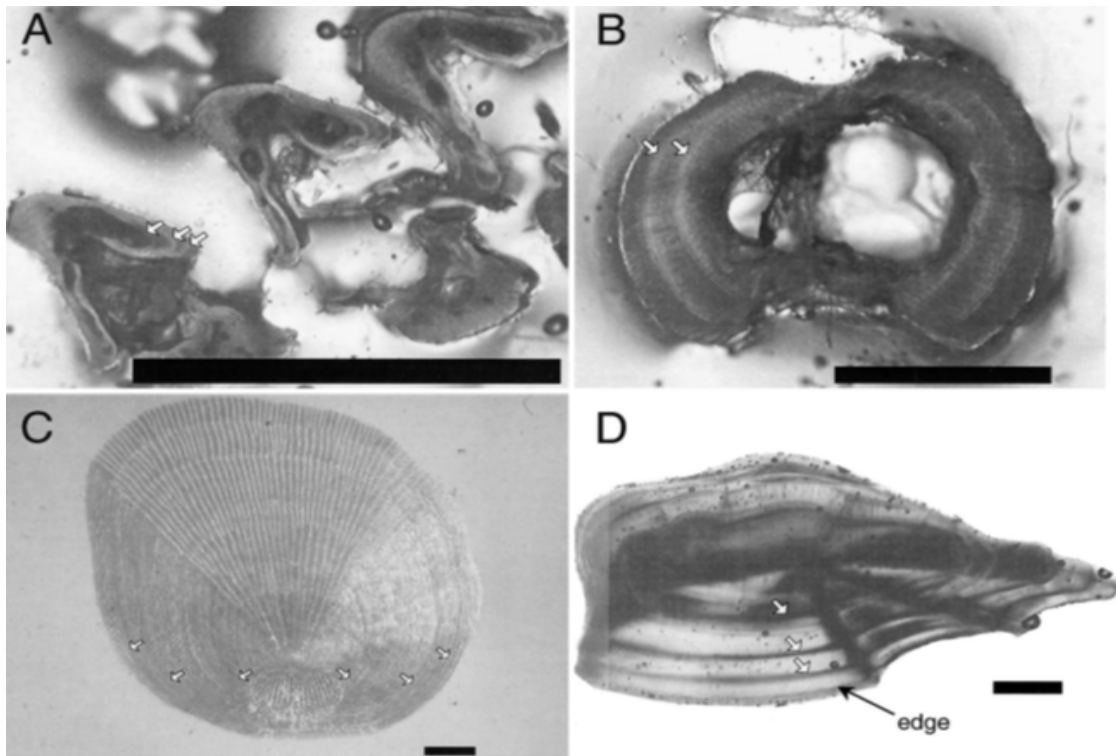


Figure 9.1.3.7 Comparative appearance of presumed annual marks on A) sectioned pectoral fin rays, B) a sectioned dorsal fin spine, C) a scale, and D) a sectioned otolith of a 657-mm-TL, 2,865-g female Spotted Seatrout. The pectoral fin ray sections show rays 6 (upper right) through 8 (lower left). The dorsal fin spine shows a section of spine 2. The edge of the otolith section is indicated in (D). Presumed annual marks are indicated by white arrows. All images were taken in transmitted light. Solid bars are 1 mm long. (Figure 1 from Ihde and Chittenden 2002).

particularly for older fish (Lowerre-Barbieri et al. 1994). Based on a thorough examination of available data, the ASMFC Weakfish Technical Committee determined that otoliths rendered more reliable age estimates. Lowerre-Barbieri et al. (1994) also examined Weakfish dorsal spines and pectoral fin rays and found that marks were “inconsistent, often blurred or impossible to follow around most of the section.” They found that, while annuli on Weakfish scales were clearer, they were still subjective in interpretation.

Barger and Johnson (1980) examined scales, vertebrae, and otoliths for Sand Seatrout, Silver Seatrout, Atlantic Croaker and Spot. They determined that for all of the studied species, sectioned otoliths were the most reliable structure for age determination.

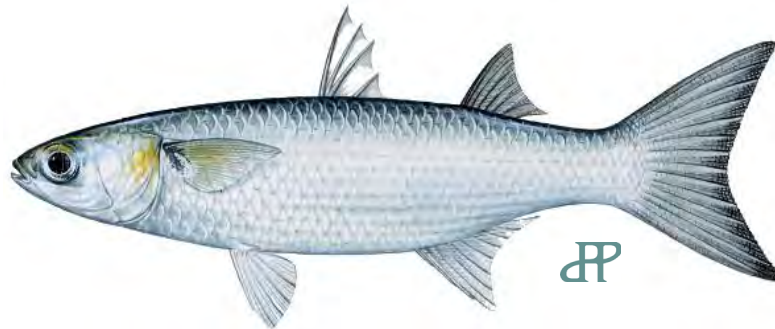
Research Needs

Considering the popularity of seatrout as a sportfish, there has been an extensive history of research for all four of these species; however, Sand and Silver Seatrout are much less well studied. Validation of annulus formation in these two species is incidental rather than direct validation through OTC or other marking techniques.

9.2 Mugilidae - Mullet



Striped Mullet *Mugil cephalus*



White Mullet *Mugil curema*

Highlights

- Otoliths in most mullet are relatively easy to locate and extract.
- Mullet otoliths are fragile; care must be taken in removal.
- Generally one otolith removal technique, 'score and break', practiced and recommended.
- Multiple otolith sectioning techniques have been used successfully.
- Annuli relatively faint but discernible.
- First distinct opaque zone forms at approximately a biological age of 1.2 in Striped Mullet and 1 in White Mullet.
- Striped Mullet generally have less than 6 annuli but may reach up to 9 (Thompson et al. 1991).
- White Mullet may reach about 7 years in Florida (Mahmoudi 2002).
- White Mullet have limited information published on ageing in the Gulf and South Atlantic and caution is recommended with the information provided.

Otolith Description

Mullet have small, fragile sagittal otoliths, which are slightly flattened and may break during extraction. The ventral surface is moderately crenate. The distal side is concave with the visible core lying in the center of the otolith (Figure 9.2.1). The sulcus runs along the proximal dorsal half of the otolith. The location of the otoliths in the neurocranium is illustrated in Figure 9.2.2.

Otolith Extraction

Any number of techniques can be used to extract the sagittal otoliths from Striped and White Mullet (Chapter 3.0, Section 3.2.1); however, the most common and simple procedure is the 'score and break' technique (Figure 9.2.3). Caution should be taken on smaller specimens (<200 mm), because this action may rupture the otic capsule and expose or expel the sagittal otoliths. Otoliths are small and may become chipped or broken if care is not taken. For example, a Striped Mullet with a 280 mm fork length has an otolith approximately 9 mm in length and 3 mm at its maximum width. Otoliths are removed with a pair of forceps and then rinsed with water.

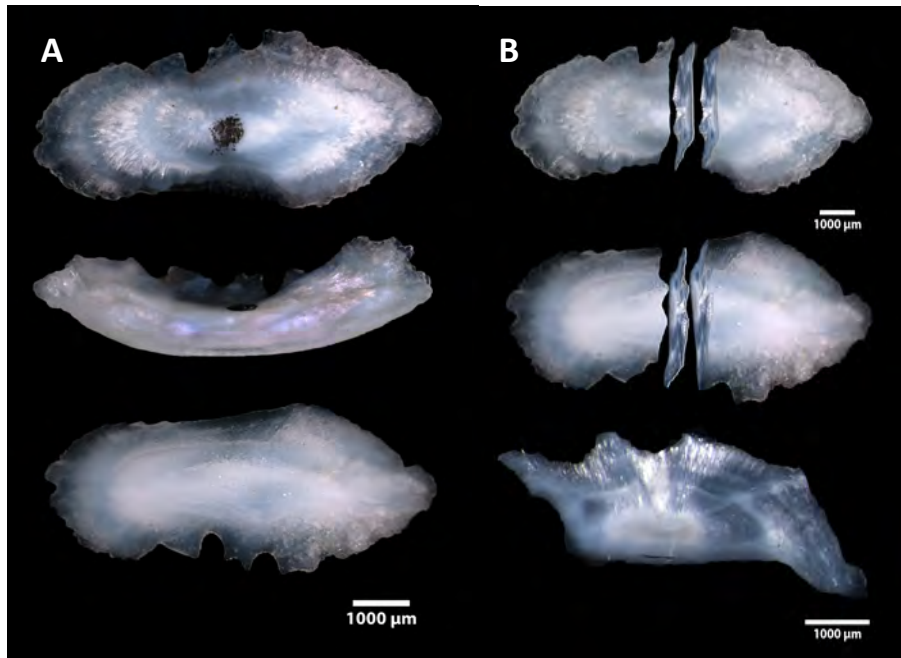


Figure 9.2.1 A) Whole otoliths of Striped Mullet proximal view with core marked (top), dorsal view (middle), and distal view (bottom) and B) Striped Mullet otolith sectioned with thin-section located and rotated showing location of cut through the core.

Otolith Processing

Mullet tend to have relatively thin and fragile otoliths and are embedded and sectioned on low-speed saws. However, any of the sectioning techniques described in Chapter 3.0, Section 3.2.5 could be used with care. Otoliths are general sectioned around 0.5-0.7 mm.

Age Determination

Striped Mullet

Striped Mullet, in the Gulf of Mexico, spawn from November to February with a peak in November/December (Thompson et al. 1989) and subsequently form a large opaque region around the core through

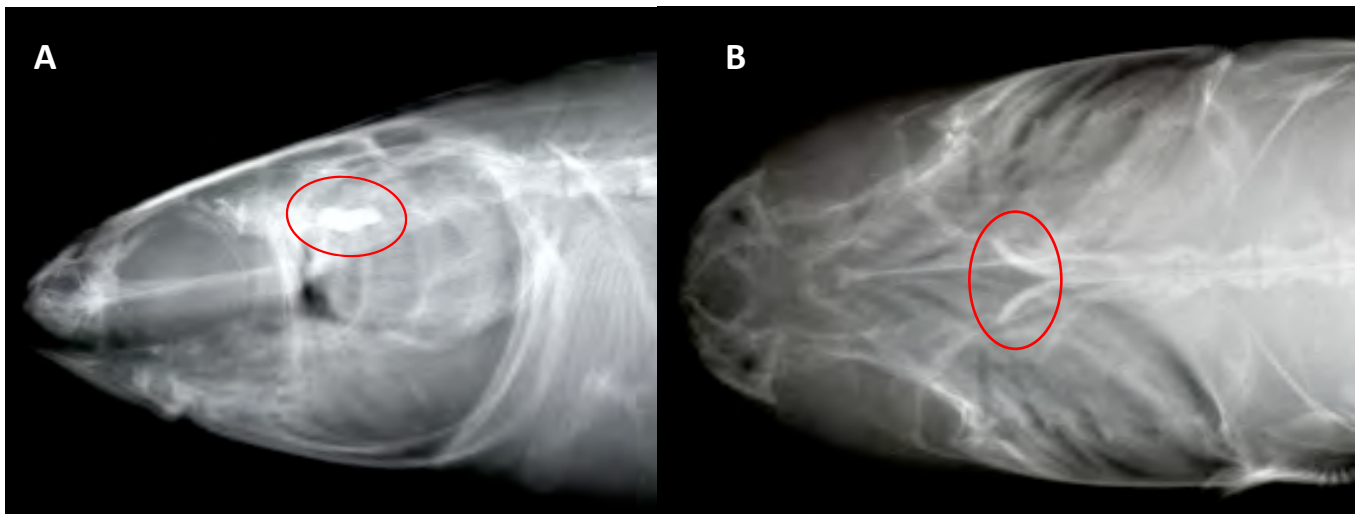


Figure 9.2.2 Location of sagittae (red circles) in the neurocranium of Striped Mullet in A) lateral and B) dorsal-ventral views.

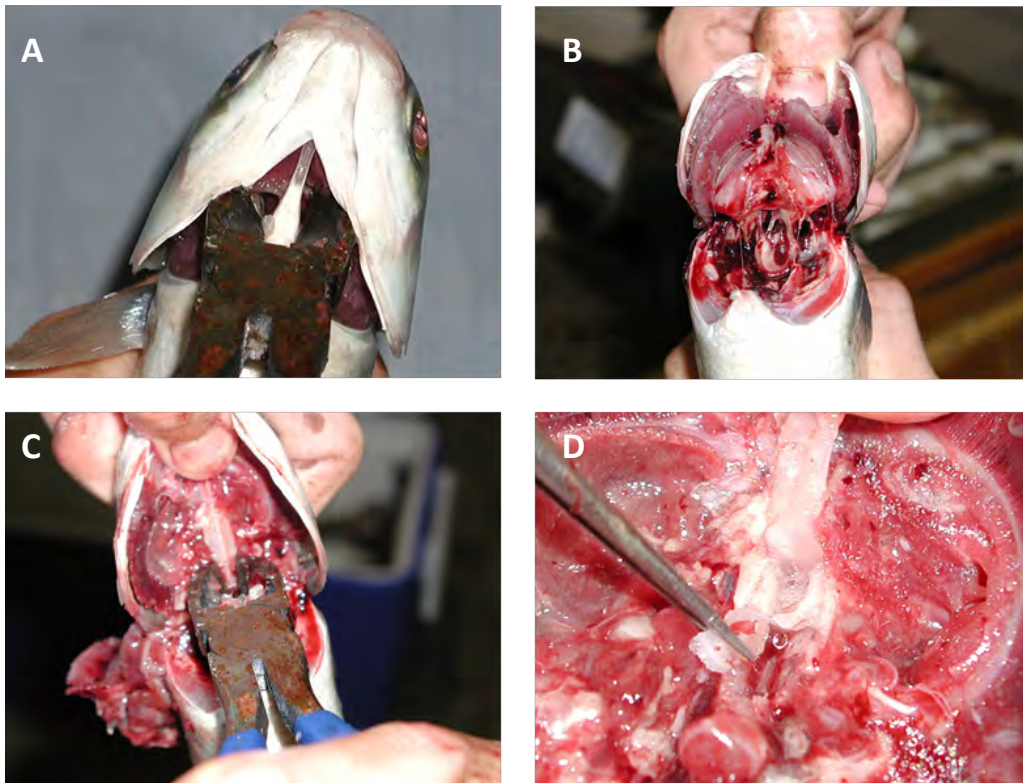


Figure 9.2.3 The 'score and break' technique for removing otoliths from a White Mullet. A) Cut the isthmus, B) pull back the cranium, C) clip the otic capsule, and D) remove the exposed otoliths.

February (Figure 9.2.4A). The core mark, or smudge, is the first annulus but the second winter mark or first true annulus is generally located further from the core, because it is formed when the fish are approximately 13-16 months of age. Spawning along the Atlantic Coast is longer than the Gulf ranging from September in North Carolina (Bichy and Taylor 2002) until February in Georgia and South Carolina (Pafford 1983, McDonough and Wenner 2003). Greeley et al. (1987) reports a peak on the Atlantic in December-January and the rest of the South Atlantic peak is from October-December. Illuminated from below, the opaque zones in Striped Mullet sections are relatively well defined (Figure 9.2.5).

Annulus formation was validated by Thompson et al. (1989) in Louisiana waters, and generally begins in January and is complete by April. Annulus formation in the South Atlantic occurs in May and June (Foster 2001, McDonough and Wenner 2003).

White Mullet

In the Mexican Gulf of Mexico, Aguirre and Gallardo-Cabello (2004) reported that spawning season lasts from February to May with a peak from February through April (Figure 9.2.4B). This agrees generally with Oren (1981) who reported spawning in the northern Gulf in April and May. Along the western Atlantic, Jacot (1920) had inferred White Mullet likely spawn from April through August with a peak in May based on the arrival of small fish to North Carolina. In South Carolina, larval White Mullet arrived in the estuary in December through May, suggesting that spawning had occurred in the fall and winter (Bozeman and Dean 1980). Richards and Castagna (1976) reported that spawning of White Mullet in North Carolina resulted in small fish (25-85mm) arriving in the Chesapeake and mid-Atlantic region in June and early July. Nickerson (1984) suggested that White Mullet spawn on the Florida East Coast from March to June with a peak in May.

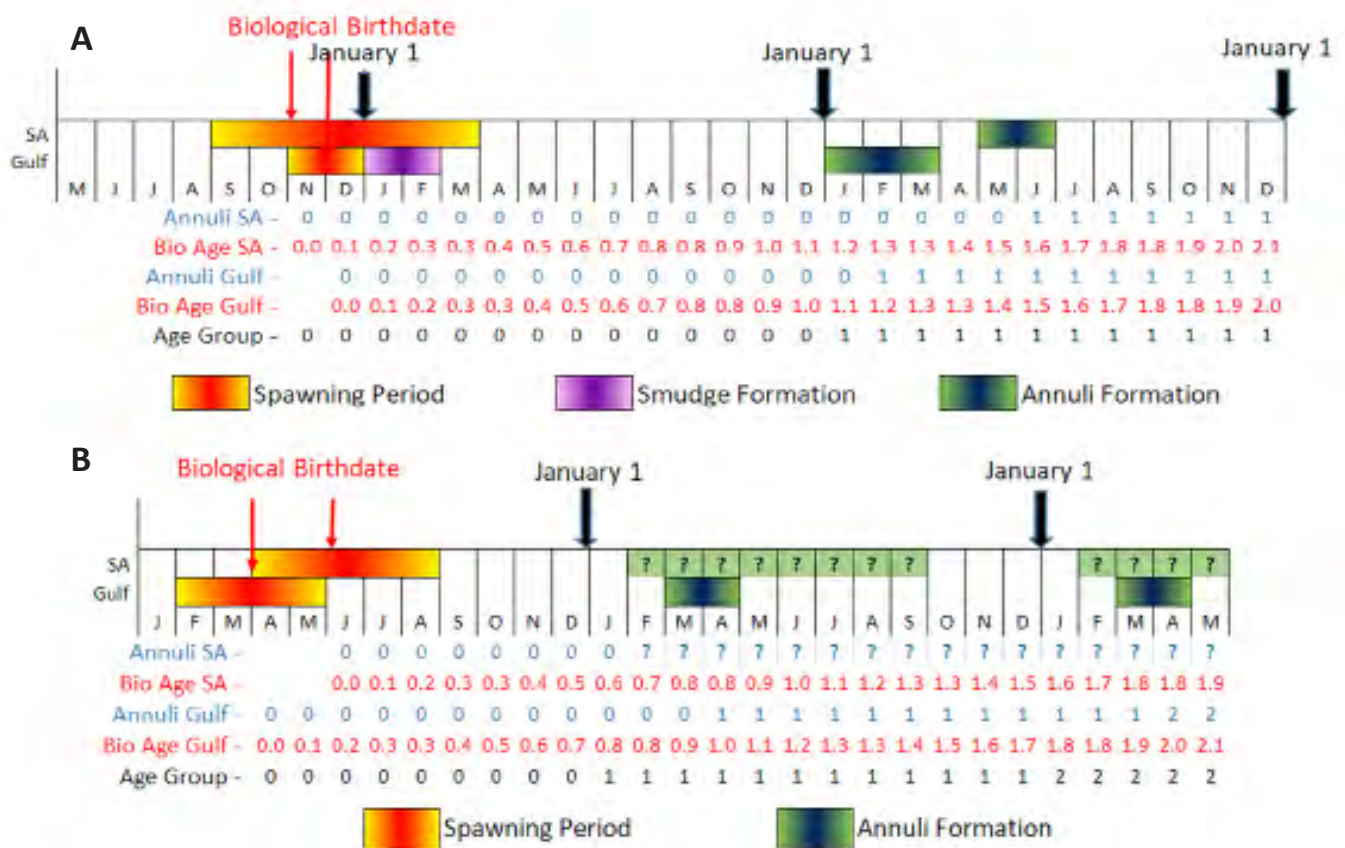


Figure 9.2.4 Spawning periodicity and age assignment timeline in the South Atlantic and Gulf of Mexico for A) Striped Mullet and B) White Mullet. **Note:** The formation and validation of annuli in White Mullet is yet to be described in the South Atlantic so caution should be used when making inferences on ages in that region.

The Alabama Department of Conservation and Natural Resources (AMRD unpublished data) indicates annulus formation in otoliths occurs in March and April. Jacot (1920) described ageing white Mullet scales in great detail, however, the study had a lack of adult specimen from north of Florida, resulting in no discernable annuli observed and no inference made regarding the annulus formation from seasonal changes or migration. Marin et al. (2003) validated daily increments on the otoliths of White Mullet juveniles younger than one years old, as a result, no annual growth zone could be derived from their study.

In the southern Gulf of Mexico, off Brazil, White Mullet annuli were validated by Santana et al. (2009). Formation occurred in January and February utilizing monthly marginal increment ratios (MIR) which follows a November-February spawning period (Figure 9.2.6). Espino-Barr et al. (2005) examined scales from White Mullet along the eastern Pacific Coast off Mexico and validated annual formation of circuli using MIA. They found that White Mullet formed annuli on scales in July and August.

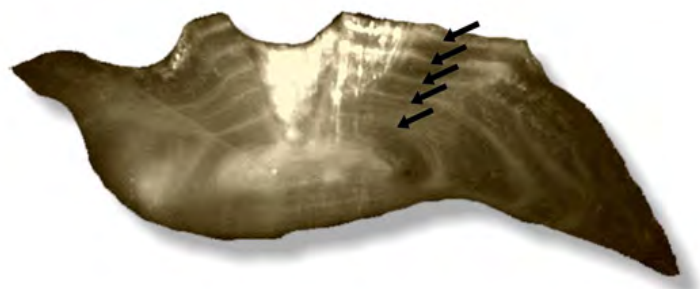


Figure 9.2.5 Age-5 Striped Mullet otolith section with annuli indicated with arrows.

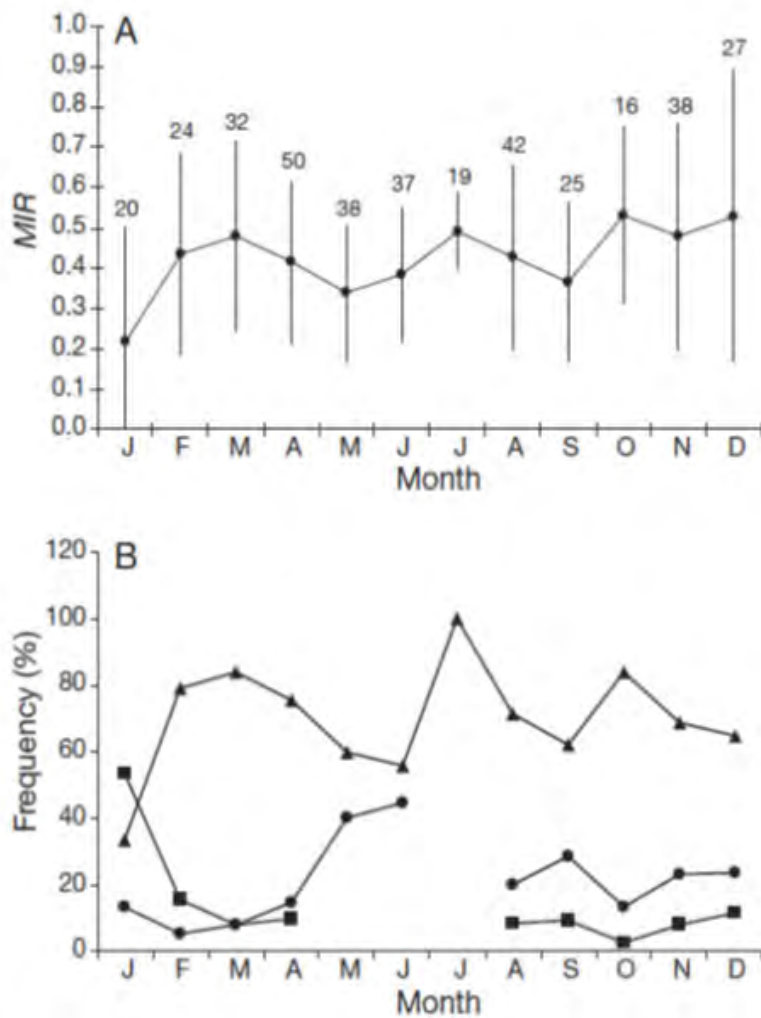


Figure 9.2.6 A) Monthly marginal increment ratio (MIR) of *M. curema* from Brazil, mean points \pm SD (vertical bars) and number of individuals sampled for each month, and B) relative frequency of individuals with the following marginal increments on the otolith edge: MIR = 0 (■); with 1 translucent zone (MIR = 0 to 0.3; ●) and opaque zone (MIR > 0.3; ▲) (Fig. 2 from Santana et al. 2009).

Other Ageing Methods

While a number of hardparts have been examined in Striped Mullet (scales, otoliths, opercula, and spines), most of the current work is conducted using sagittal otoliths (Quignard and Farrugio 1981). Quignard and Farrugio (1981) suggest that scales are the more appropriate structure to use when ageing mullet in part due to opaqueness in otolith sections. Thompson et al. 1989 reported that Striped Mullet otoliths could not be read whole due to the 'general opaqueness' of the structure. Scales were used to age mullet from the 1950s 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. Erman (1959) indicated that scales were not reliable after age-4 due to issues with the early annuli fading and obscuring in the center.

Research Needs

There is very little information available for the Gulf of Mexico and the South Atlantic for ageing of White Mullet. Most of the validation work originates outside our region and may not be applicable to

our populations. The use of alternative structures should be explored further since there is conflicting information regarding the reliability of both otoliths and scales for White Mullet.

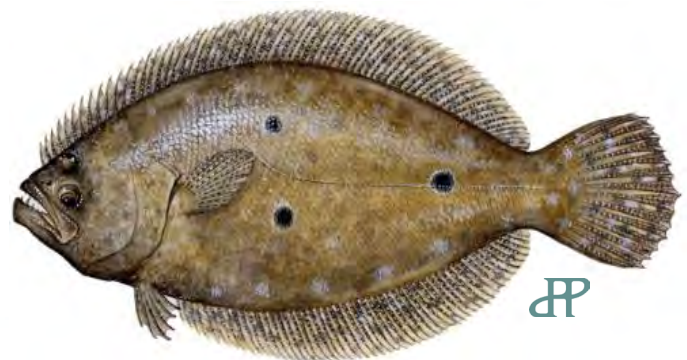
9.3 Paralichthyidae - Flounders



Southern Flounder *Paralichthys lethostigma*



Summer Flounder *Paralichthys dentatus*



Gulf Flounder *Paralichthys albiguttata*



Winter Flounder *Pseudopleuronectes americanus*

Highlights

- Flounder otoliths in general are small, fragile, but relatively easy to locate and extract.
- Because of their unique morphology, all flounder otolith pairs are asymmetrical.
- Whole otoliths, sectioned otoliths, and scales have all been accepted and can be used to accurately age most flounder.
- Most flounder have easily discernible zones and display differential growth in males and females.

- First distinct opaque zone forms at approximately one year of age.
- Flounder scales are ctenoid and very small compared to other species, but can be used to successfully age some species of flounder.
- Southern Flounder have been aged to age -8 (Fischer and Thompson 2004) in the Gulf.
- Summer Flounder have been aged to age-17 (ODU) with otoliths and age-15 (ODU) with scales.
- Gulf Flounder have been aged to age-11 (Fitzhugh et al. 2008).
- Winter Flounder are right eyed fish and scales and otolith age readings have close agreement to age-5. Otoliths have been used to age Winter Flounder to age-21 (NEFSC).

Otolith Description

Flounder sagittal otoliths have a flat arrowhead shape (Figure 9.3.1) and display morphological differences between right and left sagittae. In left eyed fish, the core of the left otolith is located more posterior to center. This is reversed in right eyed fish. 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 9.3.2.

Otolith Extraction

Sagittal otoliths can be removed from most flounder in two ways depending on the size of the specimen and whether the fish is sampled from the commercial catch and needs to remain whole

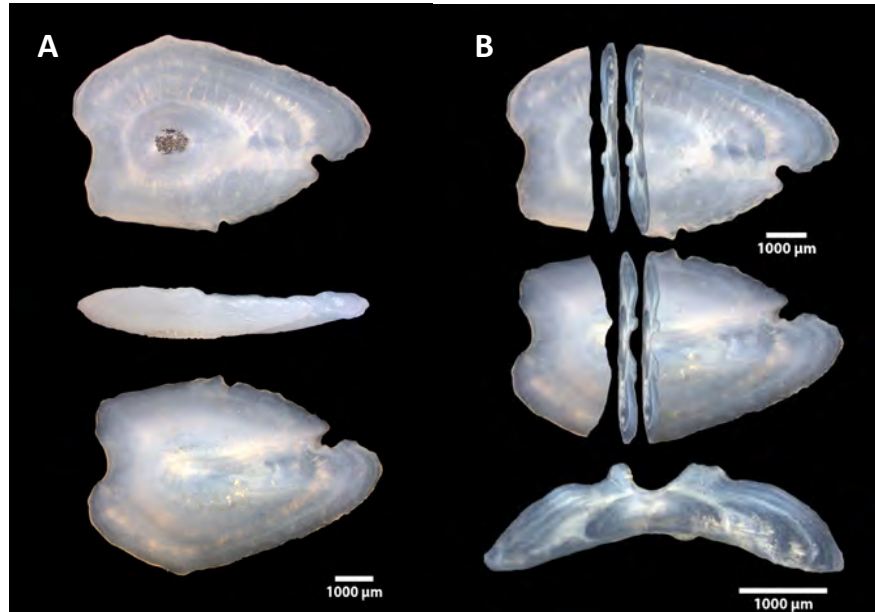


Figure 9.3.1 A) Whole otoliths of Gulf Flounder proximal view with core marked (top), dorsal view (middle), and distal view (bottom) and B) Gulf Flounder otolith sectioned with thin-section located and rotated showing location of cut through the core.



Figure 9.3.2 Radiograph of a Southern Flounder showing location of sagittae in the cranium in lateral view (red circle).

and relatively unmarked. The most common and perhaps most simple is to extract the otoliths through the operculum; however, if the fish does not need to be kept 'whole', a vertical cut at the preopercle and opercle junction works well to 'snap' the head sideways (Figure 9.3.3). In general, flounder otoliths are relatively thin so care should be exercised when extracting.

Otolith Processing

Due to the small size of flounder otoliths, the technique of sectioning whole embedded otoliths appears to provide the highest quality sections (Chapter 3.0, Section 3.2.5.2). For most species of flounder, otoliths are cross-sectioned at a thickness of approximately

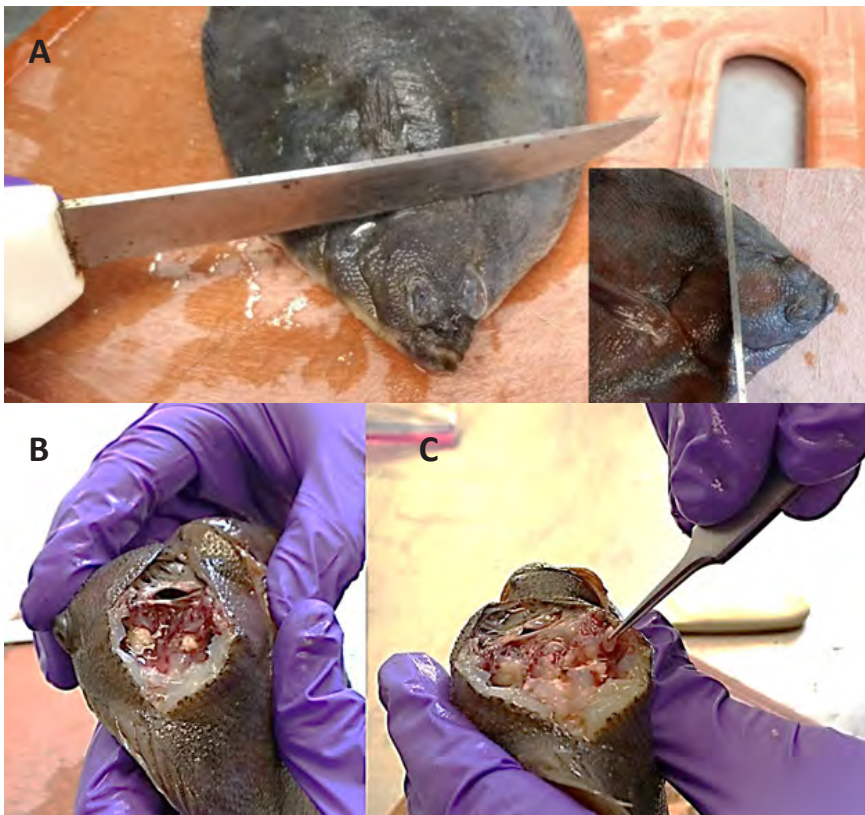


Figure 9.3.3 A) A shallow cut is made downward in the area between the opercle and preopercle through the cranium and then B) snapped laterally, C) opening the region exposing the otic capsules and sagitta.

0.5 mm to obtain the best results (Chapter 3.0, Section 3.2.6). Some labs also choose to follow a modified version of the 'bake and thin section' technique outlined in Chapter 7.0, Section 7.1.6.

Age Determination

Southern Flounder

Opaque increments are easily distinguishable on both the dorsal and ventral sides of the sulcus in Southern Flounder otolith cross-sections. 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). Annulus formation begins in the northern Gulf in January and is completed by the end of June (Figure 9.3.4). Validation of annual increments was reported using marginal increment analysis most

recently by Fischer and Thompson (2004).

Summer Flounder

Summer Flounder spawning was determined to be September to December in Chesapeake Bay and November to February south to North Carolina (Smith 1973). Warlen and Burke (1990) confirmed spawning in North Carolina by Summer Flounder based on the appearance of larvae in the estuaries in March (Figure 9.3.5). Summer Flounder from New Jersey to North Carolina spawned from September to January with a peak in October and November according to Able et al. (1990).

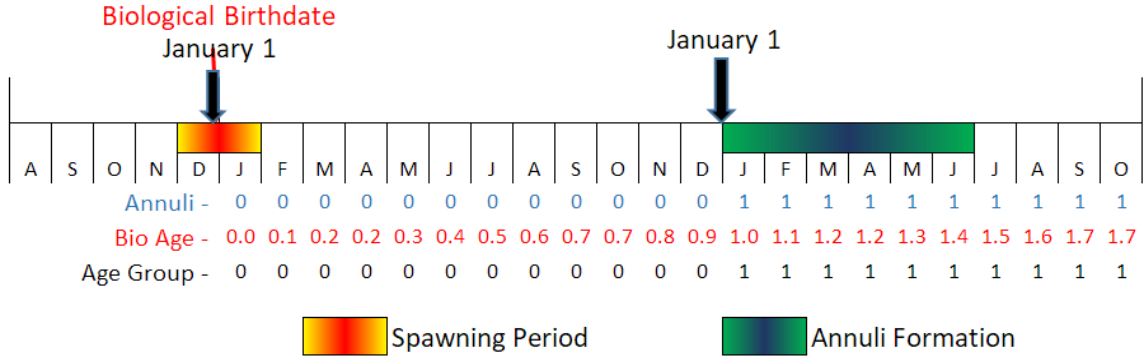


Figure 9.3.4 Timeline showing spawning period and annulus deposition ranges for Southern Flounder in the South-Atlantic and Gulf.

Smith and Daiber (1977) observed faint marks along the edge of the whole otoliths from a few young-of-year Summer Flounder collected in Delaware Bay during “winter” but didn’t count them as annuli. However, they did suggest those marks could be the first true annuli. Annulus formation was determined in New York using whole otoliths to occur around February by Poole (1961). Shepherd (1980) reported that Summer Flounder off Massachusetts also formed their first annulus very near the core in February (Figure 9.3.5), despite the fish being only a few months old. Powell (1982) reported annulus deposition in whole Summer Flounder otoliths begins in January, peaks in February-April, and ends in June in North Carolina.

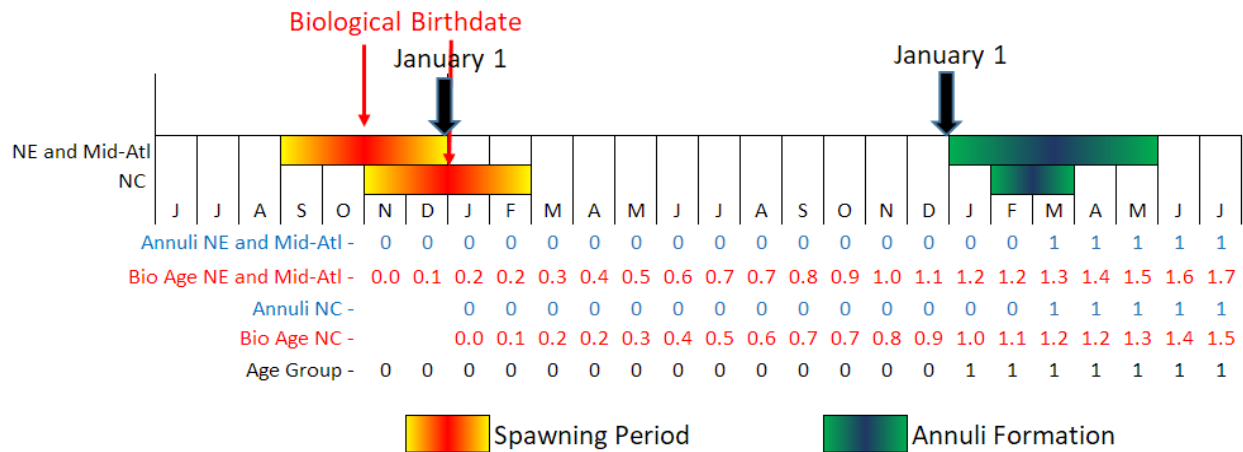


Figure 9.3.5 Timeline showing spawning period and annulus deposition ranges for Summer Flounder from New England to North Carolina.

Validation of annual increments was reported using MIA by Desfosse (1995). Annulus deposition begins in January and ends in April in the South Atlantic Bight. The northern stocks begin annulus deposition in May and ends in August (Desfosse 1995) (Figure 9.3.5). Because Summer Flounder spawn just before the time of opaque zone formation, a dark zone is often visible around the core. However, their first distinct opaque mark is deposited late during their second winter, when the fish is about 14-18 months old (Figure 9.3.6).

Gulf Flounder

Gulf Flounder can be read whole or in cross-section. Gulf Flounder exhibit fall to winter spawning, with a peak in October and November (Fitzhugh et al. 2008). Because Gulf Flounder spawn just before the time of opaque zone formation, a dark zone (or smudge) is often visible around the core but is generally ignored. Their first distinct opaque mark is deposited late during their second winter, when the fish is about 14-18 months old (Figure 9.3.7A). Annulus formation for this species is assumed to occur during the late winter and spring similar to other flounder species with most annuli completed by early summer

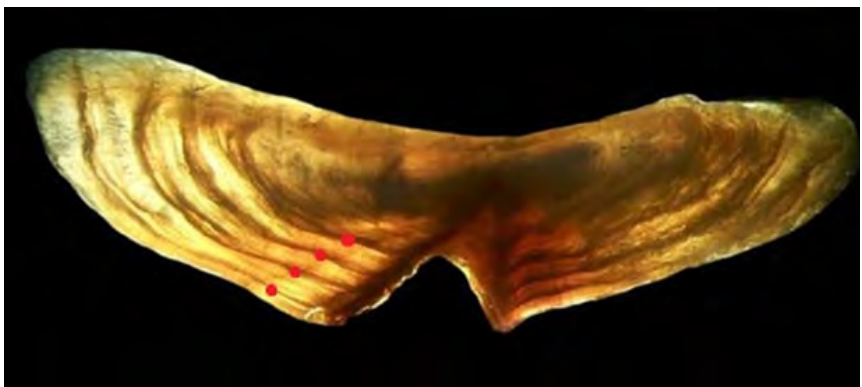


Figure 9.3.6 A baked Summer Flounder otolith section with 4 annuli marked in red.

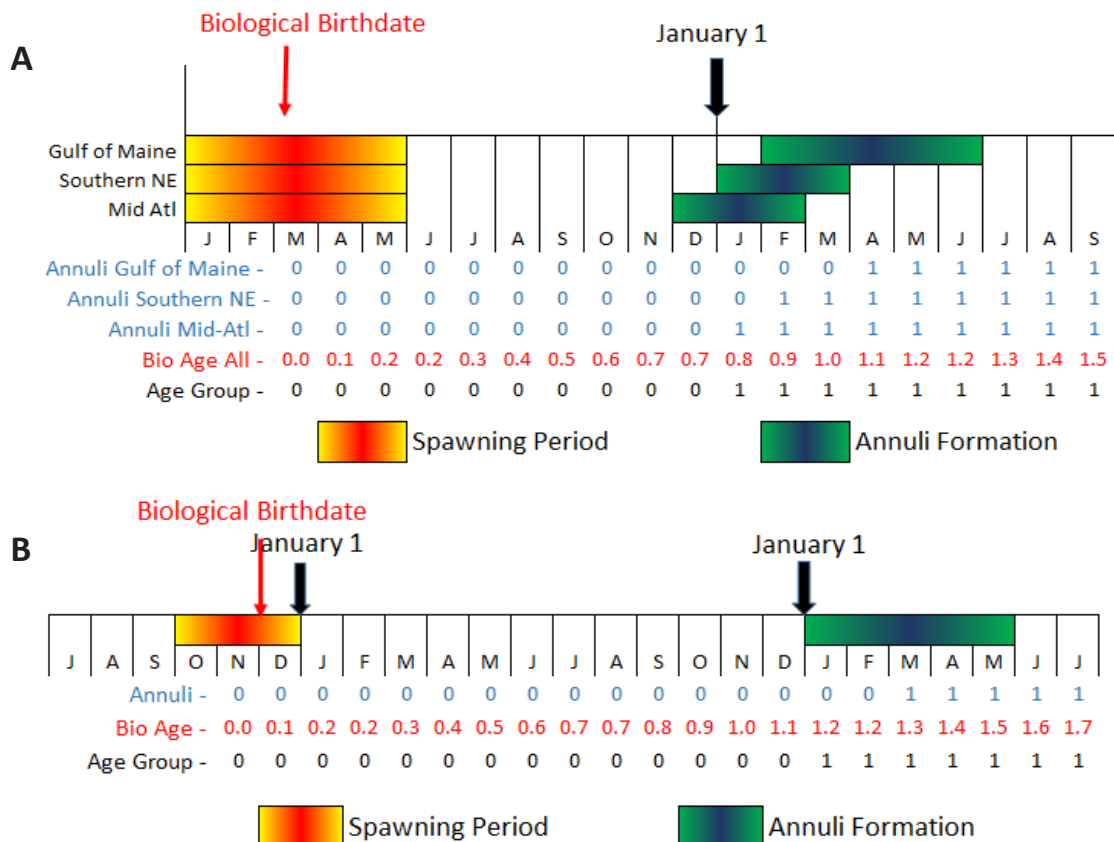


Figure 9.3.7 Timeline showing spawning period and annulus deposition ranges for A) Gulf Flounder in Gulf of Mexico and B) Winter Flounder in the north and mid-Atlantic. **Note:** Annulus formation in Gulf Flounder is based on similar flounder species and is not validated.

(Fitzhugh et al. 2008, FWRI unpublished data). No validation studies have been conducted to confirm annulus formation in Gulf Flounder however, so some caution should be taken when interpreting ages.

Winter Flounder

When using reflected or transmitted light, opaque increments can be seen on both the ventral and dorsal side of the sulcus in cross section (Figure 9.3.8). Ages are assigned based on the number of either opaque/translucent zones and season of the sample based on the January 1 birth date (Figure 9.3.7B). Annulus formation for this species occurs during the spring; most annuli are completed by early summer. Winter Flounder spawn from January through April. Because Winter Flounder annulus deposition occurs immediately after spawning, biological age is very close to age group. Interpreting the center of whole otoliths may be confusing (settling check, etc.) so care should be taken with reading annuli away from the sulcus. Checks tend to resolve into the annulus near the sulcus. Validation of annual increments was reported using MIA by Haas and Recksiek (1995).

Other Ageing Methods

Whole Otoliths

Fitzhugh et al. (2008) indicates that good age estimates may be obtained for young Southern Flounder (age-0 to age-4) when otoliths are read whole, but caution that corroboration with sectioned otoliths must be completed. MacNair et al. (2001) and Sipe and Chittenden (2001) compared whole otolith ages to sectioned ones and concluded that whole otolith ageing was adequate for young fish. Whole flounder otoliths

are viewed using reflected light, and sectioned otoliths can be viewed using either reflected or transmitted light.

Scales

Southern Flounder scales were deemed unsatisfactory for age determination, due to a lack of consistent markings (Palko 1984). It is unknown whether Gulf Flounder scales have been examined. Scales have been used to age Summer and Winter Flounder (Palko 1984).

Desfosse (1995) validated the formation of annual increments on Summer Flounder scales from Virginia using MIA and reported formation occurred in May and June. Locating the first annulus is the first step in using scales for age determination. Many scales exhibit erratic “cutting over marks” as the first annulus. The first annulus should be a complete mark across the whole scale. Care should be taken not to count checks near or within the first annulus as this will lead to over ageing.

Annular zones on Winter Flounder scales appear as changes in the circuli pattern. Zones of fast and slow growth are reflected by wide and narrow spacing, respectively, of circuli, made up of individual platelets on the sculptured upper surface of the scale. The first annulus on a scale is identified by a dense mass of winter growth (closely spaced circuli) near the focus; the end of the annulus is considered to be the outermost of these circuli (Figure 9.3.9). Sometimes pigmentation on the scale will cover the first annulus almost completely. The first annulus on many scales is barely discernible and is usually distinguished by slight changes in formation of the circuli. For all succeeding years, spring and summer growth are characterized by widely spaced circuli (rapid length accretion) and fall and winter growth by closely spaced circuli (slow length accretion). Areas of growth consisting of only a few closely spaced circuli on the scale are considered to be checks and may be ignored in assigning age. Following through to the ridges on the scales’ horizontal edges will often eliminate a check as opposed to an annulus. Scales are best aged toward a 45° angle from the focus.

Contrast between winter and summer zones tends to deteriorate toward the outer edge of scales of older Winter Flounder. After the fourth winter zone, summer growth appears to merge with the slow winter growth and the narrow growth increments may make interpretation difficult. It is recommended to use otoliths for ageing potentially older samples.

It should be noted that depending on latitude, there will be some differences in scale readability. For example, flounder from the North Atlantic exhibit clear annuli

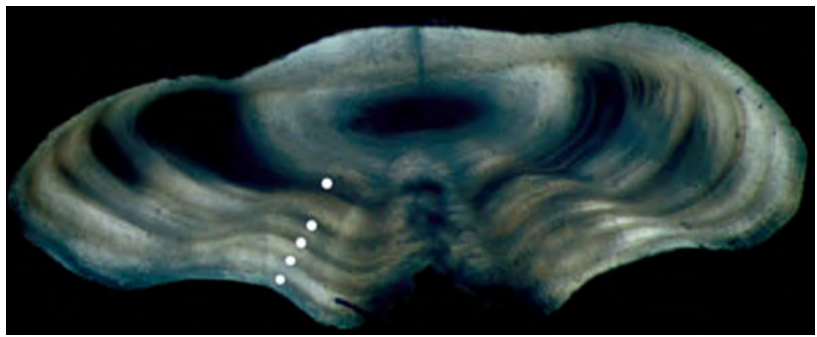


Figure 9.3.8 Winter Flounder sectioned otolith from an age-5 fish collected in February.

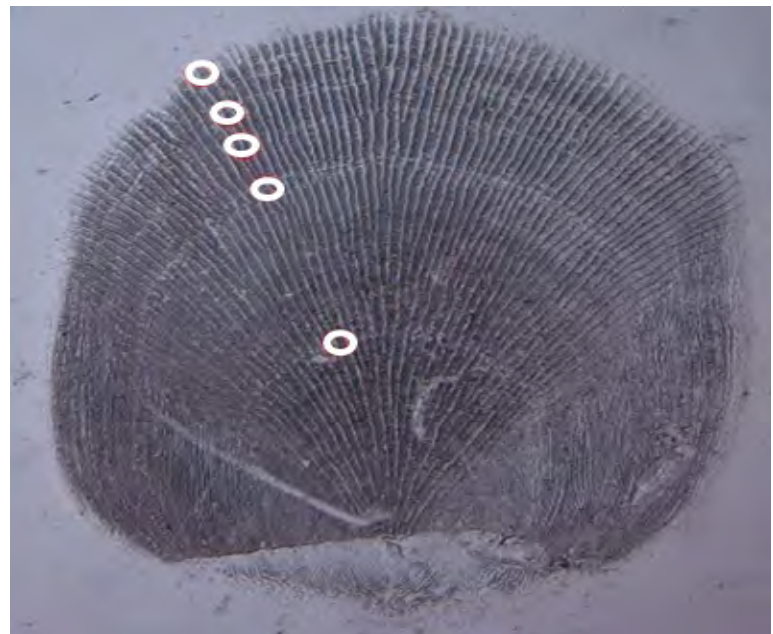


Figure 9.3.9 Scale from an age-5 Summer Flounder, white circles indicate annuli.

due to rapid scale growth. The annuli will demonstrate a consistent spacing pattern. This will make check marks or false annuli easier to distinguish.

Opercles

It is unclear whether all the flounder species have been examined for the usefulness of opercles but Sipe and Chittenden (2001) did note that Summer Flounder opercula were not viable ageing structures. They determined that opercula ages were often unclear and inconsistent with otolith determined ages.

Fin Rays

Shepherd (1980) examined dorsal fin rays from Summer Flounder from Martha's Vineyard and Nantucket Sound, Massachusetts to determine ages. He compared ray ages with whole otoliths and scales as well. Shepherd reported that the dorsal fin rays had the clearest ring formations at the ridge near the base of the ray (Figure 9.3.10). The ray was first split longitudinally and then each half was thin-sectioned (Figure 9.3.11). The closest age agreement was between fin rays and otoliths at 95%; fin rays and scales had the lowest agreement at about 77% (Shepherd 1980).

Research Needs

Establish a reference collection for Southern Flounder by stock area. A study should be undertaken to compare ages interpreted from scales and whole/sectioned otoliths for older fish. Gulf Flounder requires validation of annual increment formation and timing.

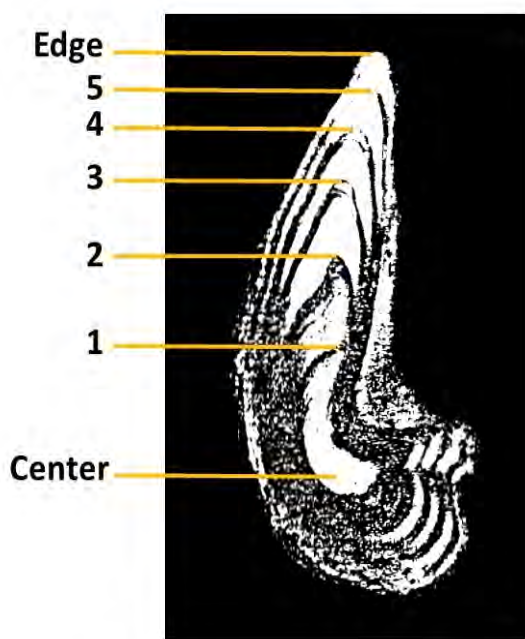


Figure 9.3.10 Cross-sectioned fin ray from an age-5 fish (Figure 3B recreated from Shepherd 1980).

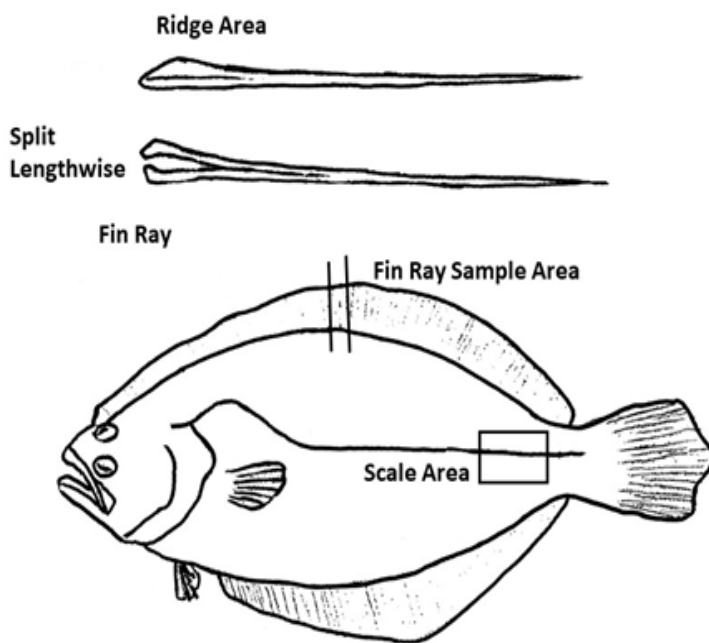
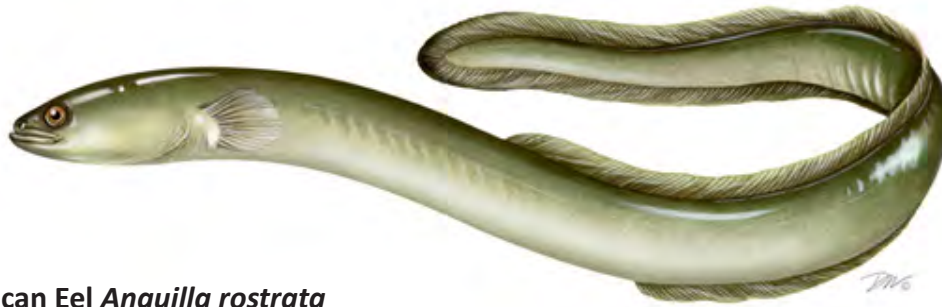


Figure 9.3.11 Illustrations of dorsal fin ray removal from Summer Flounder (Figure 2 from Shepherd 1980).

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9.4 Anguillidae - Eel



American Eel *Anguilla rostrata*

Highlights

- Otoliths are small and develop curvature with increasing age.
- The larval period in offshore waters can last from 6-12 months or longer and ends at the metamorphosis mark as the leptocephali becomes a glass eel. Glass eels are roughly age-1 when they reach continental waters.
- Age reading on the otolith begins after the identification of the transition mark.
- Age is continental age based on time since arriving in or near freshwater (from the transition mark).
- The elver stage is completed during the early stages of year one in freshwater.
- Max age of American Eels can reach 40 plus years (Jessop 1987 in Canadian waters) but typically is around 3-30 years (Helfman et al. 1987 in the mid-Atlantic), with females being larger and older than males at the time of seaward migration.

Otolith Description

American Eels have relatively small sagittal otoliths that are oval in smaller eels but as they grow they develop a curvature, becoming concave with the increase in size (Figure 9.4.1). The shape also changes with size and the sulcus and rostrum become more pronounced. They are relatively easy to find in the cranium (Figure 9.4.2).

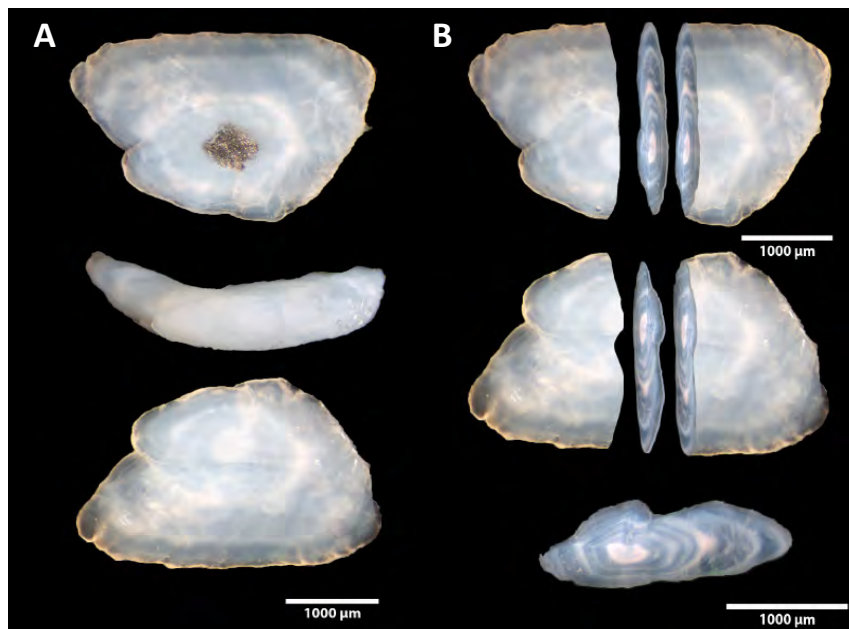


Figure 9.4.1 A) Whole otoliths of American Eel proximal view with core marked (top), dorsal view (middle), and distal view (bottom) and B) otolith sectioned with thin-section located and rotated showing location of cut through the core.

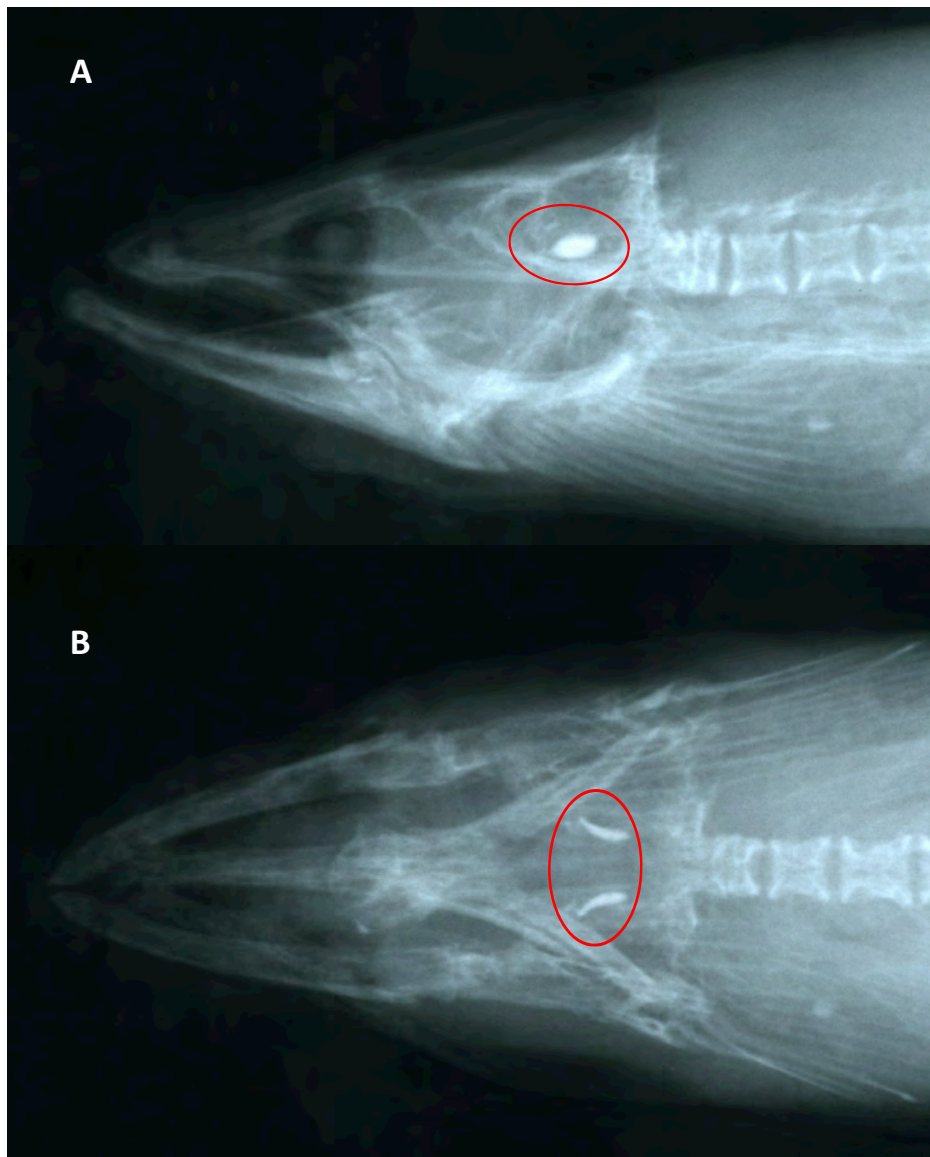


Figure 9.4.2 Radiographs showing location of sagittae in American Eel cranium (red circles) in A) lateral and B) dorsal-ventral views.

Extraction

Extraction of otoliths from the American Eel, regardless of fish size, is routinely performed by slicing through the neurocranium by one of two methods.

Oblique Cut

The preferred method is to make a diagonal cut beginning at the base of the skull moving forward (anterior) and passing below the eyes (Figure 9.4.3A). The top half of the neurocranium can be moved forward exposing the otoliths for removal (Figure 9.4.3B). In the cases where the cut was too shallow, a second cut can be made with less chance of damaging the otolith. Another distinct advantage of this method is that the angle of the cut allows the eel to be placed under a dissection scope to aid in locating the otoliths. This can be a necessity when removing otoliths from small eels even as small as 7 to 8 cm TL.

Transverse Cut

A cut is made approximately two eye-widths behind the eye from the top of the skull downward (Figure 9.4.4A). The anterior portion of the head can then be bent forward (or separated) to allow access to the

otic capsules (Figure 9.4.4B). The drawback of this method is that the otolith is not as readily visible as the preceding method and if not careful, it is possible to damage the otolith when cutting.

Otolith Processing

Upon removal, American Eel sagittal otoliths should be immediately cleaned to remove any clinging tissue that could possibly obscure the view of the core when embedded. Otoliths should be embedded in bullet molds with the medial side down (concave side down). Care must be taken to insure no air is trapped under the otolith when adding epoxy. This embedding configuration provides a more stable base and reduces the possibility of the otolith tilting when adding epoxy to the mold.

Embedded otoliths can then be sectioned on a low speed wafering saw (Section 3.4.2.1) using two blades separated by a spacer with a width of approximately 0.4 mm. The core should first be observed and marked prior to sectioning. The



Figure 9.4.3 Otolith removal using an oblique cut beginning at the base of skull.

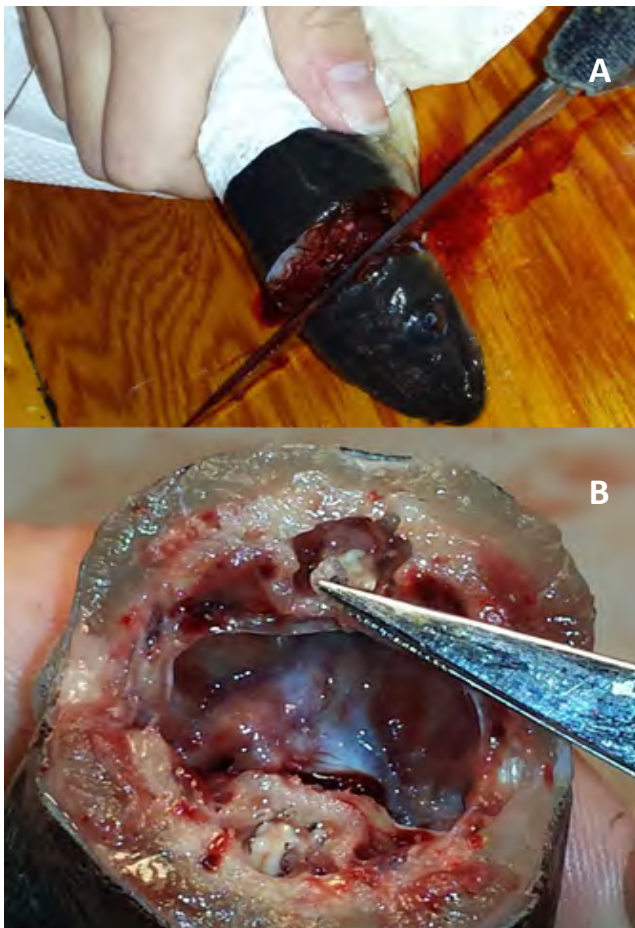


Figure 9.4.4 Otolith removal using a transverse (decapitating) cut.

marked block can then be placed in the saw chuck and the mark aligned so that the core is bracketed by the blades.

Prior to mounting the otolith section on a glass slide, using thermoplastic, any edges of the cut section with high spots (epoxy) should be trimmed with a razor blade or small fingernail clippers. The otolith can then be hand polished with a series of lapping film ranging from 12 to 9 then finally to 3 μ m to remove scratches from the otolith's cut surface.

Enhancement of growth rings can be made by first producing relief in the smooth surface of the otolith section being examined by etching (Chapter 7.0, Section 7.1.2). The section is treated with a 5% EDTA solution for 5-10 minutes and then stained by soaking in a 5% solution of toluidine blue (Figure 9.4.5) for 5-10 minutes.

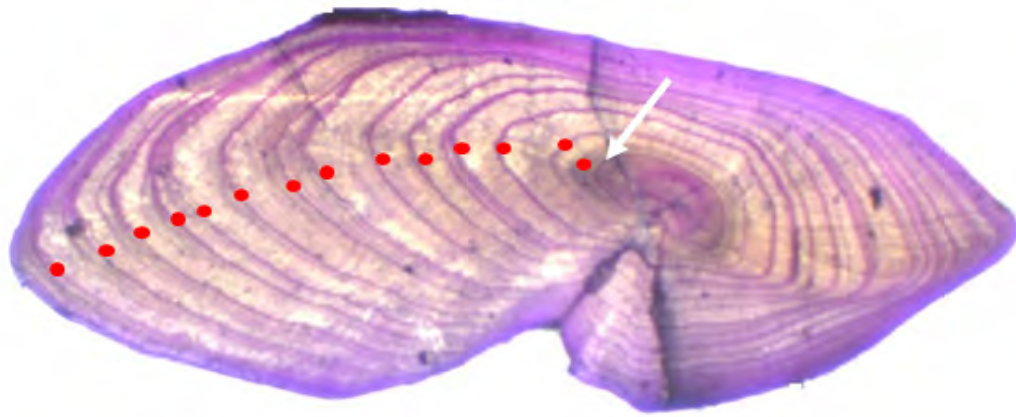


Figure 9.4.5. Transverse section of a sagittal otolith from a 14 year old silver phase American Eel. The transition to freshwater is shown by the white arrowhead and subsequent annuli are indicated by red dots. This section was etched with EDTA and stained with toluidine blue.

Age Determination

American Eels are catadromous, making extensive migrations over their life, which leads to unique issues when ageing this species (Figure 9.4.6). Adults migrate from freshwater to the sea as silver eels to spawn in the Sargasso Sea. Migration to the spawning ground occurs along the north and mid-Atlantic Coasts in the fall and spawning generally occurs from February to April far offshore (Schmidt 1925, Facey and Van Den Avyle 1987, McCleave et al. 1987; Figure 9.4.7). Larvae spend several months entrained in the offshore currents in their leptocephali form until they approach the continental shelf and metamorphose into glass eels (early juveniles) and enter freshwater becoming elvers (late juveniles) and eventually yellow eels, which represents the primary juvenile growth period for as long as 30 years or more (Jessop 1987). Reproductive eels are called silver eels as they prepare for their final migration to the spawning grounds.

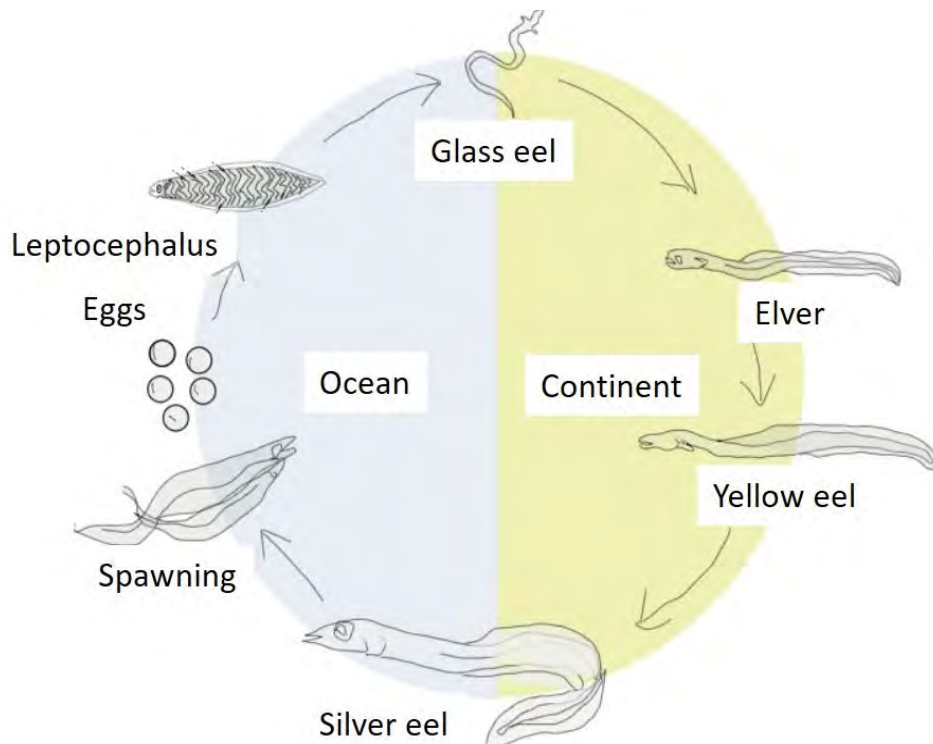


Figure 9.4.6 Life cycle of the American Eel [by Gissurardottir 2006 (Wikipedia Commons)].

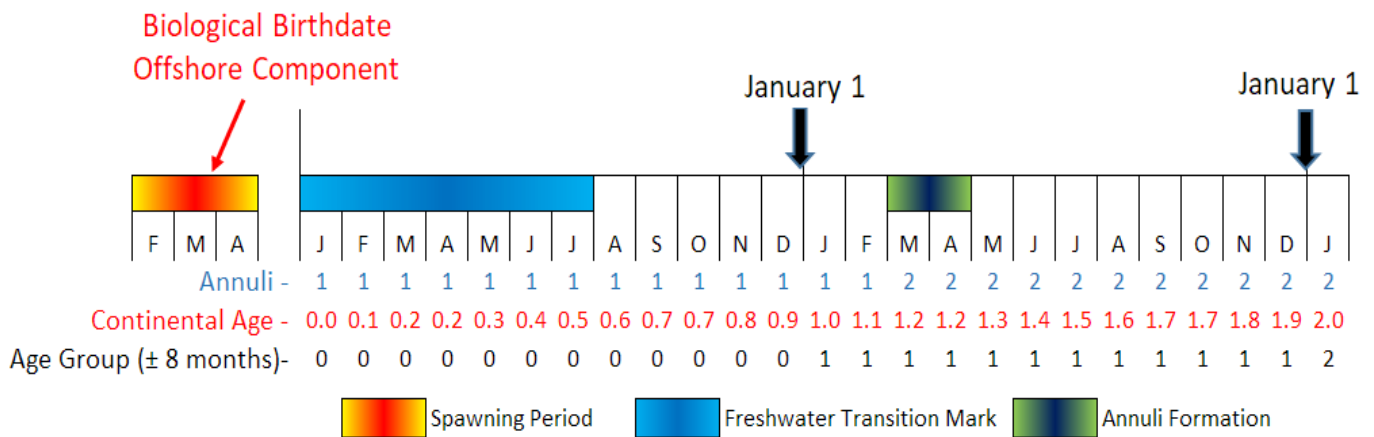


Figure 9.4.7 Birthdate assignment timeline for American Eel. Further work is needed to identify the annulus deposition period, but deposition likely occurs when water temperatures reach 10°C.

As glass eels end their extensive marine larval migration and approach or enter fresh water, a transition mark is formed on the otolith (Lecomte-Finiger 1992, Cieri and McCleave 2001). This transition mark (ring) is distinguished by being the first ring following the metamorphic mark indicating the end of the larval stage (Figure 9.4.8). This ring is used as time zero and eel age estimations are based on years post transition and exclude the larval life of the eel resulting in the ‘continental age’ (Figure 9.4.7). However, glass eels may enter freshwater over several months resulting in high variability of growth between the transition mark and the first annuli making it difficult to pinpoint an ‘average’ growth rate for year-1 eels (Figure 9.4.7).

The formation of annuli in American Eel sagittal otoliths has been validated by Oliveira (1996) examining tetracycline-injected eels marked and recaptured in the wild. Liew (1974) examined annual formation of ‘winter and summer zones’ on American Eel otoliths and frequently found what he called ‘supplementary translucent zones’ which appeared as isolated narrow bands, independent of the winter zone. Likewise, Oliveira found that a number of incomplete ‘rings’ were formed but, like circuli on a scale, could not be followed along the entire otolith. Oliveira noted that “these incomplete rings were located throughout the translucent zone bounded by complete rings.” The timing of annulus formation most likely coincides with the metabolic slowdown that occurs in eels when water temperatures fall below ≈10°C (Oliveira personal observation; Figure 9.4.7). This coincides with ‘temperature extremes’ as triggers for annulus formation suggested by Liew (1974) for American Eel and Deelder (1981) for European Eel (*Anguilla anguilla*). In warmer regions, winter temperatures may not reach levels to significantly reduce growth resulting in less clear or missing annuli in some years. Oliveira (personal observation) found that American Eel otoliths from tropical areas had less discernible annuli or annual growth patterns and did not produce reliable ages.

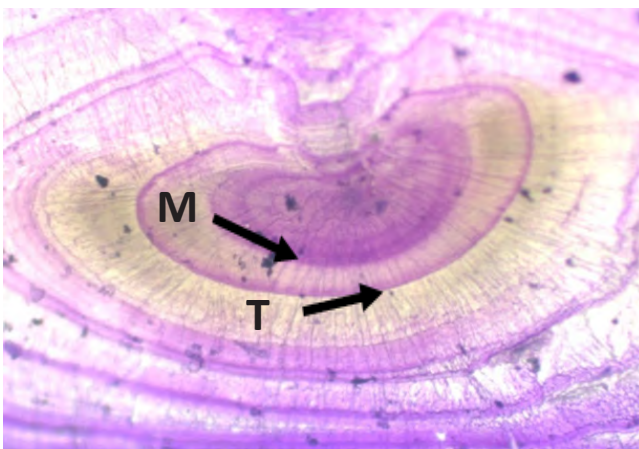


Figure 9.4.8 Transverse section of an American Eel sagittal otolith showing metamorphic (M) and transitional (T) marks.

Other Ageing Methods

Otoliths

A summary of the various techniques and comparison of methodologies for ageing anguillid eels with otoliths is summarized in Vøllestad et al. (1988).

Reading of whole otoliths has been attempted by a number of researchers with various success depending on whether the fish were slow or fast growing (Vøllestad and Jonsson 1988). Vollestad and Jonsson (1988) were able to age European Eels from age-3 to age-18 collected in Norway using their whole otoliths cleaned in 96% ethanol for 18-24 hours.

Peels from etched sagittal otolith sections have been explored by Liew (1974) and Casselman (unpublished data) but has not been widespread in its application. Liew (1974) cut and ground sagittal otoliths from American Eel and etched them with HCL for a few minutes. The otoliths were then placed in acetone and positioned on an acetate sheet which, once dried, provided the 'peel' or inverted microstructure of the otoliths surface.

The break and burn technique has been used on a limited basis for American Eel; however, its success in other anguillid species (Chisnall and Kalish 1993) warrants further examination. In general, otoliths which have been 'cut and burnt' (Figure 9.4.9) demonstrate good contrast between the annuli and could provide useful ageing information in American Eel. The modified cut and burn technique (Graynoth 1999) where otoliths are first cut and then burned is a less labor intensive process than the sectioning-staining and may be advantageous when large numbers of otoliths need to be aged. It should be noted that the ICES (2009) report noted that the break or cut and burn methods are "not appropriate for slow growing eels and it underestimates age of eels older than about five years old (Vollestad and Næsje 1988, Panfili et al. 1994)".

Scales

Scales are deeply embedded in the skin and the delayed timing of formation precludes their use in ageing.

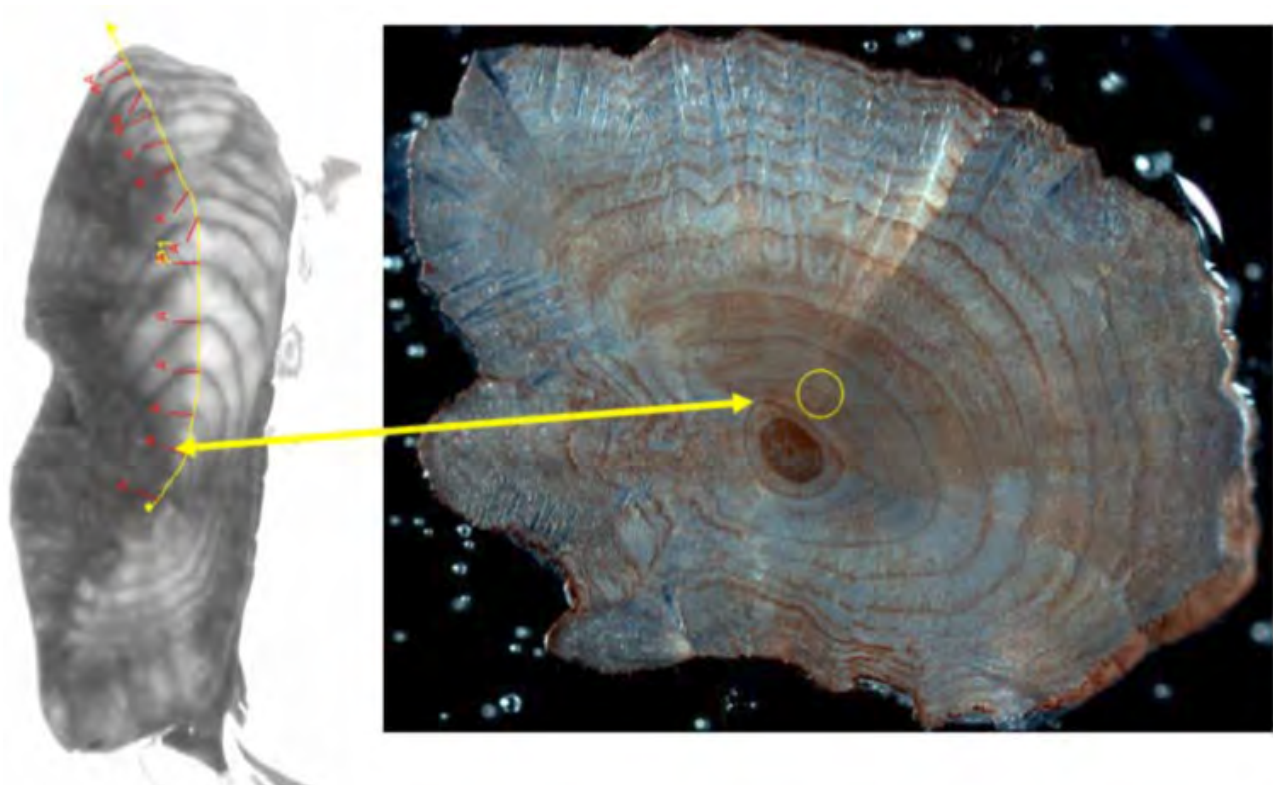


Figure 9.4.9 Examples of 'cut and burnt' age-11 European Eel *Anguilla anguilla* whole otolith (left) and a ground age-11 otolith (right). Red marks indicate annuli and yellow line represents the reading path. Arrow and circle indicate the stress check from initial tag and release in the known age fish (Figure 5.4 from ICES 2009).

Research Needs

Additional work needs to be done on annulus formation throughout the entire range of American Eels from the northeastern Atlantic to the Gulf of Mexico. There is virtually no information on American Eels from the southern U.S. waters.

The break and burn technique has been used for mass producing ages for American Eels collected in St. Lawrence River System (Verreault et al. 2017) and appeared to work reasonably well but needs further work to validate its utility.

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9.5 Moronidae - Temperate Basses



Striped Bass *Morone saxatilis*

Highlights

- Long-lived species, otoliths aged to 31 years old (ODU) and scales aged to 23 years old (ODU).
- Scales are the accepted ageing structures up to approximately 800 mm (age 10). At this length, scale edges become difficult to interpret. Scales often contain false checks which may be difficult to discern from annuli.
- Sectioned otoliths are the accepted ageing structures for specimens over 800 mm.

Otolith Description

Striped Bass otoliths (Figure 9.5.1) are relatively large and easily extracted from specimens. The location of the otoliths in the neurocranium is illustrated in Figure 9.5.2.

Otolith Extraction

Striped Bass otoliths can withstand expected impacts from otolith extraction devices without breaking. For most sizes of Striped Bass, any of the techniques in Chapter 3, Section 3.2.1 will work. On most Striped

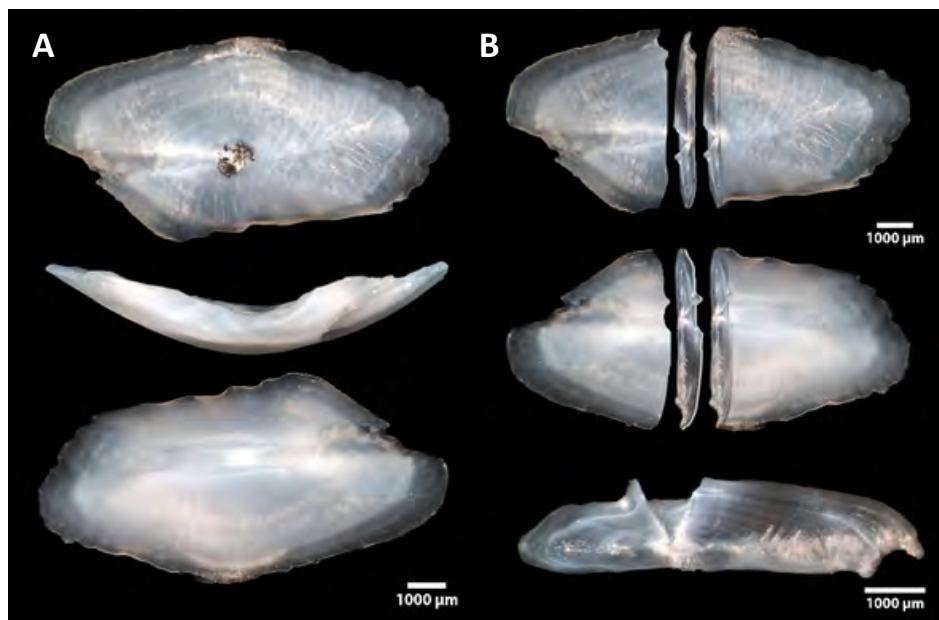


Figure 9.5.1 A) Whole otoliths of Striped Bass proximal view with core marked (top), dorsal view (middle), and distal view (bottom) and B) Striped Bass otolith sectioned with thin-section located and rotated showing location of cut through the core.

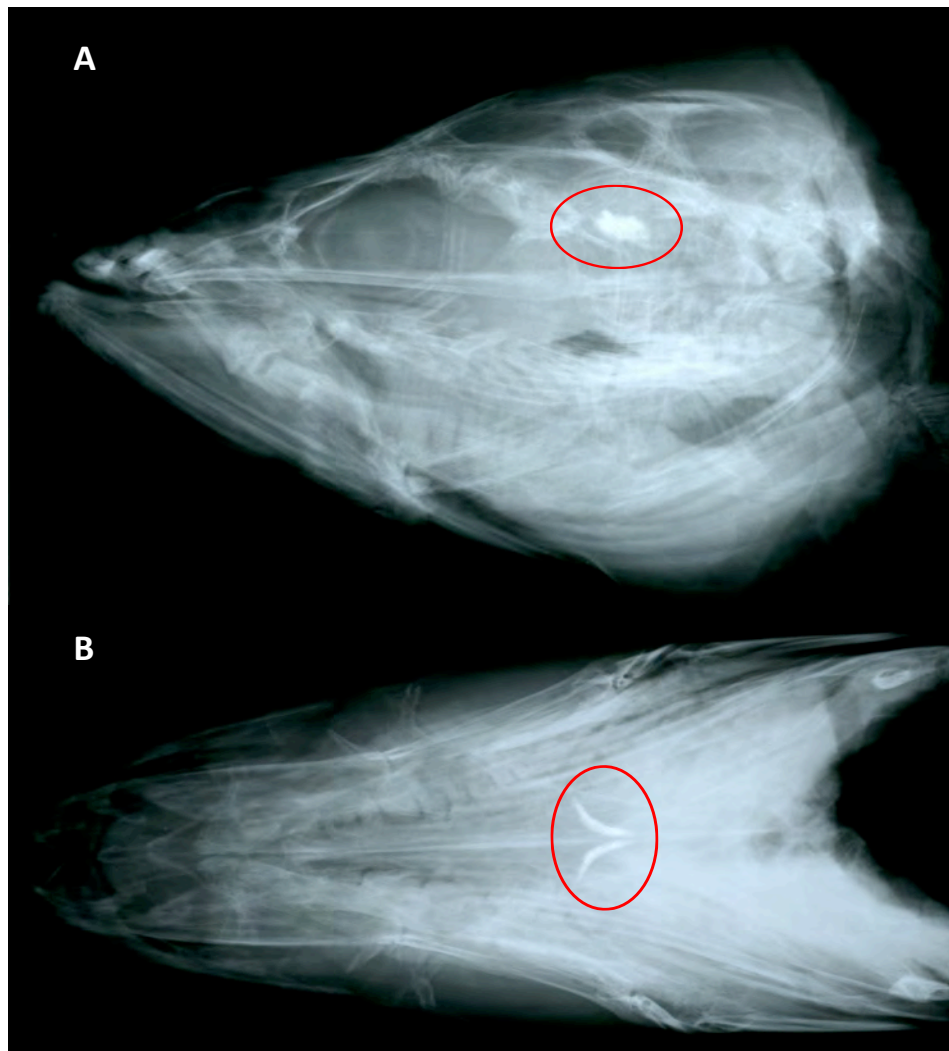


Figure 9.5.2 Location of sagittae (red circles) in the neurocranium of Striped Bass in A) lateral and B) dorsal-ventral views.

Bass, otolith removal is best accomplished using a hacksaw or electric saw and a regular knife may be used on juveniles, making a horizontal cut through the top of the brain. The Massachusetts Division of Marine Fisheries has developed a short video demonstrating the removal of the sagittal otoliths in Striped Bass (Chapter 12.0, Section 12.7). They also may be extracted through the opercular cavity, especially if visible damage must be reduced. In addition to the opercular method outlined in Chapter 3.0, Section 3.2.1, otoliths have been successfully removed from under the opercula of Striped Bass using a hole saw attached to a cordless drill. Care must be taken when storing the otoliths prior to processing. Due to the curvature of the otolith, it is prone to breakage if bound too tightly together when storing.

Otolith Processing

General sectioning in Chapter 3.0, Section 3.6 and polishing procedures noted in Chapter 7.0, Section 7.1.1 should be utilized for Striped Bass otolith processing. Critical to these procedures is the use of a precision low-speed saw. The recommended sectioning thickness is 0.5 mm and the slow speed saw is the preferred equipment but other equipment can be used based on what is available. Some labs choose to follow the 'bake and thin section' technique outlined in Chapter 7.0, Section 7.1.6, or a modified version of it, as a method of enhancing the visualization of the annuli.

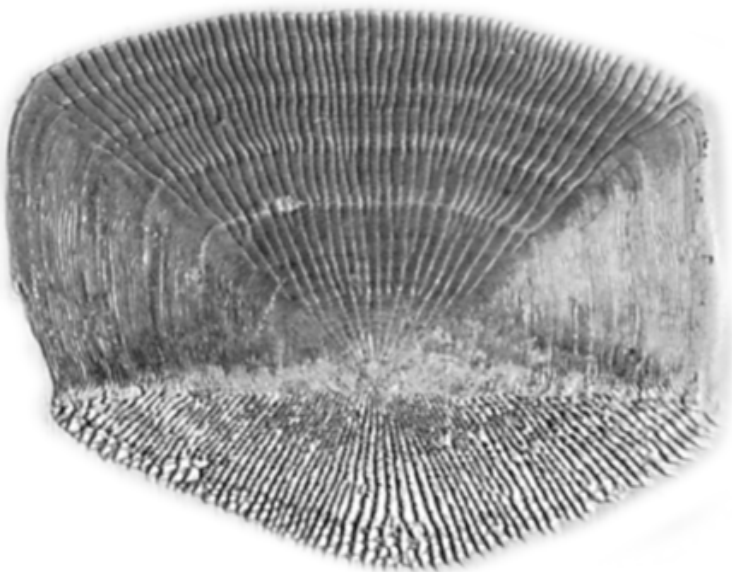


Figure 9.5.3 Striped Bass scale. *From Bobko (2002).*

Scale Description

Striped Bass scales are ctenoid, with radii in the anterior field (Figure 9.5.3). Circuli formation ceases in early winter.

Scale Removal

Scales should be removed from the side of the fish between the dorsal fin and the lateral line, and between the origin of the second and insertion of the first dorsal ray (Figure 9.5.4). Several scales (one dozen if possible) will allow ageing comparison between scales to verify false checks and allow for discards of regenerated or otherwise unusable scales.

Scale Processing

Striped Bass scales are typically used to create impressions following the methods

outlined in Chapter 4. NYSDEC uses a Carver Press[®] set at 170° F, 20,000 lbs. for five minutes, whereas, RIDEM uses the same press set at 100° F, 5,000 lbs. for 10 minutes. Similar combinations of temperature, pressure, and time may be determined through trial and error.

Age Determination

Annual growth zones on scales and otolith sections have been verified by Merriman (1941; scales to age-3) and Secor et al. (1995b; otoliths). Based on data and discussions at the March 2003 Striped Bass Ageing Workshop, it was determined that scales or otoliths may be used to age Striped Bass less than 800 mm (approximately age-10), but ideally sectioned otoliths should be used to age specimens larger than 800 mm. July 1 should be adopted as the last date to see new annuli on scales and otoliths. Fish caught in the spring are anticipated to form an annulus before that date (age assignment issue) (ASMFC 2003).



Figure 9.5.4 Region of Striped Bass to remove scales for ageing.

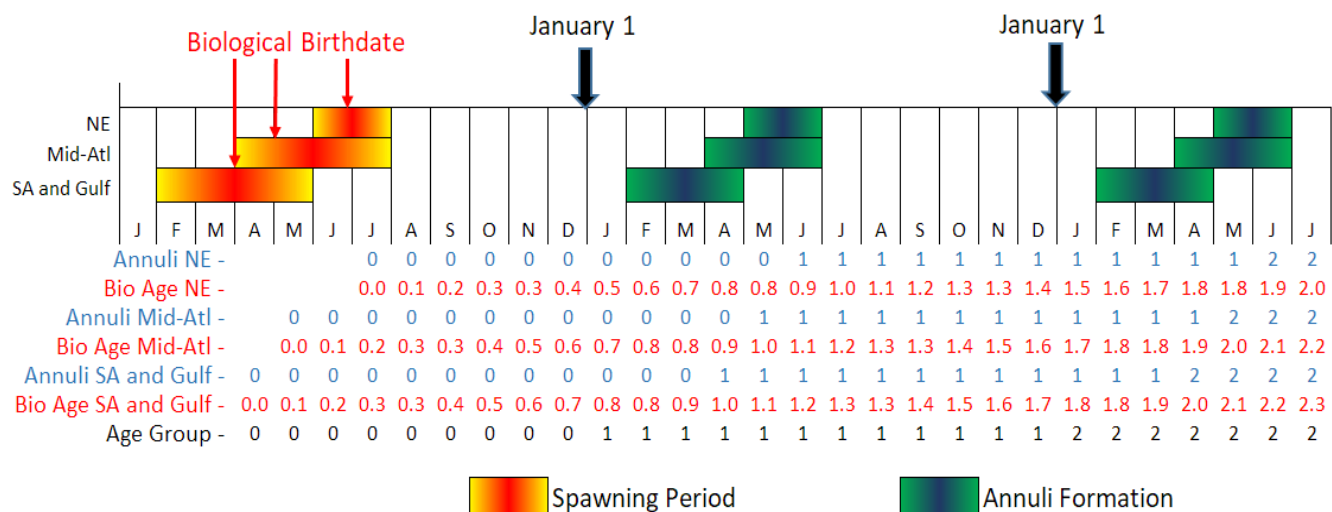


Figure 9.5.5 Spawning periodicity and age assignment timeline for Striped Bass from New England to the Gulf of Mexico.

Geographic differences are noted in spawning times for this species (Figure 9.5.5). In the southern portion of its range (FL – NC), Striped Bass spawn between mid-February and May. In the Chesapeake area, spawning occurs generally between April and early June. In the Delaware and Hudson River areas, spawning occurs between May and mid-July, and at the northern end of its range (New England – Canada) spawning occurs in June and early July (Setzler et al. 1980). In the Gulf of Mexico, Striped Bass spawn from February to May (Barkuloo 1970) with peak spawning occurring during early April to mid-May (Crateau et al. 1980).

Otoliths

Otolith annuli are concentric zones within the structure, each comprised of a translucent growth band and an opaque band. Annuli are counted along the distal edge of the sulcus acusticus (Figure 9.5.6).

Scales

Scale annuli are defined as concentric zones that are continuous around the entire anterior and lateral fields, to the baseline of the scale. Clear identification of “cutting across” should be noted throughout the anterior and lateral fields (Figure 9.5.7). Scales can also have false annuli (check marks) which do not appear in the otolith cross section. As with many other species, edge crowding is noted in older specimens.

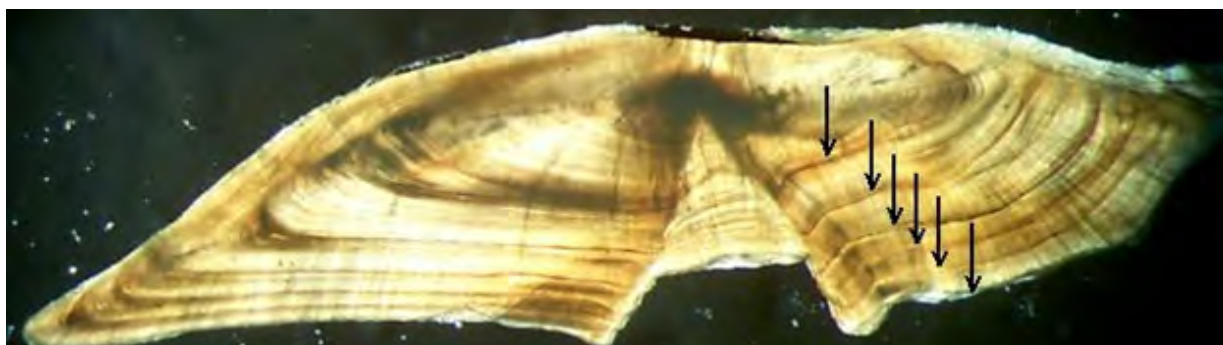


Figure 9.5.6 Otolith section from an age-6 Striped Bass. Arrows indicate annuli.

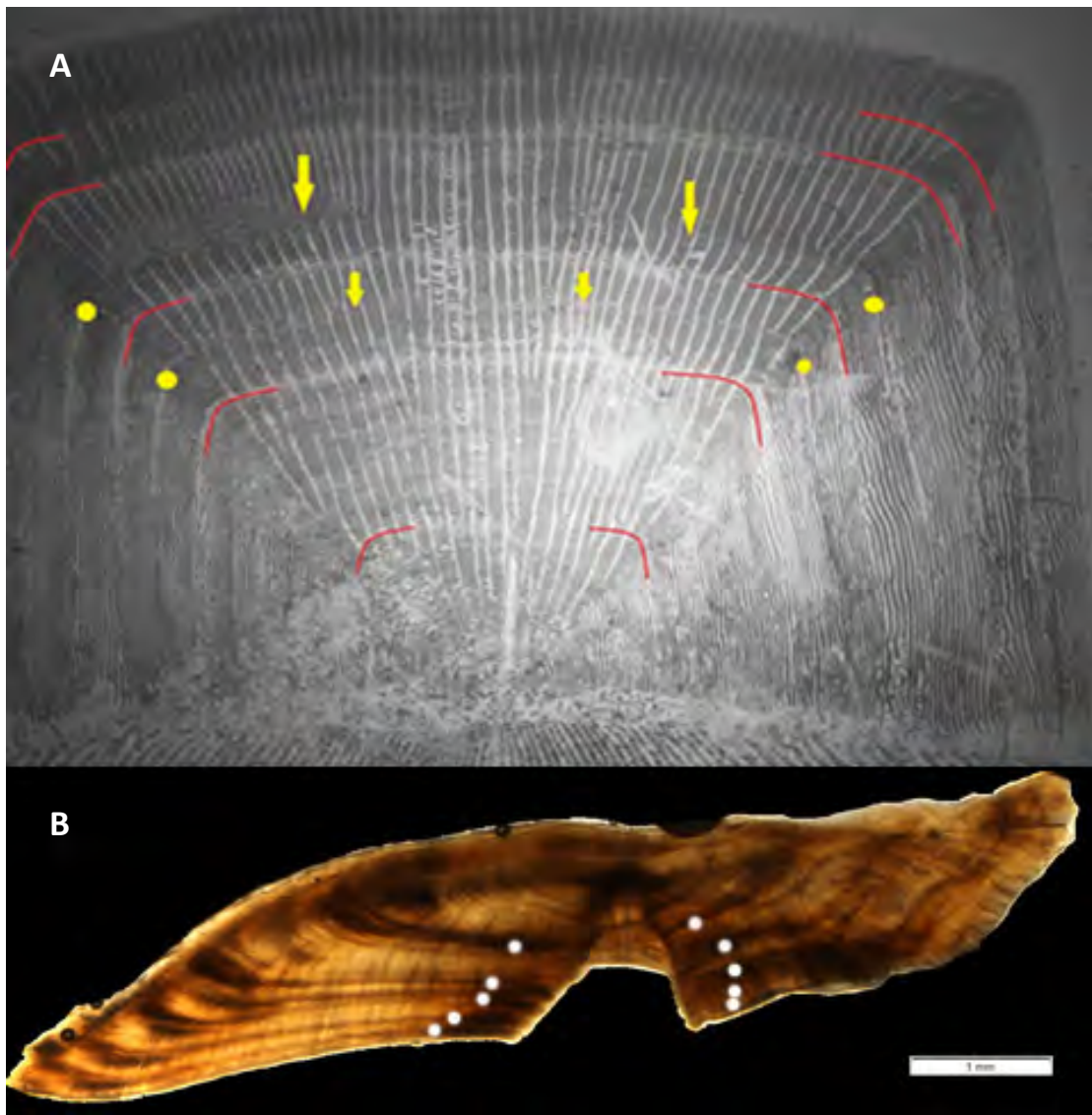


Figure 9.5.7 A Striped Bass scale and otolith section from the same fish. A) The scale shows five annuli, marked in red, and two false annuli, marked with yellow dots and arrows, B) the otolith section shows five annuli, marked with red.

Other Ageing Methods

Welch et al. (1993) compared the use of otoliths, scales, anal fin spines and rays to age Striped Bass. The ages for Striped Bass less than 900 mm TL were typically within one year for all structures. For samples larger than 900 mm, the spine and scale ages were 1.6 and 3.0 years lower than otoliths, respectively.

Research Needs

Striped Bass have been well researched, however the ASMFC 2018 benchmark stock assessment (NEFSC 2019) recommends that the collection of paired scale and otolith samples be continued to facilitate the development of the age-length key and scale-otolith conversion matrices.

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9.6 Clupeidae

9.6.1 Menhaden



Gulf Menhaden *Brevoortia patronus*



Atlantic Menhaden *Brevoortia tyrannus*

Highlights

- Scales and otoliths are both used to age menhaden but otolith/scale comparisons should routinely be conducted.
- Scales may be read directly without making acetate impressions and are the preferred method of ageing.
- Sagittal otoliths are small and fragile, so care must be taken when removing.
- Otoliths may be read whole but older fish may need to be embedded and cut.
- Gulf Menhaden live to age 5-6 (Ahrenholz 1991) but the majority of fish aged are 1-2 yrs.
- Atlantic Menhaden live to age 10-12 (Ahrenholz 1991) but the majority of fish aged are 1-3 yrs.

Scale Description

Unlike most herrings, adult Gulf and Atlantic Menhaden scales are ctenoid, where the posterior margin of Gulf and Atlantic Menhaden scales are pectinate (Figure 9.6.1.1). The anterior field is embedded in the integument. The entire scale is sculptured with fine circuli, which roughly parallel the anterior margin (June and Roithmayr 1960). A baseline is found where the anterior and posterior fields meet. Gulf and Atlantic Menhaden scales are generally thin and translucent and Atlantic Menhaden scales are generally larger than those from Gulf Menhaden. Scales are the preferred method of ageing for Atlantic and Gulf Menhaden.

Scale Removal

A blunt-edged scalpel is used to remove scales from menhaden using the techniques described in Chapter 4.0, Section 4.2. A scale patch should be taken from the median lateral band above the lateral line and below the dorsal fin (Figure 9.6.1.2). Scales taken from this location are the

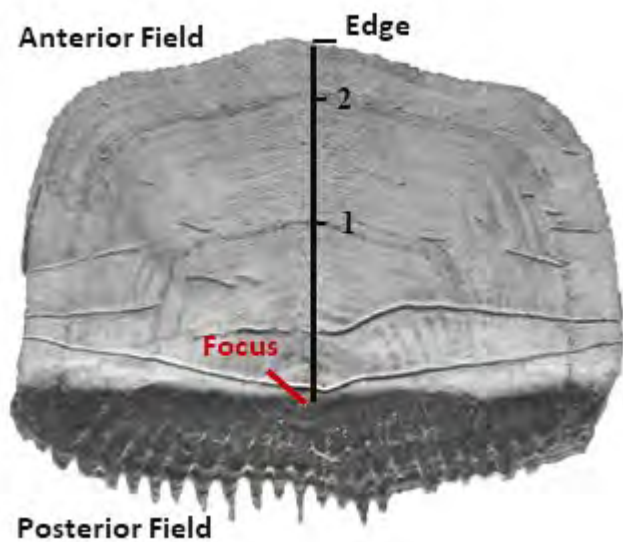


Figure 9.6.1.1 Menhaden scale showing two annuli in anterior field.

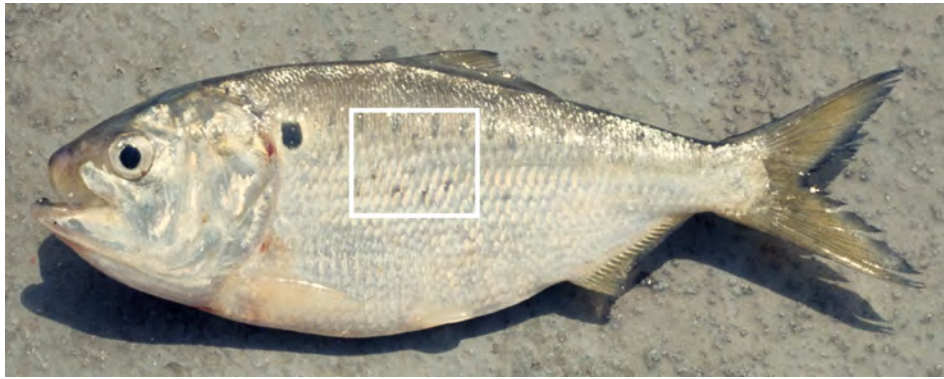


Figure 9.6.1.2 White box indicates area for scale removal on Gulf and Atlantic Menhaden.

most legible because they are the largest and the most symmetrical (nearly rectangular). The scale patch (20-30 scales) is placed in a small, labeled vial of water solution. Water solution can be made by mixing a few drops of dishwashing detergent with water in a wash bottle, which helps degrade residual slime on the scales.

Scale Processing

The scale patch is removed from the vial with recurved forceps and blotted dry on a paper towel. Scales are rubbed between the thumb and forefinger or middle finger to remove any residual integument. Individual scales are pulled from between the thumb and fingers, and then mounted between two glass microscope slides (Figure 9.6.1.3). Scales that were stored dry can be cleaned following the methods described in Chapter 4.0, Section 4.3.1.

Otolith Description

Sagittal otoliths are small and fragile with a well-defined rostrum. A translucent focus is surrounded by an opaque core and alternating concentric translucent and opaque bands, where the narrow, opaque bands are presumed to be annuli. The NOAA Fisheries Beaufort Lab began monitoring the Gulf and Atlantic Menhaden purse-seine fishery for catch size and age composition in the 1950s by sampling and looking at otoliths and scales (Nicholson 1978). While otoliths can be used to age menhaden, the Beaufort Lab determined that it was impractical to utilize otoliths to age menhaden for assessments because of the volume of fish required to be sampled. Otoliths were eliminated as the preferred ageing structure because 1) sagittae were so small and fragile (Figure 9.6.1.4A), and 2) large amounts of time and effort would be required to extract, process, and read the 10,000 or more whole or sectioned otoliths required to adequately characterize the large fishery.

Otolith Removal

Similar to most of the smaller species like the genus *Alosa*, menhaden otoliths are relatively easy to locate in the cranium (Figure 9.6.1.4B and 9.6.1.4C) but require patience due to their small, fragile nature. Extraction methods include vertical incisions down the posterior of the head or recovery on larger specimens through the operculum (Figure 9.6.1.5A-E). These techniques are further detailed in Chapter 3.0, Section 3.2.1.

Otolith Processing

Otoliths should be clean and dry prior to ageing. Otoliths freshly removed from fish will appear slightly translucent and will be much



Figure 9.6.1.3 Ten menhaden scales mounted between slides for direct ageing of raw scales.

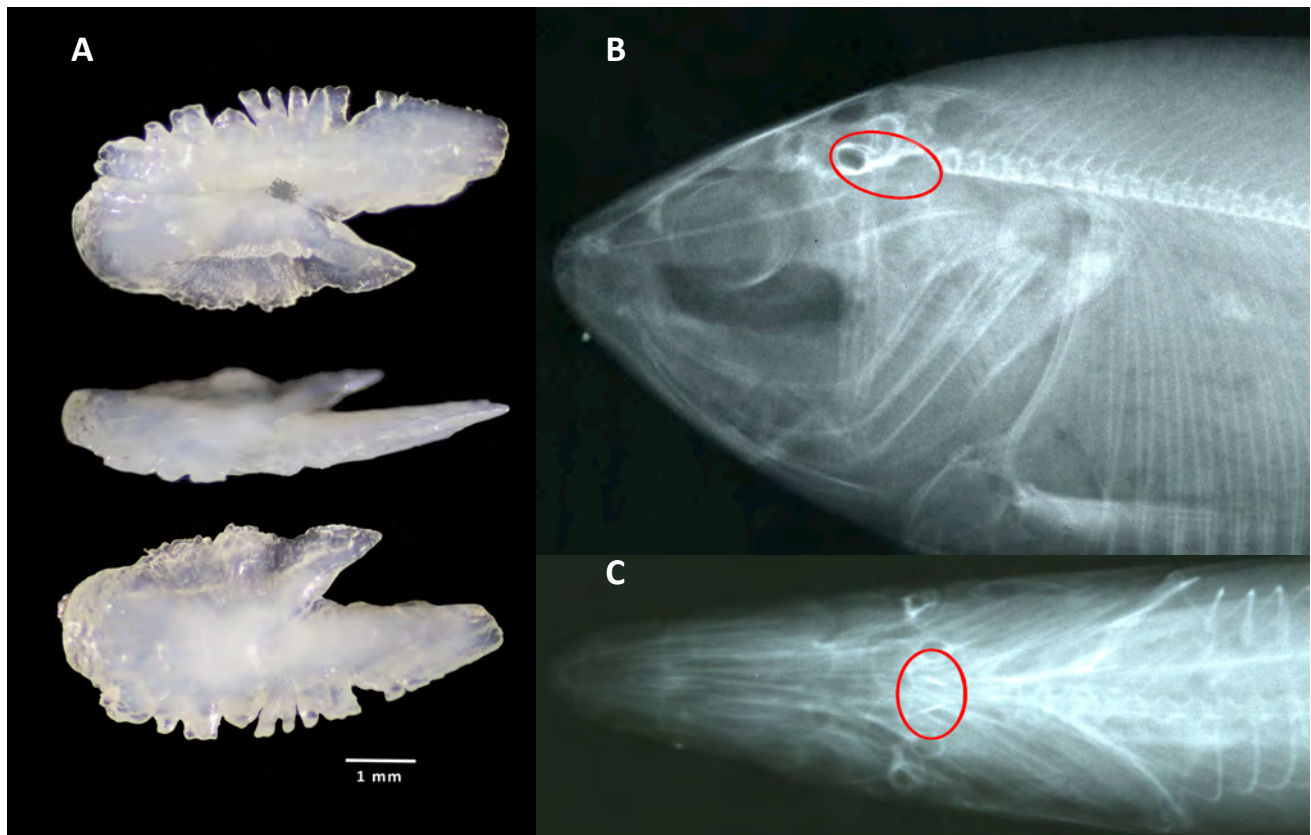


Figure 9.6.1.4 A) Whole otoliths of Gulf Menhaden proximal view with core marked (top), dorsal view (middle), and distal view (bottom). Radiographs of Gulf Menhaden showing otoliths in B) lateral and C) dorsal-ventral views (red circles).

more difficult to age than otoliths that have had several hours to air dry. Otoliths can be read whole if legible (Chapter 3.0, Section 3.2.4).

A whole otolith may be polished through the top surface to expose the annuli when covered with immersion oil or water (Figure 9.6.1.6). Gulf Menhaden are short lived so reading the otolith whole is the preferred method. Whole Atlantic Menhaden otoliths may require polishing if the older annuli begin to crowd the margin. Caution should be taken anytime a whole otolith is polished or the edge can be removed if taken too far resulting in polishing past the middle and losing annuli on the edges.

Another way to prepare menhaden otoliths for ageing is to embed and section them (see Chapter 3.0, Section 3.2.5). An embedded menhaden otolith is cut near the core and polished down to the focus rather than generating a thin-section. However, because of the size and fragile nature of menhaden otoliths, breaking otoliths is a frequent occurrence and should be considered before using this technique.

Age Determination

Scales

Gulf and Atlantic Menhaden scales can be viewed on a stereomicroscope, projector or a microfiche reader. Scales can be read dried and mounted between microscope slides and do not require acetate impressions. Annuli are defined as compressions or interruptions of uniformly spaced circuli in the anterior field of the scale, which are continuous through the lateral fields. Under transmitted light, annuli form narrow, continuous, dark bands roughly paralleling the lateral and anterior margins of the scale. Due to a lack of discernible markings, the focus of the scale is identified as the midpoint of the scale's baseline.



Figure 9.6.1.5 Otolith removal from Gulf Menhaden. A) Make a vertical cut down the pre-opercular margin. B) Remove the brain. C) Carefully remove the nerve mesh, and D) the otoliths will come out with it. An alternative is to E) make a shallow cut horizontally across the top of the brain cavity revealing the otic capsules (white arrows). The otoliths (black arrows) should be visible and can be carefully removed. **Note:** A deep cut will cut into the otoliths.

Straight-line measurements are made from the focus to successive scale annuli and the scale edge (Figure 9.6.1.1). Annuli are usually fully formed by June 1. The first annulus is usually not seen less than 1.2 mm from the focus. Each consecutive annulus is found roughly half the distance of the previous two annuli

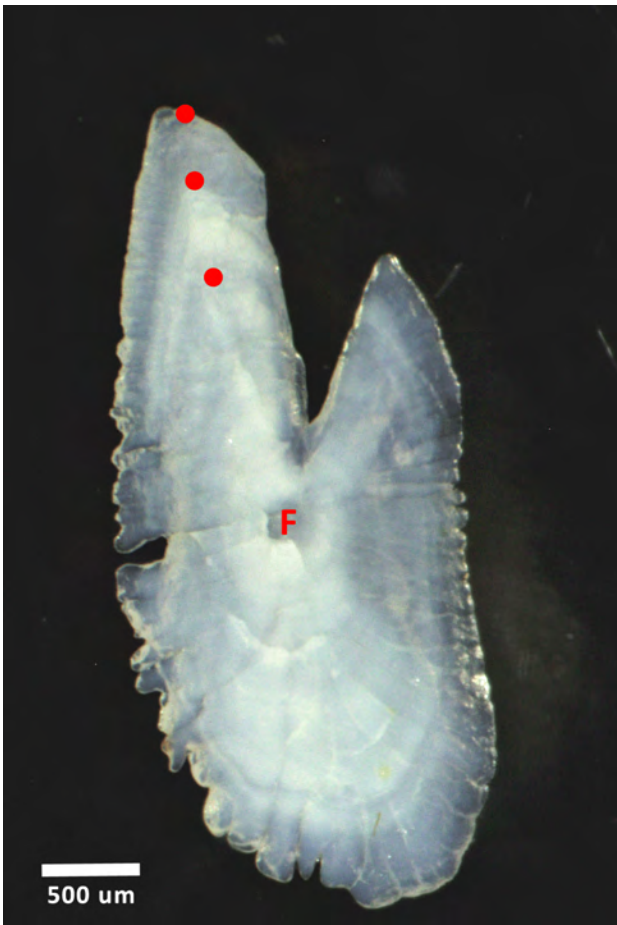


Figure 9.6.1.6 A whole otolith from an age-2 Atlantic Menhaden viewed with reflected light. The annuli and edge are indicated in red.

but crowding of annuli near the scale's edge may be seen in older fish. False annuli are not continuous, do not cross the baseline and do not appear on every scale. Some fish may be aged from length frequency (Nicholson and Schaaf 1978).

Otoliths

Otoliths can be viewed on a stereomicroscope with reflected light, dry or with a drop of immersion oil or some similar solution to enhance the annuli (Figure 9.6.1.6). Whole otoliths may also be read on a dissecting microscope against a dark background with reflected light in a small watch glass of water. Care should be taken to avoid leaving the otolith in water too long as it can be cleared, eliminating any annuli. Annuli are counted as the transition between opaque and translucent bands and should be continuous. They are usually counted from the focus outward through the rostrum and/or the postrostrum and pararostrum. Otoliths can be blurred slightly to enhance the annuli amid other structures on the surface. Producing a negative of an otolith image can have the same effect.

Gulf Menhaden

Gulf Menhaden spawn between October and April, with peak activity from December through February (Turner 1969, Fore and Baxter 1972, Warlen 1988, Brown-Peterson et al. 2017) and by convention, the birthdate for Gulf Menhaden is January 1 (Figure 9.6.1.7). Lewis and Roithmayr (1981) concluded that spawning occurs for the first time at age-1 as the fish

approach their 'arbitrary' second birthday. Lassuy (1983) suggested, however, that some large, young-of-the-year (YOY) fish may become sexually mature at age-0. Gulf Menhaden can live to age 5-6 (Ahrenholz 1991) but the majority of fish aged are age 1-2. Annulus formation on scales has been shown to occur in March/April (NOAA Fisheries Beaufort Lab) and on sagittal otoliths in May/June (Smith and Levi 1991).

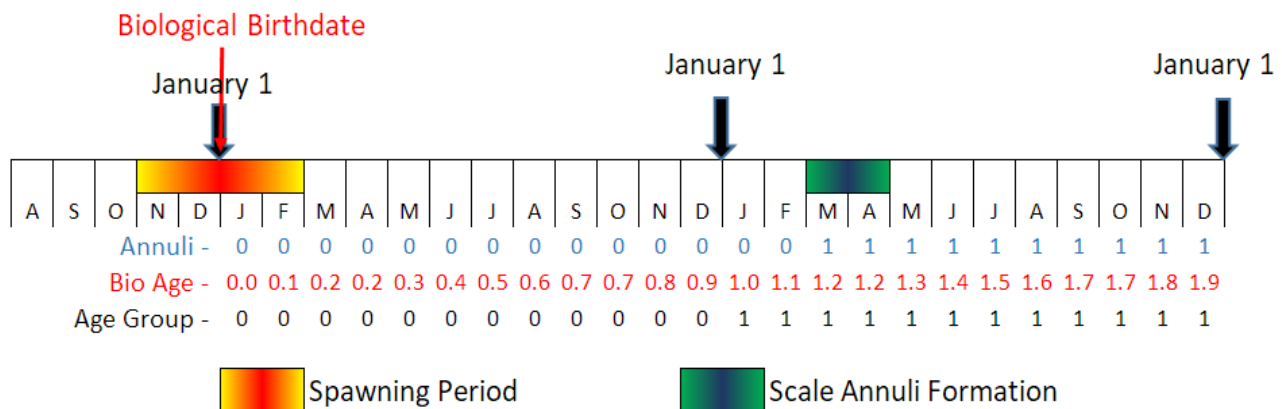


Figure 9.6.1.7. Timeline showing spawning period and annulus deposition on scales for Gulf Menhaden.

Atlantic Menhaden

Atlantic Menhaden spawn primarily from January to March in the South Atlantic Bight. In fall, spawning also occurs as fish swim southward in September, in the more northern range (Figure 9.6.1.8). Spawning intensity increases October through November as the fish move farther south and is believed to peak off the coast of North Carolina in the winter. As fish move north the following spring and summer, spawning decreases (Higham and Nicholson 1964, Kendall and Reintjes 1975, Judy and Lewis 1983, Ahrenholz 1991). Recent work sampling menhaden larvae from North Carolina to Massachusetts suggests that overall, larvae are present all months except July and August (Simpson et al. 2016) though spawning primarily occurs October through March. By convention, the birthdate for Atlantic Menhaden is March 1. Atlantic Menhaden can live to age 10-12 (Ahrenholz 1991) but the majority of fish aged are age 1-3. Annulus formation on scales and otoliths in Atlantic Menhaden occurs from February through May depending on latitude with earlier formation in the south and a slight lag to the north.

Validation

June and Roithmayr (1960) validated the formation of the first annulus in Atlantic Menhaden scales from captive fish and provided supporting evidence through scale measurements and frequency distribution modes that only one annulus is deposited each year. Kroger et al. (1974) confirmed the formation of the first annulus in captive Atlantic Menhaden. Nicholson and Schaaf (1978) reviewed the ageing of Gulf Menhaden caught in the purse-seine fishery by looking at length-frequency distributions, tags returned from juveniles in reduction plants and scale observations. They concluded that the counted scale annuli on fish aged 1-2 years old were true annuli, validating annuli formation for these ages.

Gulf and Atlantic Menhaden have long spawning seasons, so readers may observe different growth patterns on scales at different times of the year. In addition, there is great variation in length-at-age, especially in Atlantic Menhaden. To help validate the frequency of annulus formation, marginal increment analysis (MIA) was conducted for Gulf Menhaden (ages 1-2; 1998-2011) and Atlantic Menhaden (ages 1-3; 2000-2015) from the purse-seine fishery scale samples. The Atlantic data includes some samples captured outside the usual purse-seine fishery season.

Marginal increments for age-1 Gulf Menhaden increased gradually from April through September (Figure 9.6.1.9A). Similarly, marginal increments for age-2 Gulf Menhaden also increased spring through August, then slightly decreased through October (Figure 9.6.1.9B). A similar trend was seen in Atlantic Menhaden, where mean marginal increments increased through the summer. In ages 1-3, mean marginal increments increased from May through September. Age-1 Atlantic Menhaden mean increments leveled off through December before slightly increasing into January (Figure 9.6.1.10A), while age-2 decreased after December (Figure 9.6.1.10B). Age-3 Atlantic Menhaden displayed a decrease in mean marginal

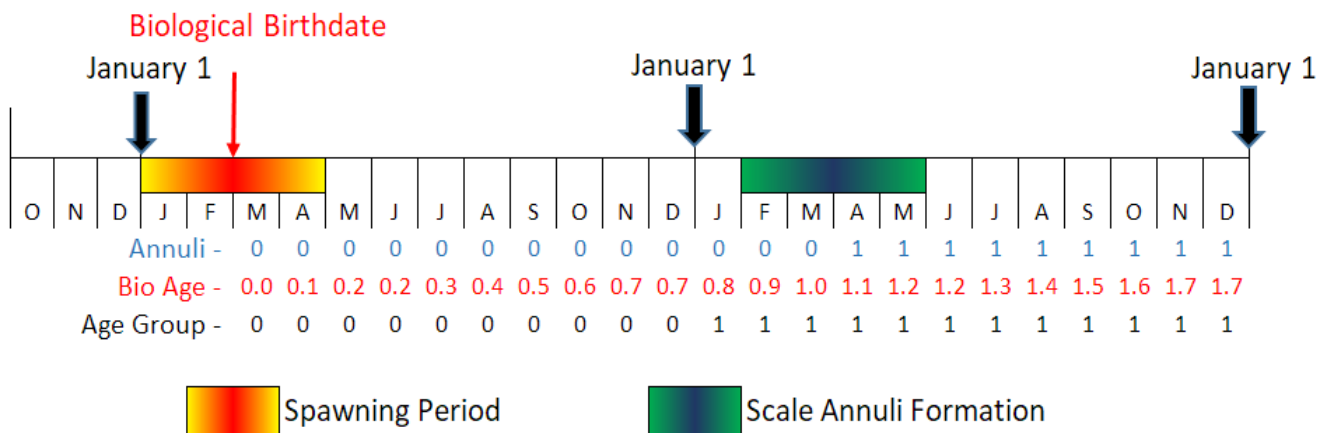


Figure 9.6.1.8 Timeline showing spawning period and annulus deposition on scales for Atlantic Menhaden along the Atlantic.

increments in October, before reaching a peak in December of 0.43 mm (Figure 9.6.1.9C). These results seem to support the assumption used in current ageing methods that both species have fully deposited their annuli by June 1.

The analysis on the ratio of mean marginal increments to the width of the previous growth indicates that the width of growth on scale given any year is roughly a half of the one in the previous year. This agrees with the general rule used by NOAA Fisheries ageing staff for reef and snapper/grouper complex fish. However, margin codes are not generally used while reading scales but margin edge is considered before assigning a final age for assessments and should be recorded using the standard scale margin coding system (Chapter 8.0, Section 8.3).

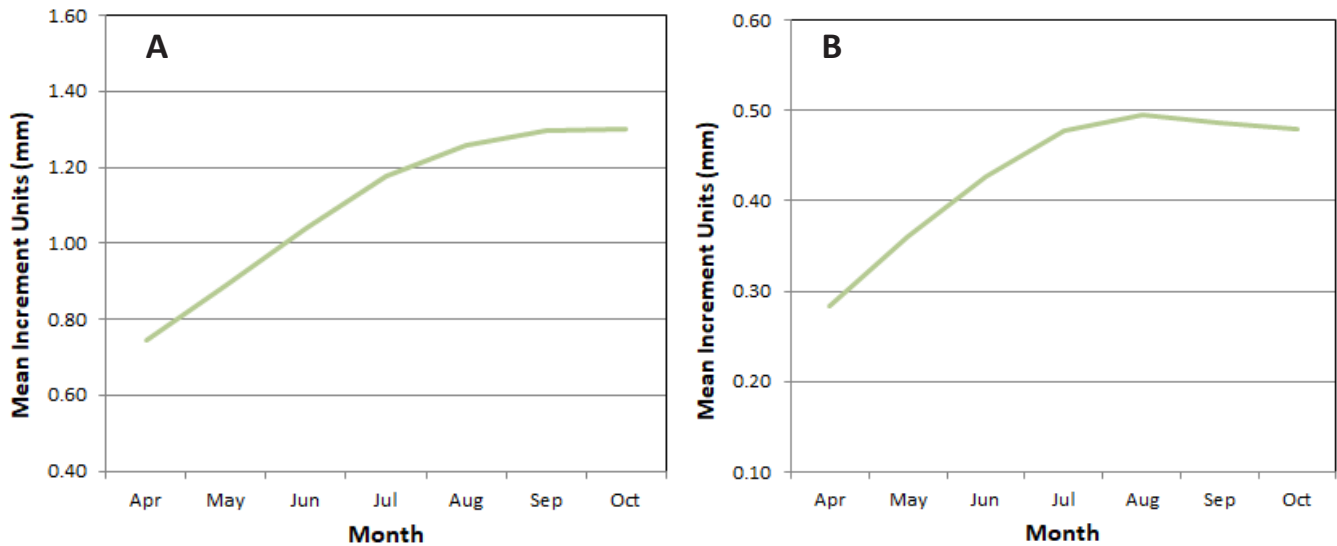


Figure 9.6.1.9 Mean marginal increments in scales for A) age-1 and B) age-2 Gulf Menhaden from the purse-seine fishery port samples, by month, 1998-2011 (NOAA Fisheries unpublished data). **Note:** y-axis scales change between panel A and B.

Scale/otolith comparisons of Gulf Menhaden conducted by the NOAA Beaufort Lab in the early 1990s (Smith and Levi 1991) concluded that scales and otoliths are valid ageing structures. They found that opaque zones formed in May and June in sagittal otoliths and were followed by a translucent zone on the edge, becoming widest during winter. A similar pattern was observed in Gulf Menhaden scales. However, age estimates differed among some paired ages when comparing scales and otoliths and may have been in part to illegible samples (Smith and Levi 1991). Old Dominion University looked at scale-to-otolith comparison with Atlantic Menhaden, indicating good agreement between paired scale and otolith age estimates age-0 and age-1 (SEDAR 2015). A more comprehensive study in the future may be conducted by Beaufort NOAA staff comparing otoliths and previously read scales collected in the 1990s.

Research Needs

More research is needed to explore paired scale/otolith age comparisons in menhaden. The same equipment should be used for each age estimate and reader agreement and processing time should be recorded while doing structure comparisons. Results from this research and considerations for production ageing will lend more insight to the best way to characterize menhaden fisheries in the future. Increased ageing and examination of bait samples from state agencies, outside the purse-seine fishery areas and season, will help identify trends and fill gaps in assessments for fishery-independent samples.

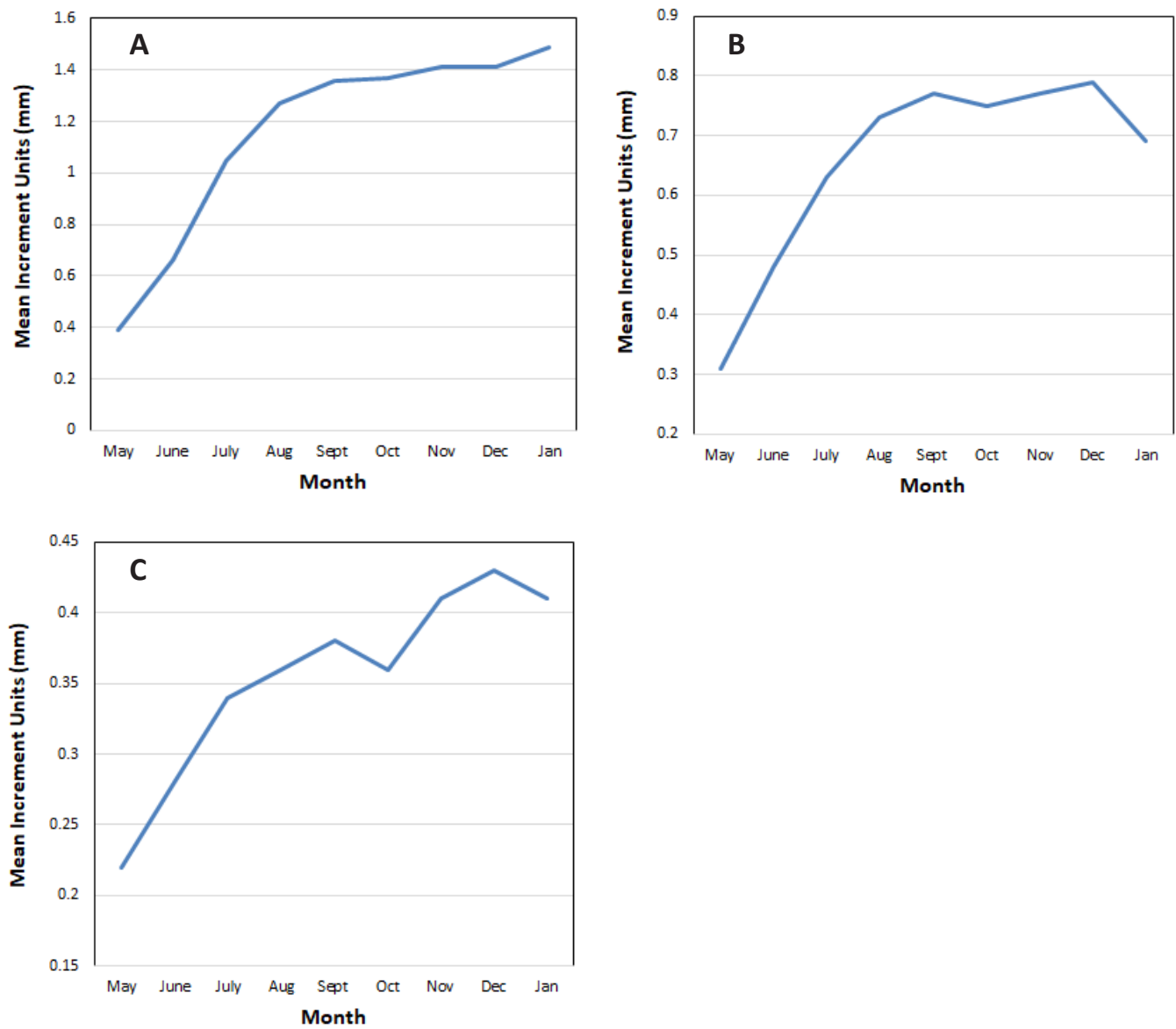


Figure 9.6.1.10 Mean marginal increments in scales for A) age-1, B) age-2, and C) age-3 Atlantic Menhaden from the purse-seine fishery port samples, by month, 2000-2015 (NOAA Fisheries unpublished data). **Note:** y-axis scales change between panels A, B, and C.

9.6 Clupeidae

9.6.2 River Herrings



American Shad *Alosa sapidissima*



Alewife Herring *Alosa pseudoharengus*



Blueback Herring *Alosa aestivalis*

Highlights

- *Alosa* otoliths are small (<5 mm) and fragile but relatively easy to remove.
- Scales and whole otoliths can be used for ageing but otolith ages are the most precise.
- Scales can provide information regarding repeat spawning.
- The majority of the fish aged are under seven years old.
- Maximum age of Alewife, 9 (MADMF), Blueback Herring, 10 (MADMF), and American Shad, 11 (MADMF), based on otoliths.

Otolith Description

Otoliths (sagittae) from the herring family are thin with a well-defined rostrum (Figure 9.6.2.1). Crystallized otoliths are seen somewhat frequently and are often broken during removal. The relative position of the sagittae in the neurocranium is illustrated in Figure 9.6.2.2. These three *Alosa* species are commonly referred to in a group as “River Herring.”

Otolith Extraction

The sagittal otoliths are thin and may break during extraction if handled roughly. Removal of both otoliths is preferred as it is not uncommon to find that one is crystallized.

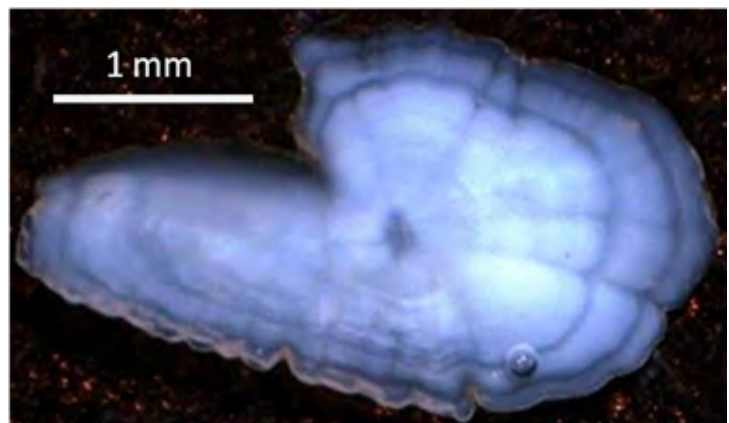


Figure 9.6.2.1 Distal view of an Alewife sagittal otolith.

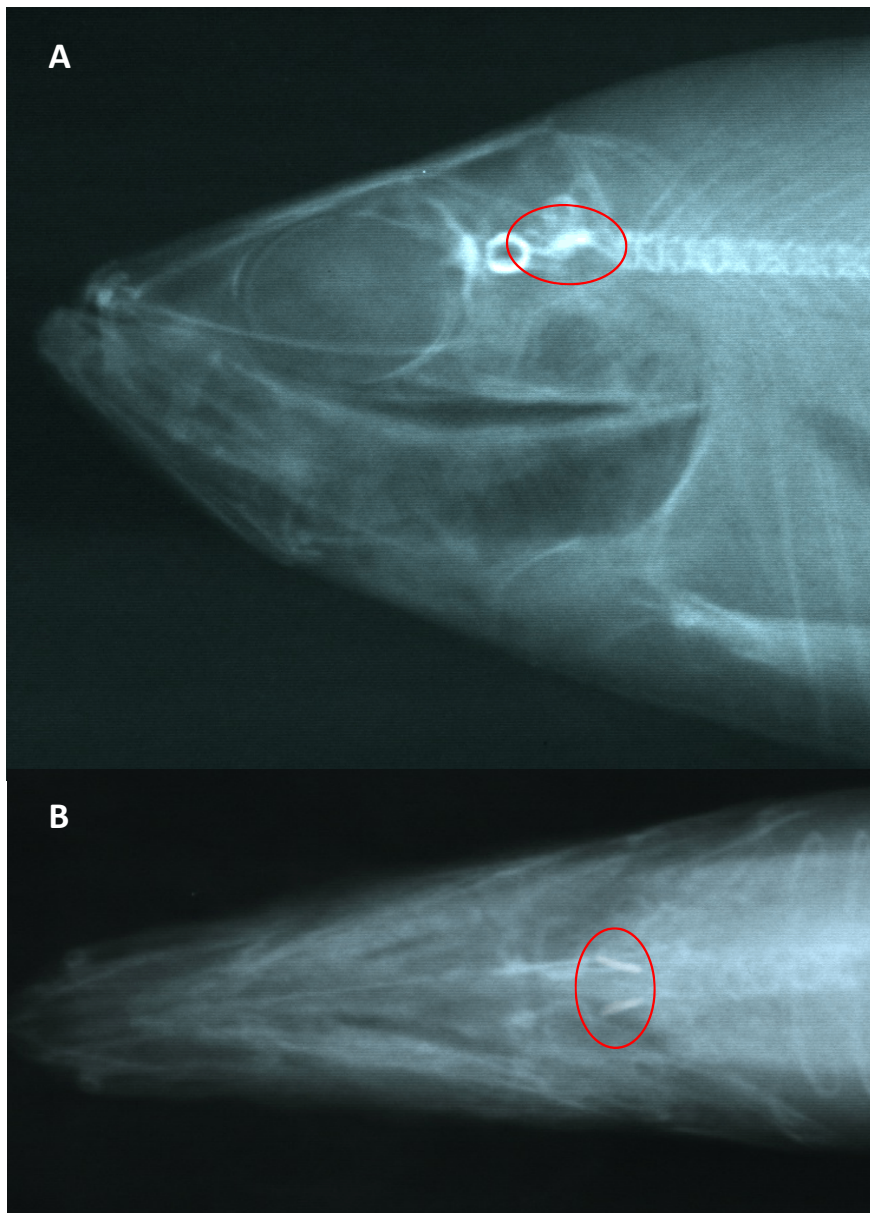


Figure 9.6.2.2 The A) lateral and B) ventral-dorsal views of sagittae (red circles) in an Alewife. Location is consistent among Alosines.

While many extraction techniques can be used, removal using a mostly horizontal cut over the eye sockets is the most simple (Figure 9.6.2.3). Once the top of the cranium is removed, the otoliths are fairly easy to get out using a pair of very fine forceps. For a video of the procedure see Chapter 12.0, Section 12.6.

Otolith Preparation

Otoliths should be clean and dry prior to ageing. Otoliths freshly removed from fish will appear slightly translucent and will be much more difficult to age than otoliths that have had several hours to air dry. The thinness of the otoliths makes them easy to read whole so no sectioning is needed. For Alosine species, whole otoliths are recommended for ageing so little preparation is necessary; make sure otoliths are clean before ageing and use water to clean off any debris.

Scale Description

Alosine scales are cycloid and have a strong boundary (baseline) between the anterior and posterior portions. The scales are thin and flexible with transverse grooves vertically (note that in all scale pictures,

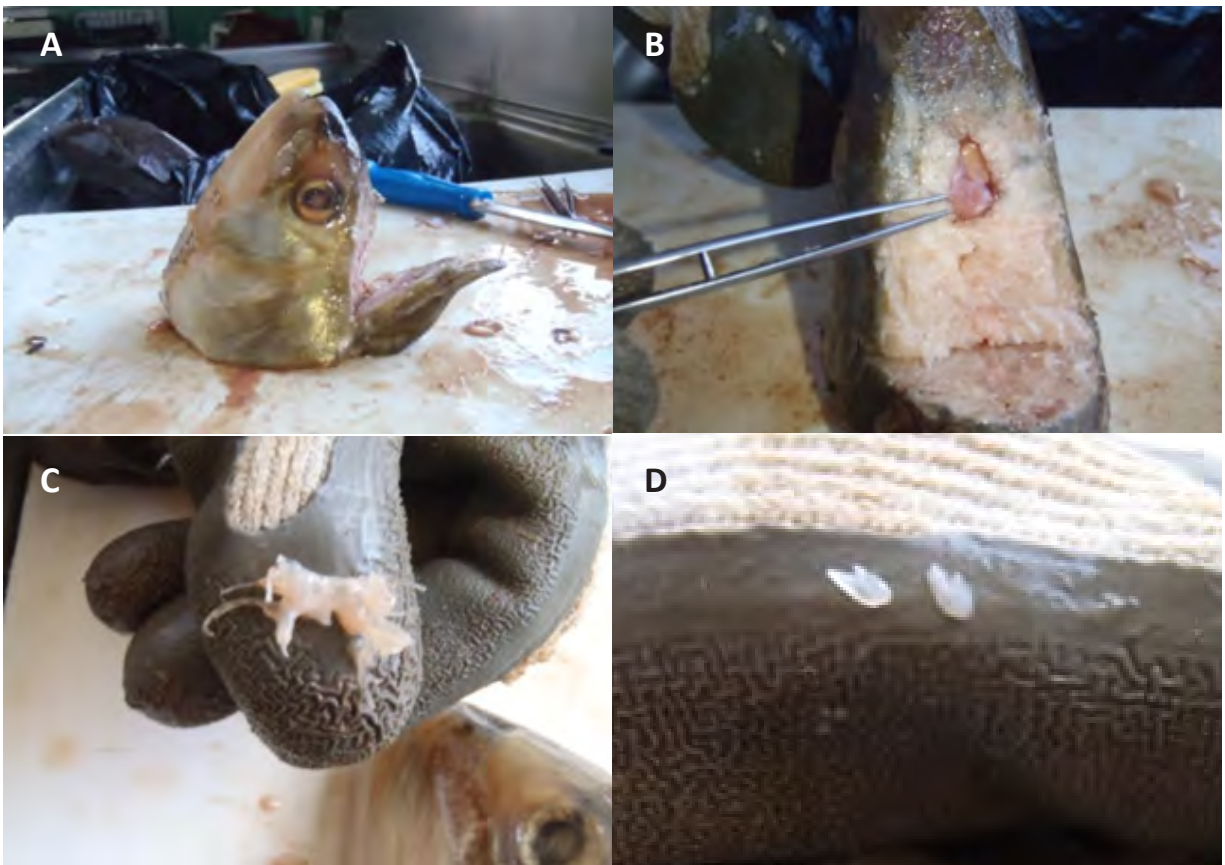


Figure 9.6.2.3 A) Make a horizontal cut across cranium above eye to B) expose brain cavity and remove it carefully. C) Extract the sacculus from the brain cavity and D) carefully remove otoliths from within the membrane.

the anterior portion of the scale is up) across the scale (Figure 9.6.2.4). Alosines are anadromous so the area closest to the center of the scale is formed in fresh water and is therefore referred to as the freshwater zone. Typically a strong check mark is laid down when the fish leave freshwater.

Scale Collection

A patch of scales should be removed from just ventral of the dorsal fin as shown in Figure 9.6.2.5. The collector should try to avoid areas of obvious damage or scale regeneration. Scales are easily collected by scraping posterior to anterior with a knife or scalpel. The knife should be wiped clean after each fish to avoid cross contamination. When working with dead fish, it is best to scrape away the mucus coating prior to removing the scale sample. This will make cleaning the scales easier.

Scale Processing

Scales need to be cleaned (Chapter 4.0, Section 4.3.1) and mounted between slides (Chapter 4.0, Section 4.3.2) before being aged. Do not put tape directly over the scales as the edges can become distorted and the scales may mold over time.

Age Determination

Otoliths

For all Alosine species, it is recommended to view whole otoliths immersed in a clearing fluid, (mineral oil, cedar oil or water work well) sulcus down, on a black background using a stereomicroscope with

reflected light. In practice, annuli are counted as the transition between the narrow translucent (dark) zones and the wider opaque (white) zones (Figure 9.6.2.6). The translucent zones should be continuous around the otolith with no breaks. Annuli are typically counted from the middle outward along the pararostrum or antirostrum. Check marks typically are not continuous, appear outside of expected growth rates, lack a defined edge or connect with translucent zone (Figure 9.6.2.6B). It should be noted that the first annulus in alosines can be the most difficult to determine. The look of the otolith inside the first annulus depends on how long the fish stayed in fresh water. It is therefore highly dependent on the characteristics of the river system where the fish was hatched. The first annulus is typically marked by a well-defined transition between hyaline and opaque zones.



Figure 9.6.2.4 A Blueback Herring scale which is typical of the Alosines.

Scales

When ageing Alosines from scales, annuli appear as continuous breakages in the circuli that continue past the baseline. The first well defined mark is usually the outside of the freshwater zone (Figure 9.6.2.7A). The first annulus is frequently weak and does not always follow the annulus criteria. In Blueback Herring and Alewife, the second annulus is typically the “strongest” looking. As a general rule, false annuli will not cross over the baseline, cannot be followed throughout the scale or cannot be seen on every scale. On older fish, annuli can become crowded together at the edge of the scale but will separate beneath the baseline. Spawning marks look like annuli but appear fuzzy and jagged above the baseline, and sometimes have resorbed over another annulus above the baseline (Figure 9.6.2.7B).

Although scales are a viable option for ageing Alosines, it should be noted that multiple studies (Duffy et al. 2012, Elzey et al. 2015) have shown bias associated with scale ages. Transverse groove counts were associated with ages of American Shad scales by Borodin (1924) and Cating (1953). However, McBride et al. (2005) discouraged the use of transverse grooves and subsequent work by Duffy et al. (2011) recommended only considering scales for ageing of American Shad if examining circuli, and not transverse

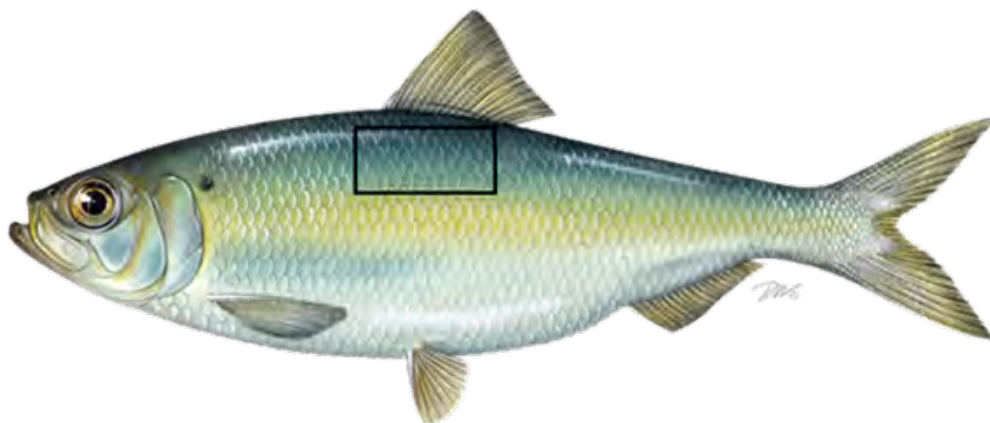


Figure 9.6.2.5 Scale collection area for Alosines.

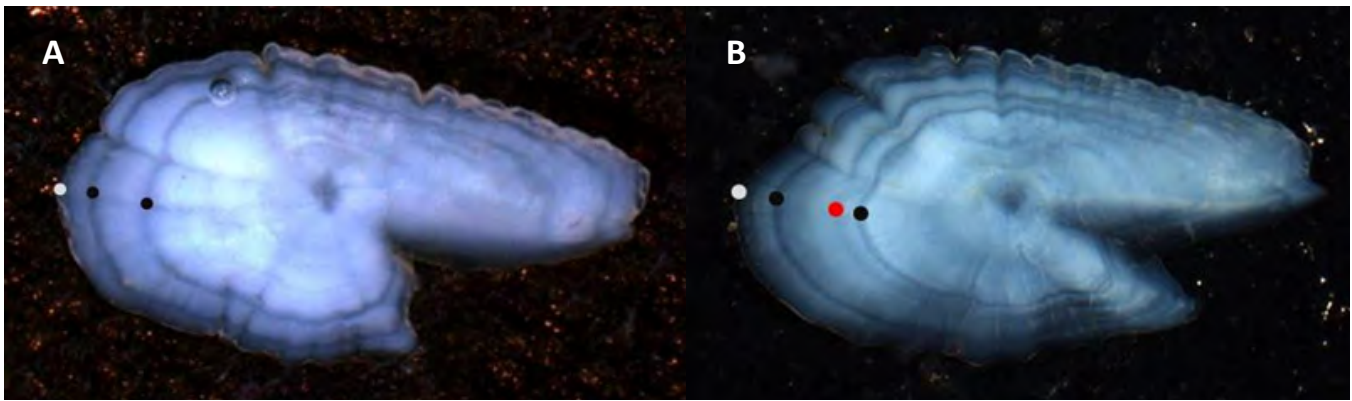


Figure 9.6.2.6 Whole Blueback otoliths from A) an age-3 and B) an age-3 with a check mark or false annuli. Black dots mark the first and second annuli, the red dot marks the check, and the white dot marks an annuli forming at the edge. **Note:** the growth between the first annulus and the check mark is not as much as expected for it to be the second annulus and the check is not continuously dark around the entire otolith.

grooves. Duffy et al. (2011) determined that the occurrence of transverse grooves was more related to the scale size and the region the specific sample originated from and not the fish's age. Transverse grooves on Alewife scales were examined using the same technique (Rothschild 1963, Marcy 1969) but was ultimately dismissed by McBride et al. (2005) and Duffy et al. (2011).

American Shad

American Shad spawn from the late winter to early spring with timing getting later moving from south to north (Figure 9.6.2.8). In their southern range, Walburg and Nichols (1967) found spawning could begin as early as mid-November but peaked in mid-January to February. Most spawning was done by March in the St. John's River, Florida. Walburg and Nichols (1967) reported spawning in Georgia and South Carolina rivers may begin as early as January but is done by the end of April. American Shad begin spawning in the Chesapeake Bay as early as mid-February and continue until mid-May and in the Delaware River, they

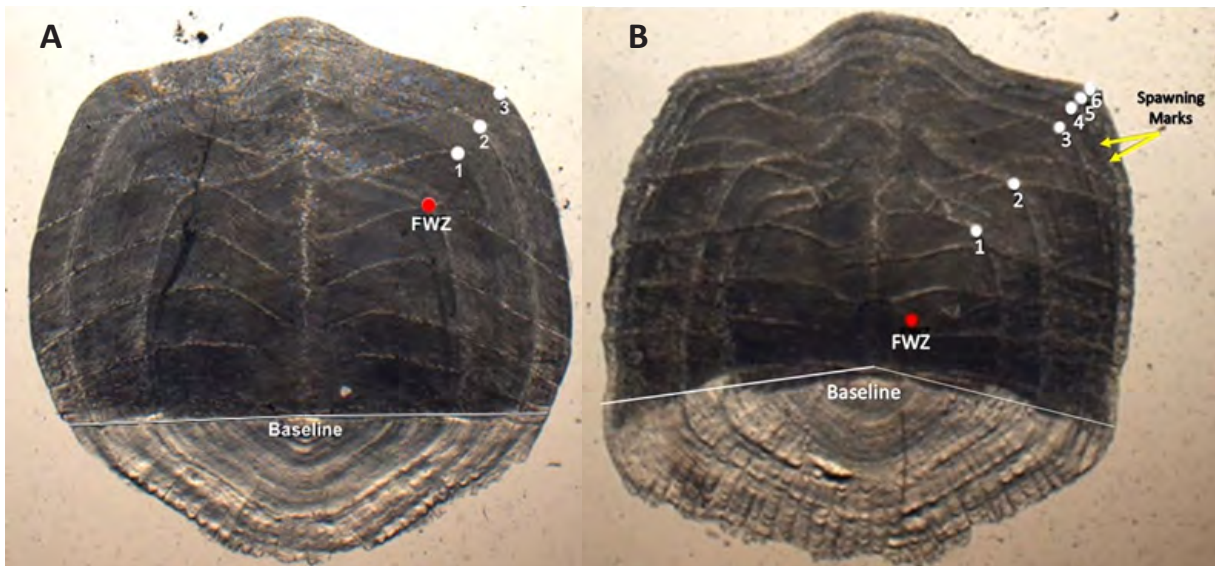


Figure 9.6.2.7 A) An age-3 Alewife and B) an age-6 Blueback with baseline, fresh water zone (FWZ in red), annuli (white), and spawning marks (yellow arrows) indicated. Note A) the straight baseline and large FWZ typical of Alewife scales and B) angled baseline with narrow FWZ in Blueback scales.

are most abundant in early May (Walburg and Nichols 1967). Elzey (personal communication) reports spawning fish entering Massachusetts waters as early as May. Spawning fish enter the Hudson and Connecticut rivers by the end of March and complete spawning by June (Walburg and Nichols 1967). Finally, in their most northern range, American Shad spawning typically begins in June and continues until July in Maine and Canada (Walburg and Nichols 1967).

Annulus formation was validated in American Shad otoliths by Duffy et al. (2012) using released hatchery fish marked with OTC but the timing of formation was only described as a ‘winter’ band. In general, American Shad that are aged are collected from the spawning period and annulus formation has already occurred in most fish (Elzey personal communication) indicating that the formation occurs only a month or two prior to the onset of spawning. An otolith/scale comparison indicated that ageing American Shad using scales led to over ageing fish age-5 and below and under ageing fish age-6 and above (Duffy et al. 2012).

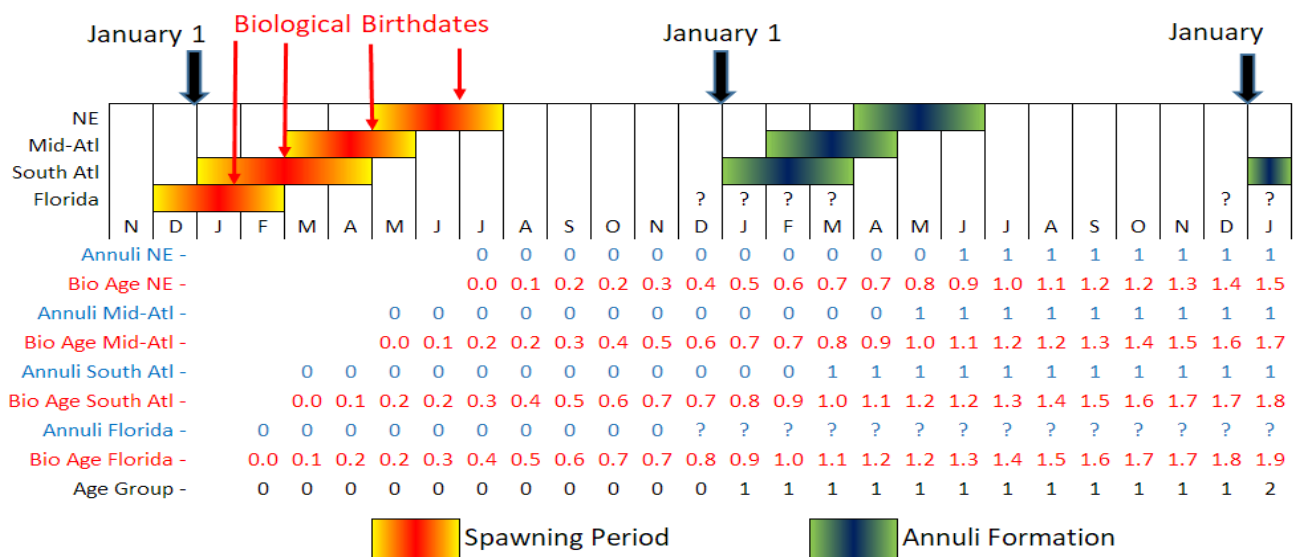


Figure 9.6.2.8 Spawning periodicity and age assignment timeline for American Shad from New England to Florida.

Alewife Herring

Alewife Herring spawning, like the other shad, occurs earlier in the south than the north and runs from late March through July (Fay et al. 1983). Walsh et al. (2005) reported eggs in North Carolina rivers in early April through late May (Figure 9.6.2.9). O’Connell and Angermeier (1997) reported spawning individuals occurred in Virginia from March through May and Alewives in Massachusetts spawned from mid-April to mid-May according to Cole et al. (1980). Elzey (personal communication) indicates that Alewife often begin spawning in Massachusetts in mid to late March as well. In the northern most range, Alewife spawning runs from early May to early June in Maine (Flagg 1977, Libby 1981). It should be noted that Alewife are nearly absent from waters any further south than about Charleston, South Carolina (Bozeman and Van Den Avyle 1989).

Annulus formation in Alewife Herring has not been sufficiently validated although LaBay and Lauer (2006) examined otoliths, scales, opercles, and vertebrae from southern Lake Michigan and determined that annuli formed on all Alewife structures after June which was when they sampled fish. Rothschild (1963) reported annulus formation, on scales, occurred concurrent with spawning which would lead to a merging

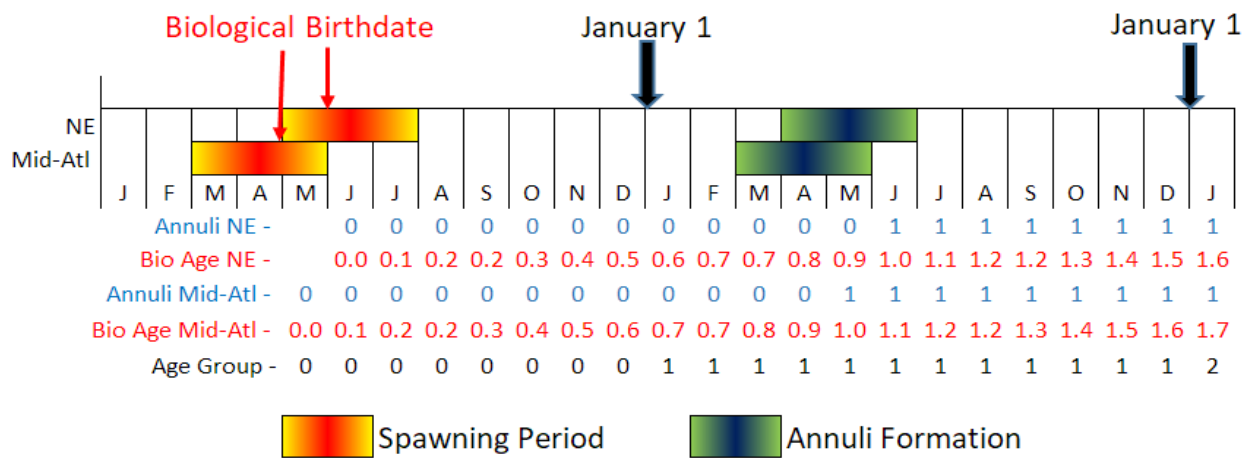


Figure 9.6.2.9 Spawning periodicity and age assignment timeline for Alewife from New England and the Mid-Atlantic (including North and South Carolina).

of the two marks in mature fish and that immature fish appeared to form annuli at the same time as the mature fish.

Blueback Herring

Blueback Herring spawning has been reported to match Alewife periodicity although typically 3-4 weeks after Alewife in the mid-Atlantic and in Maine (Fay et al. 1983, Mullen et al. 1986; Figure 9.6.2.10). Generally, Blueback spawning runs from April through mid-July along the mid-Atlantic and southern New England (Loesch and Lund 1977, Limburg et al. 2001, Schmidt et al. 2003) although Limburg et al. (2001) indicated that Blueback Herring begin moving through the locks at Troy, New York in May. This matches similar patterns seen in Massachusetts by Elzey (personal communication). Tyus (1971) reported spawning runs of Alewives in March through April to Lake Mattamuskeet from Pamlico Sound, North Carolina. In Florida’s St. John’s River, Blueback spawning began in January and ran through April according to McBride et al. (2010) which agrees with Williams et al. (1975) and Bozeman and Van Den Avyle (1989). Annulus formation has not been validated for Blueback Herring but likely occurs concurrent with spawning as in the other Alosine species (Elzey personal communication).

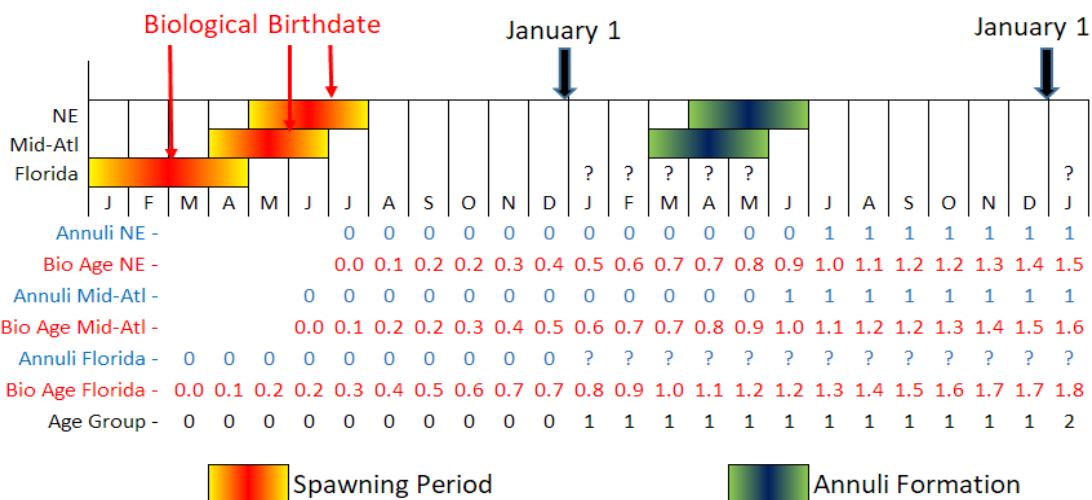


Figure 9.6.2.10 Spawning periodicity and age assignment timeline for Blueback Herring from New England and Florida (mid-Atlantic includes North Carolina). **Note:** The timing of annulus formation in Florida is unknown.

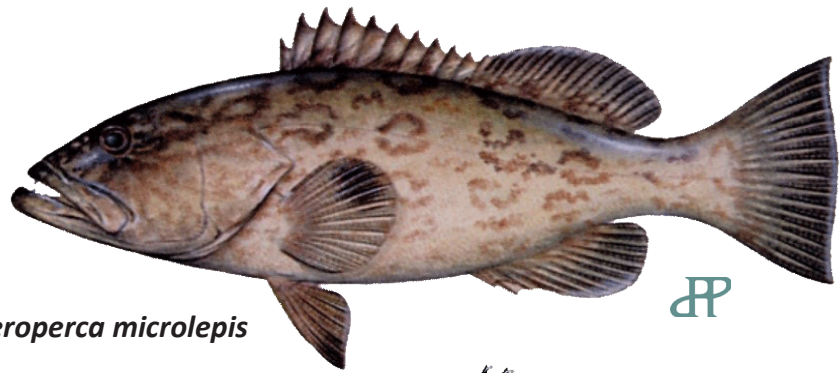
Other Ageing Techniques

Otoliths have been shown to be the preferred structure over scales for ageing Alosines by a number of researchers (Aschenbach et al. 1996, McBride et al. 2005, Duffy et al. 2011, Duffy et al. 2012). LaBay and Lauer (2011) examined four hard structures for ageing Alewife in southern Lake Michigan (otoliths, scales, opercles, and vertebrae). They found that only otoliths provided precise estimates compared to scales, vertebrae, and opercles which all led to under-ageing of old fish and over-ageing of young fish.

Research Needs

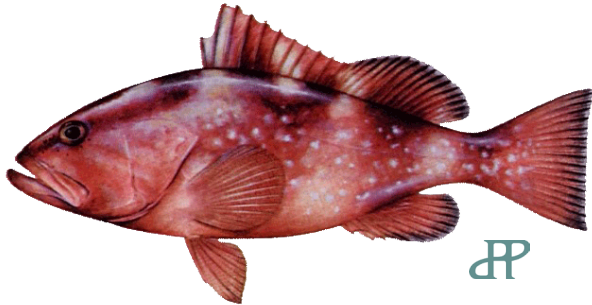
Although annuli validation has taken place with American Shad (Duffy et al. 2012), no validation has been published for Alewife or Blueback Herring. Furthermore, validation of timing of annuli deposition for all three of these species is non-existent.

9.7 Serranidae - Groupers



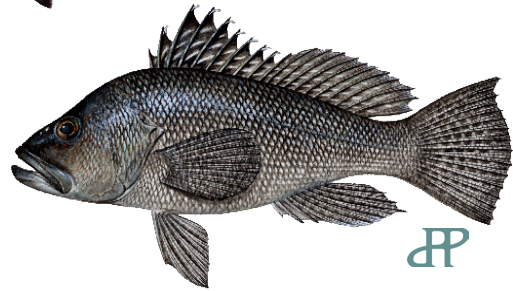
Gag Grouper *Mycteroperca microlepis*

PP



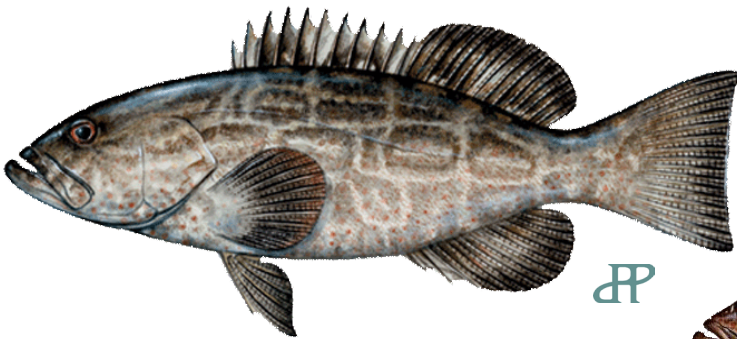
Red Grouper *Epinephelus morio*

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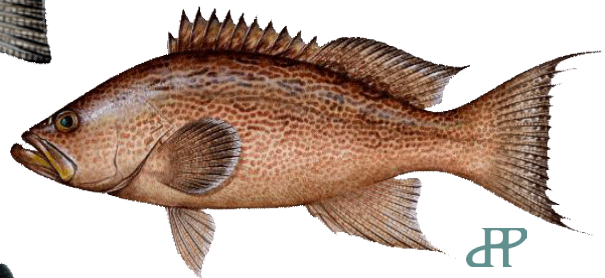
Black Sea Bass *Centropristis striata*

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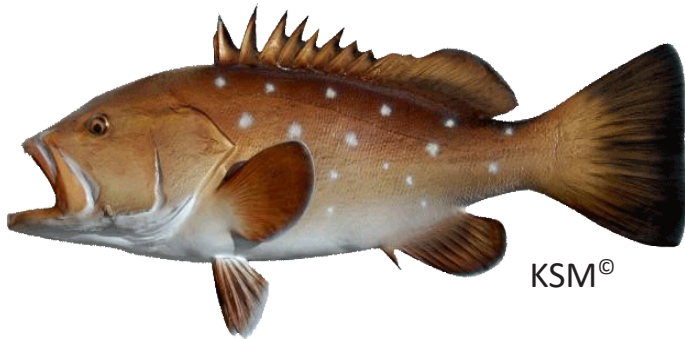
Black Grouper *Mycteroperca bonaci*

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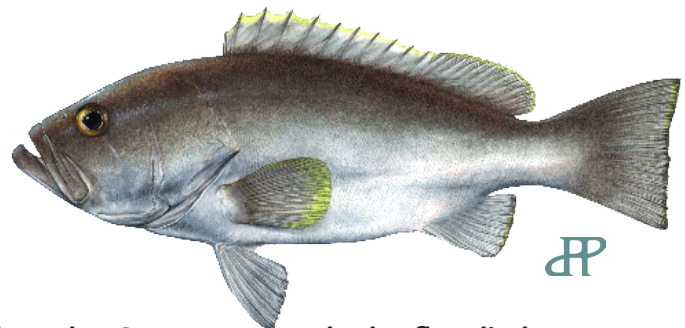
Scamp *Mycteroperca phenax*

PP



Snowy Grouper *Hyporthodus niveatus*

KSM[®]



Yellowedge Grouper *Hyporthodus flavolimbatus*

PP

Highlights

- Grouper otoliths are large and relatively easy to locate.
- Annulus formation (opaque zone) is generally complete by spring to early summer.
- In some grouper, annuli are easily identifiable in whole otoliths up to age 8 or 10.
- Grouper are moderately long lived but the majority of harvest is of younger individuals.
- Many grouper can reach ages exceeding 20 years although age-3 to age-10 are most commonly encountered.
- Black Sea Bass whole otoliths, otolith sections, and scales all used for ageing.
- Identification of first annulus in Black Sea Bass is most clearly identified in otoliths as opposed to scales (Dery and Mayo 1988).

Otolith Description

Like most of the groupers, the sagittae are relatively large, laterally compressed and have an arrow shape (Figure 9.7.1). The rostrum, anterostrum, and sulcus are easy to distinguish and locate. It is not uncommon to see protrusions or irregularities along the ventral edge of the sagittal. Smaller or younger fish otoliths can be more fragile. Deepwater groupers like Snowy and Yellowedge Grouper are long lived and their otoliths are traditionally embedded and thin sectioned, not read whole.

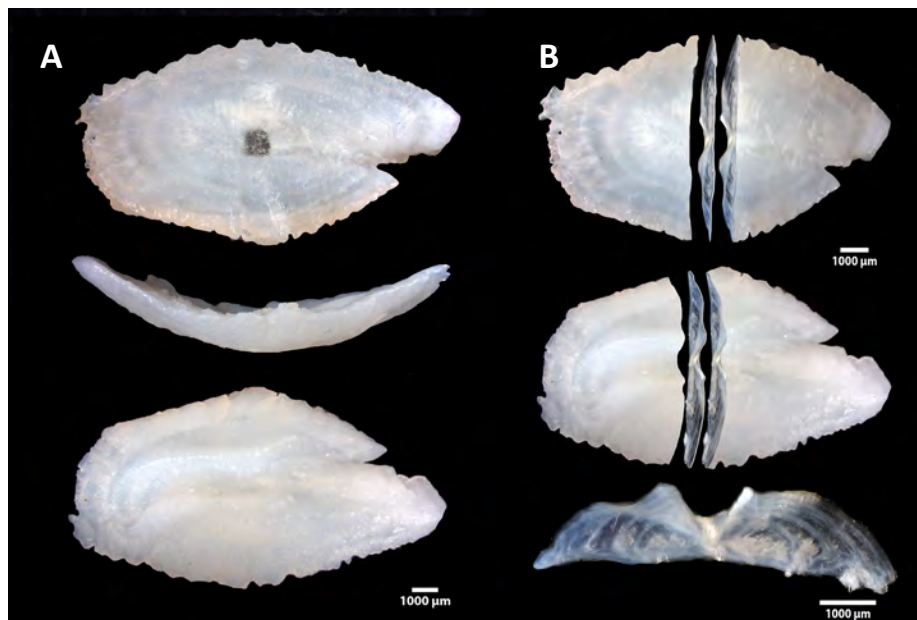


Figure 9.7.1 A) Whole otoliths of Gag distal view with core marked (top), dorsal view (middle), and proximal view (bottom) and B) Gag otolith sectioned with thin-section located and rotated showing location of cut through the core.

Extraction

Otoliths in large grouper are heavy and robust. However, the otoliths are fairly thin and fragile in younger fish, so care should be taken during removal and storage. The general location of the otoliths can be seen in Figure 9.7.2. Their size makes it easy to extract them using most of the techniques described in Chapter 3.0, Section 3.2.1. The most common is through the gill cavity near the posterior base of the skull above the gills (bottom method). It is relatively easy to cut away the surface of the exposed otic capsule with a heavy knife. In larger fish, otolith removal may be done using a saw cut made from the dorsal surface of the head to the otic capsule (top method). 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.

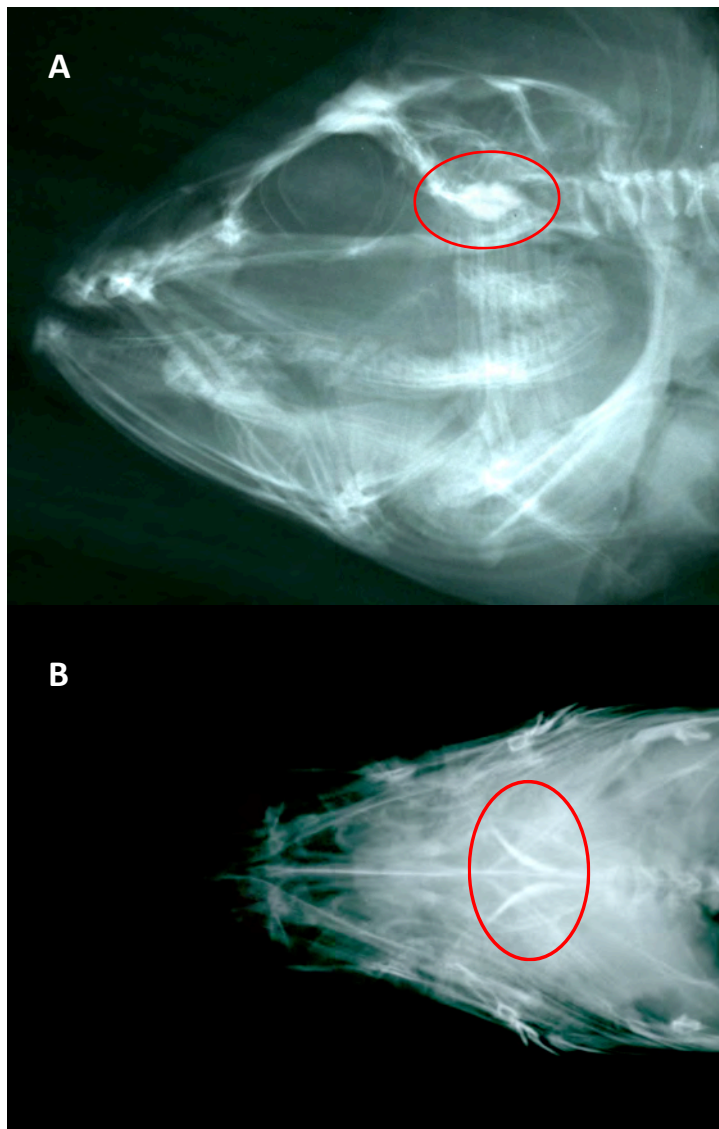


Figure 9.7.2 Radiograph of Black Sea Bass showing the positioning of the sagittal otoliths (yellow arrows) in the cranium in A) lateral and B) dorsal/ventral views.

Processing

Due to the relatively large size of most grouper otoliths, multiple processing techniques are acceptable (Chapter 3.0, Section 3.2.5). As in other species, low-speed sectioning preparation typically consists of embedding the otoliths in molds. The use of a thin sectioning machine has also been very successful with this species and the approach is the same as for other species with large otoliths (e.g., snappers and drum). For smaller otoliths, annuli can often be readily counted from whole otoliths and age estimation can be accomplished without requiring sectioning. Generally, grouper sections are processed at approximately 0.5-0.7 mm.

Age Determination

Gag Grouper

Whole Gag Grouper otoliths can be read up to about age-8 or age-9 but become increasingly difficult in fish over age-10. Multiple counting paths should be attempted in sections but unlike other species in this

manual, the best counting path for sections is often along the dorsal or ventral margins (the same plane recommended for whole otolith annulus counts). This difference may be due to the relatively thin and laterally compressed nature of Gag Grouper otoliths compared to many other species (Figure 9.7.1).

Collins et al. (1987) documented Gag Grouper in the waters off the southeastern U.S. having an annual spawning range from December to May, with the most intense period being in March and April. Additionally Hood and Schlieder (1992) verified a similar spawning period in fish from the eastern Gulf of Mexico occurring December to May, and peaking from February to March (Figure 9.7.2). Marginal increment studies in the South Atlantic Bight show that Gag Grouper along the Atlantic form annuli from May to August (Collins et al. 1987), and similar studies in the Gulf show even earlier deposition beginning in March (Hood and Schlieder 1992).

Other Ageing Methods

The method of utilizing spine and finray thin sectioning has been investigated as a non-lethal means of ageing Gag Grouper. In addition, Debicella (2005) also addresses the degree of completeness of the

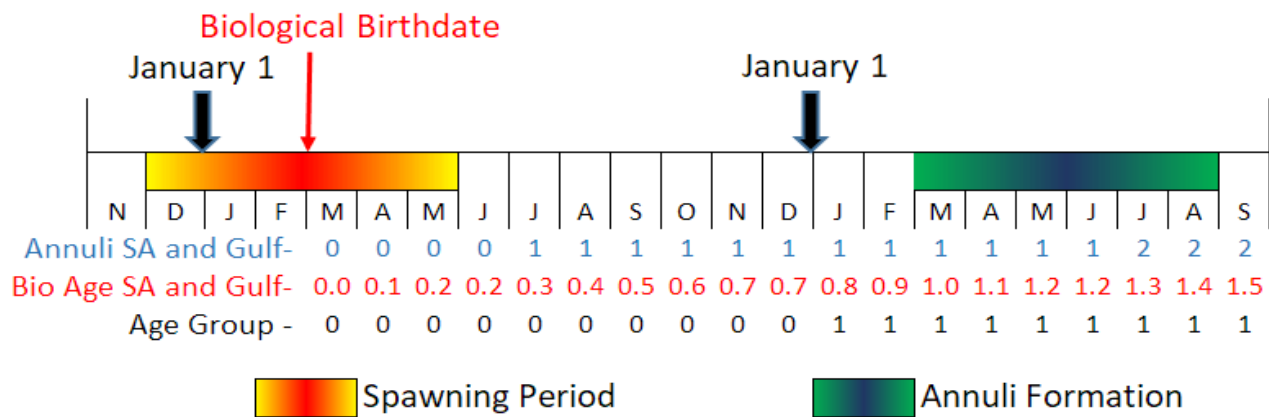


Figure 9.7.2 Timeline showing spawning period and annuli deposition ranges for Gag Grouper in the South Atlantic and Gulf of Mexico.

translucent portion of the annulus on the oblique inner portion of the fin ray since that is where the translucent zone is first laid down at least in Gag Grouper. She also discusses the importance of removing all of the skin from the fin rays. Her observations have implications for judging whether the translucent zone is “on the edge” for fin rays and spines.

Red Grouper

While small otoliths may be read whole, sectioning is more common for Red Grouper, and reading otoliths is straight forward as the rings are easily discernable. Moe (1969) and Johnson et al. (1998) documented that Red Grouper in the Gulf of Mexico spawn mainly from March through May, but may be as long as January to June. Burgos (2001) also found Atlantic Red Grouper spawning activity occurs from February to June, being most intense in April. Deposition of the opaque band typically begins in June and July (Figure 9.7.3).

Other Ageing Methods

Currently, no other techniques have been used in the Atlantic to determine the age of Red Grouper. However, researchers elsewhere in the Caribbean have attempted to use different jaw bones to estimate age, specifically urohyal bones (Gonzálas et al. 1974, Valdés and Padrón 1980) and mesopterygoids

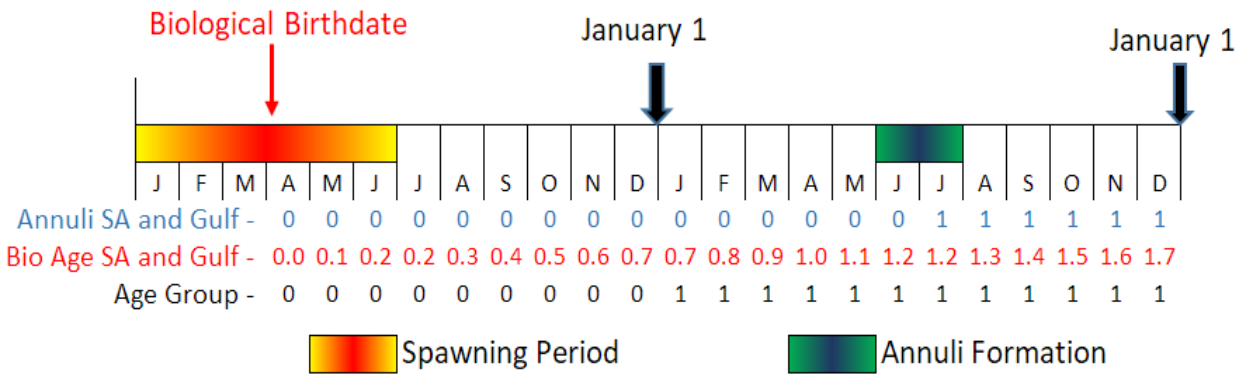


Figure 9.7.3 Timeline showing spawning period and annuli deposition ranges for Red Grouper in the South Atlantic and Gulf of Mexico.

(Rodríguez 1986). Rodríguez (1986) noted that two rings are deposited per year in mandibular bones as compared to otoliths in this species.

Black Sea Bass

The first annulus in Black Sea Bass otoliths can be difficult to identify. A weak translucent zone forms around the core of the otolith with an opaque ring forming in the first spring when the fish is 6-8 months old (Figure 9.7.4). Black Sea Bass otoliths can be read whole up to age-5; anything older should be sectioned. A common issue is the presence of check marks throughout the otolith.

The two existing stocks of Black Sea Bass in the Atlantic are divided at Cape Hatteras, North Carolina (Mercer 1978). Black Sea Bass spawning off Virginia and Maryland was estimated to be from June through July and slightly later, by a month, further north (Pearson 1941, Herman 1963, Kendall 1972). Hood et al. (1994) reported spawning of Black Sea Bass along the Florida Gulf Coast occurred in December to April. Black Sea Bass from the southern stock spawn from February to May and annuli are deposited from January to April (Mercer 1978, Dery and Mayo 1988, SEDAR 2011). Wenner et al. (1986) reported annulus formation off South Carolina occurred in April and May.

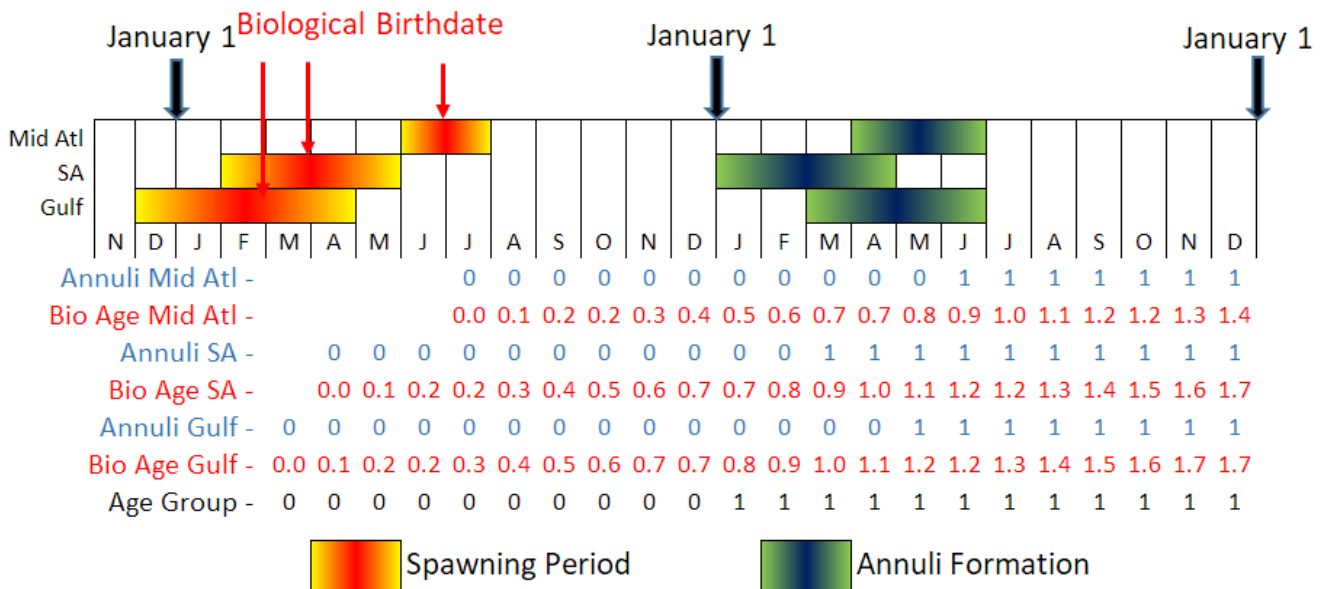


Figure 9.7.4 Spawning periodicity and age assignment timeline for Black Sea Bass from the North Atlantic to the Gulf of Mexico.

A study using OTC marking on Black Sea Bass otoliths and scales from the northern population suggests that annuli formation occurs from April to June (Robillard et al. 2017; Figure 9.7.5). Annulus formation in Black Sea Bass off South Carolina was validated indirectly using MIA by the SCDNR (unpublished data) in March to late May. Using MIA, Hood et al. (1994) suggested that annulus formation in the eastern Gulf of Mexico occurs from March through June.

Other Ageing Methods

Scales

The “cutting over mark” is interpreted as the annulus, however, there is not a cutting over mark indicating the first annulus in Black Sea Bass. The first annulus is identified as the outer edge of a zone of compacted circuli near the focus and the first cutting over mark is counted as the second annulus (Dery and Mayo 1988). Black Sea Bass scales have false cutting over marks that are not continuous.

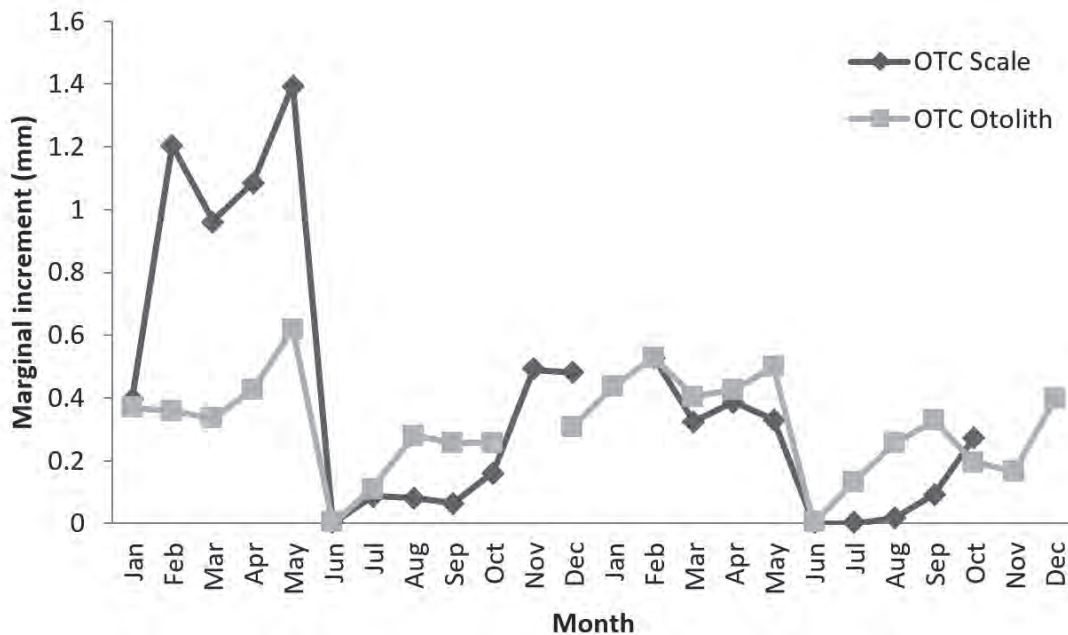


Figure 9.7.5 Mean monthly scale and otolith marginal increments measured from laboratory-held individuals injected with 50 mg/kg oxytetracycline in June 1990 and sacrificed at regular intervals during 1991 and 1992. (from Robillard et al. 2017).

Black Grouper

Black Grouper otoliths are traditionally embedded and thin sectioned, not read whole. The first annulus on Black Grouper is generally easy to identify, but in some sectioned Black Grouper otoliths, annuli are indistinct and irregular in appearance, which may make age estimation difficult. In older Black Grouper (>10 years old), annuli become closely spaced near the edge of the otolith, which can increase the difficulty in obtaining an accurate age estimation.

Black Grouper spawning in Florida may occur year round but primarily occurs in winter and early spring, with a peak from January through March (Crabtree and Bullock 1998). Ross and Moser (1995) collected larval and early juvenile Black Grouper concurrent with newly settled Gag Grouper in North Carolina supporting a March-May spawn. Crabtree and Bullock (1998) used MIA to validate Black Grouper annulus deposition for age classes up to age-7, and determined that deposition occurred from April to June (Figure 9.7.6). Manooch and Mason (1987) determined annulus formation in Black Grouper from the Florida Keys occurred in March through May.

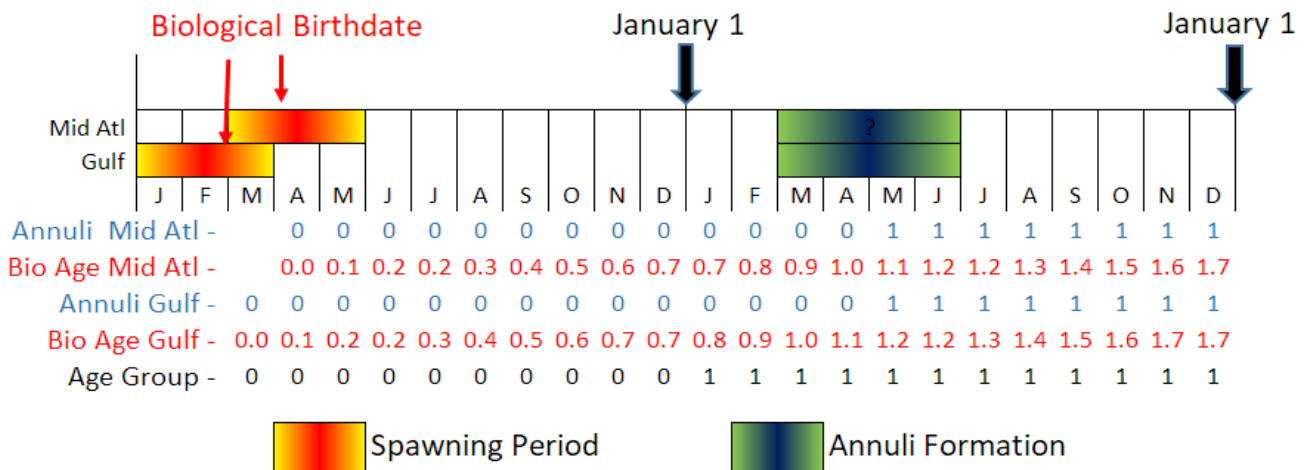


Figure 9.7.6 Spawning periodicity and age assignment timeline for Black Grouper from North Carolina to the Gulf of Mexico. **Note** there is uncertainty in annulus formation for the Mid-Atlantic. Timing is assumed from Gulf data.

Other Ageing Methods

Black Grouper otoliths have the potential to be aged whole, but no studies have been conducted to look at the reliability of this process. Aging Black Grouper with dorsal fin spines is not recommended, based on a preliminary otolith to spine comparison (Carroll personal communication).

Scamp

Scamp otoliths are similar in size, shape, and thickness to Gag Grouper otoliths but are more elongate and are typically read in thin section (Lombardi-Carlson et al. 2012). A variety of paths should be used when reading Scamp specimens, with the most effective axes being the ventromedial, dorsomedial, and the adjacent edges of the sulcus.

Scamp are protogynous hermaphrodites, and sex transition occurs at approximately 10 years of age (Lombardi-Carlson et al. 2012). Scamp in the South Atlantic spawn from February to July, peaking during the period from March to May (Harris et al. 2002; Figure 9.7.7). Similarly Scamp in the eastern Gulf of Mexico, spawn from January to June with a peak occurring in April (Coleman et al. 1996, Lombardi-Carlson et al. 2012). Marginal increment analysis performed by Harris et al. (2002) demonstrates a clear annuli deposition occurring primarily in December and January in Scamp along the South Atlantic from North Carolina to Florida. Lombardi-Carlson et al. (2012) determined the annulus formation was complete by July along West Florida and the Panhandle.

Other Ageing Methods

Lombardi-Carlson et al. (2012) determined that reading Scamp otoliths whole was impractical due to their small size.

Snowy Grouper

Deepwater groupers like Snowy Grouper are long lived and their otoliths are traditionally embedded and thin sectioned, not read whole. Annuli are often indistinct and irregular in appearance, which makes age estimation difficult, but not impossible. In older Snowy Grouper (>10 years old), annuli become closely spaced near the edge of the otolith, which can increase the difficulty in obtaining an accurate age estimation. Typical abnormalities for Snowy Grouper include crystalline areas that obscure increments and rounded opaque deformities that distort increment spacing (Wyanski et al. 2000). Also, in older

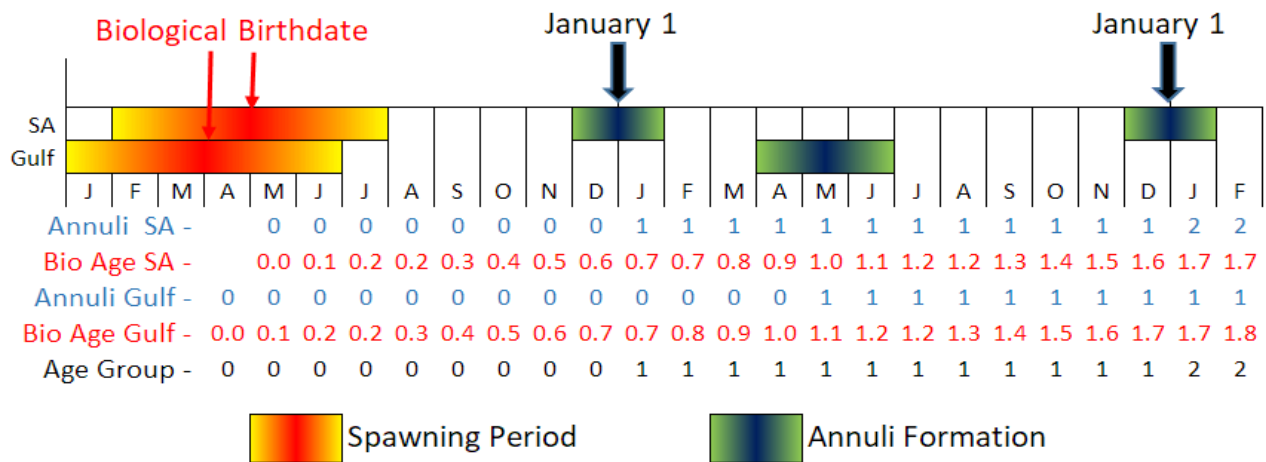


Figure 9.7.7 Spawning periodicity and age assignment timeline for Scamp from North Carolina to the Gulf of Mexico.

fish, all increments could not be counted along one axis in many specimens. Counting annuli for Snowy Grouper commenced on one of three axes (ventral, ventromedial, or adjacent to the sulcus acousticus) and shifted to another axis by following an increment to the new axis (Wyanski et al. 2000).

Snowy Grouper spawning off North and South Carolina occurs April-September, with no obvious peak (Wyanski et al. 2000, Figure 9.7.8). Sedberry et al. (2006) reported spawning from May through August. In South Florida, Moore and Labisky (1984) reported spawning Snowy Grouper were collected from April through July. Wyanski et al. (2000), used MIA to indirectly validate Snowy Grouper annulus deposition for age classes up to age-10 and determined that deposition occurred April-May. Matheson and Huntsman (1984) off the Carolinas found annulus formation in May through July. Similarly, Moore and Labisky (1984) in the Florida Keys determined that annulus deposition occurred from May to July with a peak in April and May.

Yellowedge Grouper

Similar to Snowy Grouper, Yellowedge Grouper are long lived and their otoliths are traditionally embedded and thin sectioned, not read whole. However, few Yellowedge otoliths have easily distinguishable annuli as they are indistinct and irregular in appearance, which makes age estimation difficult but not impossible (Keener-Chavis 1984, Bullock et al. 1996). In addition, annuli in fish >10 years old become closely spaced near the edge of the otolith, which can increase the difficulty in obtaining an accurate age estimation. In some instances percentages of legible specimens were below 30% with a relatively large sample size, n=590 (Keener-Chavis 1984).

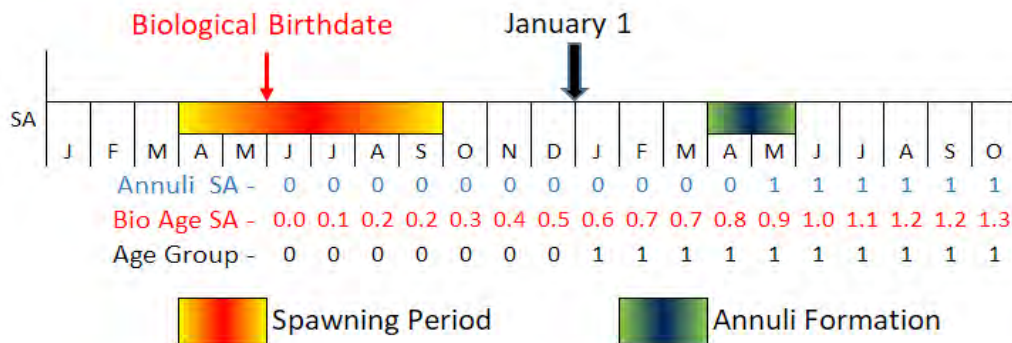


Figure 9.7.8 Spawning periodicity and age assignment timeline for Snowy Grouper from the Carolinas to the Florida Keys.

In the eastern Gulf of Mexico, Bullock et al. (1996) found Yellowedge Grouper gonadal activity peaked from May to September with female GSIs increasing to August and declining in September. Cook and Hendon (2010) reported the Yellowedge spawning season spans from March to September (Figure 9.7.9), with the spawning peak between July and September. Yellowedge Grouper in the Atlantic have a slightly shortened spawning season than those in the Gulf, occurring from April to September (Keener-Chavis 1984). More recent work (Sedberry et al. 2006) found spawning occurred off the Carolinas in August and September.

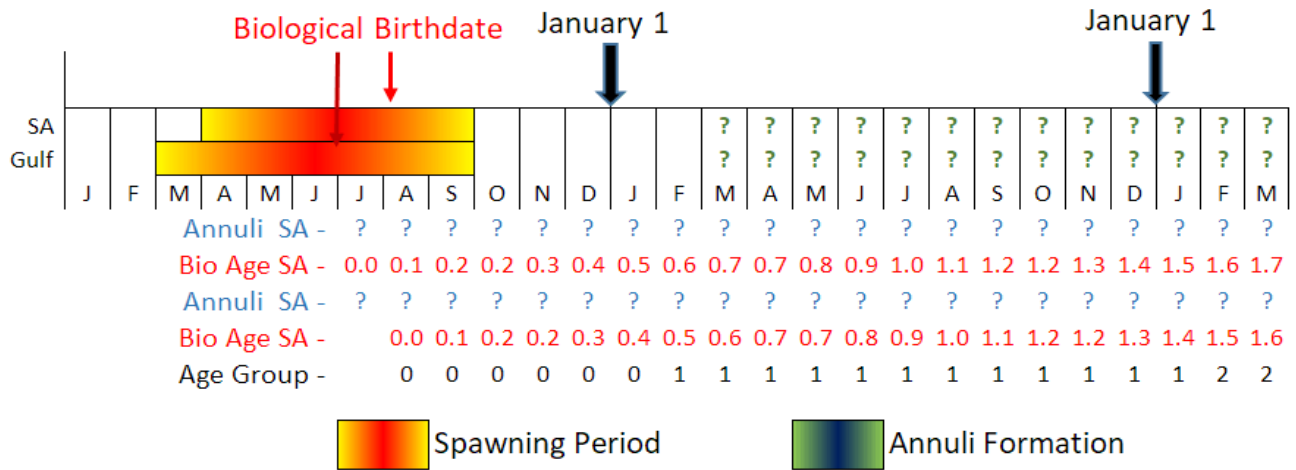


Figure 9.7.9 Spawning periodicity and age assignment timeline for Yellowedge Grouper from the Carolinas to the eastern Gulf of Mexico.

Validation has not been sufficiently conducted for Yellowedge Grouper using conventional techniques due to the high level of ageing difficulty. Keener-Chavis (1984) produced a marginal increment analyses with a limited number of readable specimens (n=184 or 27% of all specimens) but her results were inconclusive (Figure 9.7.10). Bullock et al. (1996) attempted to age otolith sections from Yellowedge from the eastern Gulf and determined that the otoliths were virtually absent of annuli. Cook et al. (2009) used bomb radiocarbon to validate ages of Yellowedge Grouper otoliths and found a higher proportion of ‘readable’ sections. Those that were compared using core analysis of trace 14C to the traditional reading in cross-section confirmed that annuli form annually in Yellowedge although they were unable to determine the timing of formation.

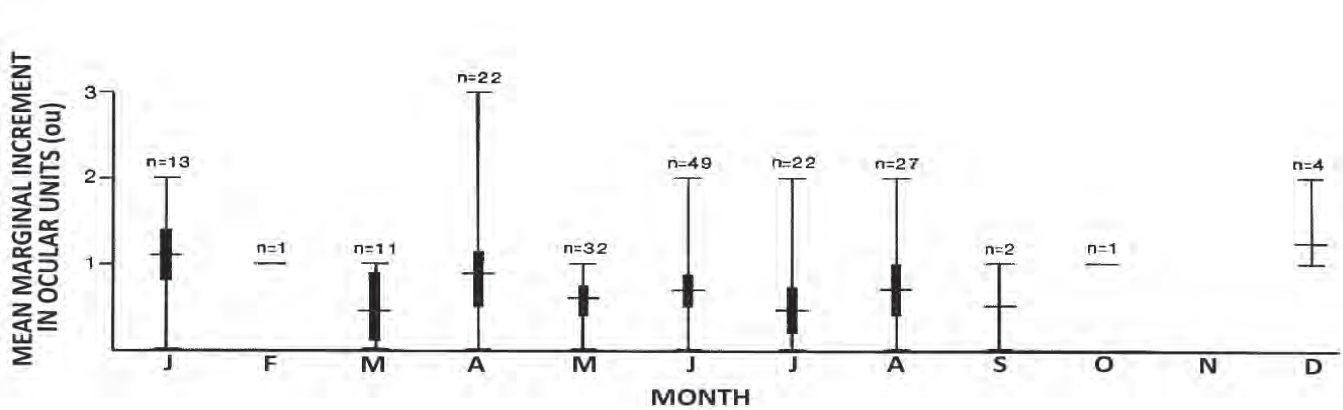


Figure 9.7.10 Marginal increment plot of Yellowedge Grouper otoliths (1 ou = 0.8mm) (Figure 8 from Keener-Chavis 1984).

Research Needs

Further work is necessary in Black Sea Bass to identify causes and frequency of check marks in otoliths and scales. Suggestions include the changing of sex from female to male, environmental factors, or migration patterns observed in northern stock fish.

Timing of annulus formation in Yellowedge Grouper needs to be determined as well as potential alternatives to sectioning otoliths which have limited success. Other structures may prove more reliable.

Expansion of the reproductive history for the less commonly encountered grouper species needs to be conducted. These include Snowy Grouper, Yellowedge, Scamp, and Black Grouper.

9.8 Lutjanidae - Snappers



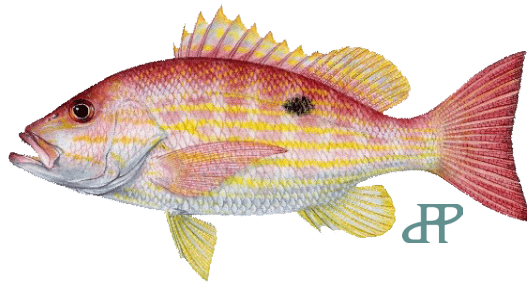
Red Snapper *Lutjanus campechanus*



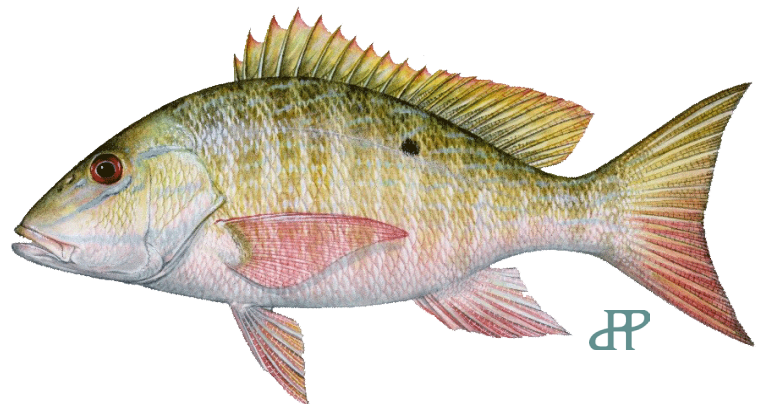
Vermilion Snapper *Rhomboplites aurorubens*



Gray Snapper *Lutjanus griseus*



Lane Snapper *Lutjanus synagris*



Mutton Snapper *Lutjanus analis*

Highlights

- Snapper otoliths in general are ovate, laterally compressed.
- Red and Mutton Snapper otoliths are much larger than the other snappers, but similar in appearance.
- Otoliths are relatively easy to locate and extract.

- In most snapper, the first increment can appear diffuse and difficult to discern.
- Opaque increment enumeration becomes increasingly difficult in older fish.
- Some snapper otoliths may have 'checking' or false annuli in between true increments that appear as incomplete (usually denser) banding, particularly on the ventral surface of the otolith. The checking appears black in transmitted light or bright white in reflected light.
- Gray Snapper can live over 25 years but most of the fishery is under age-10.
- Vermilion Snapper have been aged to 26 years but are harvested by 4-5 years.
- Mutton Snapper have been aged to 40 years
- Gray Snapper have clearer opaque zones compared to other snapper otoliths but the distance from the core to the first annulus varies (Fischer et al. 2005).
- Gray Snapper scales and otoliths have been used to age but scales are useful to age-12 at best.
- Whole Gray Snapper otoliths have been used on smaller specimens.

Otolith Description

Snapper otoliths (sagittae) are generally ovate, laterally compressed, and exhibit an indented sulcus on the proximal surface (Figure 9.8.1). The rostrum and antirostrum are distinguishable but can be quite fragile in many of the snappers.

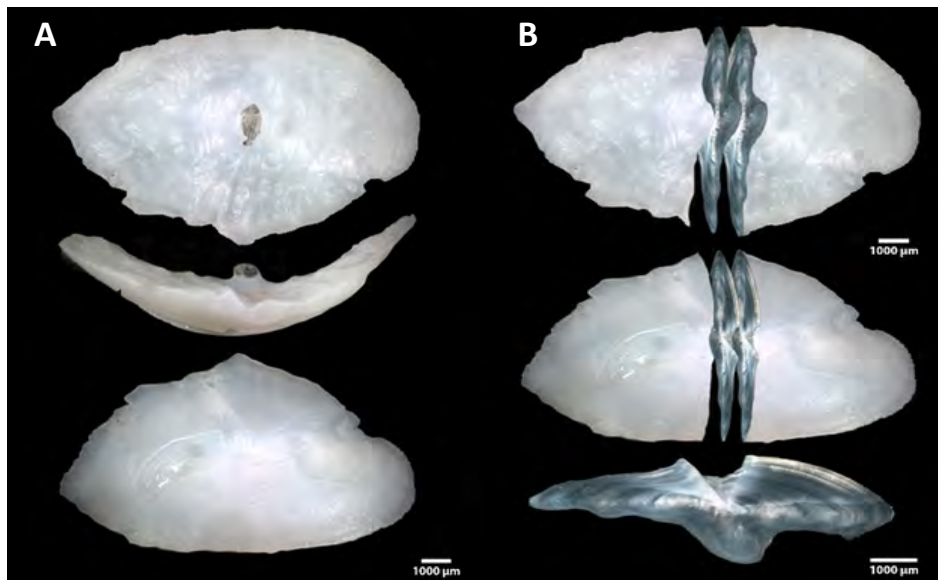


Figure 9.8.1 A) Whole otoliths of Red Snapper proximal view with core marked (top), dorsal view (middle), and distal view (bottom) and B) Red Snapper otolith sectioned with thin-section located and rotated showing location of cut through the core.

Extraction

Snapper otoliths may break during contact with certain extraction tools. The location of the sagittae in the neurocranium is illustrated in Figure 9.8.2. The otic capsule in most 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.

Any number of the methods for extraction provided in Chapter 3.0, Section 3.2.1 can be used to remove sagittae in most of the snappers. The most common is through the gill cavity since many of the specimens

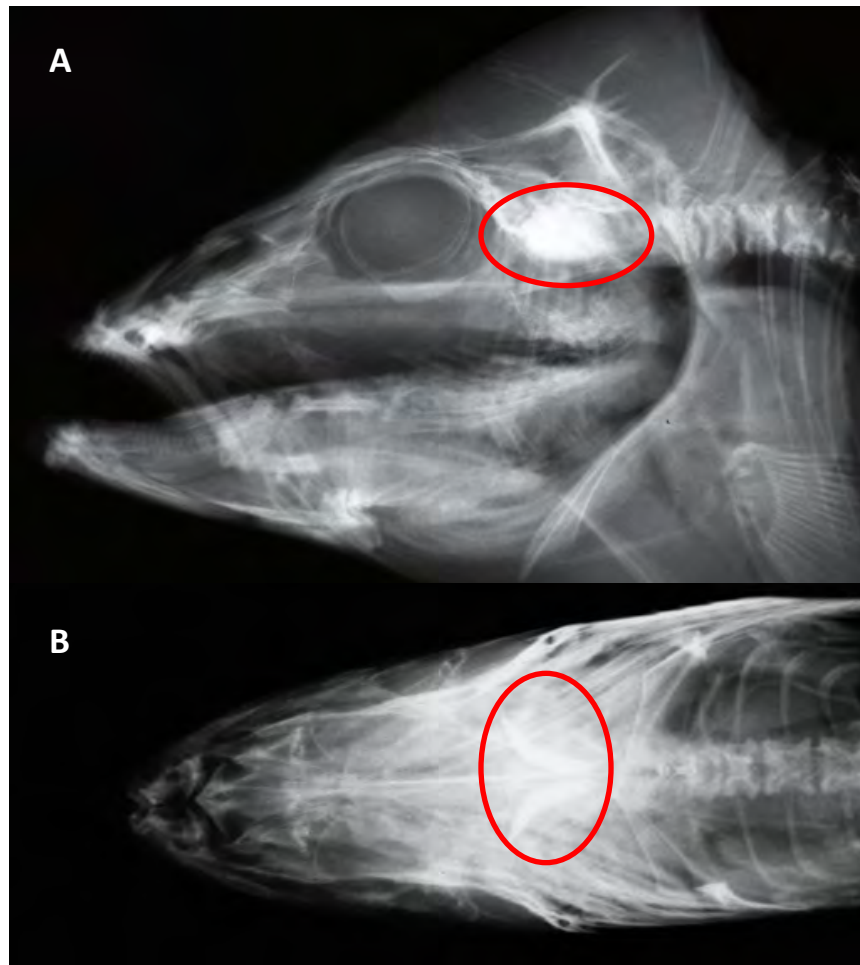


Figure 9.8.2 Radiographs showing location of sagittae (red circles) in Lane Snapper cranium in A) lateral and B) dorsal/ventral views.

sampled are from the commercial catch. On smaller and younger fish, a vertical or horizontal cut will work as well, especially if the fish is not intended for market.

Processing

Due to the relatively large size of most snapper otoliths, multiple processing techniques are acceptable whole or embedded using low or high speed wafering saws and thin sectioning machine (Chapter 3.0, Sections 3.2.5 and 3.2.6). Generally the larger snapper sections are processed at approximately 0.5 mm. Most snapper can be processed using the multiblade techniques as well.

Age Determination

Red Snapper

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 9.8.3). Due to a protracted spawning of Red Snapper, there is assumed to be considerable variation in the distance from the core to the first opaque increment, which can appear as a diffuse 'smudge.' The increment between the core and the first mark will vary depending on when during the typical spawning period that individual fish was spawned. Earlier

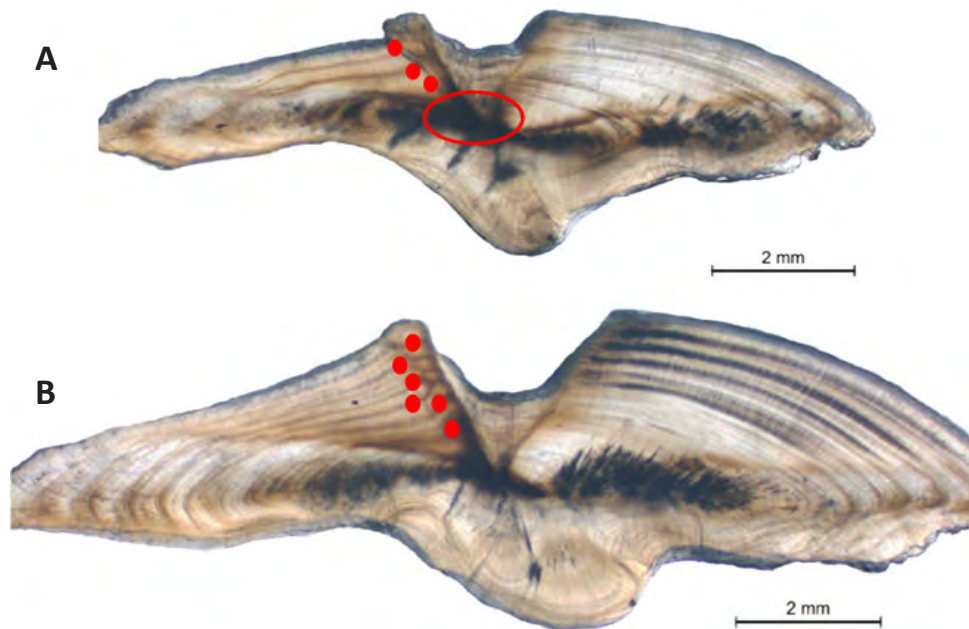


Figure 9.8.3 Sectioned Red Snapper otoliths demonstrating A) a typical first annulus near the core forming a dark zone or 'smudge' (encircled) in an age-4 and B) the first annulus further away from the core in an age-6 fish.

spawned fish will have a larger increment than later spawned fish (Figure 9.8.3). The longevity of the species also increases the difficulty in obtaining accurate age estimates of older fish. After age-10, Red Snapper somatic 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.

Spawning in the northern Gulf was reported by several authors to occur from early May through late September (Bortone and Hollingsworth 1980, Wilson and Neiland 2001, Fischer et al. 2004; Figure 9.8.4). The protracted spawning season can result in the first annulus varying in the distance from the core (see above). Red Snapper along the Atlantic Coast from North Carolina and South Florida have similar spawning to the Gulf but annulus formation occurs later (June through August; White and Palmer 2004).

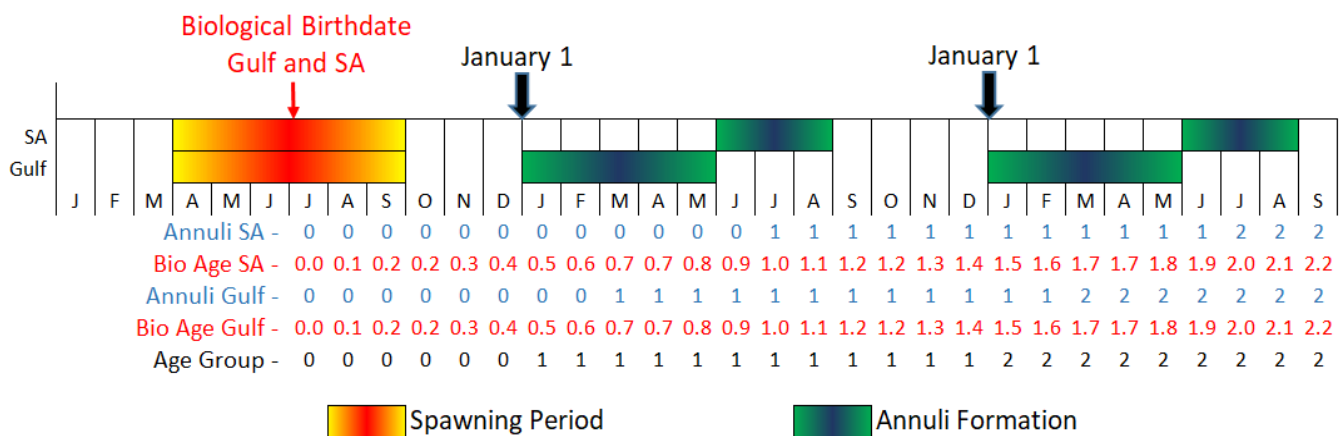


Figure 9.8.4 Spawning and annulus formation in Red Snapper in the South Atlantic and Gulf regions with an accepted birthdate of July 1.

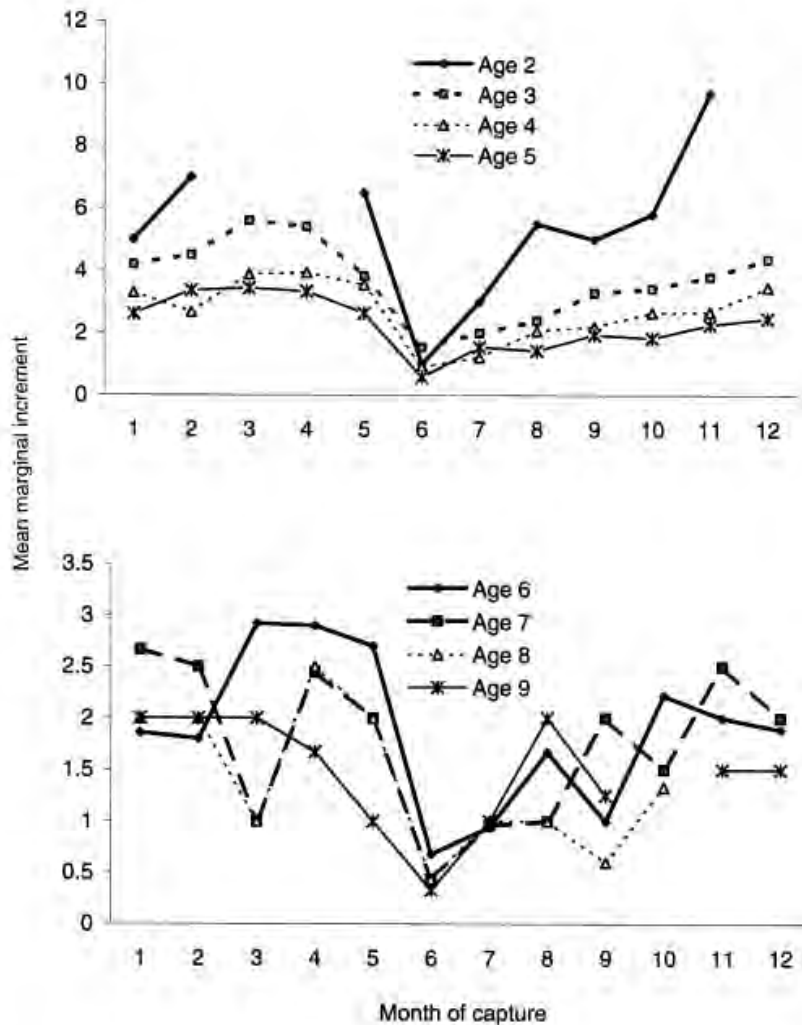


Figure 9.8.6 Mean monthly marginal increments of Gray Snapper otoliths by age along the Atlantic Coast of Florida (Figure 2 from Burton 2001).

Vermilion Snapper

As with the other snappers, Vermilion otoliths can be sectioned using any of the techniques outlined in Chapter 3.0, Section 3.2.5 or read whole in young fish, however, enumeration of annuli in Vermilion Snapper otolith sections can be challenging to inexperienced personnel. Vermilion Snapper spawning in the Gulf of Mexico and the South Atlantic occurs from May to September with some fish spawning several times a season (Grimes and Huntsman 1980, Nelson 1988, Cuellar et al. 1996, Hood and Johnson 1999). Annulus formation has been determined to occur from June to August in the Gulf (Nelson 1988, Hood and Johnson 1999) and June/July in the South Atlantic (Zhoa et al. 1997). Annulus formation in Vermilion Snapper has been validated by several studies using MIA (Zhao et al. 1997, Hood and Johnson 1999).

The problem encountered most often by readers is determining the position of the presumptive first opaque increment nearest the core which is most obvious in Gray Snapper (Figure 9.8.7). Due to a protracted spawning season (Figure 9.8.8), there is assumed to 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 near the core region if the fish was spawned in the fall or may appear as an annuli some distance from the core if a fish was spawned in early summer. In addition, Zhoa et al. (1997) noted that, occasionally, false annuli or checks were deposited close to the core that may have been the result of

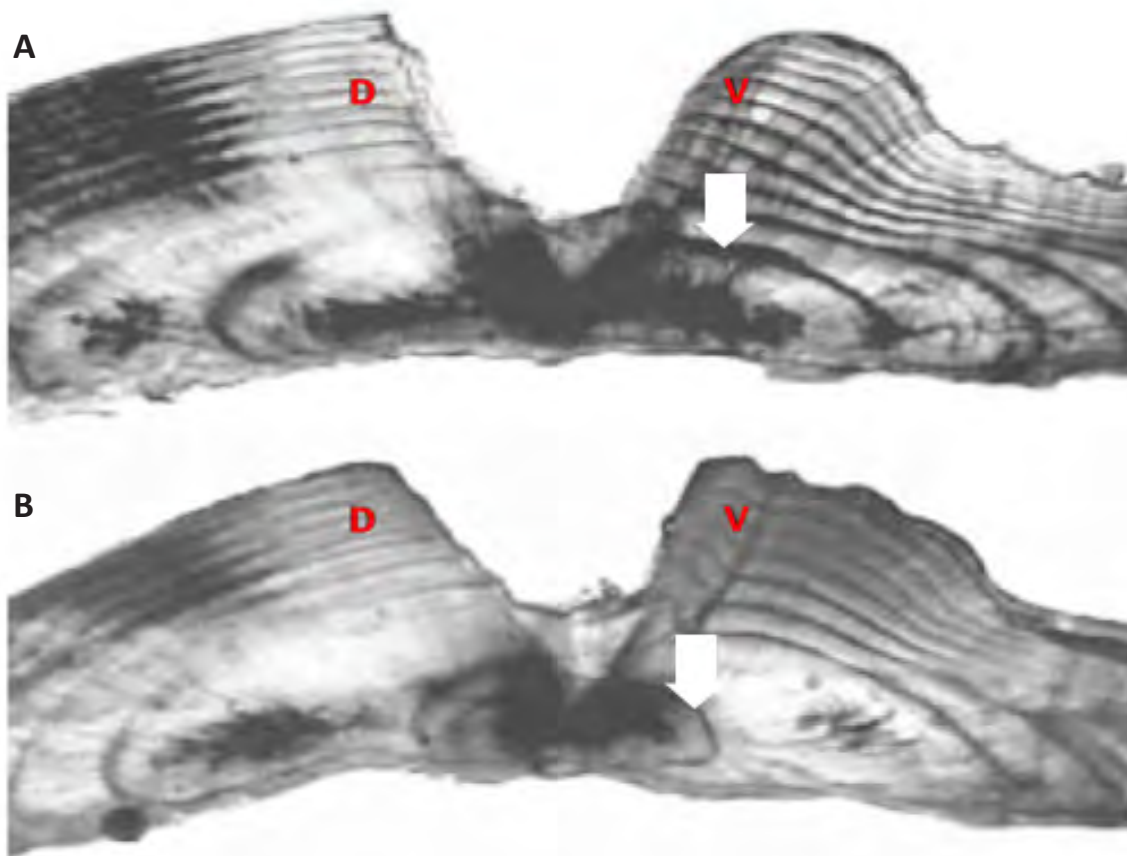


Figure 9.8.7 A) Transverse section of a Gray Snapper (*Lutjanus griseus*) otolith with first opaque zone distant from the core indicated by arrow, with 10 opaque zones and an edge condition of 4. B) A transverse section of a Gray Snapper otolith with first opaque zone close to the core indicated by arrow, with 8 opaque zones, and an edge condition of 4. **Note:** **D** indicates dorsal side and **V** indicates ventral side of otolith section (Figure 2 modified from Fischer et al. 2005).

settlement or changes in feeding habitats. Those opaque zones at predictable distances were deemed as the true annuli. The distance from the core to the distal edge of the first annulus in the otoliths of juvenile Vermilion Snapper was determined to be on average 0.5 mm. Measuring this distance can be used as a guide to help identify the first annulus (NOAA personal communication).

Other Ageing Techniques

Scales and whole otoliths have been used to age Vermilion Snapper (Grimes 1978, Barber 1989). Scales are considered less reliable because, as fish become older, scales become more difficult to interpret (Grimes 1978, Collins and Pickney 1988) compared to otoliths and therefore discouraged.

Lane Snapper

Annulus identification and enumeration is easier for this species than with other snappers, however it can still be difficult for untrained personnel, especially with respect to identification of the first annulus and presence of false annuli. The distance between the core and the first annulus varies greatly due to protracted spawning season. The first annulus can deposit close to the core if the fish was spawned late in the season. In addition, a reduction in somatic growth as fish age is reflected in the otolith deposition, making annuli appear much closer together as the fish gets older.

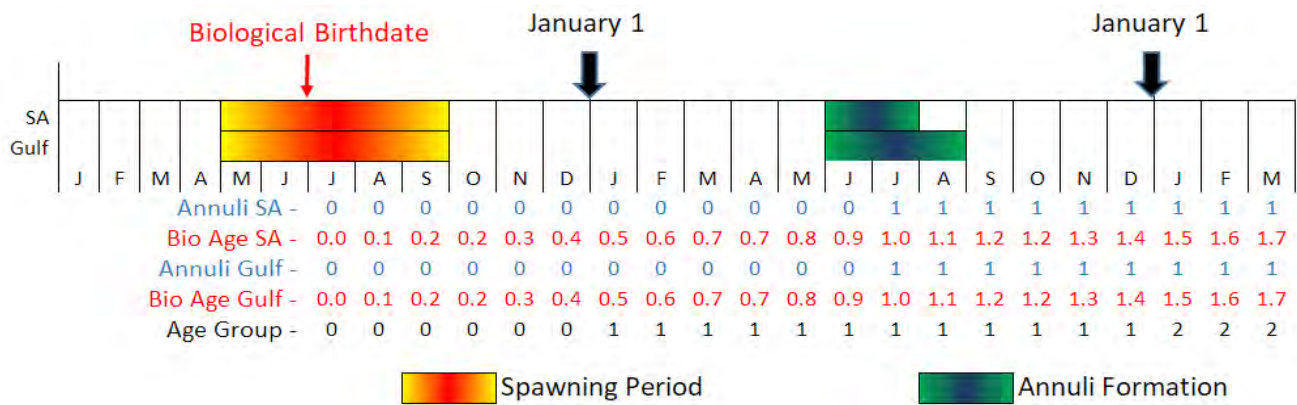


Figure 9.8.8 Timing of spawning and annulus formation in Vermilion Snapper along the Gulf and South Atlantic.

Spawning occurs from March through September; with peak spawning during June through August (Manooch and Mason 1984; Figure 9.8.9). Annulus formation occurs from April to September with a peak in June in the northern Gulf using MIA (Johnson et al. 1995). The timing is similar to other reports from Cuba and other tropical studies (Rodriguez Pino 1962, Alegria and Menezes 1970, Claro and Reshetnikov 1981).

Other Ageing Techniques

Johnson et al. (1995) also used whole otoliths to age Lane Snapper, however, they did not have high agreement compared to sectioned otoliths (69%). Manooch and Mason (1984) found whole otoliths more readable than scales but otolith sections were preferred. Manooch and Mason (1984) noted that most of the scales were either regenerated, or too thick/deformed to provide an accurate age. Dorsal fin spines may be a viable aging alternative for Lane Snapper; a preliminary study between spines and otoliths found a 91% agreement within one year (Carroll personal communication).

Mutton Snapper

Unlike other snappers, the spawning season is not protracted for Mutton Snapper. As such, the distance from the core to the first annulus for this species should be large and never have a ‘smudge’ in the core since there is minimal overlap between spawning and annulus formation. Mutton Snapper otoliths are very similar to Red Snapper in many ways, including size, core location, checking and longevity so enumeration of annuli in snapper otolith sections can be challenging to inexperienced personnel. The

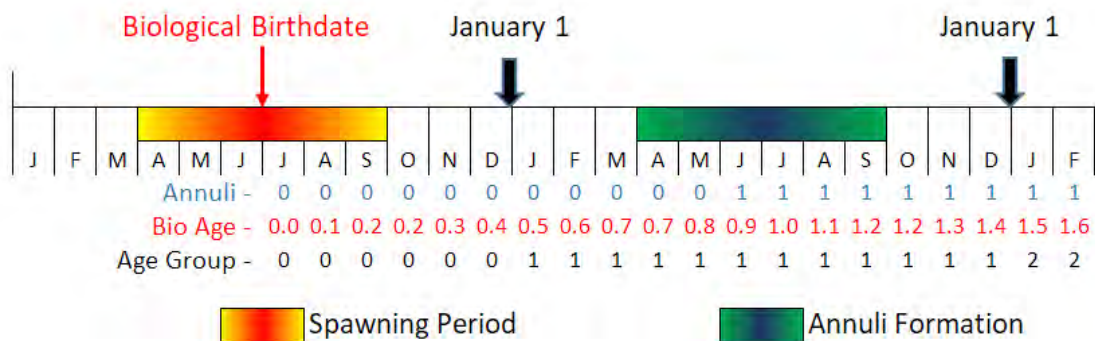


Figure 9.8.9 Spawning and annulus formation for Lane Snapper from the Gulf region.

longevity of the species increases the difficulty in obtaining accurate age estimates of older fish. After age-15, Mutton Snapper somatic 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, making enumeration difficult as they approach their maximum age of about 40.

Mutton Snapper reproduction has not been widely studied throughout the Gulf of Mexico; however, data that are available indicate a spawning season from May to July with a peak in June (Domeier et al. 1996, Burton et al 2005; Figure 9.8.10). Burton (2002) validated annulus deposition using MIA and determined that formation occurred from March through June, with a peak in May.

Other Ageing Techniques

Break and burn has not been attempted on this species in the Gulf. Whole otoliths have not been used with any success. Researchers elsewhere in the Caribbean have used urohyal bones as an ageing structure for Mutton Snapper (Claro 1983, Palazón and Gonzáles 1986).

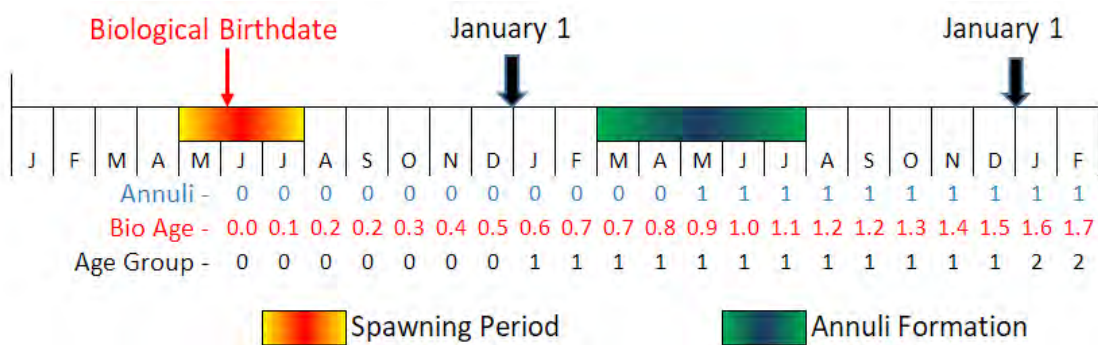


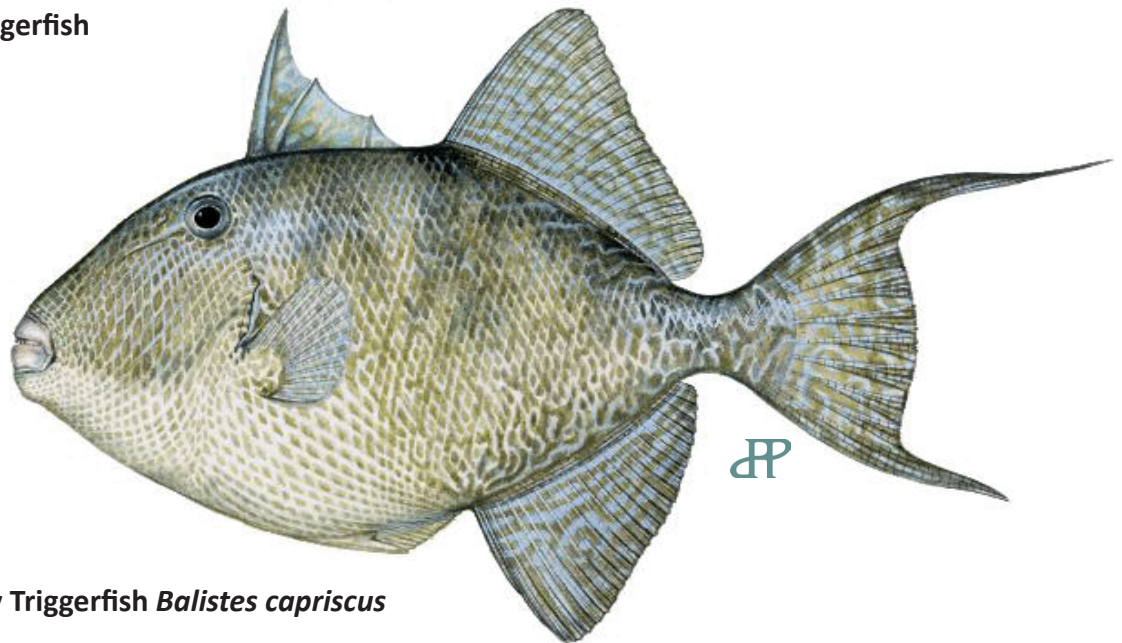
Figure 9.8.10 Spawning and annulus formation for Mutton Snapper from the Gulf region.

Research Needs

Ageing protocols and longevity have been validated for Red Snapper (Baker and Wilson 2001, Barnett et al. 2018) and Gray Snapper (Fischer et al. 2005, Andrews et al. 2020), but age validation is needed for other snapper species, especially Vermilion Snapper.

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9.9 Balistidae - Triggerfish



Gray Triggerfish *Balistes capriscus*

Highlights

- First dorsal spine commonly used for ageing.
- Spines are stored frozen or dry due to potential for specimen deterioration.
- Check marks (false annuli) occur and may be related to ontogeny, habitat shifts, and reproduction.
- Annuli in Gray Triggerfish spines often occur as doublets (two translucent zones) which are believed to be caused by limited feeding by males and females during the spawning season.
- Embedding of spines not required for sectioning.
- Otoliths small, fragile and can be difficult to extract intact.
- The maximum reported age for Gray Triggerfish is 15 for an individual of unknown sex (Burton et al. 2015), 12 for females (Johnson and Saloman 1984) and 13 for males (Hood and Johnson 1997).

Spine Description

The first dorsal spine in Gray Triggerfish is the most widely accepted hardpart for ageing (Johnson and Saloman 1984, Ofori-Danson 1989, Ingram 2001, Moore 2001, Bernardes 2002, Fioramonti 2012, Allman et al. 2015, Burton et al. 2015). The first dorsal spine is one of the more pronounced features in Gray Triggerfish (Figure 9.9.1). It is thick and elongate, lending to the common name Triggerfish as the dorsal spines are able to be locked (triggered) into an erect position as protection against predators (Matsuura and Katsuragawa 1985, Lyczkowski-Shultz and Ingram 2003). The main channel of the spine is deep



Figure 9.9.1 Dorsal fin spine from Gray Triggerfish in lateral view cross-sectioned. Cross-section of dorsal spine just above the condyle groove showing doublets and slight erosion of the core.

near the base or condyle as it supplies the vascularization for the spine. The result of the channel is a pair of lobes that eventually blend and become less pronounced further up the shaft away from the condyle. The vascularized region is the focus of the spine and the best portion with which to read annuli through the posterior lobes. However, this region can undergo resorption, obscuring, and even eliminating the first few annuli (Casselman 1983).

Spine Extraction

Removal of dorsal spines from Gray Triggerfish is relatively straightforward and can be applied to many species. See Chapter 6.0, Section 6.2.1 for a detailed description of the following methodologies.

1. Cut the membrane between the first and second dorsal spine toward the joint (Figure 9.9.2, 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 9.9.2, 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 or dry.

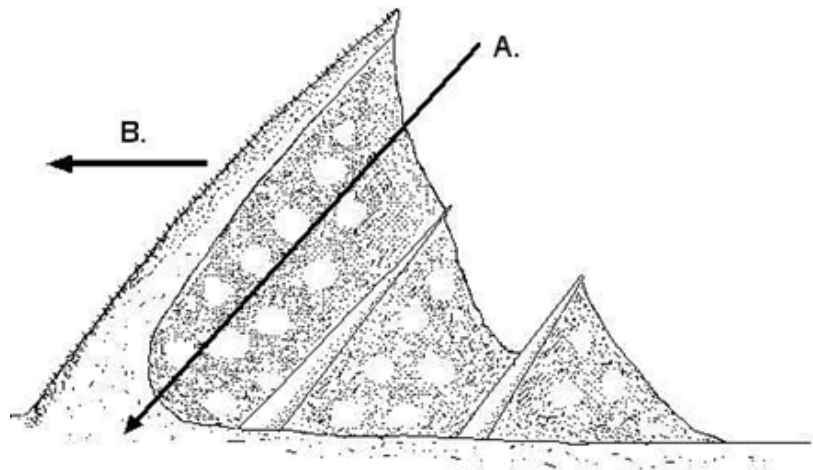


Figure 9.9.2 Cutting locations to remove first dorsal spine in Gray Triggerfish (from Ingram 2001).

Spine Processing

As noted in Chapter 6.0, Section 6.2.3, 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. Make the first cut just above the condyle of the dorsal spine is critical to ensure a readable section (Figure 9.9.3). Two techniques have been used in the Gulf for this species on the thin sectioning machine and low speed wafering saws, although any saw should suffice (see Chapter 6.0, Section 6.2.3). In addition, a multiblade technique can be used successfully on the Gray Triggerfish dorsal spine. Optimum section thickness is 0.5 mm.

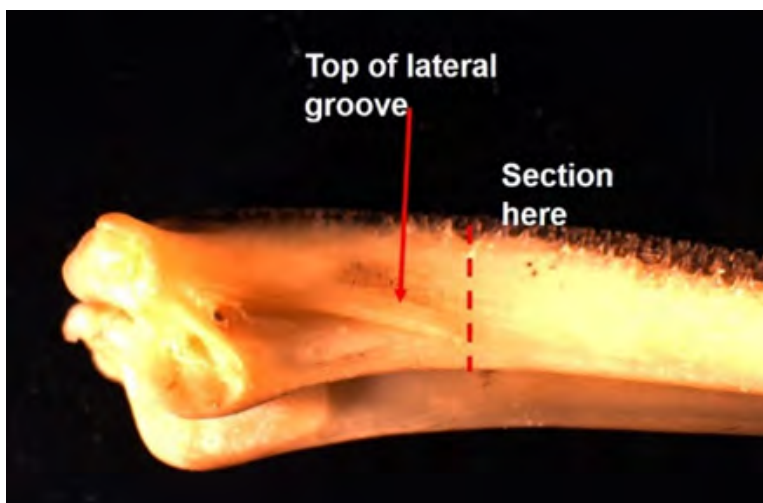


Figure 9.9.3 Location of optimal cutting plane just above the condyle of a Gray Triggerfish dorsal spine.

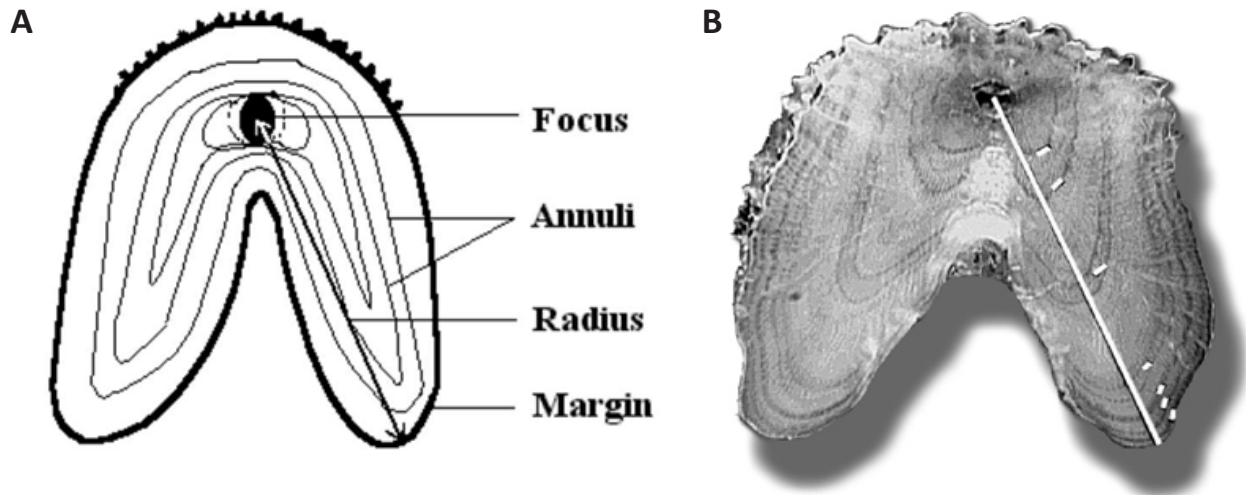


Figure 9.9.4 A) Generalized cross section of dorsal spine (from Ingram 2001). B) Cross section of an age-7 Gray Triggerfish spine indicating the core, radius, and annuli under reflected light. False annuli occur where two annuli appear with a single dash.

The summer and winter growth zones in a Gray Triggerfish spine section are opposite the pattern found in an otolith. These annuli radiate outward from the focus. The spine radius is measured as the distance from the focus to the margin of one of the posterior lobes, as seen in Figure 9.9.4.

Age Determination

Spawning season of Gray Triggerfish occurs in the Gulf region from May to August with a peak during June to July (Wilson et al. 1995, Lang and Fitzhugh 2015). In the Atlantic, spawning occurs from April to September with peak spawning May to August and a 1 July birthdate (Kelly-Stormer et al. 2017) (Figure 9.9.5).

Johnson and Saloman (1984) determined annulus formation occurred in the Gulf region from April to October with a peak in June and July; however, the authors assumed each translucent mark represented a single years growth but later work determined that the annual growth zone often includes two closely spaced translucent zones which should be counted together as one increment (Wilson et al. 1995, Ingram 2001). Kelly-Stormer et al. (2017) and Burton et al. (2015) used MIA and determined that translucent

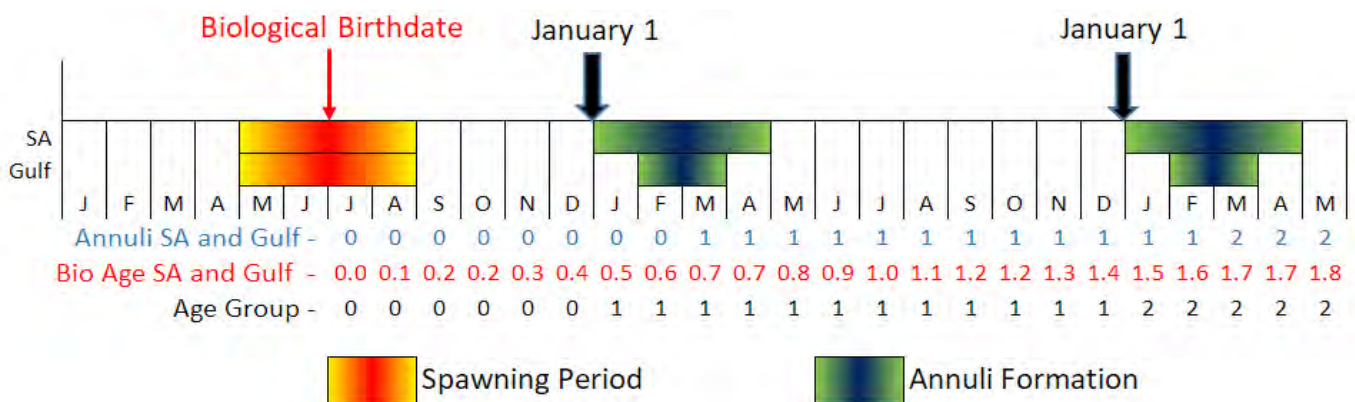


Figure 9.9.5 Spawning and annulus formation for Gray Triggerfish in the Gulf and South Atlantic regions.

zone formation was completed by July for Gray Triggerfish in the South Atlantic (Figure 9.9.6). Results from MIA and OTC marking of captive Gray Triggerfish indicate that translucent zone formation occurs in the winter and early spring in the Gulf (Allman et al. 2015) (Figure 9.9.6).

Often “false annuli” or checks in Gray Triggerfish spines are visible; annuli frequently split into doublets (two closely spaced translucent zones) (Figure 9.9.7). False annuli associated with checks that are incomplete and irregular are usually found only in one part of the structure and often not throughout the spine. 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 focus) and adult spawning events (midsummer) (Ingram 2001).

Otolith Description

The otoliths of the Gray Triggerfish are small and fragile and therefore can be difficult to locate and extract (Figure 9.9.9 and 9.9.10). Tuset et al. (2008) described the sagittae as “irregular, asymmetric, ventral area more developed, fan shaped and very fragile” and some explanation is required to understand the unique geography of Gray Triggerfish sagittal otoliths (Figure 9.9.11). The majority of previous age and growth studies of Gray Triggerfish utilized dorsal spines (Johnson and Saloman 1984, Ofori-Danson 1989, Escorriola 1991, Wilson et

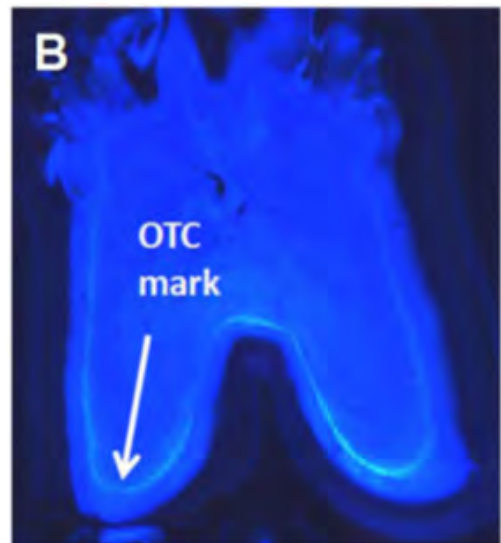
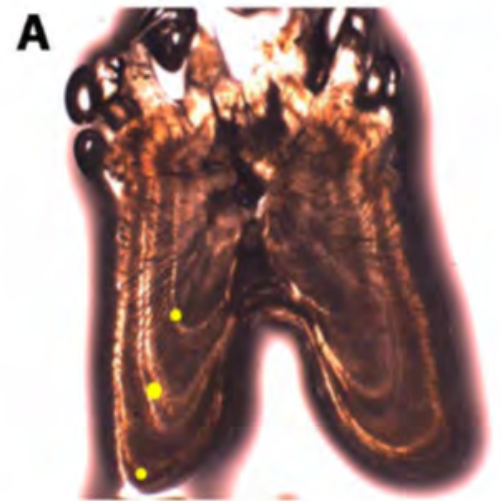


Figure 9.9.6 Digital images of OTC-marked Gray Triggerfish hardparts viewed with A) transmitted visible and B) UV light of dorsal spine. Translucent zones are marked with circles in each section. (Figure 1 A and B from Allman et al. 2015).

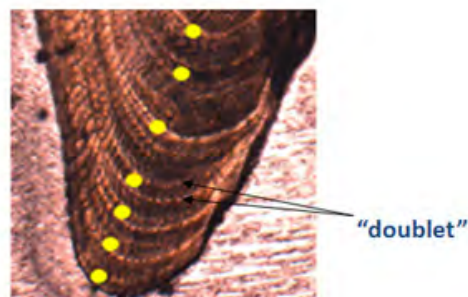
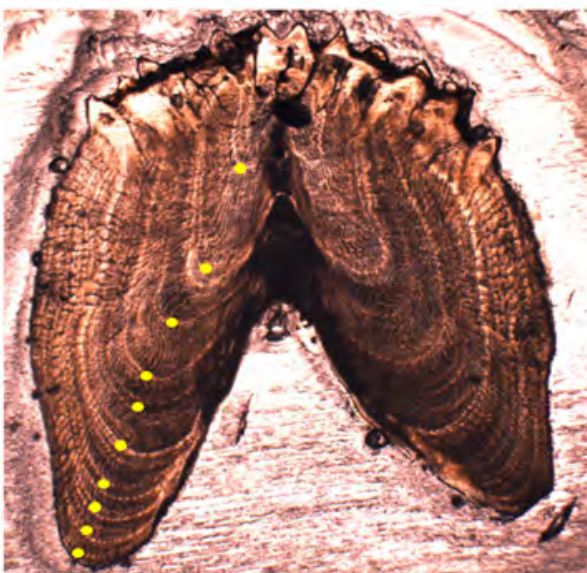


Figure 9.9.7 Example of a doublet in an age-10 Gray Triggerfish dorsal spine.

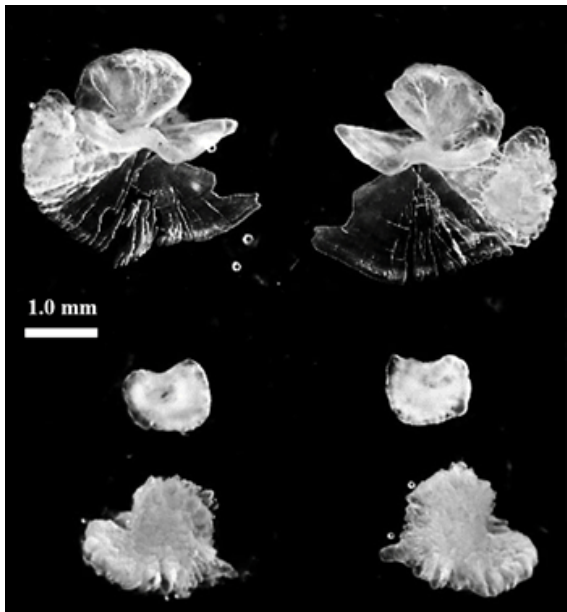


Figure 9.9.9 The three otolith pairs of Gray Triggerfish. Top to bottom: sagitta, astericus, and lapillus.



Figure 9.9.10 Radiograph of Gray Triggerfish neurocranium. Red circle approximates location of otic capsule and sagittae although the actual otoliths cannot be distinguished.

al. 1995, Hood and Johnson 1997, Ingram 2001, Kelly-Stormer et al. 2017, Allman et al. 2018). However, preliminary work by Patterson et al. (2019) used bomb radiocarbon analysis ($\Delta^{14}\text{C}$) to validate sagittal otolith age estimates for gray triggerfish and suggest that otoliths are not as difficult as originally thought to interpret and provide more accurate counts than dorsal spines.

Otolith Extraction

Sagittal otoliths in triggerfish can be difficult to remove by any technique other than cutting vertically through the entire head.

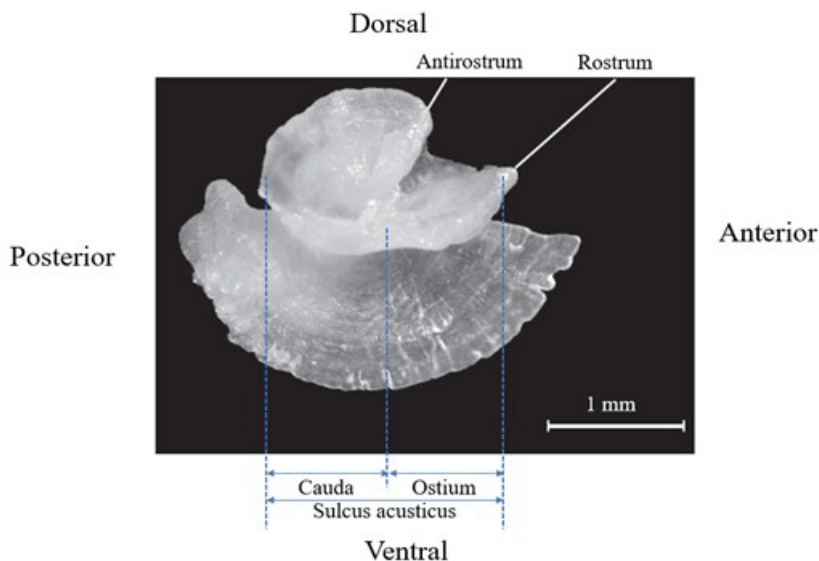


Figure 9.9.11 Left sagittal otolith (original photo from Tuset et al. 2008) with labels for the various defining structures within the otolith added (modified from Appendix 1 in Shevette and Dean 2015).

1) Use a thumbnail to locate the dorso-anterior edge of the post occipital bone (Figure 9.9.12A).

2) Line up a stout knife blade parallel to the angle of the post orbital bone just identified with your thumbnail then shift the blade 5 mm posterior of that location (Figure 9.9.12B) and make a cut down through the head (Figure 9.9.13A).

3) Turn the triggerfish head away and carefully remove the otolithic membrane from the cavity (Figure 9.9.13B). Save the entire structure with the otoliths (Figure 9.9.14) in 0.5

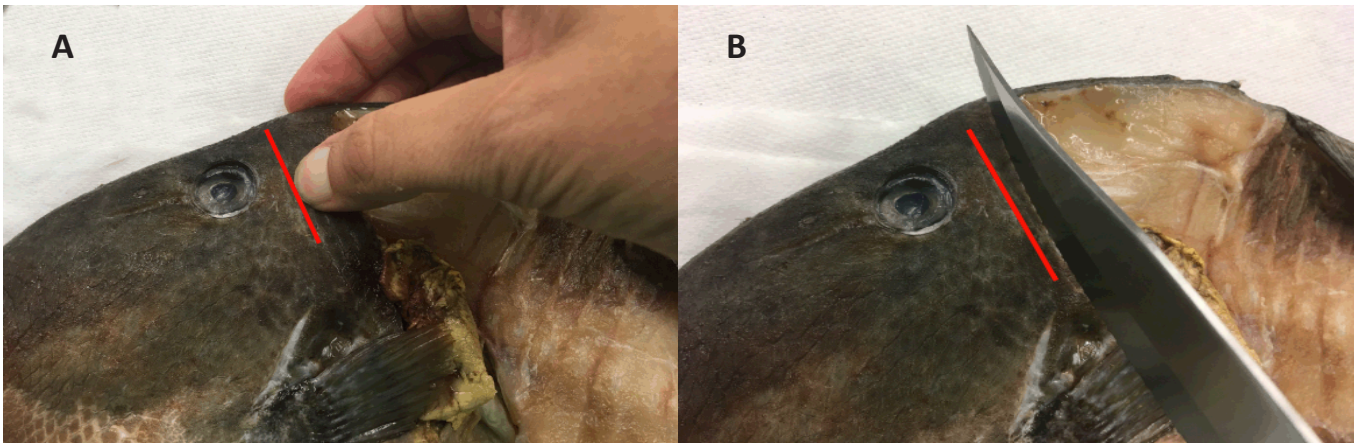


Figure 9.9.12 A) Red line indicates dorso-anterior edge of the post occipital bone. B) Cut begins a few millimeters behind the edge of the bone.

ml flip-cap plastic vials inside labeled envelopes, until they are read.

Otolith Processing

Triggerfish sagitae can be read whole. Submerging the otoliths in water against a black background allows the sagitae to be removed from the sacule tissue with the aid of a stereoscope at a magnification of 20-40X. Counts of opaque zones are made along the sicular groove under reflected light (Figure 9.9.15).

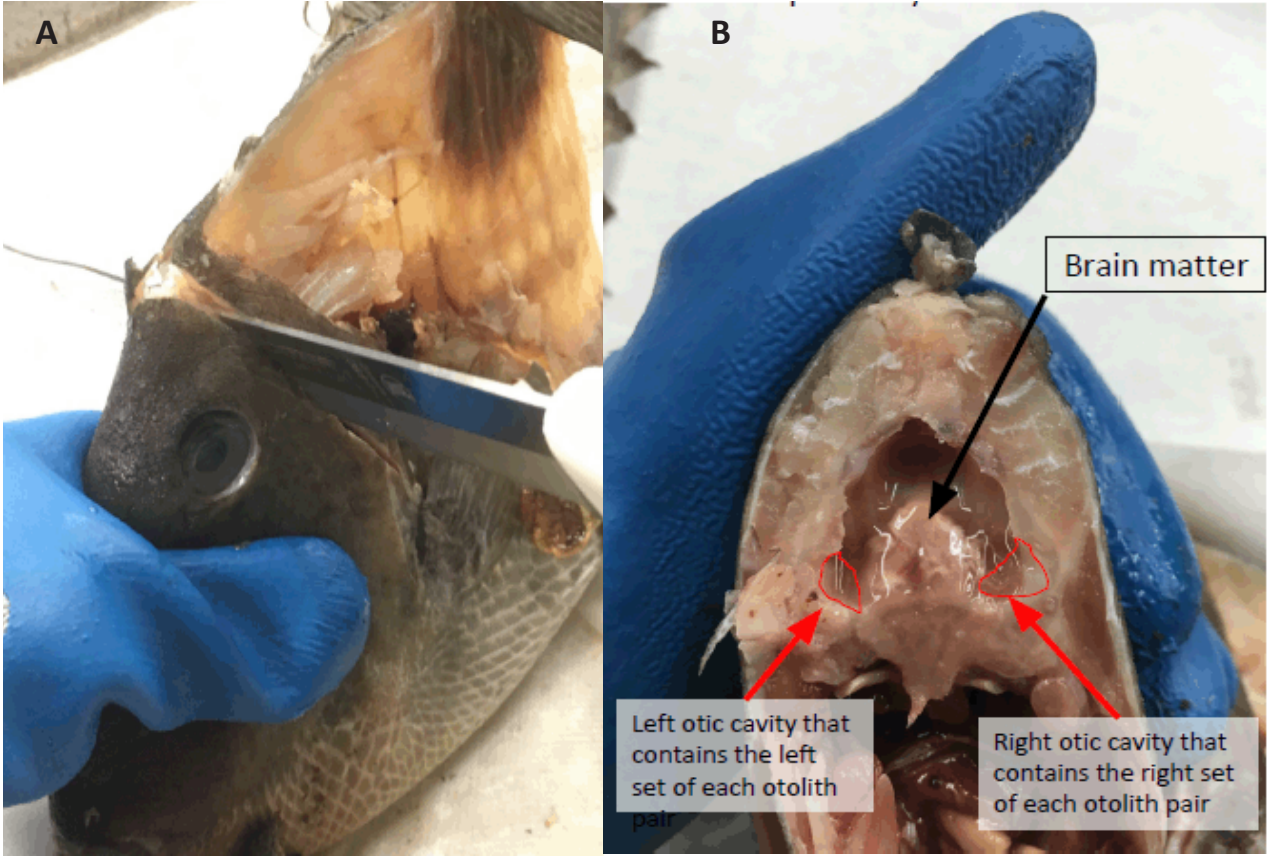


Figure 9.9.13 A) After cut is made completely through the head, B) gently remove the otolithic membrane from the otic capsule.

Other Ageing Methods

Scales have not been used in this species successfully, due to the strong insertion of the scales into the Gray Triggerfish's tough skin (Ingram personal communication). Allman et al. (2015) examined a number of structures (dorsal spines, fin rays and vertebrae) after marking with OTC to look at the usefulness of all the hardparts for ageing and validation of annuli formation. Fin rays were found to overestimate age compared to dorsal spines and vertebrae. Based on the effort to extract and process abdominal vertebrae, dorsal spines were concluded to be the best structure despite the difficulty in interpreting the translucent zones (Allman et al. 2015). A large age-validation study for Gray Triggerfish is currently underway in the Gulf region using the bomb ^{14}C chronometer to validate age estimates (Patterson et al. 2019).

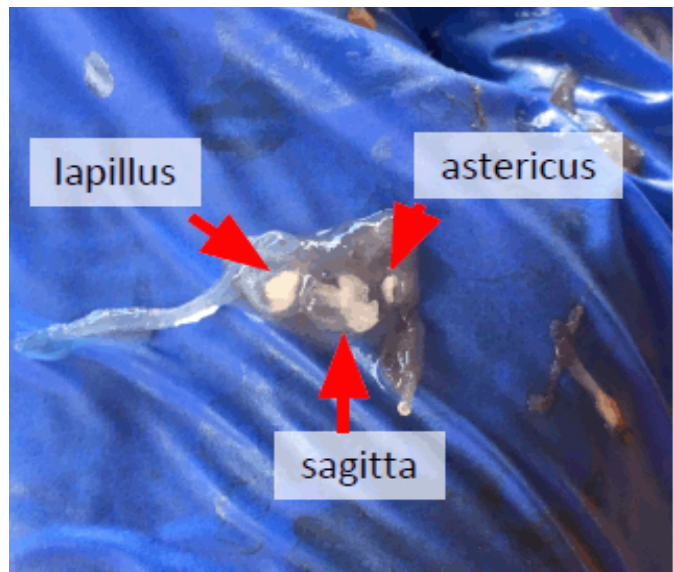


Figure 9.9.14 Freshly removed otoliths, still encased in their otolithic membrane for protection and ready for storage. Remaining tissue is removed at the time of reading.

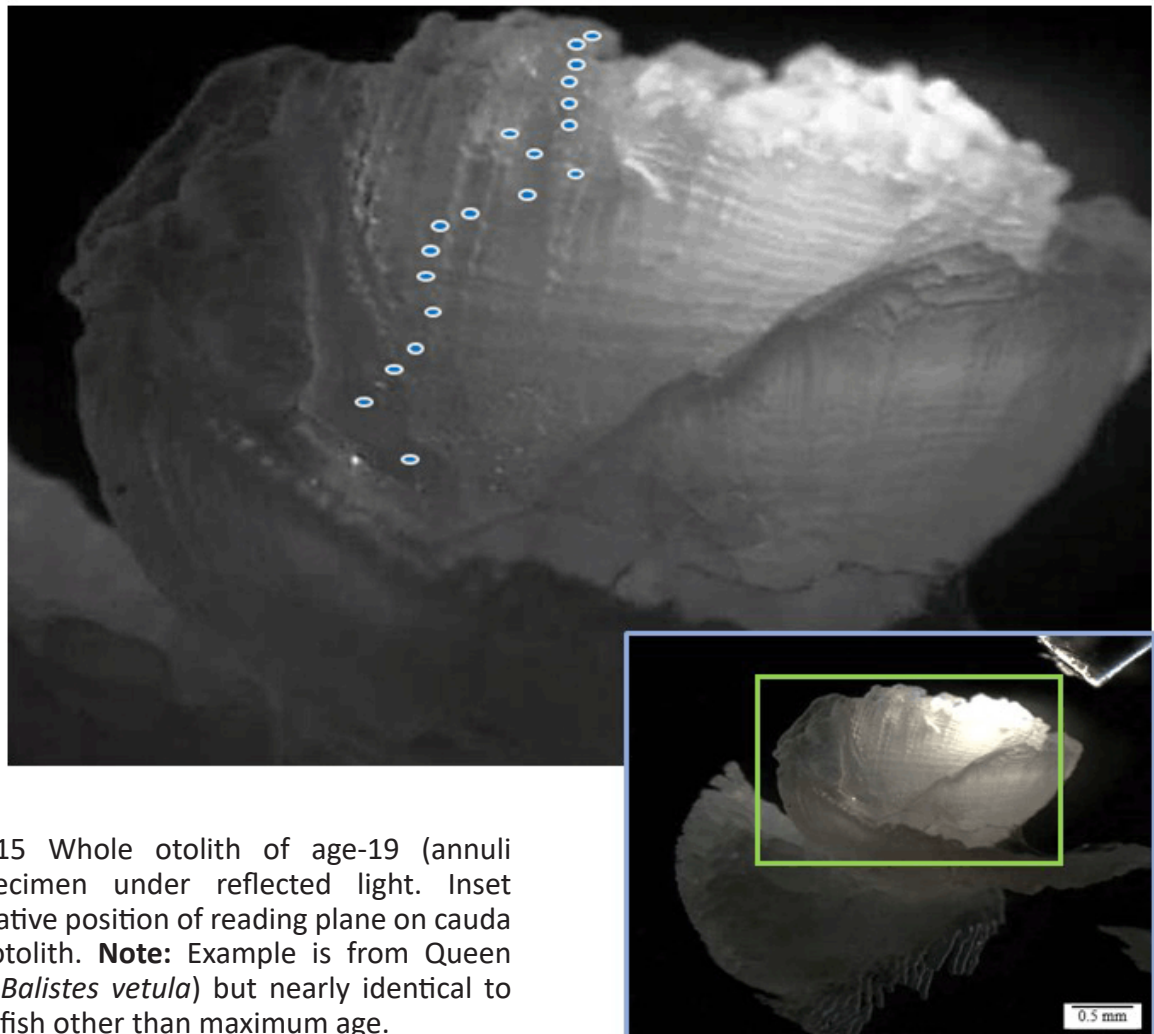


Figure 9.9.15 Whole otolith of age-19 (annuli circled) specimen under reflected light. Inset provides relative position of reading plane on cauda along the otolith. **Note:** Example is from Queen Triggerfish (*Balistes vetula*) but nearly identical to Gray Triggerfish other than maximum age.

Gray Triggerfish sagittal otoliths can be embedded and sectioned but it is difficult to get the otolith in a consistent orientation to obtain a readable section. Shervette (personal communication) indicated that only around 25% of the sections they attempted were readable. However, those that were done properly provided better counts than spine sections which tended to underage fish (Figure 9.9.16).

Research Needs

While considerable effort has been given to successfully ageing Gray Triggerfish, there are still a number of issues with various techniques. A comprehensive study is needed to determine the most reliable ageing structure for Gray Triggerfish. At time of this publication, an age validation study of spines, otoliths and vertebrae is underway in the Gulf (Patterson et al. 2019).

Ageing validation of structures is still necessary in other regions. Additional work is needed to determine the applicability of the otolith methodology for stock assessment and production ageing purposes, given fragility of the otolith and historical difficulties of extraction. Further work is necessary to identify the causes of false annuli on spine sections, which are frequently noted and currently considered to be related to larval settlement (near the focus) or due to spawning events.

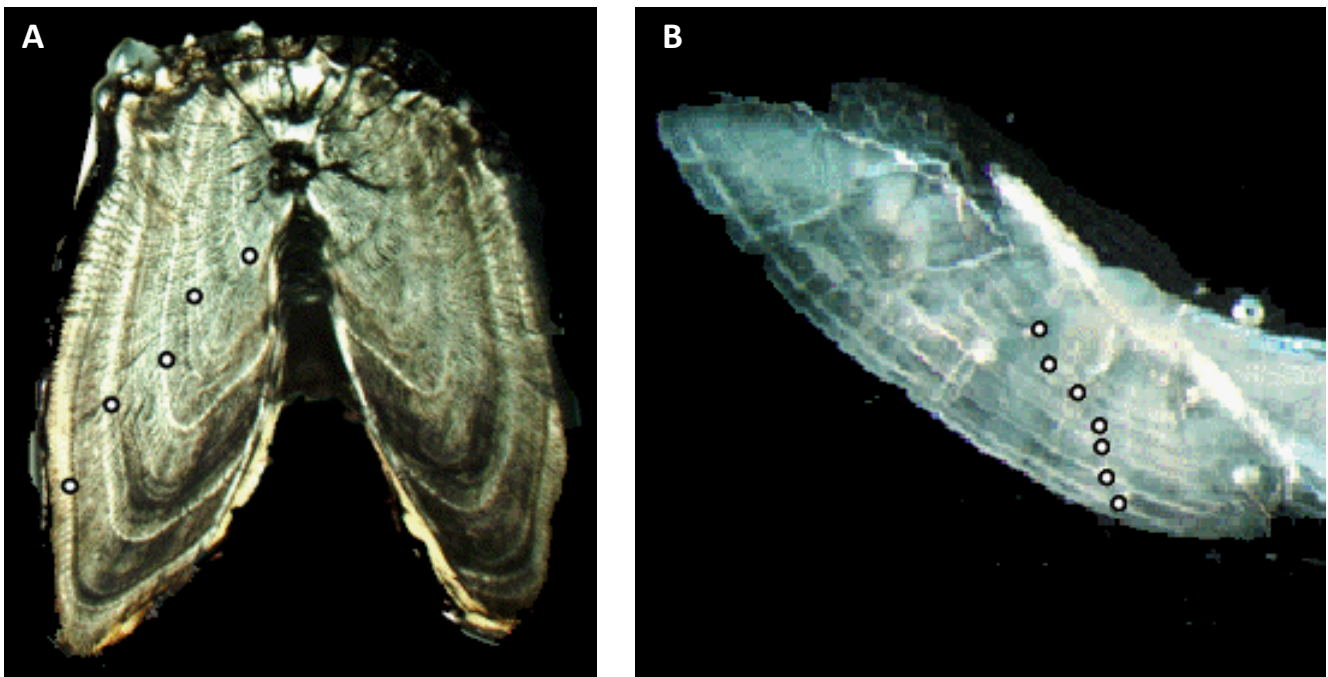
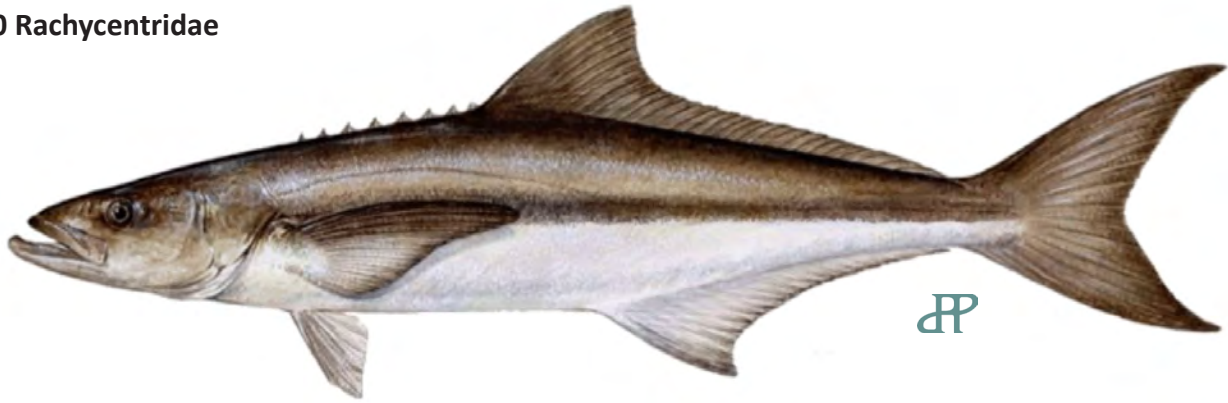


Figure 9.9.16 Sections of A) the first dorsal spine and B) the sagittal otolith from the same Gray Triggerfish (circles denote annual increments). The dorsal spine underages the fish compared to the otolith section by 2 years (age-5 vs age 7).

9.10 Rachycentridae



Cobia *Rachycentron canadum*

Highlights

- Cobia are migratory throughout their range, extending from the New York and New Jersey south through the Gulf of Mexico.
- Cobia otoliths are very small and fragile and are typically embedded for sectioning.
- Cobia otoliths can be difficult to locate and remove.
- The first annulus on sagittal otoliths of Cobia occurs at one year.
- Maximum age is around 11 years in the Gulf of Mexico (Franks et al. 1999) and 16 years in the mid-Atlantic (ODU unpublished data).

Otolith Description

Cobia are a coastal pelagic fish of the monotypic family Rachycentridae. Cobia sagittae are small, elongate, laterally compressed, and have a deeply indented sulcal groove on the medial side (Figure 9.10.1). The rostrum and antirostrum are easily distinguishable and extremely fragile due to their small size and overall thinness of the entire otolith. The relative location of the sagittal otoliths is illustrated in Figure 9.10.2.

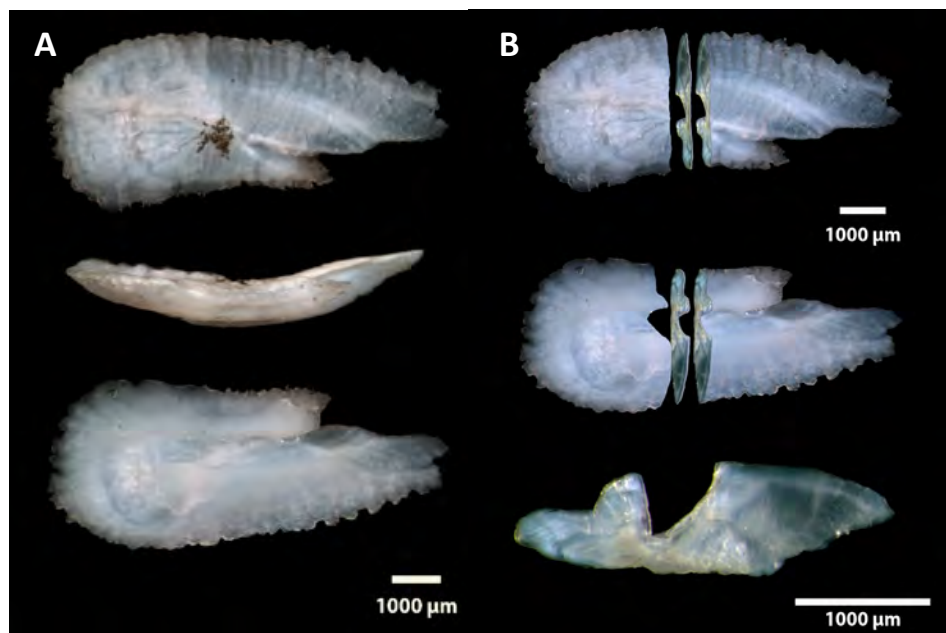


Figure 9.10.1 A) Whole otoliths of Cobia proximal view with core marked (top), dorsal view (middle), and distal view (bottom) and B) Cobia otolith sectioned with thin-section located and rotated showing location of cut through the core.

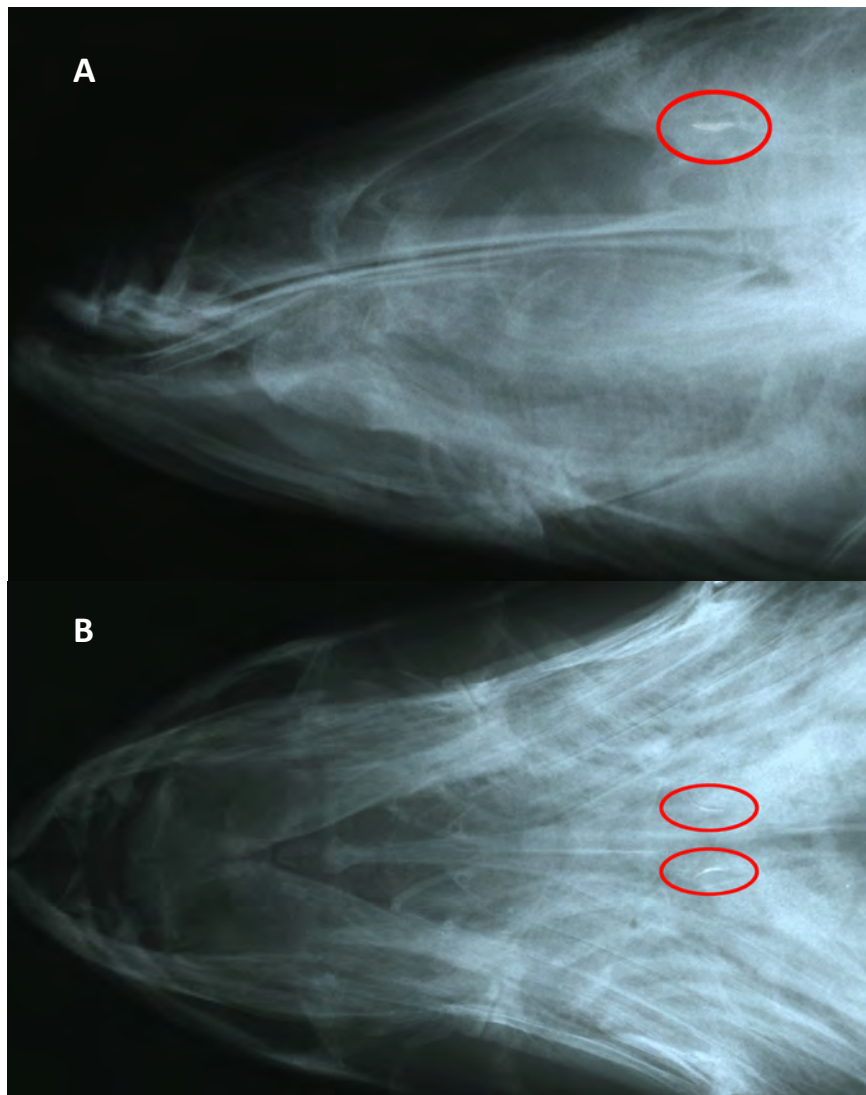


Figure 9.10.2 Radiographs showing location of sagittae (red circles) in Cobia cranium in A) lateral and B) dorsal/ventral views.

Otolith Extraction

Otolith removal in Cobia can be difficult even for experienced personnel. Due to the large size of most Cobia encountered in the recreational fishery, most researchers access the otoliths through the head with either a downward or horizontal cut. The use of a small, battery powered reciprocating saw has proven to be very effective when cutting through large heads although a handsaw or butchers saw will work as well. Most of the techniques for removing the otoliths in Chapter 3.0, Section 3.2.1 work with Cobia. The most used methods are (1) a horizontal cut across the top of the brain cavity (Figure 9.10.3A) or (2) a shallow downward cut just behind the centerline of the preopercle through the otic capsules and using the weight of the fish to snap open the rest of the capsule against the edge of a table (Figure 9.10.3B-D).

Note: The otoliths are very small relative to the size of the head making “digging” a frequent event. Care must be taken not to damage the otoliths while probing. Once the otic capsule is opened, the otoliths can be lost easily among other bone and tissue and can be swept away when rinsing head tissues with water.

Processing

Because of their small size and fragile nature, Cobia otoliths are typically embedded, however, they have

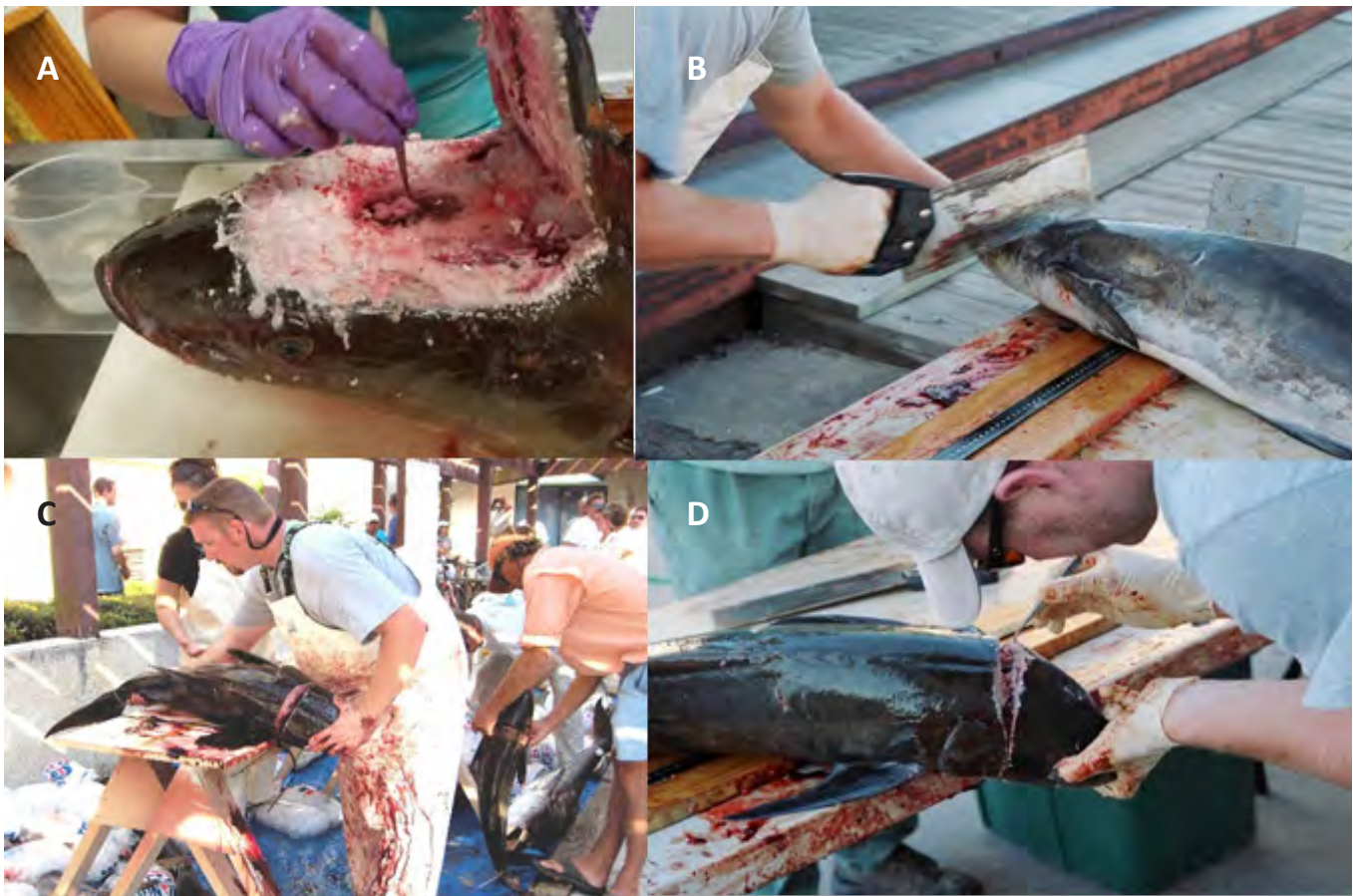


Figure 9.10.3 A) The horizontal technique and B-D) the vertical cut technique for extraction of otoliths from Cobia.

been sectioned at a variety of thicknesses from 0.3 mm (Franks et al. 1999) to 0.7mm (SCDNR personal communication). The primary saws used for Cobia otolith sectioning are the low and high speed wafering saw. The thin sectioning machine has not been used successfully with Cobia otoliths.

Age Determination

Cobia otoliths are relatively easy to read once sectioned (Figure 9.10.4). Franks et al. (1999) examined around 650 sagittae and found 25-29% were illegible but the legible otoliths had 96% agreement between multiple readers. However, they did find that annuli were not always clear and occasionally obscured along the ventral sulcal ridge in older fish (age-5+). Additional work by Hendon et al. (2004) suggests that, despite the time requirement to embed and section most Cobia otoliths, ageing in cross-section is the best approach versus spines and whole otoliths. The SCDNR indicates a much higher legibility at around 99% from South Carolina waters (unpublished data).

Cobia generally spawn between April and July with an average peak in May and June in the mid-Atlantic (Joseph et al. 1964, Hassler and Rainville 1975, Smith 1995, Brown-Peterson et al. 2001, Lefebvre and Denson 2012, SEDAR58 2018) and north as far as New Jersey and New York (SCDNR personal communication), Cobia spawn in the northern Gulf and western Florida from April through September with an average peak in May and July, respectively (Figure 9.10.5) (Brown-Peterson et al. 2001). Franks et al. (1999) validated annuli production using MIA for Cobia from the Gulf which indicates that they form a single annulus on their sagittae each year during April through August (Franks et al. 1999) which agrees with Thompson et al. (1992) off Louisiana and Smith (1995) off North Carolina. Williams (2001) assigned a June 1 formation date for Gulf Cobia in his stock assessment. South Atlantic Cobia were assigned a birthdate of June 1

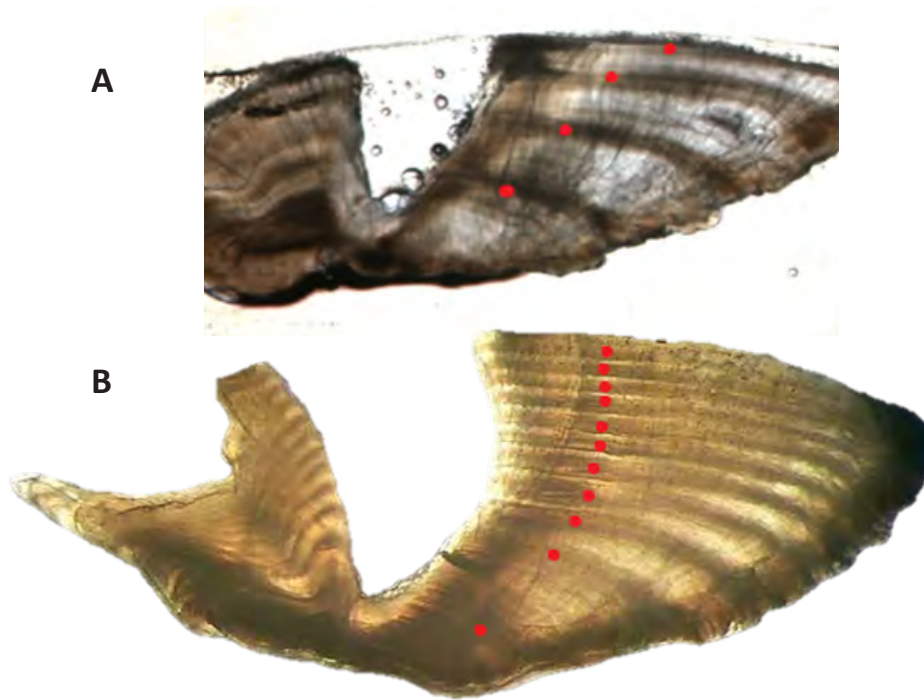


Figure 9.10.4 Transverse sections otoliths showing A) an age-4 Cobia and B) an age-11 Cobia. Annuli are indicated by red dots.

based on published GSIs (SEDAR28 2013). Williams (2001) utilized the same date for the Gulf assessment based on the overlapping spawning and MIA by Franks et al. (1999). Kalinowsky (unpublished data) and SCDNR (unpublished data) have validated annulus production exploring recaptures of conventionally and genetically tagged hatchery fish. SCDNR has a captive breeding program and has released Cobia back into their waters since 2008. Angler returns provided perfect matches between the otolith age and time at liberty from the known age hatchery fish.

Other Ageing Techniques

A number of techniques have been utilized to enhance the readability of the otoliths such as staining, immersion, and polarizing filters with limited success.

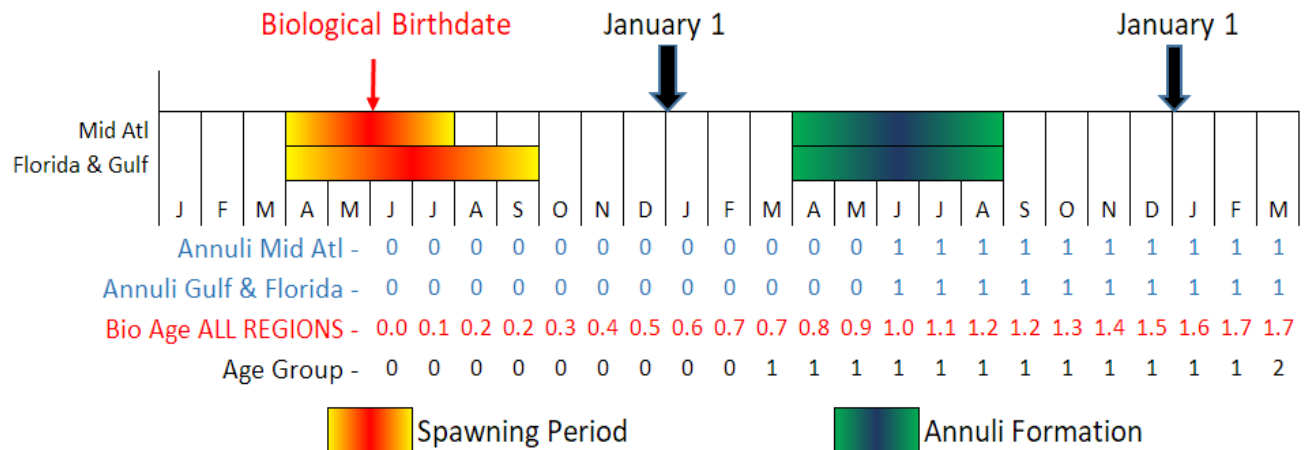


Figure 9.10.5 Birthdate assignment timeline for Cobia in the mid-Atlantic (VA to GA), and Florida and Gulf. Bio Age is the same for all regions with the accepted June 1 birthdate.

Whole Otoliths

Identifying annual rings on whole otoliths can be a formidable task to inexperienced personnel. Thin-sectioned sagittae generally reveal obvious annuli. Hendon et al. (2004) examined whole Cobia sagittae for age estimation and reported that rings were often difficult to discern (Figure 9.10.6). These authors reported that estimating age of Cobia using whole sagittae is not a recommended procedure. However,

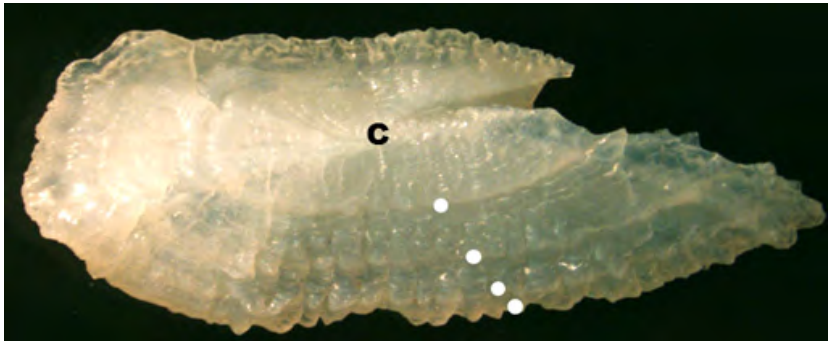


Figure 9.10.6 Whole otolith from an age-4 Cobia. Annuli indicated by white circles and C marks the core.

Franks et al. (1999) reported whole sagittal weight as a good predictor of Cobia age based on sectioned age readings of the weighed otoliths. rings observed in sectioned first dorsal spines revealed the first dorsal spine to be an unreliable ageing hard part for Cobia, often over and under estimating the age of older fish by as much as two years. Although far more practical in terms of ease of collection, ageing Cobia using sectioned spines is not recommended.

Scales

Richards (1967) used scales to determine Cobia age (Figure 9.10.8), and reported that annuli after the fifth year were difficult to recognize. Since Cobia can live between ten and 14-years, scales are not recommended for ageing Cobia samples.

Research Needs

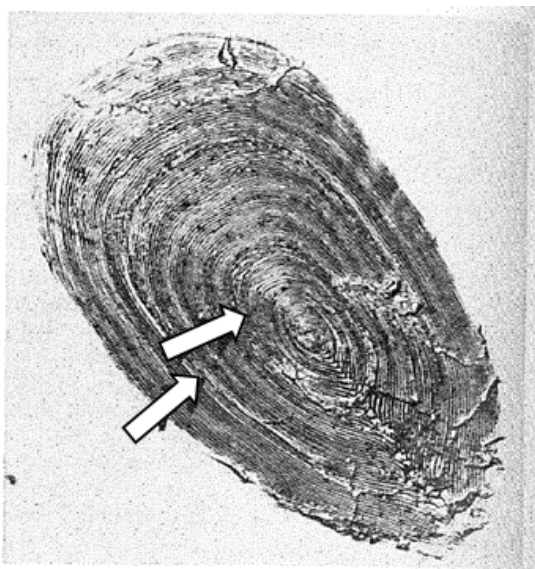


Figure 9.10.8 Location of two annuli on a Cobia scale marked with arrows (from Richards 1967).

At this time there is not a lot of research needed for Cobia specific to age determination. The standard procedures are well laid out and have a long history (Franks and Brown-Peterson 2002).

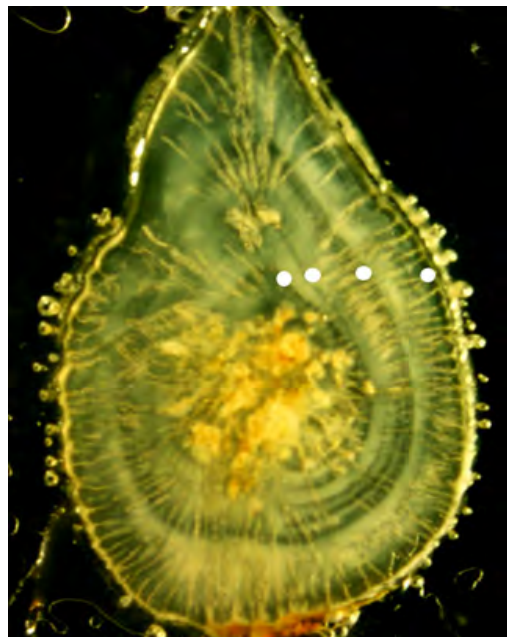


Figure 9.10.7 Cross section of an age-4 Cobia first dorsal spine (verified in otolith section) showing the vascularization of the core region and four distinct growth zones (from Hendon et al. 2004).

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9.11 Scombridae - Mackerels



King Mackerel *Scomberomorus cavalla*



Spanish Mackerel *Scomberomorus maculatus*

Highlights

- Otoliths are elongate, laterally compressed, and very fragile.
- Otoliths relatively easy to locate and remove.
- First ring may resemble a diffuse 'smudge' in otolith cross-section.
- Mackerel can be successfully aged up to age-6 using whole otoliths.
- Rings in sectioned otoliths are usually distinct in older fish.
- King mackerel can live up to 26 with the majority of ages around 2-6 years (DeVries and Grimes 1997).
- Spanish Mackerel can live to age-11 with the majority around 1-4 (NOAA personal communication).

Otolith Description

Mackerel sagittae are small, elongate, laterally compressed, and have an indented sulcus on the medial side (Figure 9.11.1). The rostrum and anterostrum are easily distinguishable and extremely fragile. The location of the otolith is illustrated in Figure 9.11.2 and is similar for most of the mackerel species.

Extraction

Otolith removal is relatively easy; therefore, any of the techniques illustrated in Chapter 3.0, Section 3.2.1 can be used. Due to the King Mackerel's size, the meatsaw technique is recommended when the condition of the head is not important (Figure 9.11.3). For Spanish Mackerel, a regular knife and standard tools can be used. The otic capsule in 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.

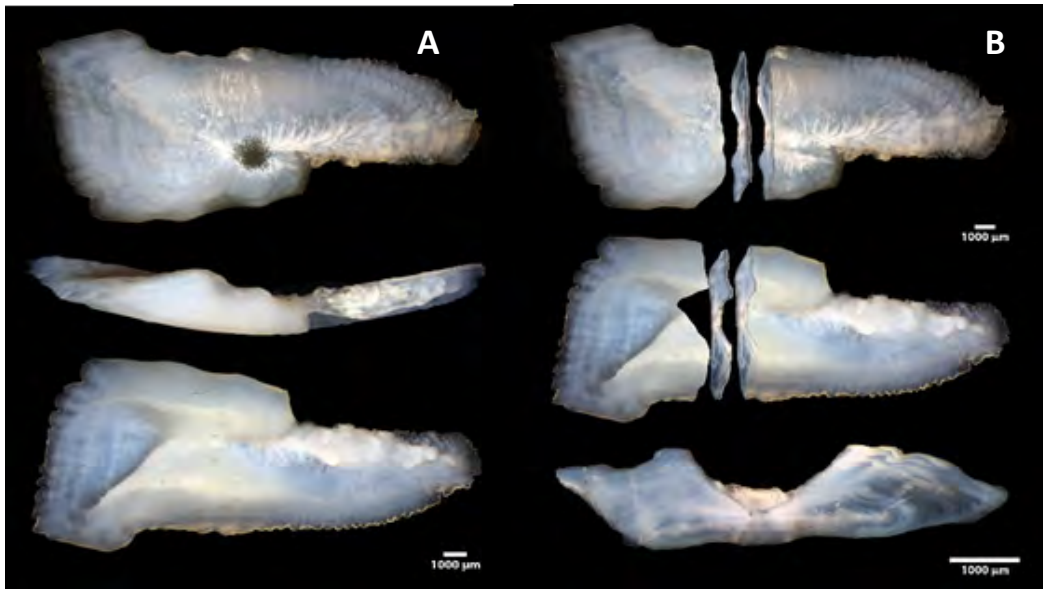


Figure 9.11.1 A) Whole otoliths of King Mackerel proximal view with core marked (top), dorsal view (middle), and distal view (bottom) and B) King Mackerel otolith sectioned with thin-section located and rotated showing location of cut through the core.

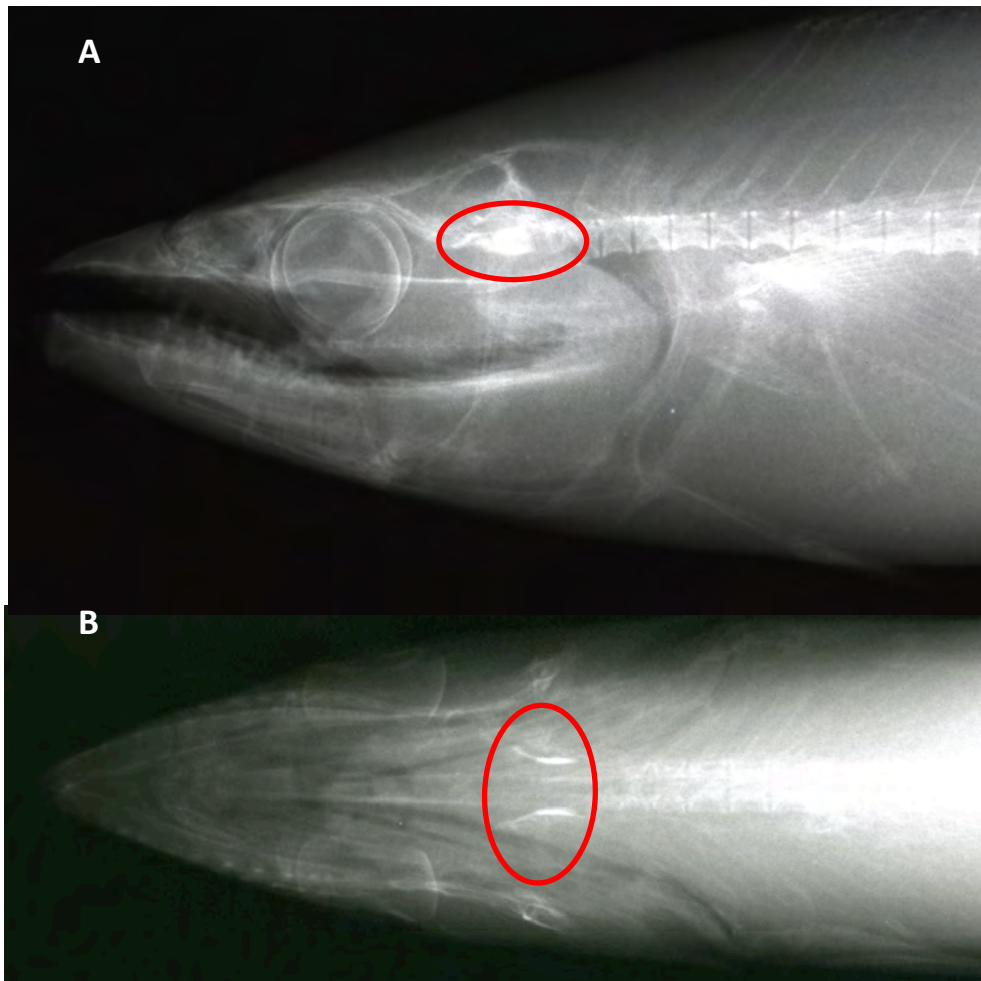


Figure 9.11.2 Radiographs showing location of sagittae (red circles) in Spanish Mackerel cranium in A) lateral and B) dorsal-ventral views.

Processing

For very young King Mackerel and most Spanish Mackerel the otoliths can be read whole. Because of their small size, unless read whole, most otoliths from larger King and Spanish Mackerel are embedded in bullet molds (Chapter 3.0, Section 3.2.5.2). 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 NOAA 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.



Figure 9.11.3 Meatsaw technique for extraction of otoliths from King Mackerel.

Age Determination

A phenomenon which can occur sporadically in King Mackerel and Spanish Mackerel otolith sections is for annuli to appear as doublets or couplets, which can lead to significant over-ageing problems if one is not careful (Figure 9.11.4). Reading the section slightly out of 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 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.

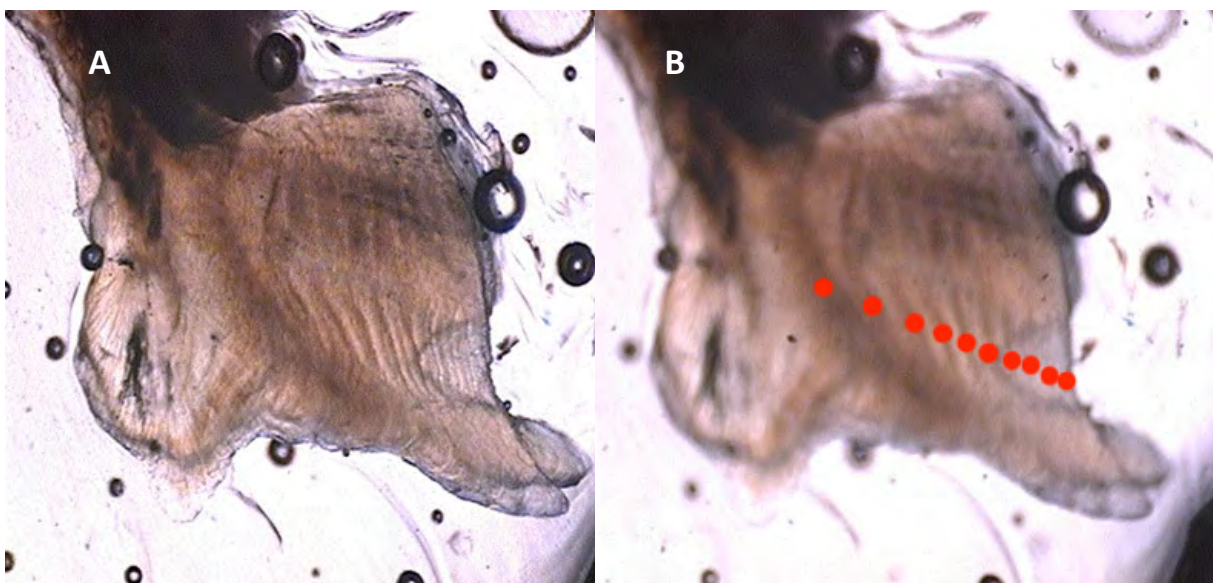


Figure 9.11.4 A) Doublets in King Mackerel otolith and B) the same section which has been intentionally blurred to minimize the doublet pattern. Red dots mark the annuli.

King Mackerel

Age determination in King Mackerel is complicated by a protracted spawning period (Figure 9.11.5) which occurs from May through October in the northern Gulf (Finucane et al. 1986) peaking in September (Grimes et al. 1990).

Late spawned fish may have a very diffuse first annulus due to an early spring annulus formation which occurs from March to May along the Florida East Coast (Beaumariage 1970). Johnson et al. (1983) reported annulus formation occurs from April to July based on fish from North Carolina to the Gulf.

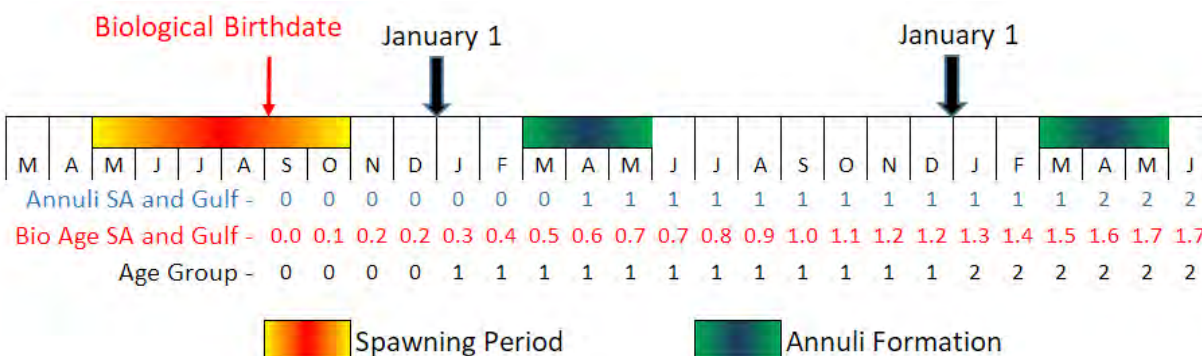


Figure 9.11.5 Timeline for King Mackerel showing spawning period and annulus formation for the Gulf of Mexico and South Atlantic. King Mackerel are aged with an accepted birthdate of September 1.

With few exceptions, small King Mackerel up to approximately age-4 are much easier to age using whole otoliths. A general rule is to use whole otoliths to age males <800 mm FL and females <900 mm FL. The following is a brief methodology for ageing King Mackerel using whole otoliths. 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 9.11.6). 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.

Annuli in sectioned King Mackerel otoliths 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 9.11.7). The first annulus is almost always the most difficult to identify, as it is often just a broad, diffuse 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. The ventral portion should be examined if the fish is very young (i.e., two or three) as sometimes the annuli will be clearer there than on the dorsal portion.

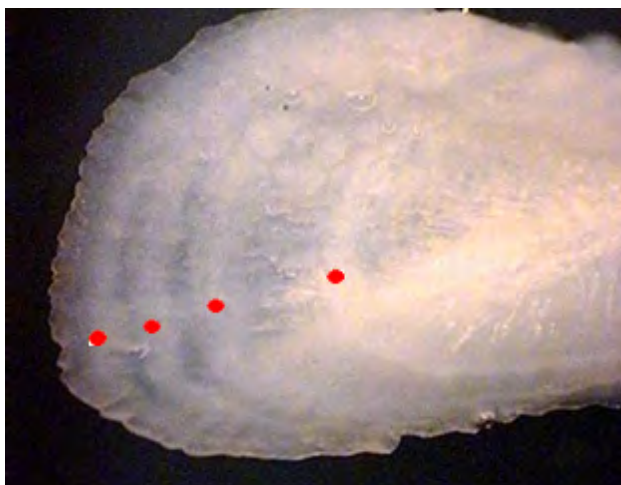


Figure 9.11.6 Whole otolith from an age-4 King Mackerel with annuli indicated by red dots.

Spanish Mackerel

Similar to King Mackerel, age determination in Spanish Mackerel is complicated by its protracted

spawning period (Figure 9.11.8). Schmidt et al. (1993) reported spawning along the South Atlantic occurred from May through October/November which agrees with studies from the northern Gulf (Powell 1975, Finucane and Collins 1986). Cooksey (1996) reported spawning by Spanish Mackerel in Chesapeake Bay occurs from June to August similar to Earll (1882) who estimated spawning in New York and New Jersey occurred from August to September. Gaichas (1997) found annuli formation occurs from May-June in the Chesapeake Bay using MIA. Schmidt et al. (1993) determined annulus formation occurring May-July from North Carolina to Florida which agreed with Powell (1975). Fable et al. (1987) found slightly early formation in the northern Gulf of Mexico from March to May.



Figure 9.11.7 An otolith from an age-8 King Mackerel sectioned on a low-speed saw. Annuli indicated with white arrows.

Similar to King Mackerel, small Spanish Mackerel up to approximately age-3 are much easier to age using whole otoliths rather than sections. Spanish mackerel females <550 mm FL and males <450 mm FL can successfully be aged whole. It should be noted, however, that specimens as large as 600 mm FL have been aged using whole and sectioned otoliths with high levels of agreement (Mareska personal communication). 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.

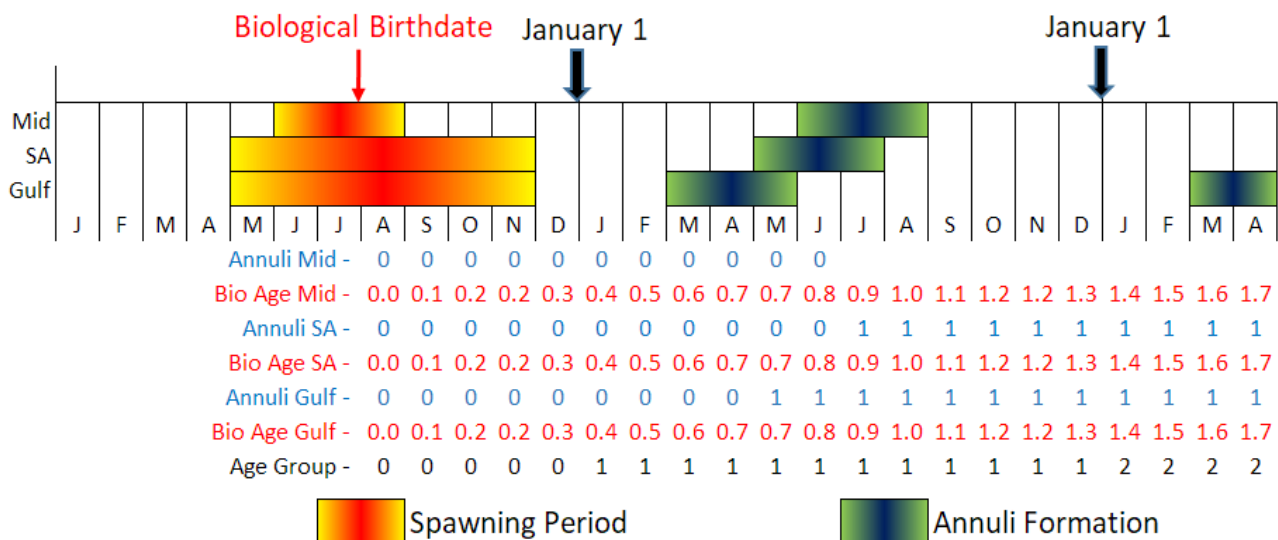


Figure 9.11.8 Spawning and annuli deposition timeline for Spanish Mackerel by region. Early spawned fish can have a mark in the core region, but it is not generally counted as an annulus.

Annuli in sectioned Spanish Mackerel otoliths are most readable in the dorsal portion, especially along the sulcus (Figure 9.11.9A). 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 9.11.9B). 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. Similar to King Mackerel, the ventral portion should be examined if the fish is very young (i.e., two or three) as sometimes the annuli will be clearer there than on the dorsal portion.

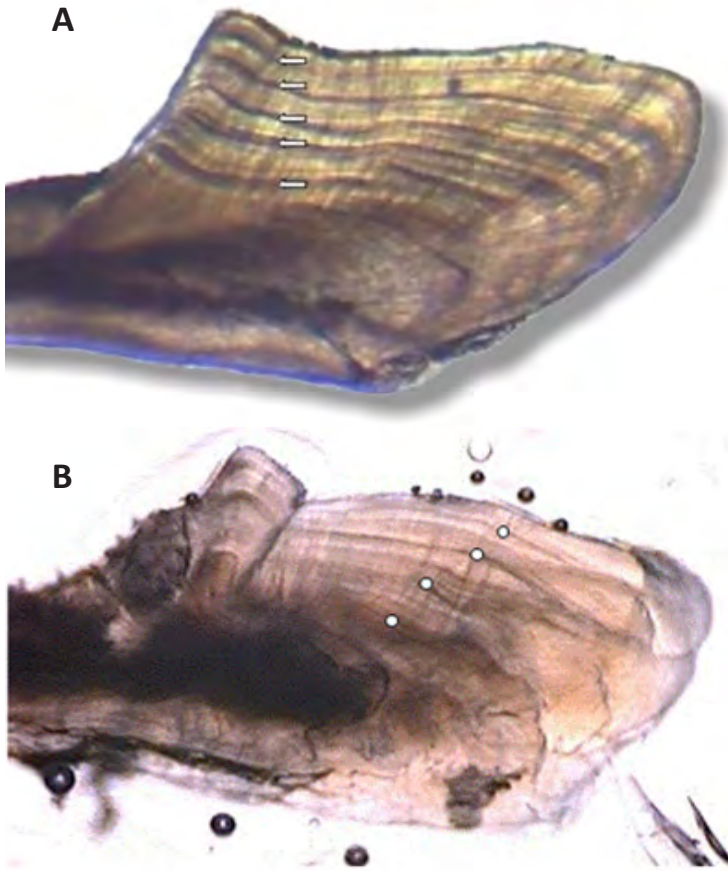


Figure 9.11.9 A) Sagittal otolith section from age-5 Spanish Mackerel. B) Sagittal otolith section from an age-4 fish with a diffuse first annulus. White dashes and dots indicate annuli.

if it is not clear on the dorsal end. Similar to King Mackerel, the ventral portion should be examined if the fish is very young (i.e., two or three) as sometimes the annuli will be clearer there than on the dorsal portion.

Other Ageing Methods

Gaichas (1997) examined sectioned dorsal spines, pectoral fin rays, and vertebra centra as options for ageing Spanish Mackerel. Gaichas determined that dorsal spines were too highly vascularized and fin rays were asymmetrical and had similar issues with a lack of central growth in the core to be utilized. Vertebral centra provided marks around the centrum but required staining with crystal violet and the growth center consisted of a hole through all the vertebrae. Gaichas' results indicate that pectoral fin rays and stained vertebrae should not be used for ageing Spanish Mackerel. Dorsal spines could be used on young fish but the vascularization and resorption of the core region resulted in under-ageing of older fish (Gaichas 1997).

Research Needs

Gulf of Mexico and South Atlantic King Mackerel are managed as separate stocks. Migration of these stocks off the coast of Florida results in a stock mixing zone. Further

research and applicable tools are needed to reliably identify catches to the correct stock for effective management.

9.12 Carangidae - Jacks



Greater Amberjack *Seriola dumerili*

Highlights

- Otoliths small and fragile, easy to break during extraction and typically require embedding.
- Annuli not always discernible requiring manipulation to read.
- The first annulus forms at about 12-15 months of age but most sections have an opaque core with a dark, lobed structure referred to as a 'butterfly wing' (Murie and Parkyn 2008).
- Very few fish older than 6 years occur in the catch but Greater Amberjack can live to age-17 (Manooch and Potts 1997a).

Otolith Description

Greater Amberjack sagittae are small, thin, and fragile (Figure 9.12.1). They have an elongated rostrum that breaks off easily, so care must be taken in extraction, as well as in the storage method (i.e., do not store them in coin envelopes). The otoliths have a very deep sulcus, which makes them prone to breaking

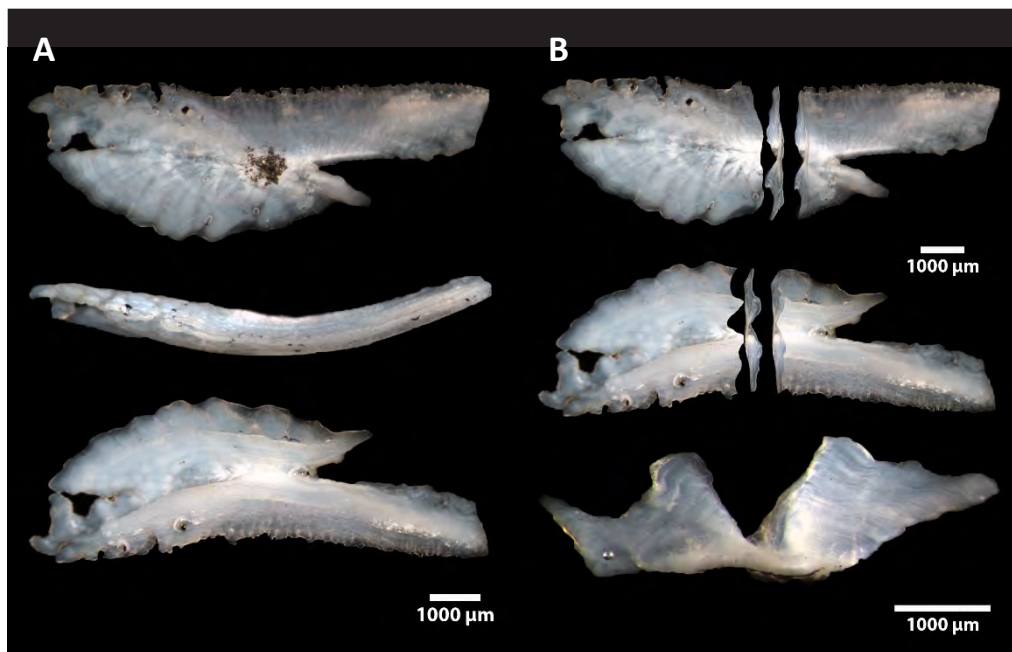


Figure 9.12.1 A) Whole otoliths of Greater Amberjack proximal view with core marked (top), dorsal view (middle), and distal view (bottom) and B) Greater Amberjack otolith sectioned with thin-section located and rotated showing location of cut through the core.

in half during sectioning unless embedded. The location of the otolith in the neurocranium is illustrated in Figure 9.12.2.

Extraction

Otolith removal in Greater Amberjack is not easy. The otic capsule in Greater Amberjack is located directly behind and under the brain making it difficult to get into it through the gill cavity, although it can be done. The otoliths are small and fragile, making it easy to damage them during extraction; however, while any of the techniques illustrated in Chapter 3.0, Section 3.2.1 can be used, a few tend to be easier than others.



Figure 9.12.2 Location of sagittae (circle and arrows) in the neurocranium of Greater Amberjack in A) lateral and B) dorsal-ventral views.

The recommended approach is to cut vertically down through the head using the hacksaw technique or horizontally across the top of the neurocranium.

Processing

Greater Amberjack otoliths need to be embedded in bullet molds prior to sectioning due to their small size and deep sulcus (Chapter 3.0, Section 3.2.5.2). It is particularly important for a Greater Amberjack otolith to be epoxied with its long axis parallel to the long axis of the mold and its surface parallel to the bottom of the bullet mold. Once the epoxy is hardened the core must be marked using a dissecting scope (i.e., not by eye). Sectioning can then be done using standard methods (Chapter 3.0, Section 3.2.6.2), primarily using a low speed saw cutting at about 0.3-0.5 mm thickness. When sectioning, make sure to mount the block with the otolith perpendicular to the blade.

Age Determination

Like many of the pelagics, one of the difficulties 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. Positioning the otolith in the embedding mold correctly will reduce the amount of tilting necessary to read the sections. In addition, otoliths in this species can have either indistinct annuli or annuli comprised of a series of checks. While difficult, Greater Amberjack can be aged when viewed in thin section. Annual deposition of opaque zones has been validated through marginal increment analysis (Manooch and Potts 1997b, Harris et al. 2007) and using OTC-tagged fish that were subsequently recovered (Thompson et al. 1999).

Beasley (1993) estimated spawning for Greater Amberjack in the northern Gulf of Mexico (off Louisiana) peaked in April to June using GSIs. This was confirmed when Murie and Parkyn (2008) examined GSIs in both male and female Greater Amberjack and found spawning reaches a maximum in March and April from fish collected throughout the Gulf of Mexico. Spawning of Greater Amberjack was determined by Harris et al. (2007) to occur primarily along the Atlantic Coast of Florida in April and May despite having examined fish from Georgia and North Carolina as well (Figure 9.12.3).

Burch (1979) utilized marginal increment analysis to determine that Greater Amberjack from South Florida formed annuli between February and April (Figure 9.12.3). Manooch and Potts (1997b) determined that the annulus formation in the Gulf occurred between March and May. Thompson et al. (1999) injected Greater Amberjack with OTC and estimated annulus formation off Louisiana occurred between November

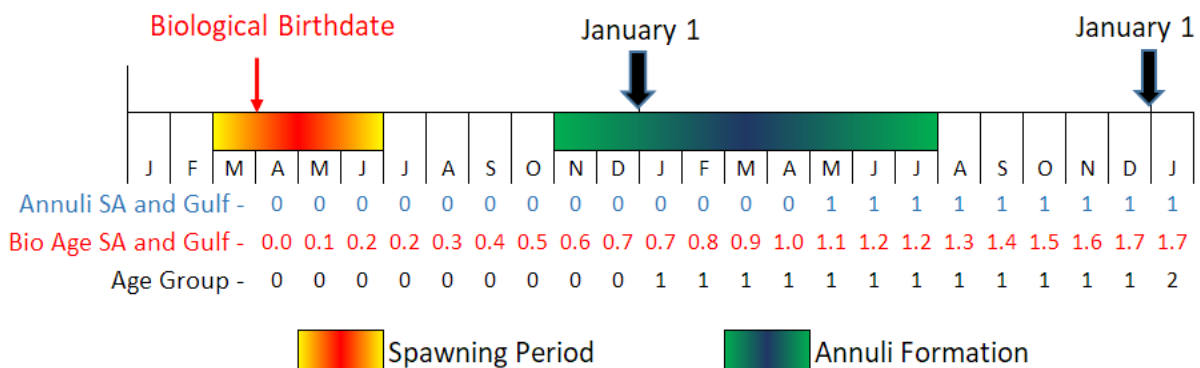


Figure 9.12.3 Birthdate assignment timeline for Greater Amberjack. Age and year group based on biological birthdate (April 1). A mark (butterfly wing) can occur close to the core; however, the first true annulus does not occur until the fish is actually a year old.

and March. Murie and Parkyn (2008) reported that annulus formation can occur from April to August in the Gulf of Mexico, with the first annulus demarcated by a distinct translucent zone following the opaque core and 'butterfly wing' (Murie and Parkyn 2008) making the formation of the first readable annulus around 12 to 15 months (Figure 9.12.4).

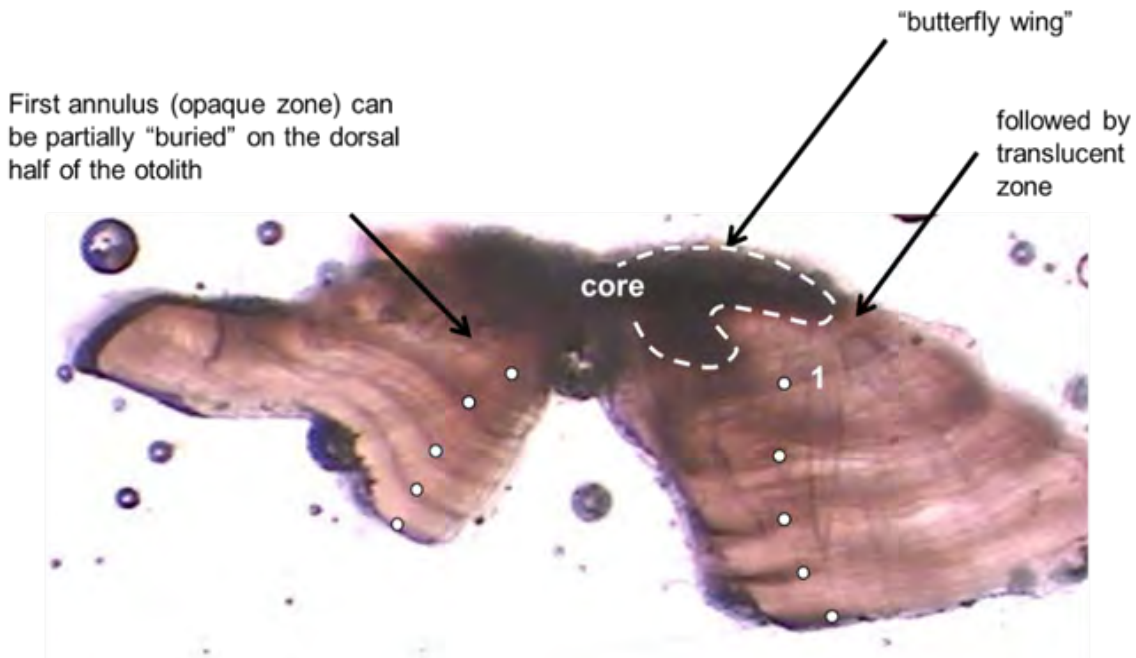


Figure 9.12.4 Otolith section of an age-5 Greater Amberjack showing position of the first annulus.

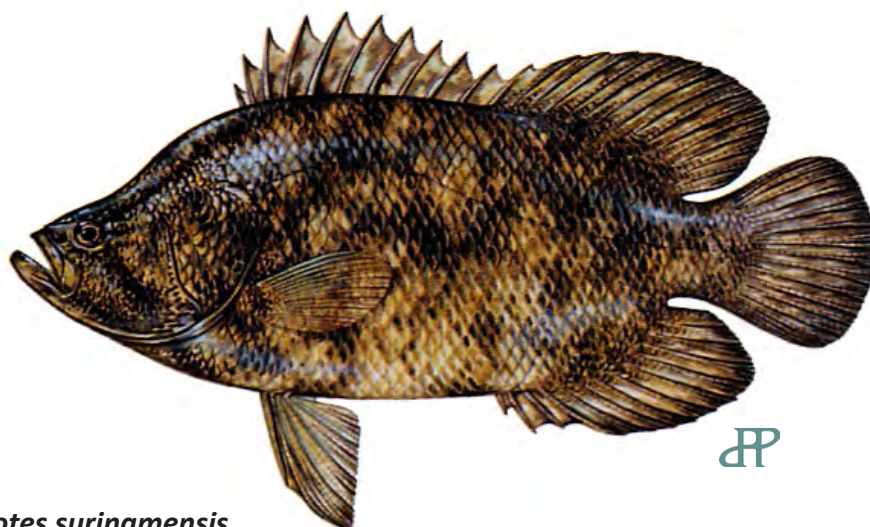
Other Ageing Techniques

Whole otoliths were not readable according to Manooch and Potts (1997b). Thompson et al. (1999) determined that whole otoliths lacked translucence even when immersed in clove oil or glycerin. In addition, Thompson et al. (1999) were unsuccessful in sectioning dorsal and anal spines and vertebrae. Burch (1979) used scales to age Greater Amberjack in South Florida, although Manooch and Potts (1997b) considered scales to be unreliable for ageing Amberjack due to edge erosion. Murie (personal communication) is investigating the use of fin rays to age Greater Amberjack but does not currently recommend their use.

Research Needs

In general, there is a need for more age and growth data in all the other *Seriola* species. In Greater Amberjack, there is a need to still validate the ageing methods for older fish due to the dark area along the sulcus which makes it difficult to see the older annuli further from the focus.

9.13 Lobotidae



Tripletail *Lobotes surinamensis*

Highlights

- Tripletail are unique in that they range worldwide through tropical and subtropical oceans, and with the exception of a sister species along the Eastern Pacific, little is known about this species.
- Tripletail sagittal otoliths are ovate and fragile with a deep sulcal groove.
- Otoliths are relatively easy to locate and extract; however, there are mixed results in legibility. Franks et al. (1998) and Strelcheck et al. (2004) found them unsuitable, while Parr et al. (2018) and Jefferson et al. (in press) were able to reach agreement between readers.
- Spines have been used with mixed success. Franks et al. (1998) and Strelcheck et al. (2004) did not reach agreement between otoliths and spines, while Parr et al. (2018) and Jefferson et al. (in press) did.
- Scales have also been used to age Tripletail (Merriner and Foster 1974).
- The maximum reported age of Tripletail along the U.S. Atlantic coast is age-6 for males and age-7 for females (Armstrong et al. 1996).
- The maximum reported age of Tripletail in the U.S. Gulf of Mexico is age-4 for males and age-5 for females (Jefferson et al. in press).

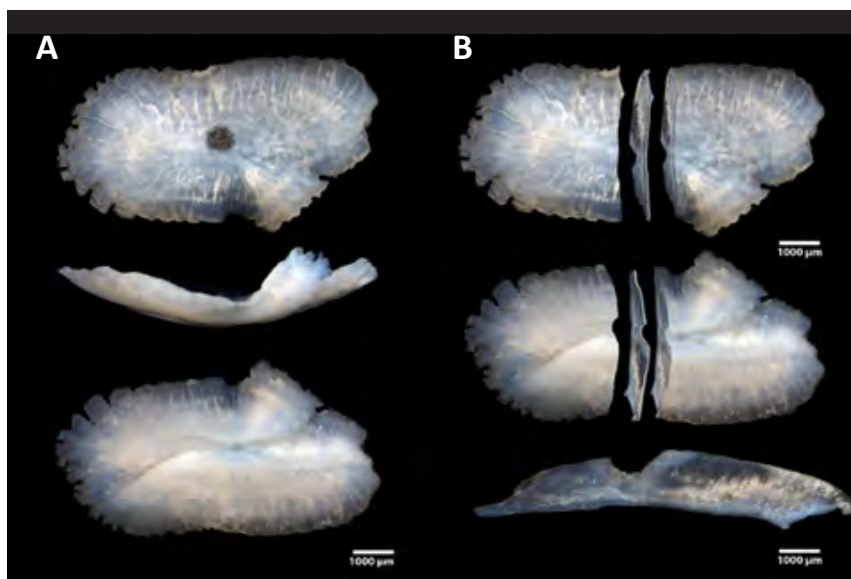


Figure 9.13.1 A) Whole otoliths of Tripletail proximal view with core marked (top), dorsal view (middle), and distal view (bottom) and B) Tripletail otolith sectioned with thin-section located and rotated showing location of cut through the core.

Otolith Description

Tripletail otoliths (sagittae) are ovate and relatively fragile (Figure 9.13.1). Removal is not difficult and a number of methods can be used. The margins of the otolith are highly crenellated or serrated and chip or break easily. Care should be taken in processing as well as long-term storage. The location of the otoliths in Tripletail is illustrated in Figure 9.13.2.

Otolith Extraction

Otoliths in Tripletail can be removed easily. The sagittae are readily accessible through the operculum but can be removed by horizontal or vertical cuts through the head using any of the techniques illustrated in Chapter 3.0, Section 3.2.1. 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; however, the 'pop the top' technique is appropriate for most sizes of Tripletail not returning to the marketplace.

Otolith Processing

Due to the small size of Tripletail otoliths, embedding is the most common technique for sectioning. Generally Tripletail sections are produced at approximately 0.5 mm.

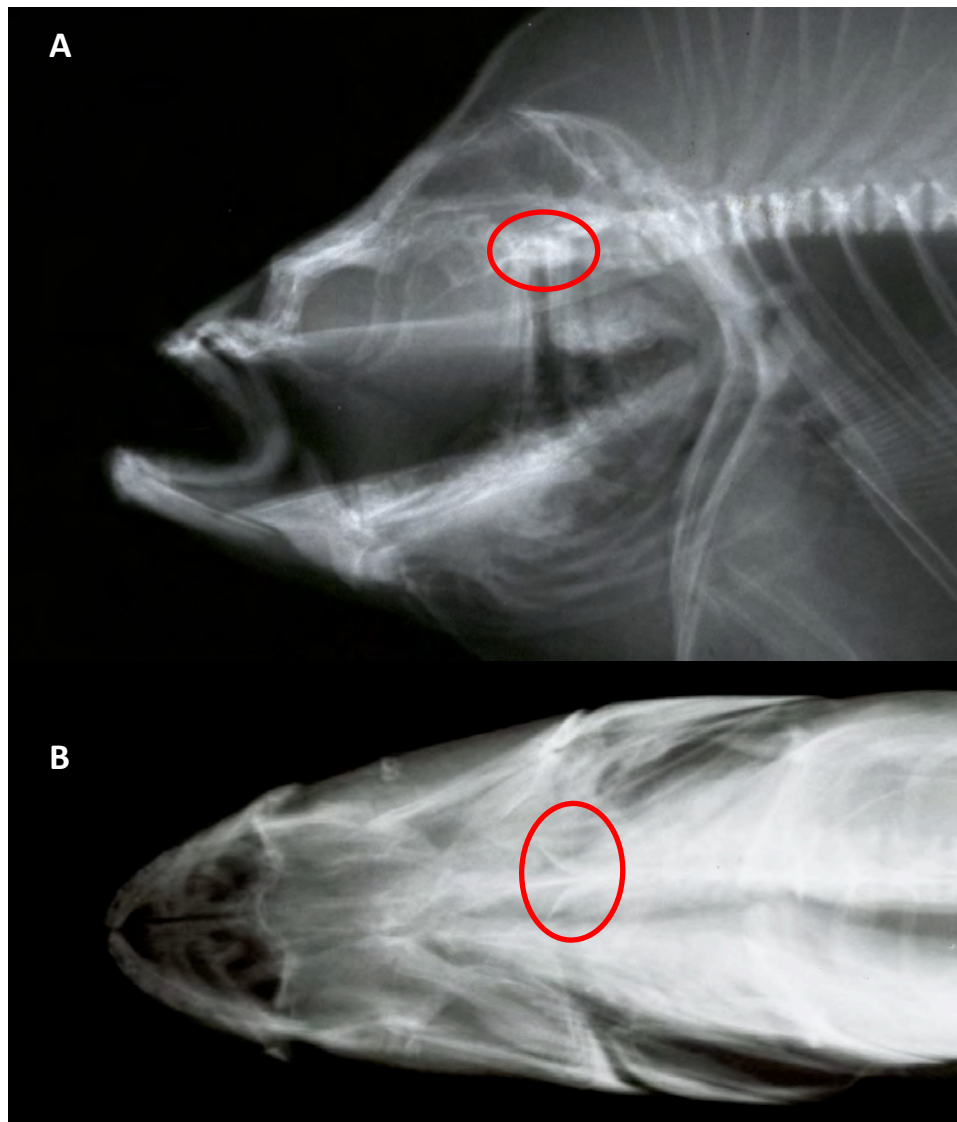


Figure 9.13.2 Radiographs showing location of sagittae in Tripletail cranium (red circles) in A) lateral and B) dorsal-ventral views.

Dorsal Spine Removal

Dorsal spines, including the condyle base, are removed using the general techniques outlined in Chapter 6.0, Section 6.2.1 (Figure 9.13.3). Spines can be placed in bleach for a maximum of two minutes or boiled for one minute to loosen excess tissue and skin and scraped with a scalpel and forceps or brushed with a toothbrush prior to storage or processing. Unlike other species, the first dorsal has generally been used in most of the published studies on ageing Tripletail (Franks et al. 1998, Parr et al. 2018).

Dorsal Spine Processing

Tripletail spines may be embedded in molds or they may be mounted whole and sectioned. Sections should continue up the shaft until legible thin sections are generated. Sections are then mounted similarly to otolith sections.

Age Determination

Tripletail are believed to have a protracted spawning period in the Gulf, with male Tripletail running ripe from May through September and females having late-staged ovaries from June to August, peaking in July (Brown-Peterson and Franks 2001). Parr et al. (2018) noted annuli forming on the edges of some otoliths in early June off Georgia. However, Jefferson et al. (in press) hypothesized that otolith annulus deposition likely occurs during early spring in the northern Gulf of Mexico based on margin code analysis of fish caught in that region from May through October. With limited data, spawning and annulus formation may overlap, suggesting that Tripletail annuli may represent actual birthday increments in U.S. Atlantic waters; however, this does not appear to be the case in the northern Gulf of Mexico. Actual validation using otoliths or any other structure has not been accomplished for the species (Figure 9.13.4).



Figure 9.13.3 First dorsal spine location, removal, and final product ready for sectioning.

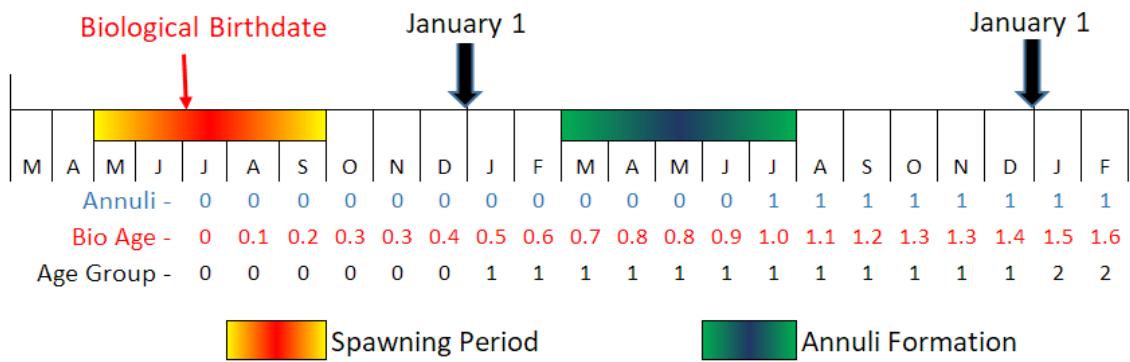


Figure 9.13.4 Spawning and estimated annulus deposition periods in Tripletail from the Gulf and Georgia waters.

Otoliths

While some banding can be seen in both otolith and spine sections, annulus production has not been validated for this species. Generally, enumeration of Tripletail annuli in otolith sections is straightforward when they can be seen, but some sections are simply illegible. Parr et al. (2018) and Jefferson et al. (in press), who had 90% and 95% initial reader agreement for otoliths, respectively, reported that rings in cross-sections are often very thin and appear closely stacked (Figure 9.13.5). Both studies also found higher between-reader agreement and lower between-reader APE for otolith-based ages compared to spine-based ages.

Spines

Translucent bands can be found on sectioned dorsal spines; however, many of the marks are doublets and triplets, similar to triggerfish, making counting marks difficult. According to Franks et al. (1998), a ring in a cross-sectioned spine is defined as two and occasionally three small, conspicuous, adjacent translucent rings separated from each other by a small opaque zone.

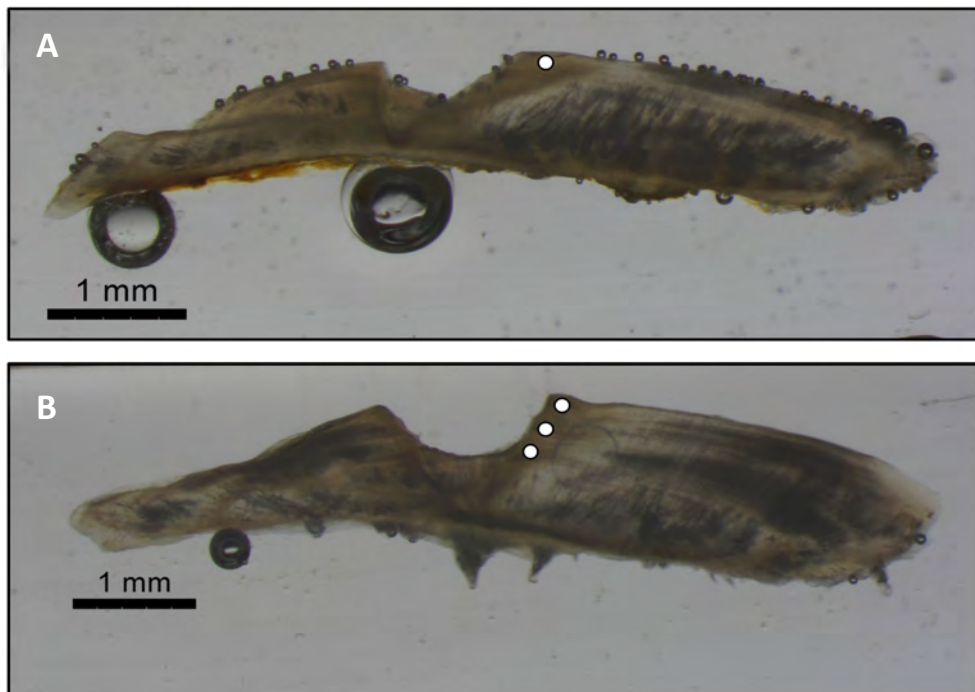


Figure 9.13.5 Images of otolith sections from A) an age-1 and B) an age-3 Tripletail captured from the northern Gulf of Mexico [Figure 4 (panels C and G) from Jefferson et al. (in press)].

In Tripletail, Franks et al. (1998) determined that the first annulus is the second doublet or triplet (multiples), whereas Strelcheck et al. (2004) decided that the first annulus is the first multiple. By comparing corresponding otoliths and spines from the same individuals, Parr et al. (2018) determined that the first multiple should be ignored in Tripletail younger than age-2, but should be counted in older individuals. Jefferson et al. (in press) only skipped the first multiple if it appeared too close to, or didn't completely encircle, the spine's core (Figure 9.13.6). They found no discernable pattern for skipping with age class. Moreover, their method produced the highest percent agreement between otolith- and spine-based ages when compared to the methods of Franks et al. (1998) and Strelcheck et al. (2004).

As with other dorsal spines, as the fish gets older, significant core resorption can occur, thereby obscuring and eliminating the early annuli. Even with these difficulties, Parr et al. (2018) and Jefferson et al. (in press) found strong agreement (84% and 80%, respectively) between Tripletail sagittal otoliths and first dorsal spines.

Other Ageing Methods

Whole Otoliths

The ageing of whole Tripletail otoliths has not been attempted in the Gulf (Franks unpublished data; Figure 9.13.7).

Scales

Tripletail have relatively small scales, which have been used successfully to estimate age along the U.S. South Atlantic to age-3 (Merriner and Foster 1974); however, in comparison to other structures, scales have generally been thought to be less accurate, although problems do occur with otoliths and spines as well. Scales were prepared as plastic impressions and aged from projected scale images on an Eberbach microprojector. Criteria for presumed annuli were cutting over and proximity of circuli. Merriner and Foster (1974) did note that they were unable to confirm or validate annulus formation due to the temporal availability of Tripletail in North Carolina waters.

Fin Rays

Franks et al. (1998) evaluated a variety of spines and fin rays as ageing structures for Tripletail in the Gulf, including dorsal spines #1-5, anal spines #1-3, the left pelvic spine, and the first anal ray. Among the structures they examined, the first dorsal spine provided the most reliable age estimates except for in older fish, followed by the first anal spine. The rest of the structures were not legible for age estimation.

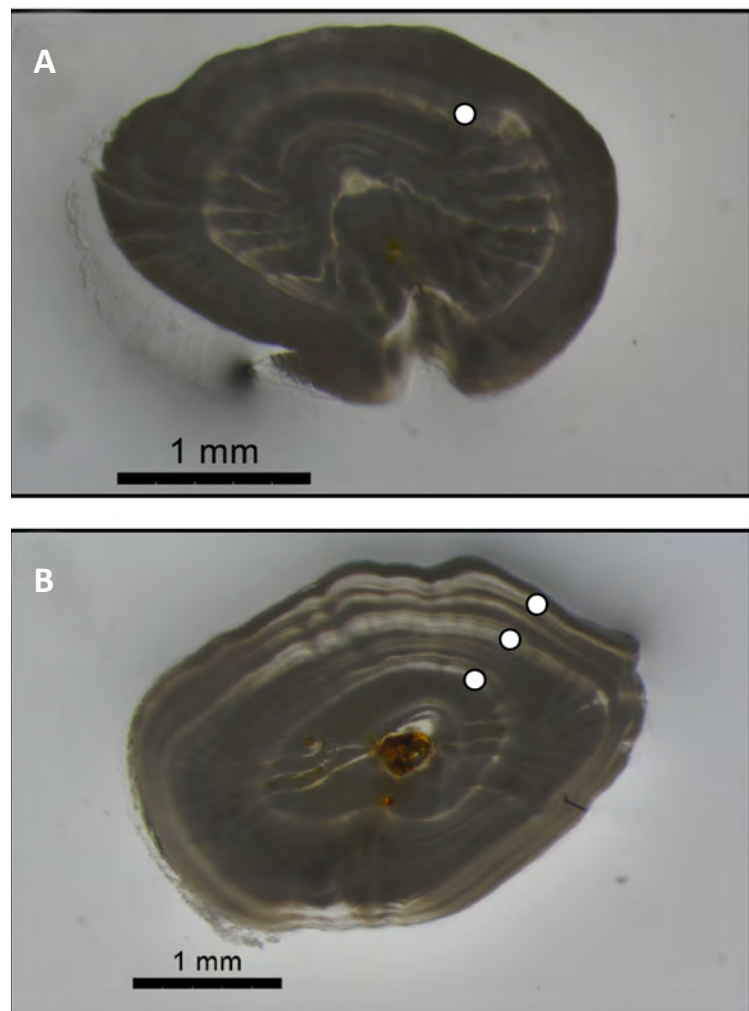


Figure 9.13.6 Images of spine sections from the same individual Tripletail in Figure 9.13.5. A) represents an age-1 individual and B) represents an age-3 individual [Figure 4 (panels D and H) from Jefferson et al. (in press)].

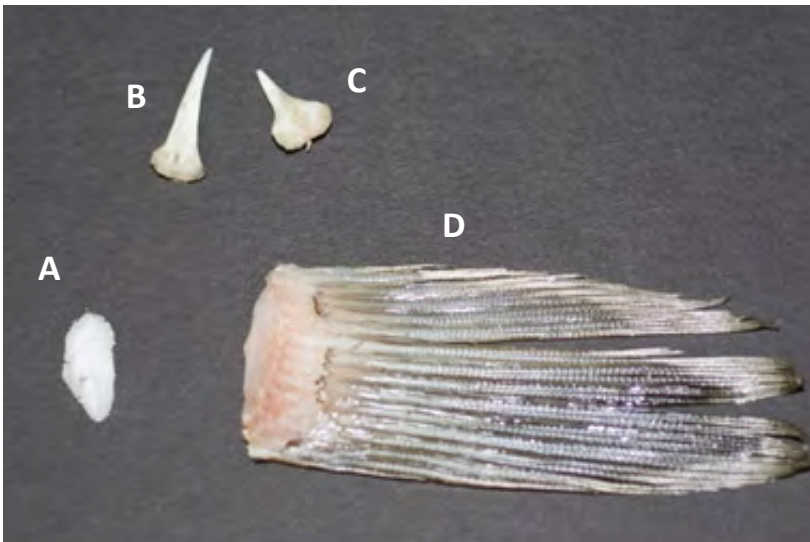


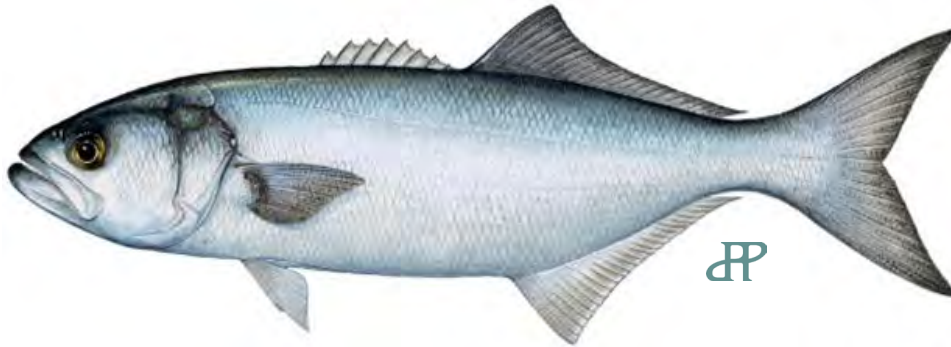
Figure 9.13.7 The various structures from Tripletail that are or could be used for ageing include the A) sagittal otolith, B) first dorsal spine, C) first anal spine, and D) pectoral fin rays.

Research Needs

As noted in VanderKooy (2016), while considerable effort has been given to successfully ageing Tripletail, there are still a number of issues with the techniques used to date. A number of studies have aged Tripletail using spines, scales, and otoliths, but each has its own difficulties. In addition, there has been no validation of annulus formation to date. Part of the difficulty in ageing Tripletail may be related to their narrow temperature preferences and propensity to remain at or near the surface. These factors combined could lead to reasonably consistent growth throughout the year by reducing the clearly defined slow and fast growth periods used to age structures in other species.

Other aspects of Tripletail biology and ecology remain understudied or unknown. First, the timing of spawning is based on GSIs in fish from nearshore waters, but the most reproductively active females have not been examined, as it is believed that they occur more offshore (Brown-Peterson and Franks 2001). Also, spawning locations are unidentified and seasonal migration patterns are poorly understood. The lack of biological samples from Tripletail caught offshore and/or during the winter months contributes to most of the existing knowledge gaps concerning this species.

9.14 Pomatomidae - Bluefish



Bluefish *Pomatomus saltatrix*

Highlights

- Otoliths are elongate, laterally compressed.
- Otoliths are fragile; care must be taken during removal.
- Burning the otolith, either whole or sectioned, is successful in enhancing annuli.
- First ring is diffuse and often marked by a crenulation on the ventral surface.
- Maximum age is reported to be 13 (NEFSC, VMRC).

Otolith Description

Bluefish sagittal otoliths are elongate, laterally compressed and have an indented sulcus on the proximal surface (Figure 9.14.1). The rostrum protrudes past the antirostrum and is elongated in larger otoliths. The otoliths are very fragile and the rostrum can easily be broken off.

Extraction

Otolith extraction in Bluefish can be difficult due to the fragility of the otoliths. Any of the techniques in Chapter 3.0, Section 3.2.1 can be employed; however, some techniques are more successful than others. The otic capsule in Bluefish is relatively thick and is located directly behind and under the brain thus making it difficult to get to through the gill cavity (Figure 9.14.2). The preferred method of extraction

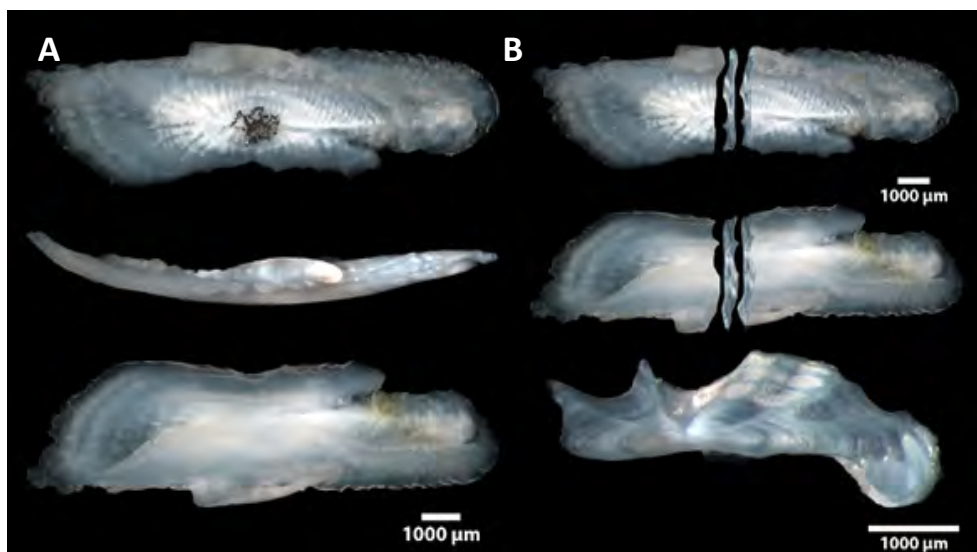


Figure 9.14.1 A) Whole sagittal otoliths of Bluefish proximal view with core marked (top), dorsal view (middle), and distal view (bottom) and B) Bluefish otolith sectioned with thin-section located and rotated showing location of cut through the core.

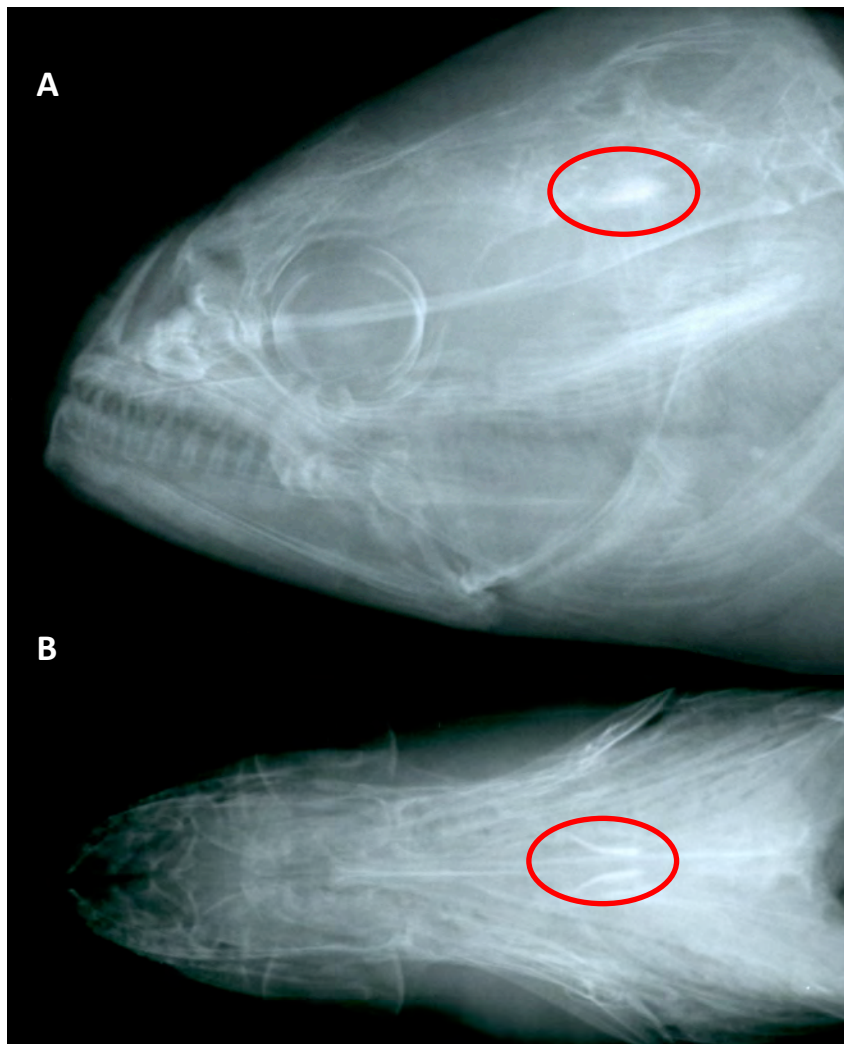


Figure 9.14.2 Radiographs of Bluefish showing location of sagittal otoliths (red circles) in A) lateral and B) dorsal-ventral orientation.

for Bluefish is to use the top method where a horizontal cut is made through the upper neurocranium. 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.

Otolith Preparation

Sectioning preparation varies among labs, but all have similar methodologies. Because the sagittal otoliths are fragile, most are embedded before sectioning on low speed saws (Chapter 3.0, Section 3.2.6.2). Sections are cut in series or using multiple blades on a single pass. Most labs directly follow, or have slightly modified, the ‘bake and thin section’ technique outlined in Chapter 7.0, Section 7.1.6 prior to

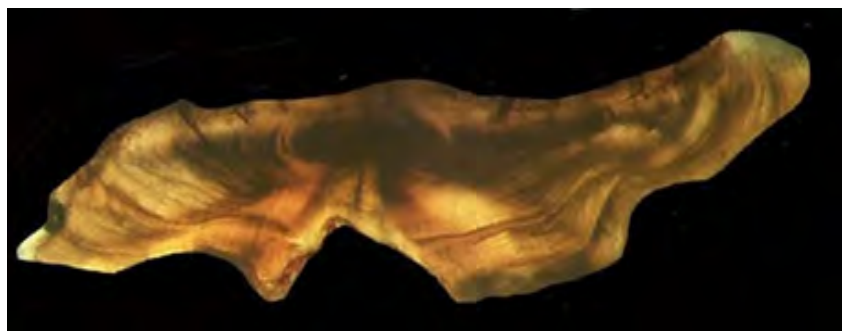


Figure 9.14.3 A sectioned-and-baked age-2 Bluefish sagittal otolith. Note the deep caramel color that is achieved from baking prior to sectioning.

embedding and sectioning (Figure 9.14.3). Bluefish sections are generally cut at about 0.5 mm thickness.

Age Determination

Bluefish otolith sections should be read using transmitted light. Annuli are most visible along the sulcal groove on the dorsal portion of the otolith. As with other fast-growing fish, the first annulus often appears more diffuse than subsequent annuli. The first annulus is sometimes distinguished by a crenulation, as described by Robillard et al. (2009); this crenulation often serves as a landmark for the first annulus, and can help in identifying it (Figure 9.14.4). Double rings are sometimes seen in older fish. If these rings join together at the edge (sulcal groove or outer edge), then they are counted as a single annulus; if they remain separate, they are counted as two annuli. Robillard et al. (2009) indirectly validated annulus formation for fish ages 1-8 by using marginal increment analysis (MIA).

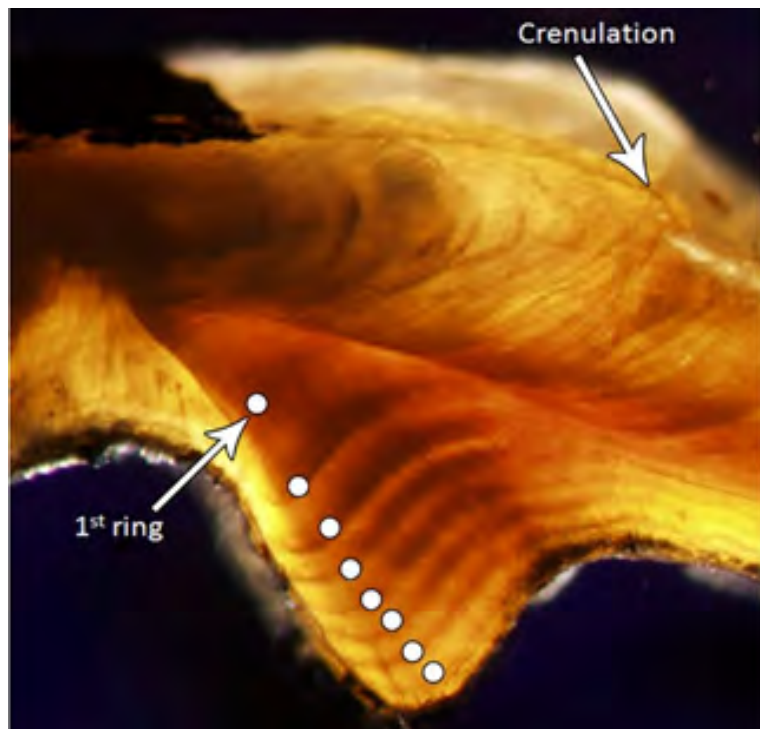


Figure 9.14.4 Sagittal otolith section from age-8 Bluefish. White dots indicate annuli and arrows mark the first annulus and associated crenulation.

Bluefish are characterized as iteroparous spawners with indeterminate fecundity and spawn continuously during their spring migration (Robillard et al. 2008). Bluefish spawn from June to August between Massachusetts and Cape Hatteras, and from March to May between Cape Hatteras and Florida (Kendall and Walford 1979). Taylor and Able (2006) likewise reported Bluefish spawning along the New Jersey Coast occurred between early July and early September (Figure 9.14.5).

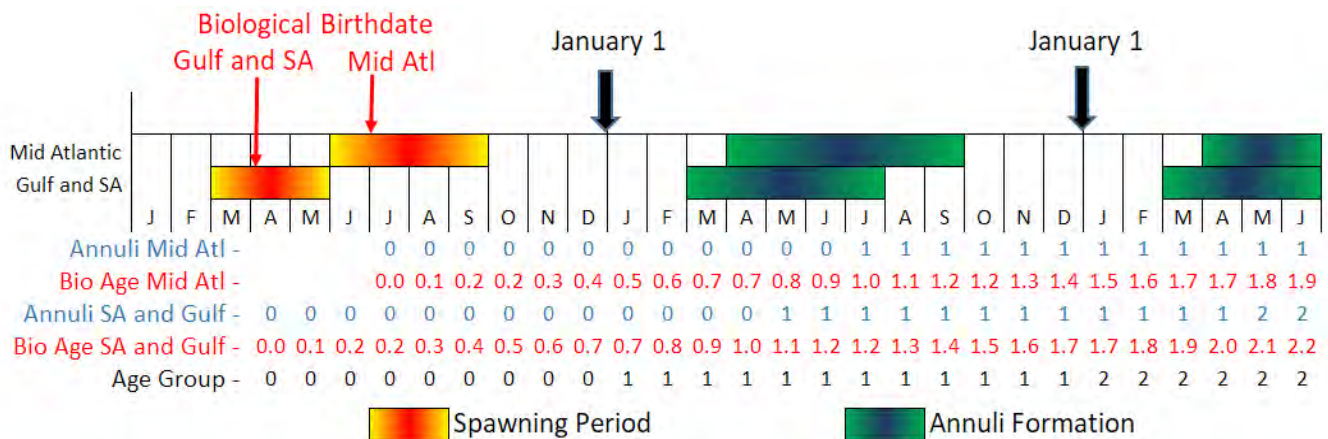


Figure 9.14.5 Birthdate assignment timeline for Bluefish. Age and year group are based on a biological birthdate of July 1 in the Mid-Atlantic and April 1 in the South Atlantic and Gulf. Early spawned fish can have a mark in the core region, but it is not generally counted as an annulus.

Barger (1990) determined annulus formation in most Bluefish from the Gulf of Mexico and from South Carolina to Florida occurs around March and April although there were a small percentage of fish that had opaque margins as late as July. Robillard et al. (2009) determined annulus formation occurred in April/ May until September along the Atlantic Coast from New York to Florida which concurred with previous studies (Barger 1990, Terceiro and Ross 1993).

Other Ageing Techniques

Historically Bluefish have been aged using scales; however, the use of scales as an ageing structure has fallen out of favor. Publications have found that reader agreement is higher with otoliths than scales (Sipe and Chittenden 2002, Robillard et al. 2009). Barger (1990) compared sectioned Bluefish otoliths to whole otoliths as well as scales, scale impressions, and vertebrae. Barger (1990) found good agreement with whole otoliths compared to sections but progressively worse agreement compared to scale impressions, vertebrae, and scales respectively. Sipe and Chittenden (2002) also examined Bluefish scales and dorsal spines and determined that neither structure was useful for ageing fish beyond age-4.

Research Needs

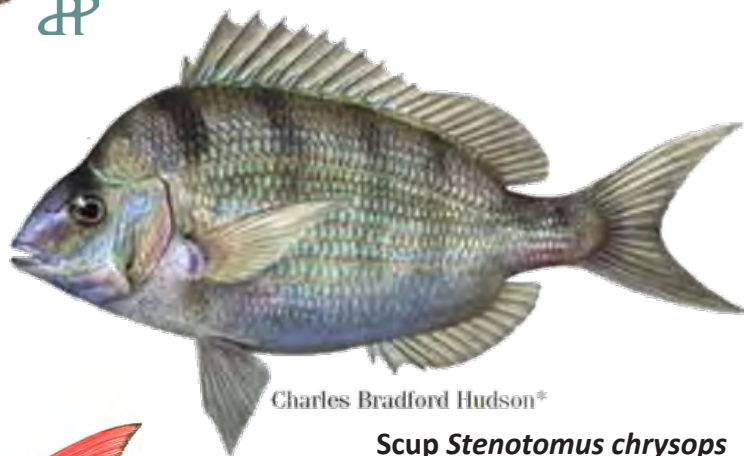
There are no gaps in our understanding of ageing in Bluefish at this time.

9.15 Sparidae - Porgy



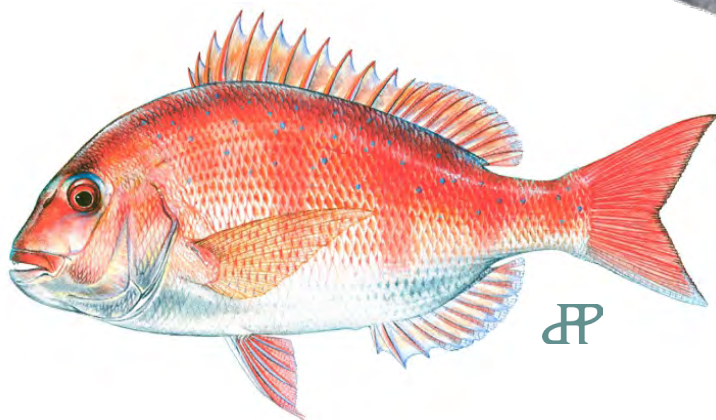
Sheepshead *Archosargus probatocephalus*

AP



Charles Bradford Hudson*

Scup *Stenotomus chrysops*



Red Porgy *Pagrus pagrus*

AP

Highlights

- Porgy otoliths are slightly ovate, laterally compressed, and arrowhead shaped.
- Porgy otoliths are easy to locate and extract using a variety of methods and multiple processing/sectioning techniques can be used.
- Scup can be aged using both otoliths and scales.
- Sheepshead are primarily aged using otoliths.
- Red Porgy are primarily aged using otoliths.
- Maximum age for Sheepshead varies by region ranging from age-17 (AL), age-20 (LA), age-20-25 (West and East FL), age-26 (SC), to age-40 (VA).
- Maximum age for Scup can vary from age-14 to age-20 depending on region (Dery and Reardon, 1979).
- Maximum age for Red Porgy is 25 years, (SEDAR60 2020).

Otolith Description

Sagittal otoliths from the porgy group are relatively large, ovate, laterally compressed, and exhibit an indented sulcus on the proximal surface (Figure 9.15.1). The rostrum and anterostrum are distinguishable by the separation created by the ostium at the mouth of the sulcal groove. The location of the sagittae

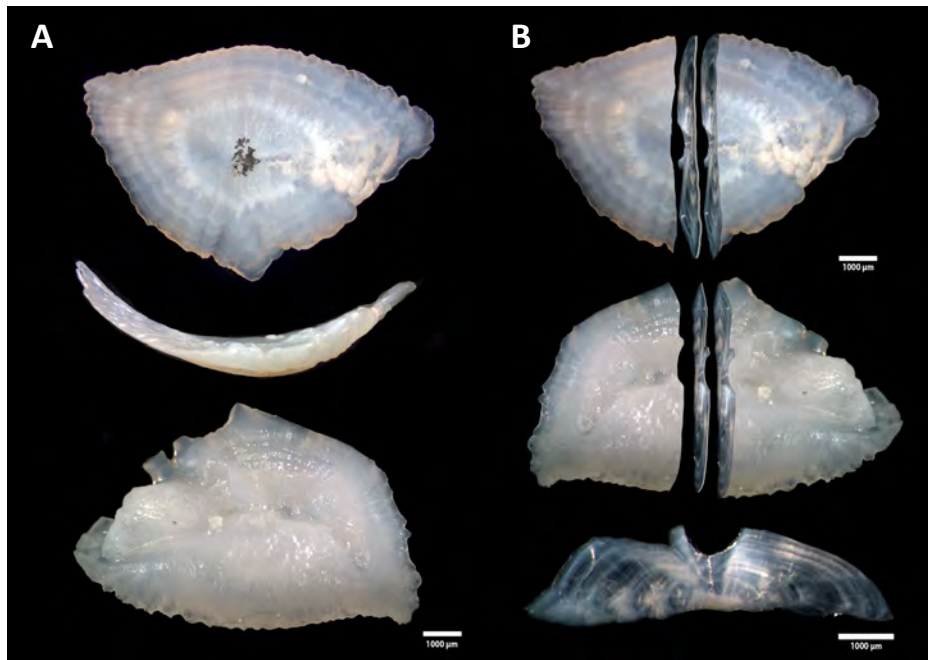


Figure 9.15.1 A) Whole sagittal otoliths of Sheephead proximal view with core marked (top), dorsal view (middle), and distal view (bottom) and B) Sheephead otolith sectioned with thin-section located and rotated showing location of cut through the core.

in the neurocranium (otic capsule) is illustrated in Figure 9.15.2 and is similar for all sparidea.

Otolith Extraction

Compared to other species, porgy otoliths are not terribly fragile, but caution should be taken during extraction as they may break during contact with certain instruments. Any technique can be used to remove otoliths (Chapter 3.0, Section 3.2.1) but the two primary extraction methods include removal through the gill cavity, especially when sampling the commercial catch and a vertical cut through the head, just behind the centerline of the opercula (decapitation). Either technique is acceptable but care should still be taken not to damage the otoliths.

Otolith Sectioning Guidelines

Due to the relatively large size of most porgy otoliths, multiple processing techniques are acceptable when sectioning otoliths. The technique chosen will likely reflect available equipment. Porgy sections are generally processed at approximately 0.5 mm across all techniques (Chapter 3.0, Section 3.2.7).

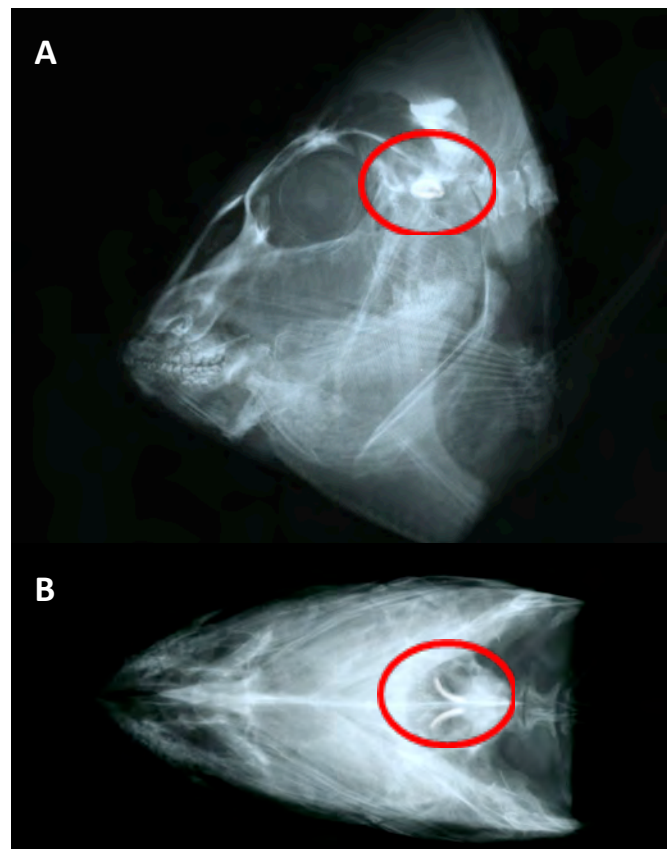


Figure 9.15.2 Radiographs of sagittae in the neurocranium of Scup in A) lateral and B) dorsal-ventral views.

Scale Description and Extraction

Porgy scales are ctenoid. The annuli encompass the whole scale but are most distinguishable on the anterior portion of the scale. Annuli appear darker and thicker than the circuli also encompassing the scale (Chapter 4.0, Section 4.1) to the “cutting-over” mark where annuli can be best observed. Many scales (30-50) should be removed from a single specimen using standard techniques (Chapter 4.0, Section 4.2; Figure 9.15.3).



Figure 9.15.3. Scales should be removed from porgy within the area highlighted with the red box. This region is below the dorsal fin near the middle of the body where scales first form during growth.

Scale Processing

Raw scales are not recommended for ageing porgy. Scales are often too thick and opaque, especially on larger specimens, to clearly observe all of the annuli on the scale directly. Most scales are read from impressions in acetate (Figure 9.15.4). Either a roller press or Carver® Laboratory Press can be used to imprint the scales to an acetate medium (Chapter 4.0, Section 4.3.3). Once removed, the scales should be scrubbed clean of any debris and then mounted with the textured side facing the acetate. Regenerated scales should not be considered for ageing purposes. After examination of the individual scales, a minimum of five scales should be selected for processing. The Carver® Laboratory Press has been recommended and is the preferred scale imprinting method. Each species requires a different formula of time, pressure and heat to press and imprint the sample properly.

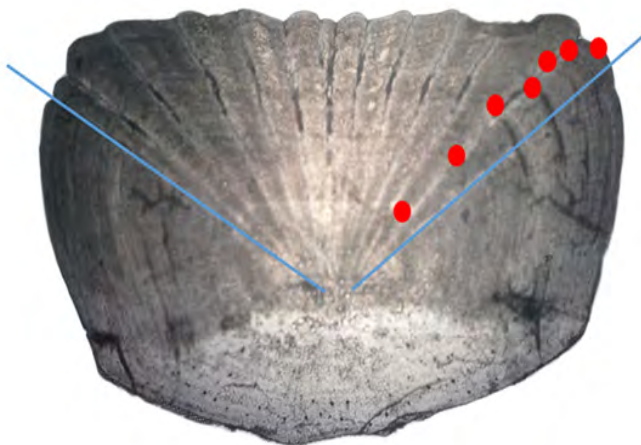


Figure 9.15.4 Annotated heat pressed Scup scale, Age-7. Annuli counted on a line around 45° from the baseline annotated with the blue lines.

Age Determination

Sheepshead

Enumeration of Sheepshead annuli in otolith sections is straightforward with the exception of the first ring (Figure 9.15.5). Sheepshead spawn offshore from February through April with a peak in March and April (Wilson et al. 1988), and from December to June in Atlantic (Murdy and Musick 2013). The period of annulus formation is from March through June in the northern Gulf (Beckman et al. 1990) and from March to July in Atlantic (Murdy and Musick 2013). The

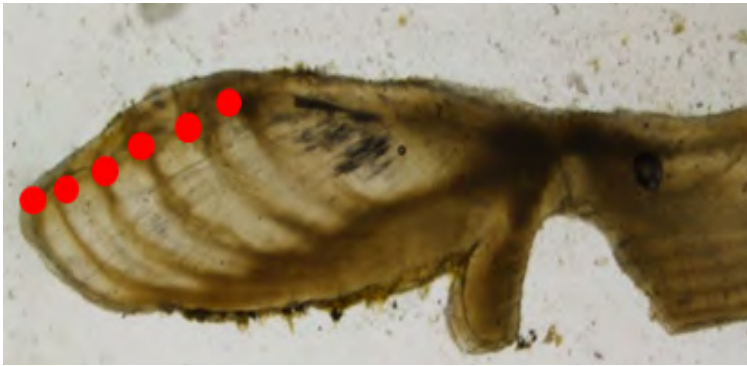


Figure 9.15.5 Annotated section of a Sheepshead otolith, age-6. Final annulus located near the edge of otolith .

coincidence of ring formation and spawning can lead to dark cores in early spawners and opaque cores in late spawners (Figure 9.15.6A). In general, it is accepted that the core mark is not interpreted as a true annuli (Dutka-Gianelli and Murie 2001). Dutka-Gianelli and Murie (2001) validated annulus formation in Sheepshead using chemical marking.

Scup

Scup spawn from May through August with a peak in June (Figure 9.15.6B; Morse 1978, Ferraro 1980, MAFMC 1996). Scup migrate

south and to deeper water as water temperatures decrease in December and January and begin return migration in around May which is hypothesized to result in annulus formation (Morse 1978, Hamer 1979). After the observed annulus formation in late spring to early summer, the summer growth appears on the edge of the otolith. Growth will appear faster on otoliths than scales according to Dery and Rearden (1979).

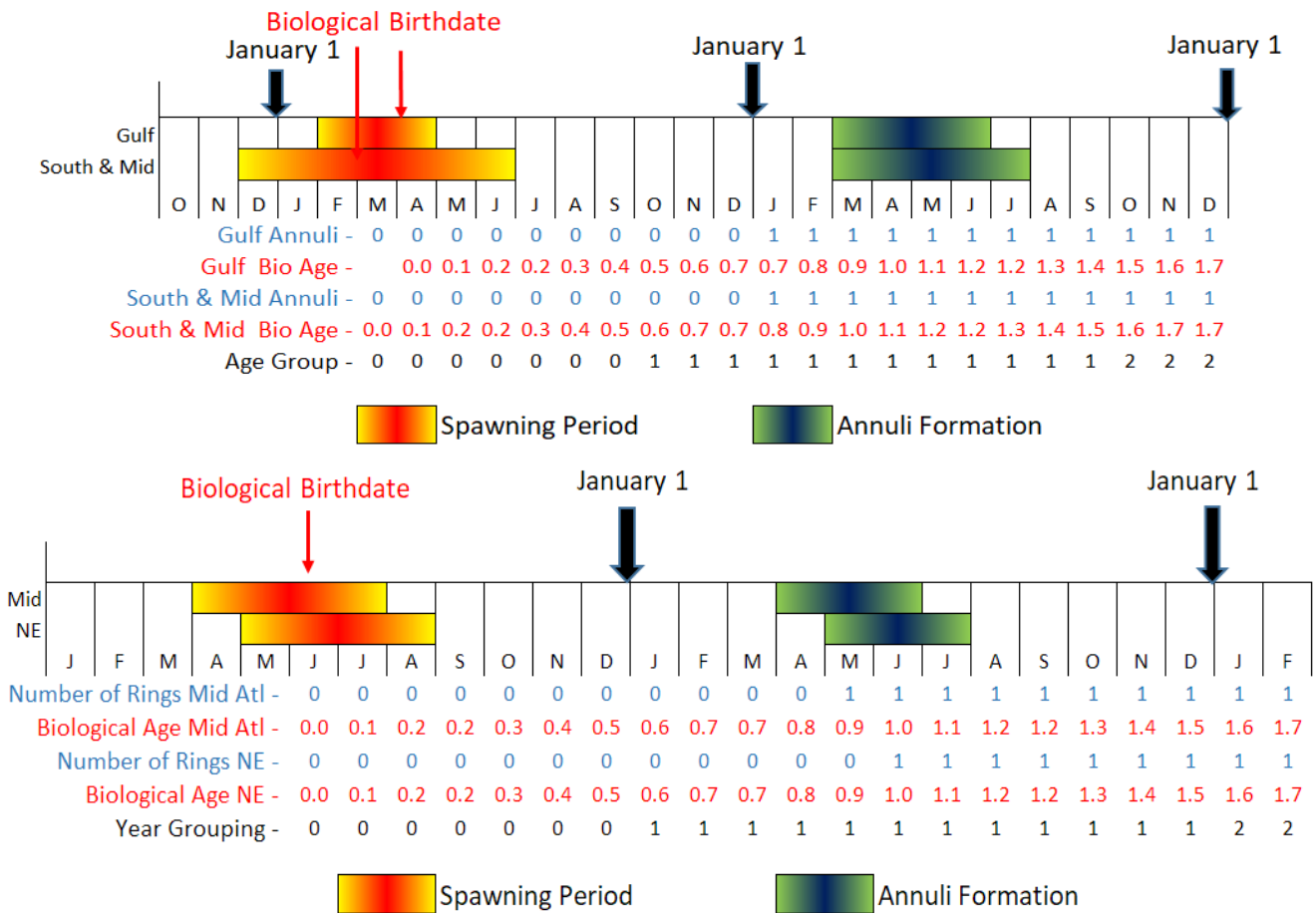


Figure 9.15.6 Spawning periodicity and age assignment timeline for A) Sheepshead in the Atlantic and Gulf of Mexico and B) for Scup along the mid Atlantic Coast to New England. **Note:** There is more commonly a dark core in Sheepshead than a distinct region near the focus.

Otolith Sections

A continuous opaque line running from the sulcal groove to the outer edge of both sides of the otolith is interpreted as an annulus (Figure 9.15.7). The first annulus is defined as the first visible opaque ring *after* the first visible hyaline zone. Young-of-the-year scup in the mid-Atlantic and New England grow very little in their first winter resulting in an annulus close to the core (Bigelow and Schroeder 1953).

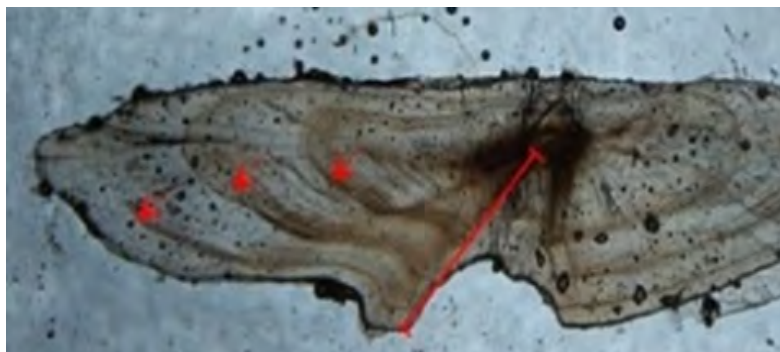


Figure 9.15.7 Annotated sectioned of Scup otolith age-3.

Whole Otoliths

Whole otoliths may also be used to age Scup. Whole otoliths are scrubbed clean, submerged under ethanol, glycerin or water in a watch glass, and viewed under a compound microscope using reflected light. Hyaline gaps with an opaque edge are interpreted as annuli (Figure 9.15.8).

Scales

Locating the first annulus is the first step in using scales for age determination. Many scales exhibit erratic “cutting over marks” as the first annulus (Figure 9.15.4). The first annulus should be a complete mark across the whole scale. Care should be taken to avoid counting the many check marks near the focus of the scale surrounding the first annulus. There may be difficulty ageing scales older than two years old (Hamer 1979).

Red Porgy

Red Porgy ages from the Gulf have been determined using whole and sectioned otoliths (Hood and Johnson 2000) and scales (Nelson 1988). On the U.S. Atlantic Coast, ageing information was provided by a number of authors using both otoliths and scales (Manooch and Huntsman 1977, Collins et al. 1996, Harris and McGovern 1997, Potts and Manooch 2002)

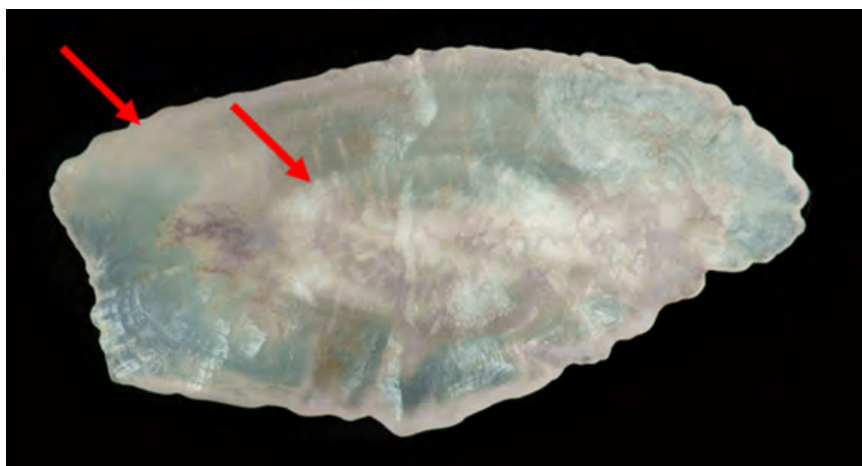


Figure 9.15.8 Opaque annuli are annotated with red arrows. **Note:** the second opaque annulus is on the edge of the otolith. The opaque annuli are separated by faster growth, observed here as the clear hyaline area.

Spawning in Red Porgy occurs from January to April along the Florida Gulf Coast (Hood and Johnson 2000) and December through February in the Florida Panhandle (DeVries 2006). Peak spawning of Red Porgy occurs during January – March along the U.S. South Atlantic, though it can extend from mid-November through mid-April (SEDAR01 Update 2006; Daniel 2003) (Figure 9.15.9). Hood and Johnson (2000) validated annuli formation in the Gulf of Mexico using marginal increment analysis (MIA) and confirmed formation

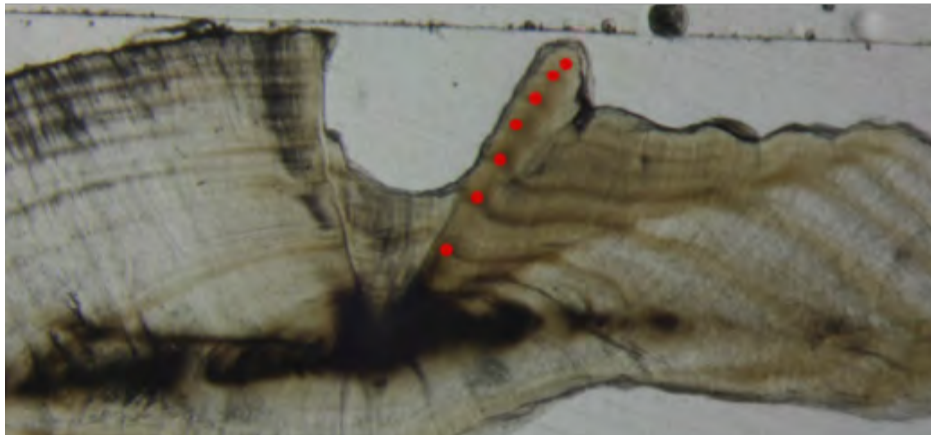


Figure 9.15.11 Annotated section of Red Porgy otolith, age-7.

Other Ageing Methods

Break and burn has been used successfully with a number of other porgy species but has not been attempted for Sheepshead or Scup. Based on the size of the otolith, this technique may warrant further investigation. Scales have been used in the past to age Sheepshead, but when compared to otoliths, scales are found to underestimate age in specimens age-3 and older. Manooch and Huntsman (1977) utilized scales to determine ages in Red Porgy. Legibility was an issue since many scales are regenerated in this species although they did get some agreement between otoliths and scales.

DeVries (2006) used the break and burned technique described by Christensen (1964) to age older Red Porgy that could not be read whole and underaged fish.

Research Needs

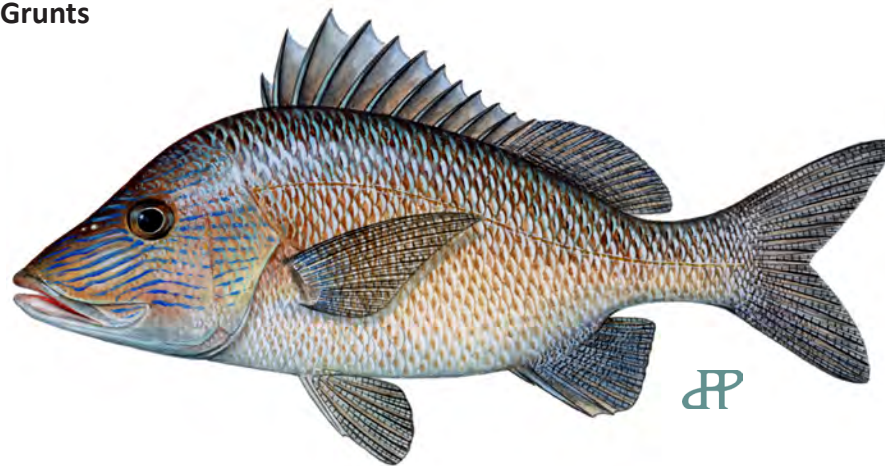
Further validation efforts need to occur for Scup. Specific year round regional sampling supplemented with marginal increment analysis should be performed to validate either and/or both otoliths and scales. Other possibilities for validation can be conducted with captive rearing and Oxytetracycline (OTC) or other chemical marking. The ASMFC has conducted ageing workshops focusing on Scup in 2014 and annual quality assurance and quality control workshops since 2016 exploring ageing both otoliths and scales for Scup. Despite not specifically citing Scup, generally otoliths are preferred over scales by designated agency agers attending these workshops (ASMFC 2019).



Figure 9.15.13 Section from a Red Porgy hatched and reared for 1.33 years (hatched date March 2015; death date July 2016). The top panel illustrates the true first annulus (A) and the late summer/early fall check mark (C). The line indicates the 2.34mm measurement to the first annulus from the bottom of the “V” of the sulcal groove out to the outer edge of the opaque zone in the dorso-ventral plan on the dorsal side. The bottom panel demonstrates the Calcein mark on the otolith from March 2016, which appears near the timing of opaque zone formation.

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9.16 Haemulidae - Grunts



White Grunt *Haemulon plumieri*

Highlights

- Otoliths are relatively large and robust and are easy to locate and extract
- Annuli are clear and easy to distinguish
- Maximum age to 27 years using otoliths (SCDNR unpublished data); 18 years (west coast FL)

Otolith Description

Sagittae from White Grunt are ovate, laterally compressed structures that exhibit an indented sulcus acousticus on the proximal surface (Figure 9.16.1). Some crenulations are formed along the ventral margin. The rostrum is broad and short and the postrostrum is blunt. The location of the sagittae in the neurocranium is illustrated in Figure 9.16.2.

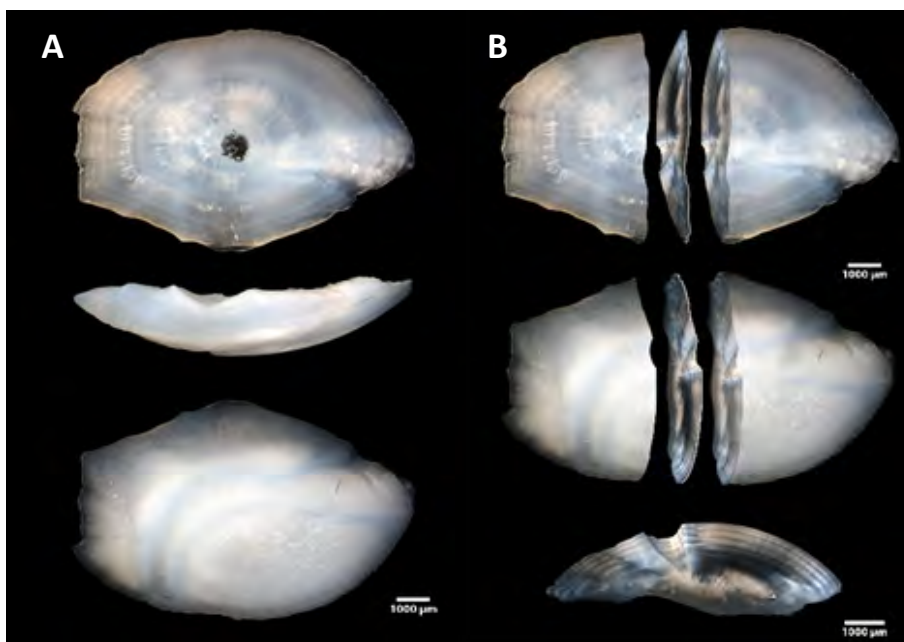


Figure 9.16.1 A) Whole otoliths of White Grunt proximal view with core marked (top), dorsal view (middle), and distal view (bottom) and B) otolith sectioned with thin-section located and rotated showing location of cut through the core.

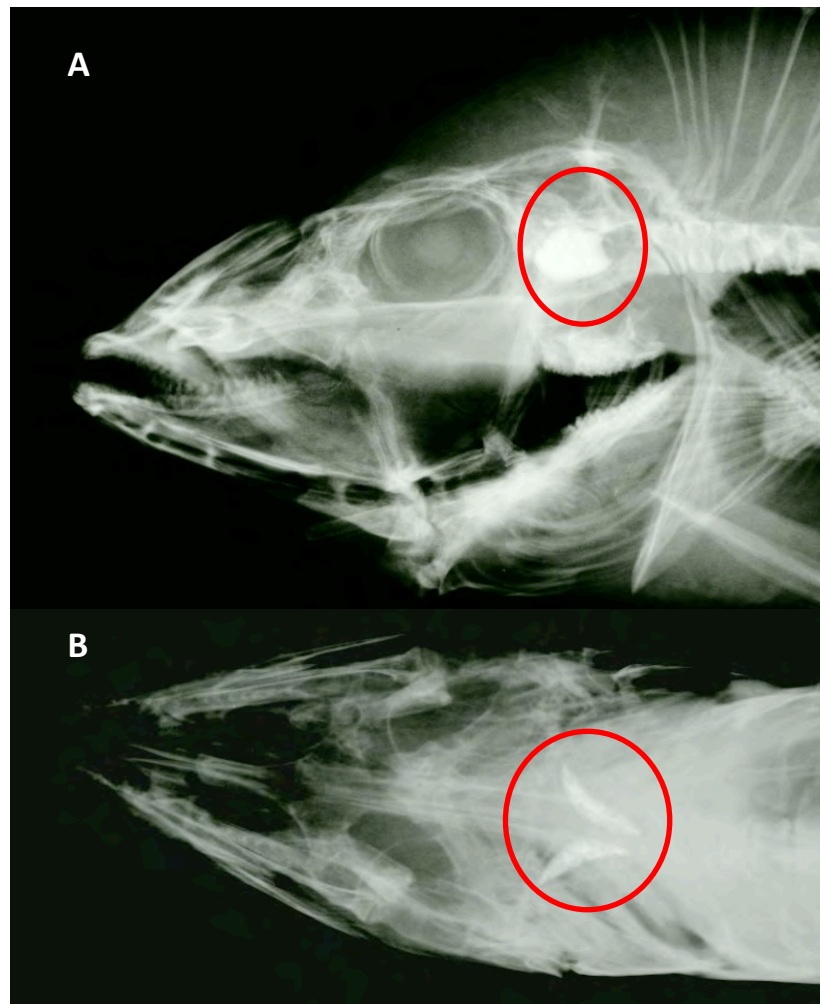


Figure 9.16.2 Location of sagittae (red circles) in the neurocranium of White Grunt in A) lateral and B) dorsal-ventral views.

Extraction

White Grunt otoliths may be extracted using any of the methods outlined in Chapter 3.0. The otic capsule is located near the posterior base of the skull behind the gills. If the fish is intended for market then the otoliths can be extracted via the gills (Chapter 3.0, Section 3.2.1). The ventral surface of the otic capsule is easily discernible once the gills are removed. The capsule surface is thin, can appear transparent, and is relatively easy to chisel away and expose the otoliths, which can be removed using forceps. For routine sampling, the easiest and quickest approach is to cut vertically down through the head using the hacksaw technique. Once the otic capsule is breached (i.e., sawing sound changes), then the nose of the fish can be pushed down, cracking the head open across the saw line. Usually the two sagittae are exposed by this motion and can easily be removed using forceps.

Otolith Processing

Due to the relatively large size of White Grunt otoliths, multiple processing techniques are acceptable (Chapter 3.0, Section 3.2.5) and the technique chosen will likely reflect available equipment. The core of the otolith is easily discernible on the whole otolith using a dissecting microscope. Whole White Grunt otoliths can be thin-sectioned using either a sectioning saw or a Hilquist saw (Chapter 3.0, Section 3.2.6.2). Generally, White Grunt sections are processed at approximately 0.5 mm thickness.

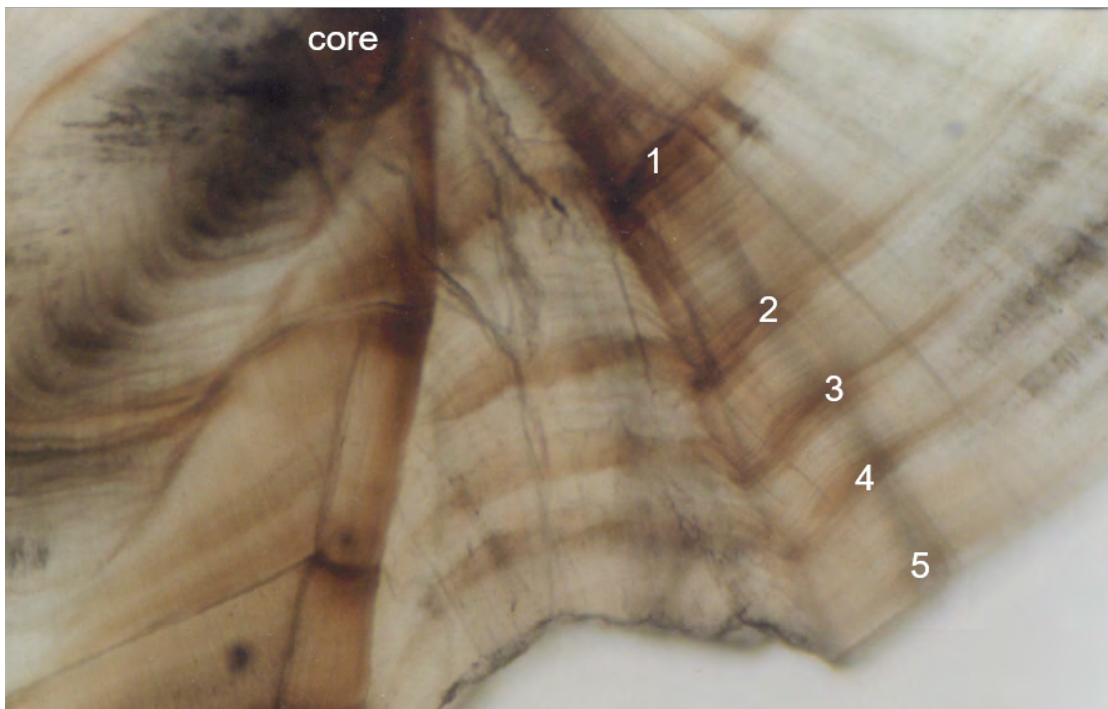


Figure 9.16.3 Transverse cross-section of an age-5 White Grunt viewed using transmitted light.

Age Determination

Enumeration of annuli in White Grunt otolith sections is easy even with inexperienced personnel. The problem encountered most often by readers is determining the position of the presumptive first opaque increment nearest the core (Figure 9.16.3). Due to a protracted spawning season (Figure 9.16.4), there is assumed to be considerable variation in the distance from the core to the first opaque increment, which can appear as a dark diffuse area. In addition, after age-10, White Grunt somatic growth slows markedly in fish from southeastern Florida (Padgett 1997) and the west coast of Florida (Murie and Parkyn 2005), resulting in a decrease in the accretion rate of material in the otolith. The opaque rings therefore appear closer together in older fish (Figure 9.16.3), making their identification difficult.

Padgett (1997) determined White Grunt spawning off North Carolina and South Carolina is typically from March through September, with a peak in May through July (Figure 9.16.4). Murie and Parkyn (2002)

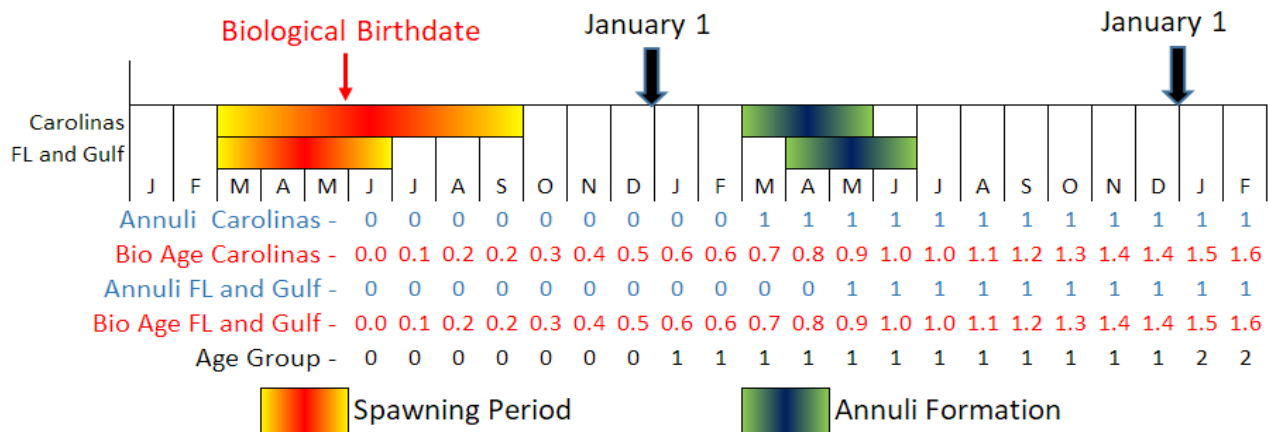


Figure 9.16.4 Timeline showing spawning period and annuli deposition ranges for White Grunt in the South Atlantic and eastern Gulf.

found spawning females off the west coast of Florida during April to June, with a peak in May and June. Padgett (1997) also validated annulus formation using marginal increment analysis (MIA) of White Grunt otolith thin sections and determined formation occurs in March and April in the Carolinas. Potts and Manooch (2001) examined MIA for fish from the Atlantic Coast of Florida and found formation in March – June with a peak in May. Murie and Parkyn (2005) validated formation in fish from the Gulf Coast of Florida using both MIA and chemical marking with oxytetracycline and found annulus formation in May.

Other Ageing Methods

Scales have not been used successfully for White Grunts (Potts and Manooch 2001). Whole otoliths of White Grunt can be used for ageing juveniles or fish <8-10 years old, but after ~10 years surface reading of whole otoliths underestimate the age of the fish (Murie and Parkyn 1999; Figure 9.16.5A). White Grunt can also be aged using the break-and-burn technique because their otoliths are relatively large and robust (Figure 9.16.5B). Otoliths can be scored through their core using a diamond-marking pen and then snapped in half using finger pressure, or cut in half using a sectioning saw, and then burnt using an alcohol flame. This method is useful for production ageing when otolith measurements are not required and appears to be unbiased up to at least 16 years of age (i.e., oldest White Grunt aged using the method) (Murie and Parkyn 1999).

Research Needs

At this time there is nothing required for White Grunt. It has been suggested that White Grunt could serve as a “control” for all agers because they are so reliable to age accurately. The species is a good candidate for training and QA/QC.

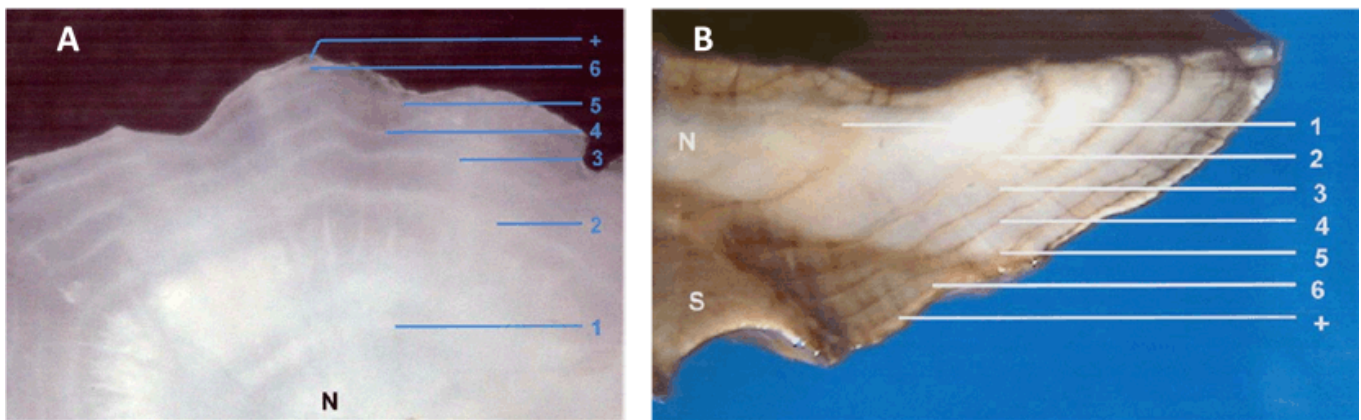


Figure 9.16.5 Surface view under reflected light of A) a whole otolith of a 6-year old White Grunt in black background (N=core) and B) a cross-sectioned break-and-burnt otolith of the same age.

9.17 Labridae



Tautog *Tautoga onitis*

Highlights

- Opercles are the standard structure for ageing because they are very easy to process and can be read with or without magnification; however, otoliths are a more reliable structure for age determination (ASMFC 2012).
- As Tautog age, the first one or two annuli on the opercle become difficult to discern due to thickening of the bone near the center of growth.
- Sagittal otoliths are very small compared to body size and, with enough practice, relatively easy to remove.
- Annuli on sagittal otoliths are easily discernible when baked and sectioned.
- Pelvic spines are a viable option for non-lethal ageing (Elzey and Trull 2016).
- Tautog are a relatively long-lived species with a maximum observed age of 31 (ODU unpublished data).

Opercle Description

Tautog opercles are roughly triangular in shape (Figure 9.17.1). The dorsal and anterior edges intersect at the articular apex, a thickened structure that contains the cup of a ball and socket joint that serves as the hinge point for the operculum. The center of the articular apex corresponds to the center of bone growth and thus serves as the origin for radial and annular measurements (Le Cren 1947, McConnell 1952, Bardach 1955, Cooper 1967). The outer surface of the opercle is convex and the inner surface concave, with both surfaces tapering to a thin, delicate edge along the ventral margin. This thin ventral margin is where new growth is most apparent.

Opercle Extraction

When dissecting the opercle from a Tautog, care must be taken not to damage or cut through the articular apex (center of growth), the anterior or dorsal margins (helpful for differentiating annuli from checks), and especially the ventral margin (the most prominent area of new growth). For additional details on removal, see Chapter 5.0, Section 5.2.1.

There are other methods for removing the opercle.

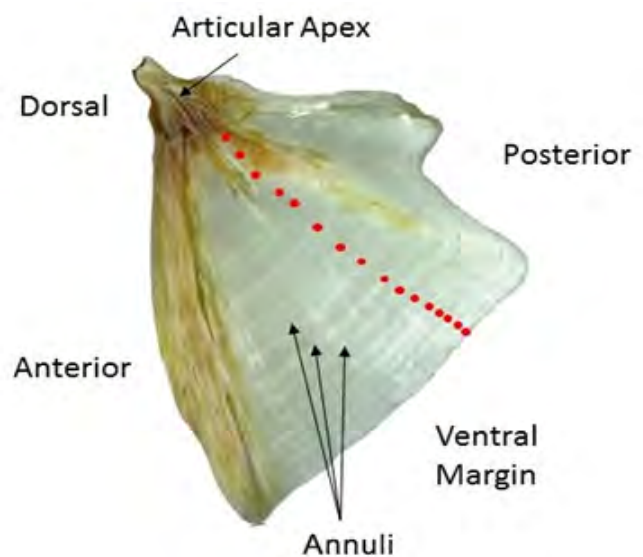


Figure 9.17.1 An opercle from a Tautog cleaned and ready to be aged.

A fast technique, which results in very little attached tissue, is well illustrated in a video by ODU's Center for Quantitative Fisheries Ecology (Chapter 12.0, Section 12.7).

Opercle Processing

Boil the opercle in water to loosen the tissue that is attached, then use a small brush to remove the softened tissue. Rinse the opercle with clean water and allow to air dry for 24 hours. Then store dry in labeled coin envelopes. See Chapter 5.0, Section 5.2.2 for a more detailed process for cleaning opercles.

Otolith Description

Tautog sagittal otoliths are very small relative to their body size, generally smaller than 7 mm. Their sagittae have a sulcal groove that extends from the posterior edge to the anterior edge. The rostrum, postrostrum, and antirostrum are easily discernible. The primordium, or core, can be located where the edges of the sulcal groove are closest (Figure 9.17.2).

Otolith Extraction

The location of otoliths in the neurocranium is illustrated in Figure 9.17.3. Tautog sagittal otoliths are small and delicate; some patience and practice is required for their removal. Because of their location and fragility, removal by the top method (Chapter 3.0, Section 3.2.1) is recommended.

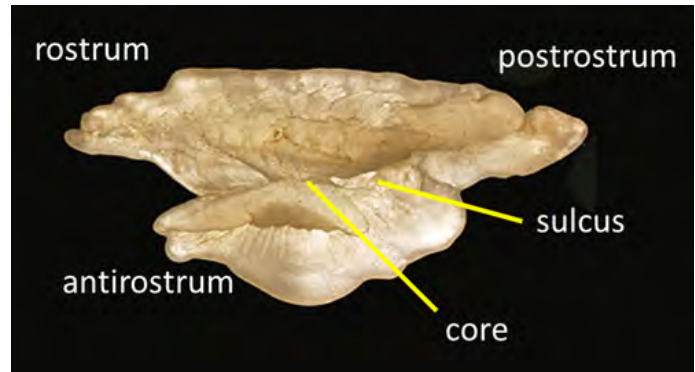


Figure 9.17.2 Proximal side of unbaked sagittal otolith.

Make a lateral cut above both eyes using a saw or filet knife (Figure 9.17.4). A perfect cut would reveal three components of the brain: two optic lobes and one cerebellum (Figure 9.17.5). The sagittal otoliths are surrounded by the sacculus, located behind the optic lobes, and below the cerebellum on the left and right sides. Using tweezers, gently insert them downward into the cavity until the tips of the tweezers touch the bottom of the cavity. Gently close the tweezers and pull the whole sacculus out. The sagittal otolith will come out with the sacculus (Figure 9.17.4). If the brain has been damaged it may be easiest



Figure 9.17.3 Location of sagittal otoliths in the neurocranium of Tautog in A) lateral and B) dorsal-ventral views.

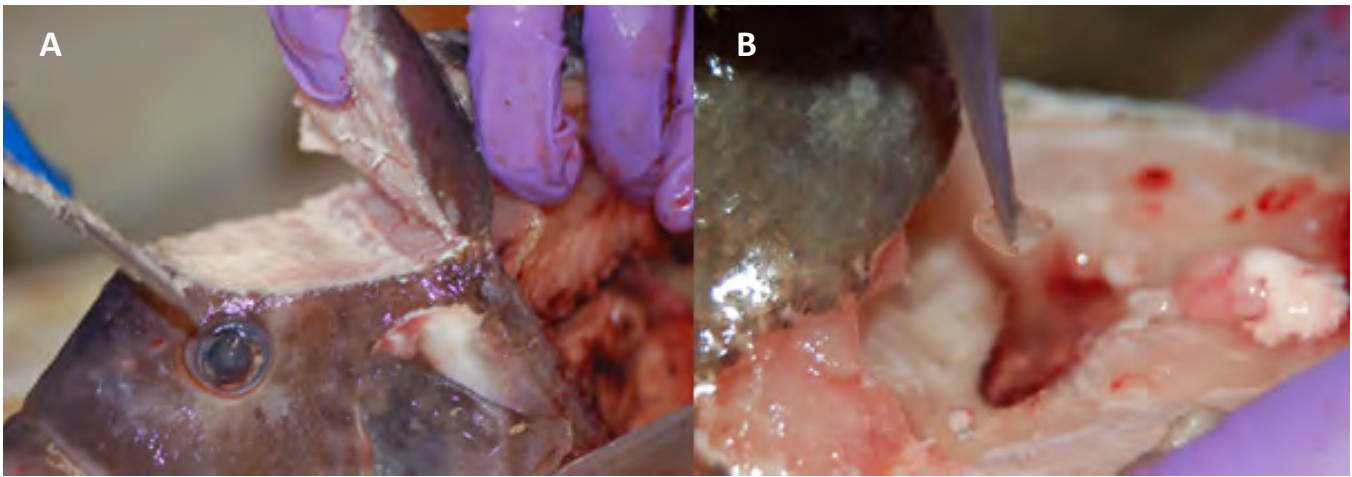


Figure 9.17.4 A) Lateral cut above both eyes. B) Otolith (still in sacculus) being removed from brain cavity.

to clean out the brain case prior to searching for the otoliths. *Use caution*; otoliths may be accidentally removed during cleaning.

ODU's Center for Quantitative Fisheries Ecology developed a video demonstrating the process of removing sagittal otoliths from Tautog (Chapter 12.0, Section 12.7).

Otolith Preparation

Tautog otoliths need to be baked prior to embedding in resin. Most labs follow a modified version of the 'bake and thin section' technique outlined in Chapter 7.0, Section 7.1.6. The otolith, like most, is cross-sectioned through the focus or core (Figure 9.17.6). The baked section is typically caramel in color which enhances the annuli (Figure 9.17.7).

Age Determination

Tautog spawning has been observed in Long Island Sound from mid-May to early September (LaPlante and Schultz 2007), in the New York Bight from June to early August (Malchoff 1993), and from April to mid-June in the lower Chesapeake Bay (White et al. 2003). Annulus formation occurs April through July (Figure 9.17.8; Hostetter and Munroe 1993). Because of the long spawning period, young-of-year fish hatched in May have a considerable growth head start on fish hatched in September. Thus, at the time of annulus formation the following spring, it is possible for a fish born in May to be considerably larger (and have a larger first annual increment) than a fish born in September. Due to these factors, correct identification of the first annulus can be challenging.

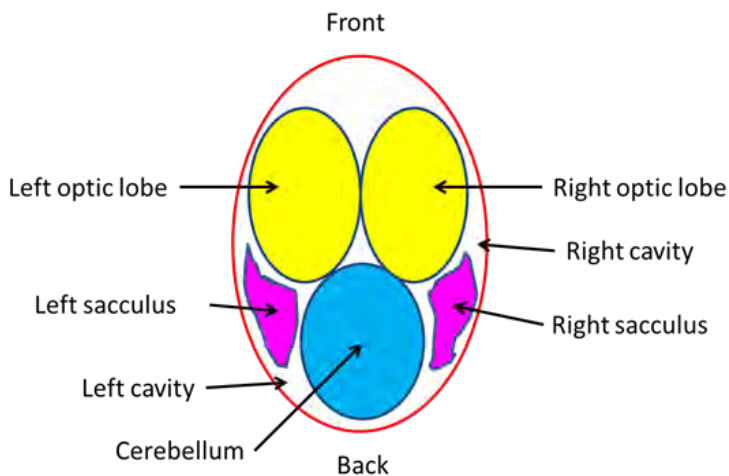


Figure 9.17.5 Diagram of the position of the otoliths in relation to the brain in a Tautog.

Opercles

Hostetter and Monroe (1993) validated the annual periodicity of zone formation in opercula with marginal increment analysis.

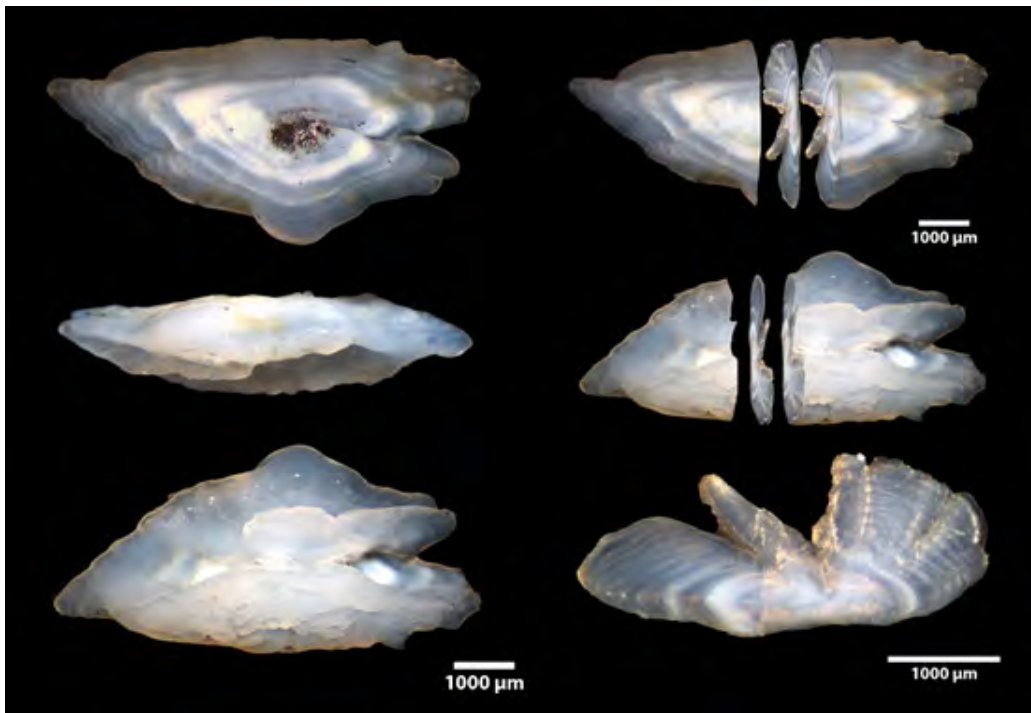


Figure 9.17.6 A) Whole otoliths of *Tautog* proximal view with core marked (top), dorsal view (middle), and distal view (bottom) and B) *Tautog* otolith sectioned with thin-section located and rotated showing location of cut through the core. **Note:** this otolith has not been baked.

Although *Tautog* opercles can be read with reflected or transmitted light, most ageing is done with transmitted light (window, overhead light, microprojector), with and/or without magnification. While magnification will reveal more detail, viewing without magnification often presents a clearer annular pattern. Magnification using a microprojector is best for discerning subtle annuli closest to the articular apex (age-1 and -2). A combination of both methods is helpful with difficult bones. Growth zones appear with sharp definition from translucent to opaque zones. Check marks can be distinguished from annuli by whether or not they are continuous onto the margins of the opercle. It is often helpful to examine both opercles in tandem to aid in deciphering annuli.

The first, and sometimes second, annuli are often obscured as the opercle thickens with growth (Figure 9.17.9; ASFMC 1995, ASFMC 2012, Gottschall personal communication). The identification of such phantom annuli must usually be inferred from the size of the opercle, the distance to the first observable annulus, and experience expecting where the annulus would be relative to other annuli. If measurements are being taken for back-calculation, such annuli are assigned a missing value if counted.



Figure 9.17.7 Baked and sectioned otolith from an age-6 *Tautog* with annuli indicated.

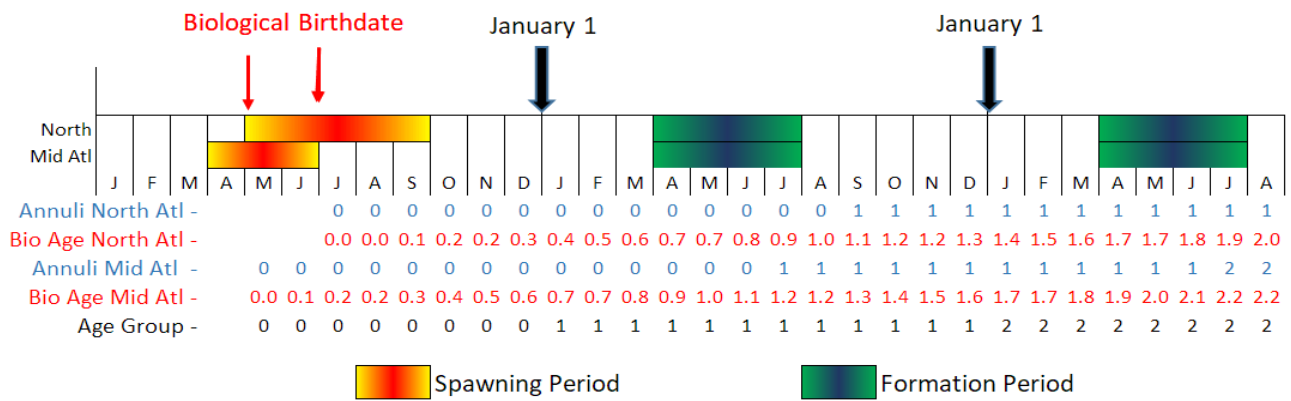


Figure 9.17.8 Spawning periodicity and age assignment timeline for Tautog in the mid-Atlantic (VA to GA), and northeastern US.

Otoliths

Annual periodicity of growth zones in Tautog otoliths has not been validated. Comparison of age estimates from opercles, which have been validated, and sectioned otoliths do result in systematic bias, though this bias appears to be driven by difficulties identifying the first annulus in thick opercles of older fish as opposed to differences in periodicity of growth zone formation between structures (Elzey and Trull 2016). A well sectioned otolith will provide a clear view of the core, annuli, and sulcal groove (Figure 9.17.7). The first annulus is generally easily discernible and has a larger radius than the core.

Other Ageing Methods

Pelvic Spines

Tautog have a single spine on the leading edge of each pelvic fin. Pelvic spines have minimal vascularization as compared to dorsal spines. Elzey and Trull (2016) reported that pelvic spines provided ages as precise as opercles and otoliths. Compared to opercles and otoliths, the biggest advantage is that "before" spines can be removed from live fish as well as from dead fish without interfering with marketability.

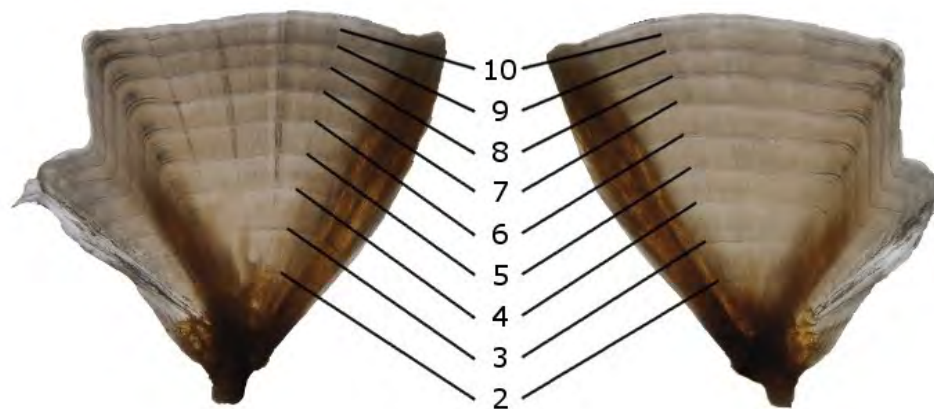


Figure 9.17.9 Annuli on particularly well-formed Tautog opercles. The first annulus is not visible and the bones are judged to be from a 10-year old fish.

Pelvic spines are easily excised by cutting as close as possible to the body of the fish with a sturdy pair of scissors or wire cutters. A knife can be used to cut the tissue between the spine and the fin rays on live fish to minimize tearing. It is important to cut the spine as close to the fish as possible so that the first annulus is not missed. See Chapter 6.0, Section 6.2 for discussion of spine growth.

Pelvic spines are placed in boiling water for 1-2 minutes. Excess flesh is removed with a small brush and the spine is allowed to air dry for at least 24 hours. The spine is then embedded in epoxy and sectioned with a low speed isomet saw. Sections are approximately 0.75 mm thick. Several sections are cut starting from the base of the spine and working distally. The sections are adhered to microscope slides with liquid cover slip.

Pelvic spines are typically viewed with transmitted light under a compound microscope at 100-200X magnification. A sectioned pelvic spine will have alternating opaque and translucent zones (Figure 9.17.10). One pair represents one year of growth. The first annulus (transition from translucent to opaque) is less obvious than subsequent annuli and can be partially obscured by vascularization. A section too close to the base of the spine will show more vascularization and a section too far out will miss the first annulus. Examining multiple successive sections eliminates this problem.

Whole Otoliths

Tautog otoliths can be aged whole. Unbaked whole otoliths can be placed in a dish of fluid (Chapter 7.0, Section 7.2.2) on a dark background and illuminated with reflected light (Figure 9.17.11). This technique saves time over baking and sectioning otoliths but has limited utility in older fish as the annuli can become too close together near the edge to distinguish and in especially thick otoliths, the first annulus can be hard to see. With a limited age range, Elzey and Trull (2016) found no bias between whole and sectioned otoliths. Elzey (unpublished data) suggests sectioning otoliths where whole ages yield ages of nine years or more but cautions that growth patterns in different geographical regions may vary, impacting the age where sectioning is necessary.

Scales

Scales have been determined to be unreliable structures for ageing Tautog (Cooper 1967, Hostetter and Munroe 1993, Elzey and Trull 2016).

Research Needs

Validation studies are needed for otoliths and pelvic spines. Further evaluation of whole otoliths, by age, would be useful to determine reliability of a structure that requires less processing time than baked and sectioned otoliths.

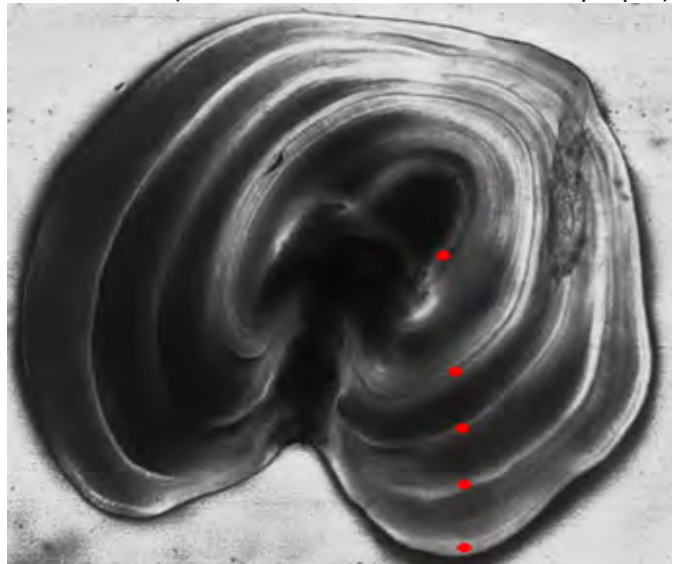


Figure 9.17.10 A pelvic spine from an age-5 Tautog with annuli marked by red dots. The first annulus is less clear than the others and the fifth has formed near the edge.

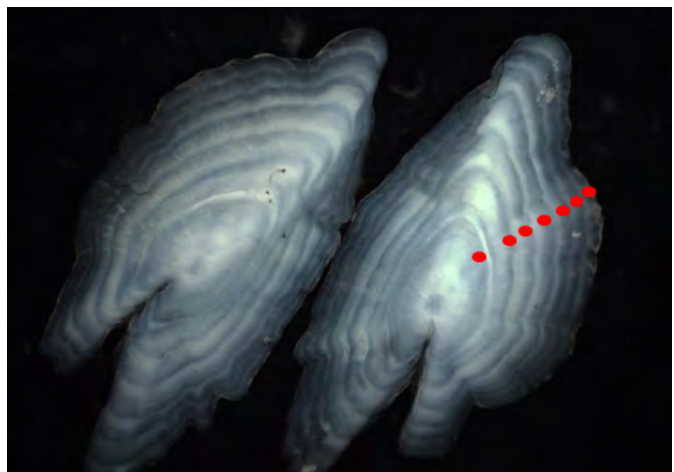


Figure 9.17.11 Whole sagittal otoliths from an age-7 Tautog.

10.0 Glossary of Terms Used in Age and Growth Studies

Ageing terminology in practice can be ambiguous and many terms are often used to refer to the same thing. Common terms used outside this manual are included in parentheses next to the term defined in this glossary, but inclusion here is probably not comprehensive. Sources used to compile this glossary include: Summerfelt and Hall 1987, Secor et al. 1991, Kalish et al. 1995, C.A.R.E. 1997, ODU/VMRC 2001 as well as agreement from the multiple agencies contributing to the development of this document.

A

accuracy - the closeness of a measure or estimated value to its true value. Used in age reading to describe how close an age estimate is to the true age.

age - the time from birth to capture, measured in years, months, days or other units and expressed in whole numbers.

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

ageing – the process of estimating the age of a fish.

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

annual growth zone - all growth on a structure which forms during one year; usually consisting of an opaque zone and a translucent zone.

annulus (pl. annuli) - the transition from one annual growth zone to another. The optical appearance of these marks depends on the structure and the species.

anterostrum - 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 age of a fish based on the time elapsed from estimated birth date to date of capture expressed in years and fractions of years or decimal equivalents.

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

blind reading – the process of estimating age of a fish by visual assessment of growth zones and margin development with no knowledge of other characteristics from which age could be inferred.

C

calendar age - the age of a fish based on time elapsed from the first day of a calendar year rather than the birth date of the fish, to date of capture expressed in years and fractions of years.

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

check (false annulus) - a discontinuity (e.g., a stress-induced mark) that forms on a structure used for age estimations. Checks usually correspond with a slowing of growth. Checks do not form annually but reflect various environmental or physiological changes.

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

cohort – a 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, has a protracted spawn, or some other periodicity; does not imply year-class.

confidence – a measure of how an age reader feels about their age estimate.

continental age - the age of an Anguillid eel based on the time elapsed from entering freshwater or near shore habitats, It does not include the oceanic larval phase of the life history.

core (focus) - the primordium of the otolith. The hypothetical or real point of origin of a structure used for age estimation; the center of growth.

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

crystallized (vateritic) otolith - an otolith displaying alternate forms of calcium carbonate; age estimates are generally not provided due to missing or disrupted annuli.

D

deposition – the process by which minerals and proteins are accreted on the surface of an otolith thereby causing growth of the otolith.

E

edge code – see margin code

F

false annulus – see check

focus – see core

formation – accumulation of material at the edge of a structure used for estimating age. Frequently used to discuss growth of a particular growth zone; opaque or translucent. Sometimes used interchangeably with deposition.

H

hard part - any calcified structure in a fish which increases in size relative to the overall growth of the fish and may be useful for ageing.

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

margin (edge) - a term used to describe the most recent growth at the edge of a structure used for age estimation.

margin code – a subjective code used to describe the amount of growth that has occurred since the last formed annulus.

marginal increment - the zone beyond the last identifiable estimation mark at the margin of the ageing structure; frequently expressed in relative rather than quantitative terms, i.e., as a fraction or proportion of the last complete annual growth zone; see margin code.

marginal increment analysis (MIA) – an analysis (verification method) of the marginal increments throughout a full year to verify the periodicity of growth zones and deposition period on an ageing structure. MIA is considered indirect validation of an ageing method.

N

nucleus – see core.

O

opaque growth zone - the region of a structure used for age estimations that interferes with the passage of light and therefore appears dark relative to adjacent translucent growth zone(s) when using transmitted light; appears bright under reflected light. The opaque and translucent growth zones together form the annual growth zone.

opercular series - group of bones that makes up the operculum.

operculum (pl. opercula) - entire flap that opens to allow water to go over the gills.

opercle (pl. opercles) - the individual bone that is typically used for estimating age.

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 (i.e. repeatability); in age reading, it relates to the variability of age estimates 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.

R

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

read (age determination) - the process of estimating the age of a fish by visual assessment of growth zones and margin development.

readability - a measure of how good a particular sample is for age estimation in relation to an ideal sample.

reading axis - the path along which annuli are counted.

regenerated scales – a scale which replaces one previously lost that does not possess the same annual markings and cannot be used for age estimation.

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.

sagitta (noun, pl. sagittae; adjective, sagittal) – generally the 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 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 consisting 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 annual growth zone, analogous to a check; causes the annulus to appear as two or more closely spaced zones.

sulcus acusticus - commonly called sulcus or sulcus groove; a longitudinal sculptured groove extending down the medial surface of a sagittal otolith along 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.

T

translucent growth zone - the regions of a structure used for age estimation that allow a greater passage of light relative to the opaque zones. Appears dark with reflected light, and bright with transmitted light.

U

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

V

validation - the process of proving that the growth zones used for age estimations are accurately related to age so they can be used to assign an age to a fish; often used to refer to proving that zones interpreted as an annulus are deposited annually.

verification - the process of evaluating the precision of a particular age estimation method. Akin to, but not to be confused with, validation.

vaterite – an alternate form of calcium carbonate. Seen in crystallized otoliths.

Y

year class - fish spawned or hatched in a given year. Often preceded by the year the fish were spawned (e.g., the 2017-year class).

Z

zone – a distinct area or region of a structure used for age estimation.

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12.0 Appendices

12.1 Standardizing Field Techniques

12.1.1 Labeling of Specimens

Life history and stock composition data (size, age) are critical components of stock assessments and are largely obtained from biological samples taken during scientific surveys, by port agents, and by observers working aboard fishing vessels. The first and most critical component in the collection of biological data and hardparts for ageing of fishes is keeping track of samples and being able to reconnect the biotic and abiotic data tied to that sample.

Insuring that the biological samples are effectively connected to field data and the originating sampling program is dependent on the care taken during packaging and labeling. When multiple laboratories are responsible for tissue processing (ageing, reproduction, DNA, diet, condition, health, etc.) consistency and care in packaging and labeling is critical. Sharing samples is the trend as more specialized and technical approaches are being taken. Sometimes workloads have to be shared between agencies and labs due to production-style hardpart processing and ageing. Consistent and careful labeling insures that the information generated by the processing laboratories can make its way back to the originating programs and databases.

12.1.2 Standard Field and Lab Labeling Methods

Whenever biological samples (otoliths, scales, fins, spines, tissues, organs) are taken from a selected individual, a unique identification number must be assigned to ensure that all the samples can be linked back to the original specimen. This identification number can include any number of codes related to date of capture, specific sampling program, gear, and species. In addition, a number of other data elements related to environmental conditions and morphometric and or condition data must be recorded for each fish. (e.g. temperature, salinity, depth, collection date and time; location; source (fishery-independent, roving creel, fish house); gear type; length (total, standard, or fork); weight (total or gutted); and sex).

Note: *it is critical with all these data elements that the unit and interval of the measurement are recorded as well (imperial or metric, inches/feet, mm/km, etc).*

Standardizing sample packaging and labeling across a diverse array of agencies and programs is highly desirable but is a major challenge due to often divergent work priorities and funding. Thus, achieving such standards is an important ongoing task for the inter-agency coordination bodies and councils.

Minimally, archived otoliths (or any tissue) must be assigned a unique identification number that refers to the individual fish which comprises an individual record in a database and may include the other vital information related to the collection. A unique number does not have to include all the collection information, it just has to connect the biological sample to the fish record where information such as species would be found.

The move to electronic fisheries data collection systems is advancing with good reason and should be promoted (Van Tamelen 2004). Coinciding with the development of these systems, numerous biological samples could be delivered with barcodes and electronic data files as opposed to hand written tags and field sheets.

12.1.3 Barcode Tags

Barcodes are already being implemented by some state (FWC) and federal agencies (NOAA) and a standardized code structure should be promoted to the greatest extent practicable (Figure 12.1.1). The

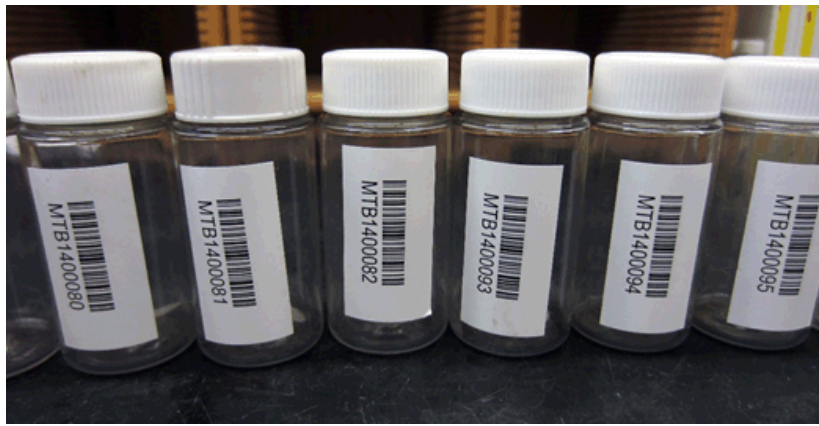


Figure 12.1.1 Example of barcode tags used by the FWC to track fishery independent samples.

benefits of barcodes are well proven.

Some key criteria for a bio-sample barcode structure may include:

1. Barcodes need to be unique for each specimen (a requirement) and can serve as the unique identification number, but should be able to be produced at multiple locations given simple rules. Thus, barcodes should be equally applicable for personnel working out of a truck at a fish dock as well as aboard the most modern survey ship.
2. The use of barcodes should reduce the error and time taken and subsequently enhance all of the following:
 - a. labelling at the point of collection in the field,
 - b. registration of the specimen record in the database(s) and
 - c. physical inventory and subsequent labeling of processed materials during laboratory workflow.
3. Barcodes should incorporate the minimum amount of information necessary.
4. Barcodes can be pre-printed for ease of use at the point of sample collection. A barcode sequence could be ordered as specialty labels from a commercial vendor, or could be produced at the program level using inexpensive printers and distributed to samplers.
5. Barcodes should be as compatible as possible across federal and state agency laboratories and programs.
6. The barcodes should be expandable and remain relevant over time.
7. The barcodes should be backwards compatible—that is an archived specimen taken in years past may be readily assigned a barcode.
8. Code 128 symbology is recommended for 2D labels. But as barcode reading technology is changing over time, it is advisable to include alphanumeric representation of the code as well as the symbology on all labels.

12.0 Appendices

12.2 Ageing Parameters and Their Usefulness

12.2.1 Back Calculation

Fish growth is usually examined by fitting data to a length-at-age relationship, which can be used to estimate length for a given age. However, fishery-dependent sampling may be biased, which reduces the accuracy of the length-at-age relationship. Many times only older fish are available for examination (i.e., large fish from tournaments or dockside sampling of commercial catch). Size limits may also impede collections of fish representing the full size range of the population. This biased sampling of the population is problematic because growth rates change throughout the life of the fish. In long-lived species when small specimens are rarely encountered, the growth rates of young fish are of particular interest. In these cases, length at a given age can be estimated from a technique referred to as back-calculation.

Back-calculation of annual growth is used to understand the growth history of fish based on the assumption that there is a relationship between the otolith radius and fish length. If the relationship of otolith radius and fish length is linear, then an estimate of fish length relative to a location (i.e., growth ring) on the otolith can be calculated. The linear relationship of otolith radius and fish length is validated by regressing a series of otolith radii against the fish lengths for samples that cover as many ages/lengths as possible, given the available data. Assuming that the relationship is linear, lengths are estimated for each age using the following equation:

$$L_e = \frac{D_r}{D_m} * L_t,$$

where L_e is the estimated length,
 D_r is the measured distance from the core to a chosen growth ring,
 D_m is the measured radius of the otolith, and
 L_t is the total length of fish at capture.

This equation gives an estimate of length for each chosen growth ring. If each ring represents an annual growth increment, estimates of length can be calculated for several ages on each otolith. This method is called the 'direct proportion' method. Further refinement of the above equation includes the Y-intercept from the regression of total length and otolith radius:

$$L_e = \frac{D_r}{D_m} * L_t + y,$$

where L_e is the estimated length,
 D_r is the measured distance from the core to a chosen growth ring,
 D_m is the measured radius of the otolith,
 L_t is the total length of fish at capture, and
 y is the y-intercept from the otolith radius-fish length regression.

This technique is 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. The Fraser-Lee method adjusts for any fish length obtained prior to otolith growth. Other similar methods have been used with the intent of partitioning the variance into age effects and length effects. DeVries and Frie (1996) provide a detailed description of the above methods.

12.2.2 Otolith Growth Models

Otoliths grow not only as a function of fish size, but also in response to changing environmental variables (Neilson and Geen 1982, Jones 1992). The relationship between otolith growth and somatic fish growth is often assumed to be constantly proportional, but the validity of this assumption has been questioned (Secor and Dean 1989, Casselman 1990). For example, slower-growing larval and juvenile Striped Bass (*Morone saxatilis*) tended to have larger otoliths relative to fish standard length than did faster-growing individuals (Secor and Dean 1989). Based on the evidence for variation in the otolith-fish size relationship, otolith growth should be used as an indicator of fish growth only after examining the species-specific relationship.

The influence of environmental variables on otolith growth has been extensively studied using daily growth increments. Observations of consistent daily increment formation, even under constant light or dark conditions, suggest that the frequency of increment deposition remains constant except in the case of extreme stress (Radtke and Dean 1982, Campana and Neilson 1985, Gauldie and Radtke 1990). Multiple studies have demonstrated that the diel formation of growth increments is entrained to photoperiod (Tanaka et al. 1981, Campana 1984, Wright et al. 1992). Although the frequency of increment formation does not change, the width of increments can vary due to environmental factors such as feeding activity, temperature, and dissolved oxygen (Weisberg 1993, Morales-Nin 2000).

Temperature is a major environmental factor contributing to growth variability in otoliths. Annulus deposition patterns reflect differential fish growth rates due to seasonal changes in water temperature (Campana and Neilson 1985). Short-term fluctuations in water temperature can be detected in daily growth increments (Campana 1984, Neilson and Geen 1982, Bestgen and Bundy 1998). A positive relationship between temperature and otolith growth has been demonstrated through laboratory experiments. For example, Arctic Char (*Salvelinus alpinus*) reared at different temperatures had increasing mean daily otolith growth rates related to increasing water temperature, even above the optimal temperature for somatic growth (Mosegaard et al. 1988). Temperature-dependent otolith growth was also observed in larval Atlantic Cod (*Gadus morhua*) otoliths (Otterlei et al. 2002). The relationship between temperature and otolith growth is more difficult to detect in field-collected samples.

In addition to temperature, other environmental factors have been shown to influence otolith growth. Variability in daily increment widths from juvenile Starry Flounder (*Platichthys stellatus*) otoliths was related to temperature, salinity, and tide cycle (Campana 1984). Dissolved oxygen is correlated with temperature and salinity, and further influences otolith growth. For example, juvenile Black Seabass (*Centropristis striata*) otolith growth rates were reduced in hypoxic conditions, and the relationship between somatic growth and otolith growth rates was altered (Hales and Able 1995). In addition, daily growth was highly variable and reduced in larval European Smelt (*Osmerus eperlanus*) otoliths at oxygen levels below 13.5 mg-1 (Sepulveda 1994). Feeding activity, or prey density, is commonly cited as a factor that regulates otolith growth rates (Neilson and Geen 1982, Moksness et al. 1995).

Several methods have been published for understanding otolith growth variability. However, few published studies have reported models describing inter-annual otolith growth variability. In one such example, the effects of inter-annual temperature variation on annulus formation were examined in adult Atlantic Cod (*Gadus morhua*) otoliths (Pilling et al. 2007). Timing and rate of otolith annulus deposition were related to North Sea water temperature using multiple linear models. The results from Pilling et al. demonstrate the value of using historical otolith collections to understand climate change impacts. With the existence of long-term otolith collections and imaging technology, otolith growth data are accessible for analysis. These analyses may represent a novel approach to understanding variability in fish growth.

12.2.3 Otolith Weights

There are occasional needs to validate field data which may have been recorded incorrectly or transcribed and key entered with errors. Additional data elements such as raw otolith dry weights can be used to verify errors in such things as fish lengths or weights by using standard analysis for outliers. When ages do not fit the typical age at length estimates, otolith weight may be a way to verify an error and reduce the number of samples excluded simply due to transcription errors. For example, the processor could go back to the otolith envelope or field data sheet and determine if the age was incorrect or the morphometric information was incorrect.

Red Drum can be used as an example of the relationship between whole-body somatic growth and growth in otolith weight, and the way in which this information can be used to detect outliers. Starting in 2003, TPWD began recording otolith dry weight along with other field-based data points. This has resulted in 4,820 Red Drum specimens for which otolith dry weight (mg), total body length (mm) and age have been recorded simultaneously (in this case the data were constrained to individuals < 5 years of age). There is a strong statistical relationship between otolith weight and body length, and predictably this relationship is consistent across age classes (Figure 12.2.1). Figure 12.2.1 also demonstrates the method of using otolith weight against body length and age to identify potential data outliers (indicated by black arrows). In this case outliers were identified qualitatively, but quantitative model-based approaches can also be used.

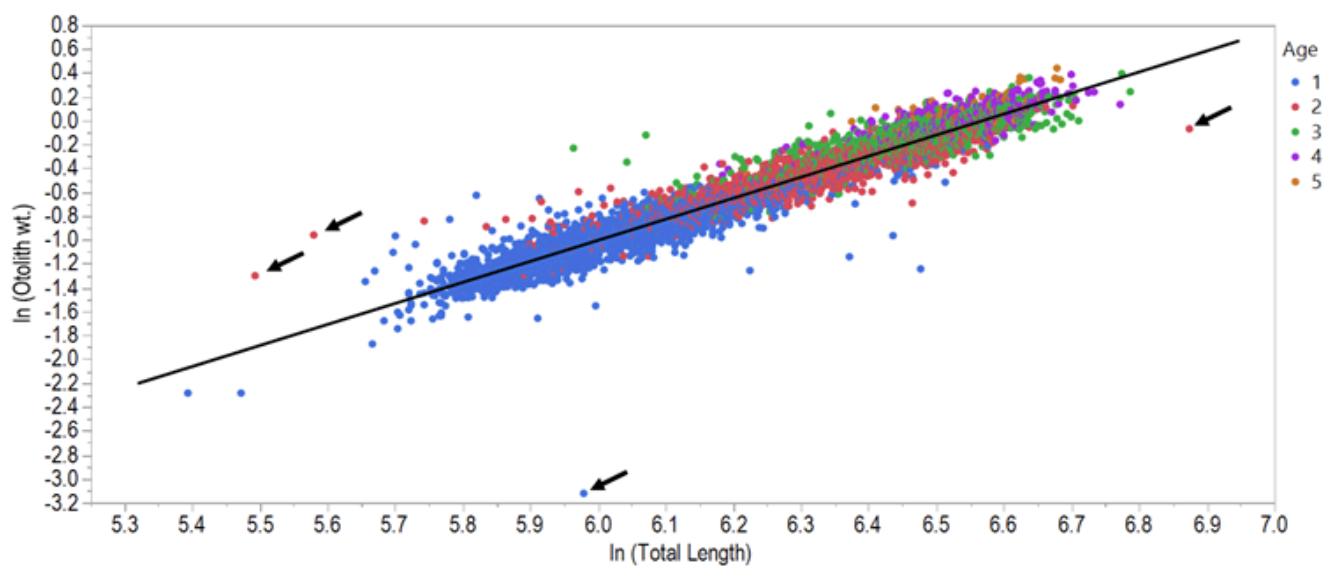


Figure 12.2.1 Relationship between total body length and otolith weight in Red Drum, ages 1-5 (TPWD unpublished data).

Otolith weight is simple to measure, and can be easily added to the front end of any otolith processing protocol. Additionally, assuming that statistical validation is conducted up front, otolith weight can be used as a proxy for total body length or age in some situations. In the case of Red Drum, growth in otolith weight is very similar at-age to growth in total body length (Figure 12.2.2).

12.2.4 Isotopic and Chemical Analysis of Otoliths

As noted in Chapter 2.0, Section 2.3, the microchemistry in the otolith can be used to provide insight into the life history and habitat usage of fish over time. Proportions of various elements and isotopes within the annuli can help identify ontogenetic shifts in foraging habitat, seaward migrations and relative locations within systems throughout the history of the animal. Several isotopes are used to look at salinity and

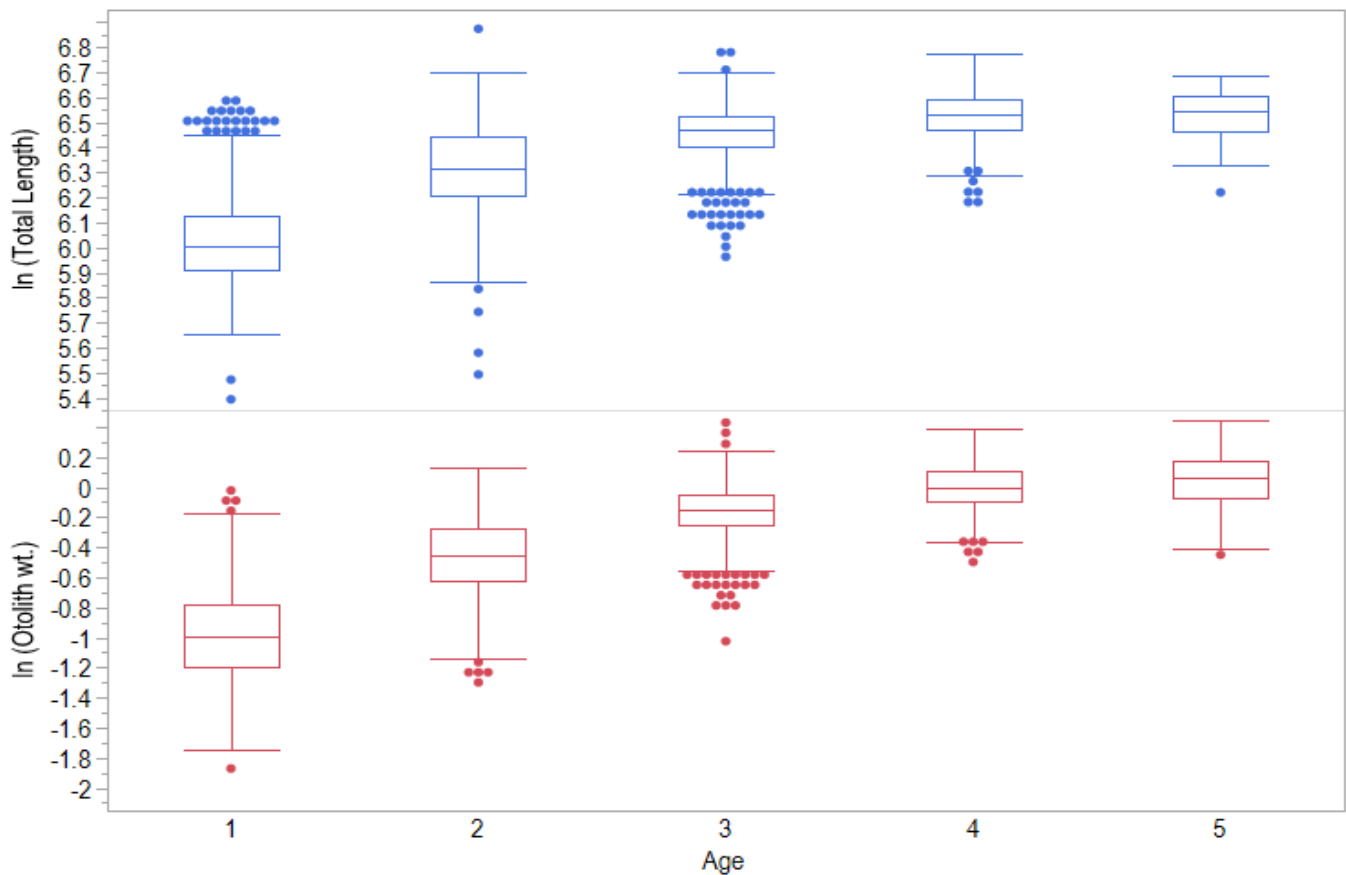


Figure 12.2.2 Age and growth of otolith weight (g) and total body length (mm) in Red Drum, ages 1-5 (TPWD unpublished data).

temperature of the environment the fish was subjected to. Strontium (Sr) and barium (Ba) are inversely proportional in the freshwater/saltwater systems (Figure 12.2.3). A switch in either isotope can indicate a departure or a return to a higher or lower salinity water body. In other words, higher Sr concentrations are typically incorporated into otoliths of fish living in higher salinity waters and higher Ba is found in the otoliths from fish living in freshwater environments (Campana 1999). Other isotopes can elucidate additional patterns that would normally be impossible to validate other than through tag and recapture studies or electronic tracking and monitoring.

The earliest use of otolith chemistry included the use of bomb radiocarbon for dating long lived otoliths for validation purposes. The concept is based on the natural uptake of carbon in all living things which were extant during period of above-ground nuclear tests which began in 1945 and continued through the 1950s and 1960s. Prior to the testing, ^{14}C (the radioactive form of ^{12}C) was limited in the environment. Immediately following the tests, the levels of measureable ^{14}C increased significantly and has had a continuous decay rate which can be used to validate the age estimates if very clean samples are taken from the otolith. Organisms that were alive through the testing period or born after, show a very specific bomb testing evidence along their tissues including otoliths and plotting the ratio of $^{14}\text{C}/^{12}\text{C}$ in carbon-based hardparts can pinpoint specific years along the life of that organism (Figure 12.2.4). Radiocarbon has been explored in fish, corals, and mollusks as well as marine and terrestrial mammals and trees. The rate of decay for ^{14}C is predictable and therefore relatively easy to match along the hardpart and uptake of environmental ^{14}C ceases at the point of death providing additional information as to when an organism died.

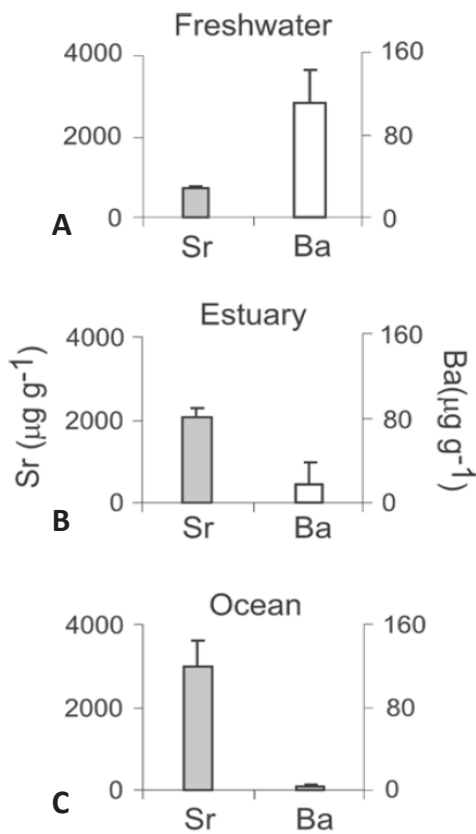


Figure 12.2.3 Reference strontium (Sr) and barium (Ba) mean concentrations of otoliths of *Micropogonias furnieri* calculated for A) freshwater and C) coastal environments and estimated for B) the Patos Lagoon Estuary, in southern Brazil (Fig. 5 from Albuquerque et al. 2010).

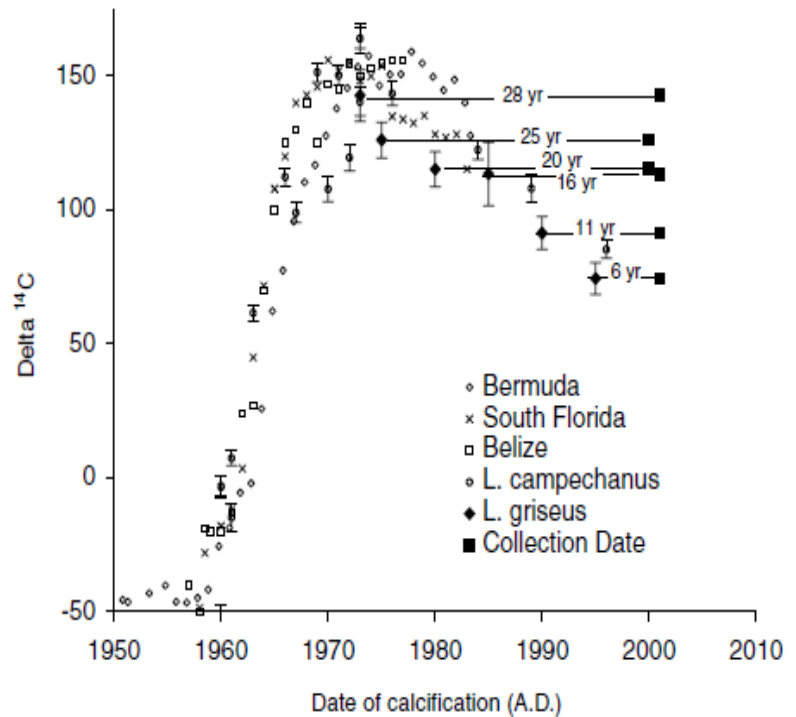


Figure 12.2.4 Plot of radiocarbon (^{14}C) values versus date of calcification for Gray Snapper (*Lutjanus griseus*) (Fischer et al. 2005) and Red Snapper (*Lutjanus campechanus*) (Baker and Wilson, 2001) from the northern Gulf of Mexico and from corals off Bermuda (Druffel, 1989), South Florida (1989), and Belize (Druffel, 1980). Solid squares (■) indicate collection dates for the Gray Snapper samples (n=6) and the ages listed are the estimated ages as read from the otolith sections. (Figure 3 from Fischer et al. 2005)

12.2.4.1 Micromilling

Jointly, otolith elemental and stable isotope signatures have been employed to examine fish environmental history, migrations, population connectivity, and the percentage of recruits sourced from different nursery habitats or regions (Patterson et al. 1998, Gillanders and Kingsford 2000, Rooker et al. 2001, Hanson et al. 2004, Patterson et al. 2004a and 2004b, Hamer et al. 2005).

To accomplish these various applications, otolith elemental and stable isotope composition can be assayed in one of two general ways: analysis of whole otoliths or cores (solution-based elemental analysis or pulverizing whole otoliths for isotope analysis) or microsampling some portion of an otolith (laser-ablation for elemental analysis or microsampling otolith powder from a given transect for isotope analysis). Either approach may be appropriate depending on the question that is being addressed. The chemical signature obtained from examining a whole otolith integrates the chemical signature over the entire life of the fish. Analysis of a mechanically extracted core or core material from an adult otolith would reveal the integrated otolith chemical signature imparted during the juvenile period.

Core extraction is required in this approach to assay an otolith's chemical signature that corresponds to the nursery period. A computer-driven micromill machine can be used to extract the core area from an adult otolith (Figure 12.2.5). The micromill moves in three dimensions (x, y, and z) and will allow multiple passes to be milled along a predetermined pattern to a predetermined depth as established by the operator. The first part of the milling process requires taking a transverse thin section (~ 1.5 mm thickness) that includes the otolith's core and then mechanically extracting only the nursery or juvenile material. Implicitly assumed is that reducing the otolith's core from a 3-dimensional to an essentially 2-dimensional structure does not affect the core's chemical signature. The extracted core is then cleaned and pulverized into a fine powder. One-half of the powder can be analyzed for stable isotopes and the other half of the powder can be dissolved and analyzed for trace elements. Analyzing the core from one otolith for both stable isotopes and trace elements will allow the other otolith to be used for ageing the fish.

Microsampling, whether via laser ablation or mechanical micromilling, differs from analysis of whole otoliths or extracted cores in that only a relatively narrow transect across an otolith is sampled. For microsampling the juvenile chemical signature contained within an adult fish's otolith core region, a transverse thin section (~ 1.5 mm thickness) is prepared first. Then, a laser is employed to ablate otolith material along a narrow (e.g., 50 μm wide) transect sampled across an adult otolith's core region, or a micromilling device is used to mechanically sample a trench across the core region. In either case, the first assumption made is that reducing the otolith's core from a 3-dimensional to an essentially 2-dimensional structure does not affect the core's chemical signature. But a further implicit assumption made is that the chemical signature assayed along the narrow (e.g., 50 μm) transect across the otolith thin section's core region reflects the chemical signature of the entire core region.

Laser ablation of a transect across the core area of a thin section prepared from an adult fish's otolith is the most commonly used method for microsampling the elemental composition of juvenile chemical

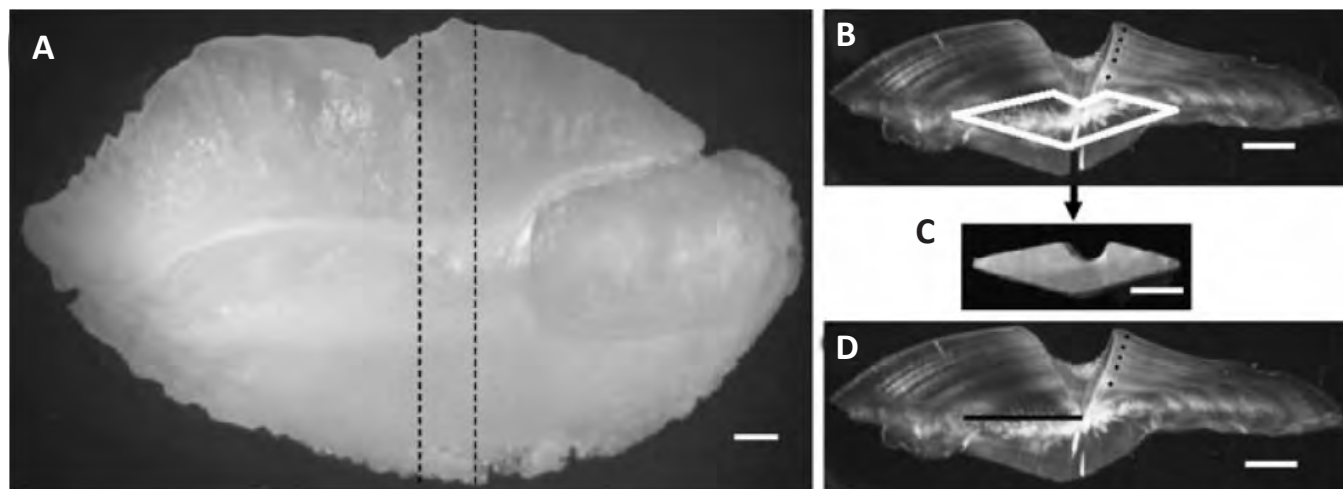


Figure 12.2.5 Digital images of whole Red Snapper sagittae, otolith sections, and extracted cores. All images are shown at a common scale; white bars on each image scale to 1 mm in length. A) is a left sagitta from a 563 mm total length Red Snapper, with dashed vertical lines indicating a 1.5 mm wide core section centered on the otolith's primordium. B) is of a transverse thin section extracted from otolith 1a, with annual opaque zones indicated with black dots and a white outline of a pattern used to extract the age-0 core (C) with a micromill. D) is the same thin section as (B) but depicting a hypothetical 50 μm wide \times 2.3 mm long path across the otolith section's core. Modified from Fig. 1 from Barnett and Patterson 2010.

signatures. This approach utilizes a high-powered laser to ablate the surface of an otolith, which results in vaporization of the material (Thomas 2004). The vaporized material then is analyzed with inductively coupled plasma mass spectrometry (ICP-MS) once inert Argon (Ar) gas sweeps the vaporized otolith material into the machine's plasma.

The other method for microsampling is performed by using the micromill. Using this method, the operator establishes a predetermined pattern that includes the number of passes and depth to be milled which will allow sample material to be milled as a powder which can then be analyzed for stable isotopes using an isotope ratio mass spectrometer.

12.2.5 Historic Ageing Structures

Historic (archeological) otoliths have been found in the remains of many previous human populations and historical fishing-based communities. The presence of pre-Cambrian otoliths in Native American middens along the Gulf Coast have provided baseline chemical signatures prior to the arrival of Europeans and the industrialization of the coast. These middens are essentially trash heaps left by ancient peoples which typically include fish and other wildlife bones, shells, and the material recovered can be dated reasonably well. Larger otoliths of the more common species utilized by the native people can be determined from digging into these middens and in many cases, the otoliths can be measured, aged, and even have useful chemical processing. Additional materials include fish bones and spines which can be identified and explain patterns in fishing effort, angler preferences, and general abundances of previous populations.

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12.0 Appendices

12.3 Vertebrae

12.3.1 Introduction

The vertebral column in fish is the central skeletal structure and is made up of vertebrae which house the spinal cord. An individual vertebra consists of a centrum, the round, central portion of the vertebra, a series of arches which extend from the top and bottom of the centrum, and several processes which originate from the centrum and/or arches (Figure 12.3.1). The arch at the top of the centrum is the neural arch. The arch underneath the centrum, when present, is the hemal arch.

In some fish that lack hard parts such as otoliths or scales useful for ageing (i.e., many cartilaginous fish), vertebral centra can be used to derive growth information (Cailliet 1990). Vertebrae have also been used in some bony fishes where other traditional ageing structures have proven less useful. Many researchers working with a variety of species have utilized centra and found regular growth patterns which have been assumed to be annual patterns (Mather and Schuck 1960, Van Utrecht and Schenkkan 1972, Caddy and Butler 1976, Armstrong et al. 1992, MacNeil and Campana 2002, Liu et al. 2009, Elzey et al. 2015). Each pair of wide/narrow bands is assumed to represent an annual growth cycle and the translucent bands are counted along the edge of the centrum as the annuli (Figure 12.3.2), but this assumption needs to be validated for all species, life stages, and geographic regions. It is generally hypothesized that broad, opaque bands are formed in the summer months and thin, translucent bands are formed in the winter months. It is important to keep in mind that width descriptors (wide vs. narrow) and optical descriptors (opaque vs. translucent) can vary depending on preparation techniques, viewing techniques, and species. Many chondrichthyan species have been shown to have a band closest to the center of the centrum which looks like an annulus but is actually formed during gestation (i.e. birth band; Figure 12.3.2) and should not be included in band counts. Bands near the edge, or margin, of the centrum can be notoriously difficult to discern, particularly if the edge is damaged during processing.

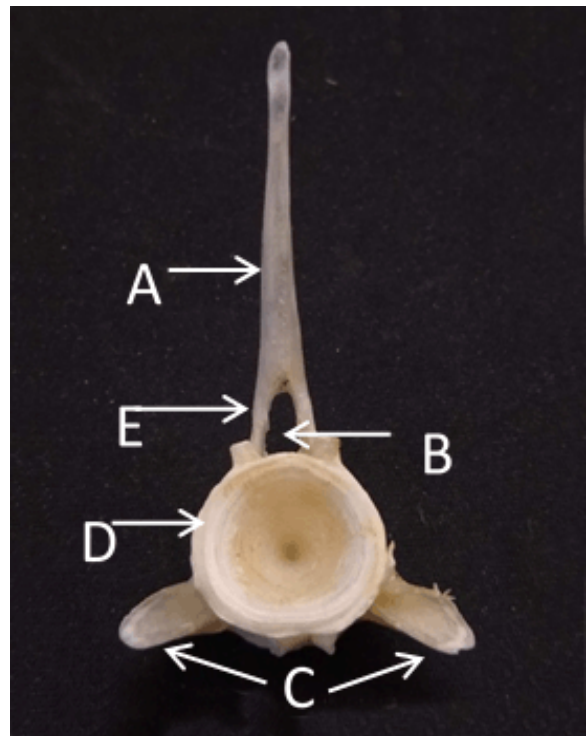


Figure 12.3.1 A Striped Bass (*Morone saxatilis*) precaudal vertebra located anterior to the vent. Features include the (A) neural spine, (B) neural canal, (C) parapophyses, (D) centrum, and (E) neural arch. Precaudal vertebrae lack a hemal arch.

Validation studies on ageing vertebrae have had mixed results, highlighting the need to validate assumed periodicity of growth patterns. Bomb radiocarbon dating has been used to validate vertebral-derived ages in several shark species (Campana et al. 2002, Ardizzone et al. 2006, Kneebone et al. 2008, Ong et al. 2020). Marginal increment analysis and edge analysis are other methods that have commonly been used to “semi-directly” validate, or verify, vertebral-derived ages (Cailliet et al. 2006, Joung et al. 2018, Liu et al. 2018). However, vertebrae have also been found to be unreliable for ageing some species (Lee et al. 1983, Natanson and Cailliet 1990, Bank 2016) and are no longer used for assessing Monkfish (*Lophius* spp.) due to inconsistent interpretation of presumed annuli near the centrum margins (Richards 2016). Further, recent work by Natanson et al. (2018) has questioned the use of vertebrae as an ageing structure for several shark species, hence the inclusion of vertebrae information as an appendix in this manual.

Natanson et al. (2018) found that centrum band formation is related to somatic growth and may only be loosely correlated with age. This relationship may explain the formation of more than one band pair annually in some young, fast growing animals (Wells et al. 2013) and result in fewer than one band pair annually in some older, slower growing animals. The authors note their findings do not necessarily repudiate past vertebrae validation studies, but do indicate greater uncertainty in the relationship between centrum band counts and age.

12.3.2 Preparing Vertebrae for Ageing

12.3.2.1 Vertebrae Removal

Vertebrae samples are removed in two ways depending on the intended use of the animal. If the external appearance of the fish is important, the vertebrae can be removed by cutting up through the body cavity (Figure 12.3.3). If appearance is not important, the vertebrae can be removed through the dorsal surface of the fish. Cuts are made through the dorsal surface and multiple vertebrae can be removed from a single location along the spinal column. Natanson et al. (2018) found that band pair counts can vary along the vertebral column due to a relationship between centrum size and body girth. This variation is species-specific and

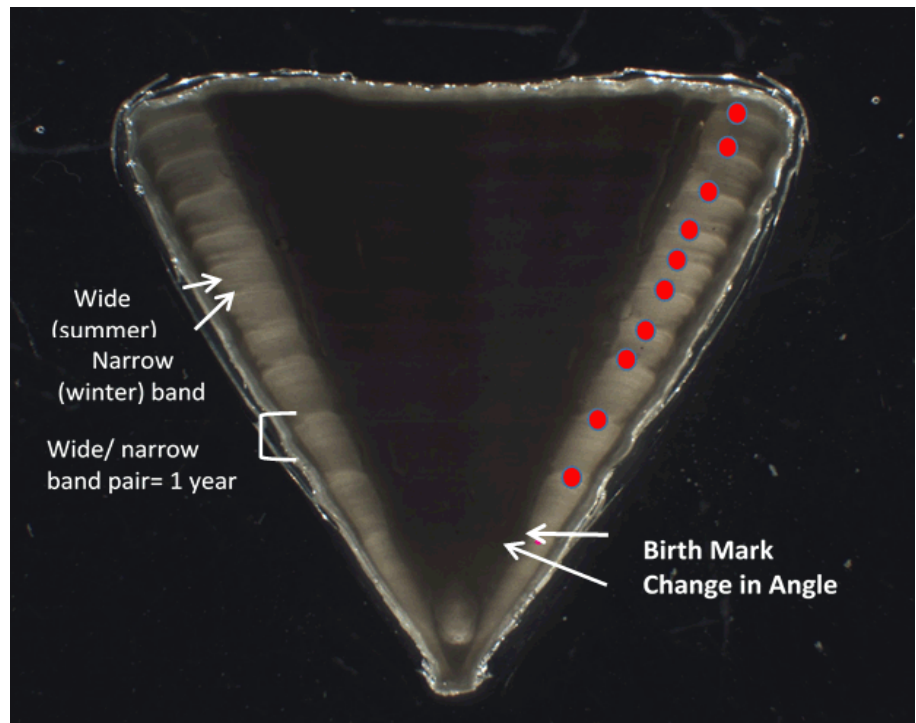


Figure 12.3.2 One half of an age-10 Lemon Shark (*Negaprion brevirostris*) centrum cross-section showing growth bands and 'birth band' under transmitted light.

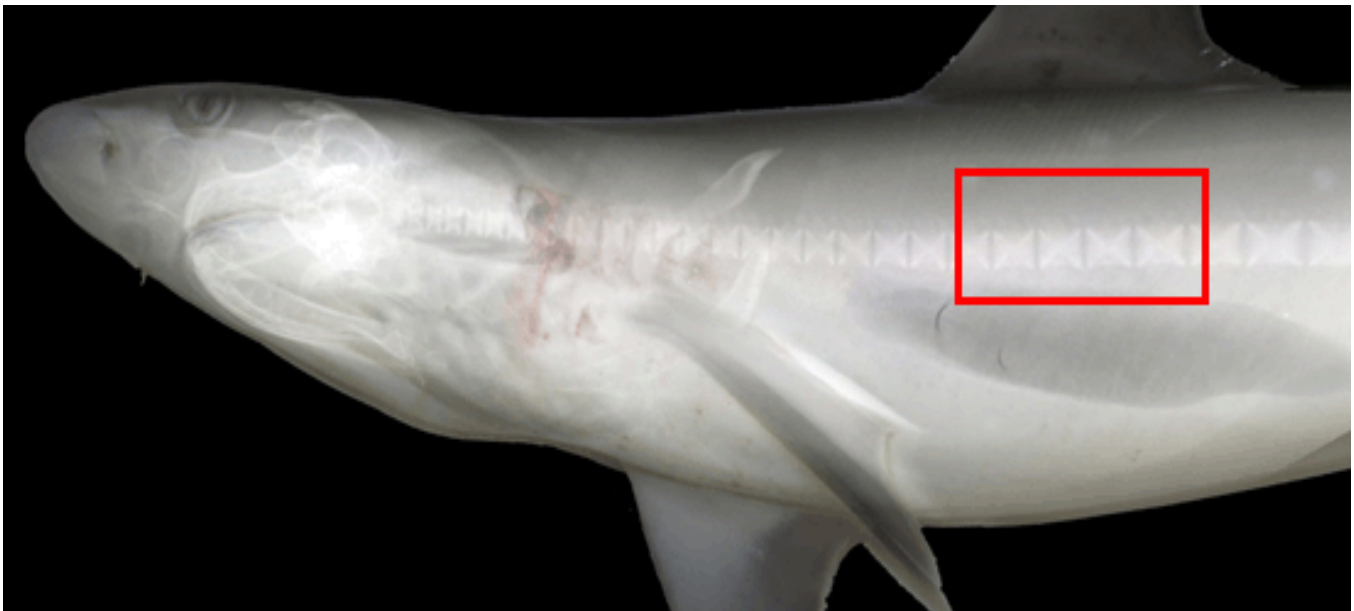


Figure 12.3.3 Common location to remove vertebrae, indicated by the red box, through the pleuroperitoneal cavity of an Atlantic Sharpnose (*Rhizoprionodon terraenovae*) directly below the first dorsal fin.

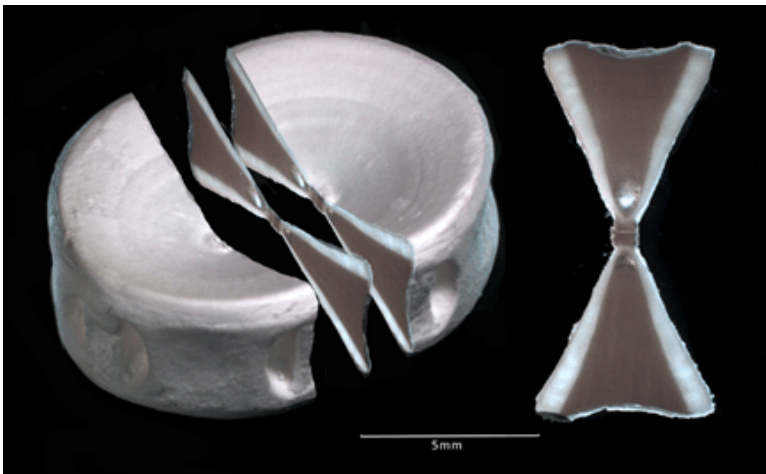


Figure 12.3.4 Three-dimensional rendering of a sectioned centrum from a Silky Shark (*Carcharhinus falciformis*) showing the orientation of the section to be used for ageing and where it is removed from the centrum.

should be considered when determining location(s) of vertebrae to be removed. However, between 5 and 10 vertebrae are often removed just below the dorsal fin (Figure 12.3.3). Once vertebrae are removed, excess tissue around the section should be cut away with a sharp knife.

12.3.2.2 Cleaning Vertebrae

Multiple species of fish have been aged using whole centra (Armstrong et al. 1992, MacNeil and Campana 2002, Elzey et al. 2015), but ageing centrum sections (Figure 12.3.4) is the more common ageing method. Techniques for cleaning vertebrae can vary slightly depending on whether they will be examined whole or sectioned. The first step in either method is to begin with fresh or fully thawed samples. Using a knife, carefully remove excess tissue from

around the spinal column so as not to damage the vertebrae. For vertebrae to be viewed whole, cut off the neural arch and spinal cord, as well as the hemal arches, or transverse processes, so that you are left with just the round centra (Figure 12.3.5). The individual centra can now be separated using a scalpel or knife. It is important to cut cleanly between the centra so as not to damage the edges. Carefully clean away any excess tissue from the edges. Alternatively, the vertebrae can be separated from each other prior to removing the arches and processes. The choice of whether or not to remove the arches is lab and species-specific.

Vertebrae to be sectioned can be cleaned as noted above; however there is some advantage to leaving the arches and processes attached for easier visualization of the correct sectioning plane later. Some labs have shown preference for not separating the vertebrae from each other prior to sectioning. This ensures that all sections are in the same orientation.



Figure 12.3.5 Once mostly clean, use a knife to separate into individual centra.

Care should be taken when cleaning to not cut into the centrum or scrape the edges. If too much of the edge is scraped away, growth bands along the edge may be damaged which can result in an inaccurate band count when ageing.

If further cleaning is required, centra can be immersed in 5% hypochlorite solution to help remove excess tissue (Figure 12.3.6). Immersion times vary by species so centra should be monitored closely to avoid degradation. Alternatively, a 3% solution of hydrogen peroxide has also been used to aid in tissue removal. The centra can be left soaking in hydrogen peroxide for 24 hours or more (Elzey et al. 2015).

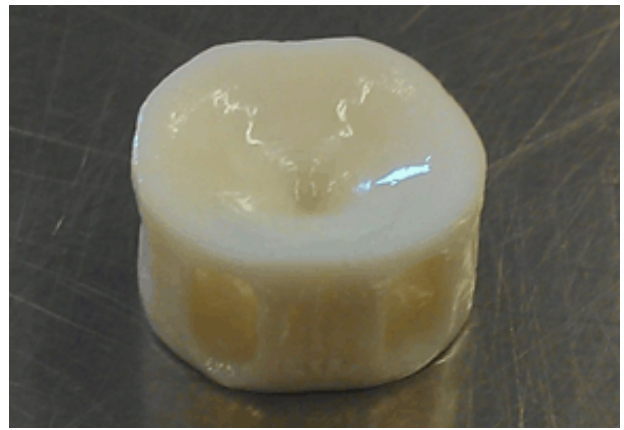


Figure 12.3.6 A properly cleaned centrum ready for ageing whole and/or sectioning.

12.3.2.3 Temporary Storage Prior to Examining or Sectioning

Once clean, centra can be stored prior to examining or sectioning. Storage technique is highly lab and species-specific. Freezing centra is an easy and effective method for most species. Many teleost samples can be dried without causing damage, but samples from chondrichthyan fishes often crack or warp when dried. To prevent damage, many of these samples are stored in 70% ethanol. However, some skate species have been aged from dry centra successfully (Sulikowski et al. 2005) so individual species and techniques should be evaluated prior to processing.

12.3.2.4 Examining Whole Vertebrae

Bands can be seen on the anterior and posterior conical surfaces of the centra (Figure 12.3.7). Several techniques can be used to enhance visualization of the bands including baking, staining, immersion in a fluid such as water or oil, and changing the viewing angle. As always, validation studies should be conducted for each species to ensure bands counted are in fact annuli.

12.3.2.5 Sectioning Vertebrae

Whether examining vertebrae from a teleost fish or a cartilaginous fish, the centrum of the vertebra is typically sectioned directly through the center, resulting in a 'bowtie'-thin section that is mounted on a glass slide and read using a microscope (Figure 12.3.2). Sectioning of vertebrae is typically performed on a low speed wafering saw. Many of the same techniques outlined in Chapter 3 for sectioning otoliths can be used for vertebrae as well. Although the optimal sectioning plane is subject to differ between species, the lateral plane (side to side) is often preferred (Figure 12.3.8). The thickness of the section is also species-specific. The resulting section should have a bowtie shape. The section can then be affixed to a slide or further processed. If multiple processing techniques are to be used, the halves of the bowtie can be separated and used differently (Figure 12.3.9).

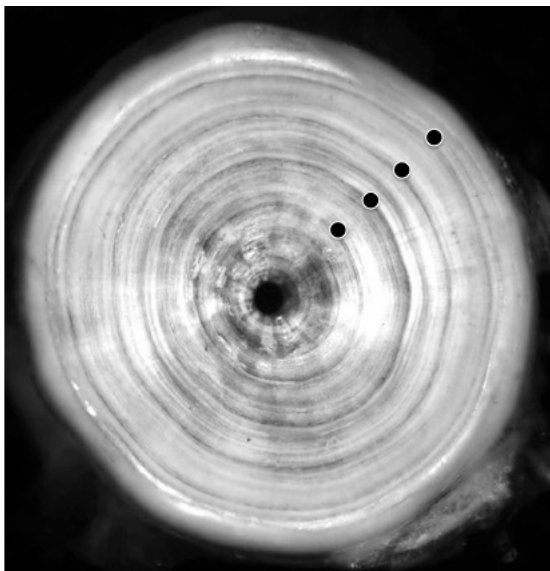


Figure 12.3.7 Whole centrum from an American Shad (*Alosa sapidissima*) with four annuli marked by black dots.

12.3.2.6 Examining Sectioned Vertebrae

Translucent bands are counted along the corpus calcareum, the translucent region on the edges of the centrum section, starting with the first translucent band past the birth band (Figure 12.3.3). Many of the enhancement techniques



Figure 12.3.8 The proper orientation for sectioning most vertebrae.

outlined in Chapter 7 can be used to help visualize the annual growth bands on centrum sections (Figure 12.3.8). The best enhancement technique will be species-dependent but success has been achieved by baking, staining, and histological techniques.

12.3.3 Long-Term Storage of Vertebrae

Dry teleost centra can be stored in containers indefinitely. Due to potential cracking, most chondrichthyan centra should be kept wet and can be stored frozen or in 70% ethanol. Once sectioned and mounted, centra can be handled similar to mounted otolith sections or scales. It is recommended to store mounted sections in slide boxes in a climate-controlled area. Evaluation of different mounting media should be considered as some may be more suitable for long-term storage than others.

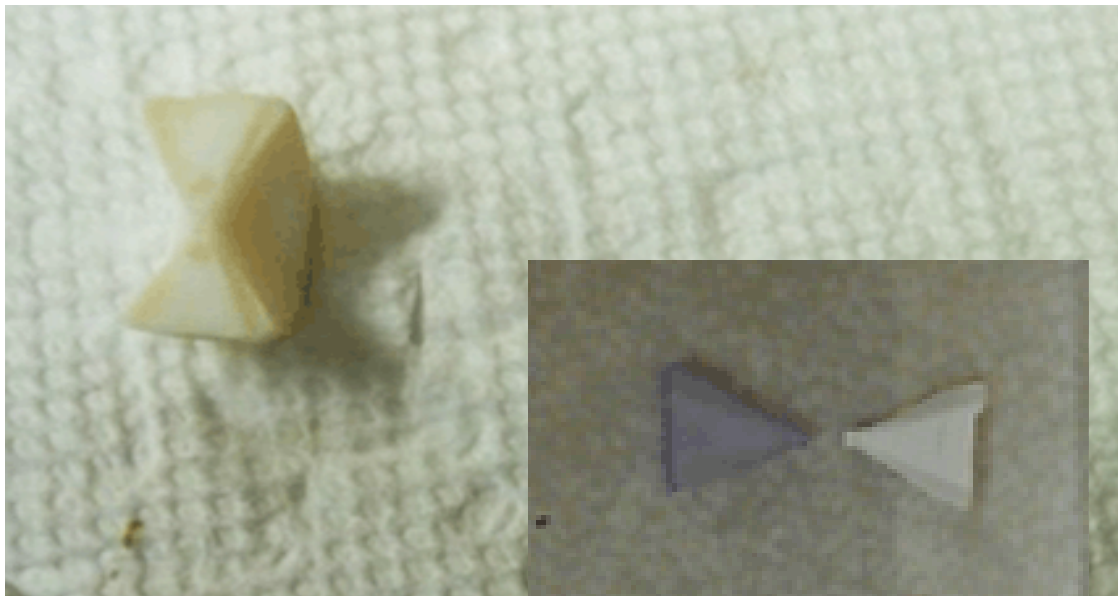


Figure 12.3.9 Bowtie from cross-section of a centrum. Bowtie can be separated if multiple post-sectioning processes need to be performed. Centrum sections may also be stained, as was done with crystal violet in this image.

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12.0 Appendices

12.4 High Speed Otolith Sectioning System (Benetec® L250)

The Benetec® L250 (Benetec® Ltd. United Kingdom) is a high speed, self-contained sectioning saw that allows for sectioning multiple otoliths at the same time (Figure 12.4.1). Otoliths are marked through the nucleus, aligned in rows, and embedded between layers of a resin mixture in aluminum trays. The resin composition is a mixture of both casting and flexible unsaturated polyester resins, a curing agent and black pigment dispersion. Most of the samples are aged using reflective light so the black pigment (carbon dust) added to the resin creates maximum contrast for the white otolith sections for easier annular interpretation. There are two blocks in a tray and up to six rows in a block. A video camera and monitor are used to aid in alignment of the otoliths as they are being set in the trays. Once the resin block has cured, it is placed in the cutting vice of the L250 and sectioned. For the Woods Hole lab, the species sectioned with this saw are Atlantic Cod (*Gadus morhua*), Haddock (*Melanogrammus aeglefinus*), Pollock (*Pollachius virens*), Bluefish (*Pomatomus saltatrix*), Silver Hake (*Merluccius bilinearis*), Scup (*Stenotomus chrysops*), American Plaice (*Hippoglossoides platessoides*), Winter Flounder (*Pseudopleuronectes americanus*), and Summer Flounder (*Paralichthys dentatus*).



Figure 12.4.1 Benetec® L250 high-speed saw. Sectioned blocks are rinsed with water and placed in protective trays until they are labeled and secured to a large slide from which they will be aged.

12.4.1 Marking Otoliths

Mark through the nucleus of the otolith on the proximal side with a thin line (Figure 12.4.2). This mark must be straight so it aligns to the grooves on the sides of the tray and the line on the monitor. Wrap the mark around to the distal side since this side will be face up when mounted. For longer otoliths, such as Cod, Haddock, Pollock, and Silver Hake, the posterior end needs to be clipped so it does not interfere with the otoliths in the preceding and subsequent rows.

Mark all of the otoliths in this fashion. The number of otoliths embedded in a block depends on the species and size of the otoliths. This results in a range from 40-100 otoliths per block. After all the otoliths are marked, they are placed in a cell tray in rows mimicking the order they will be embedded in the molds.

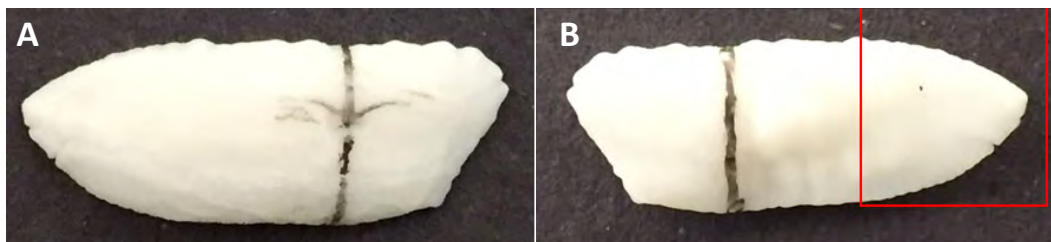


Figure 12.4.2 A) The nucleus is found on the otolith's proximal side and, using a pencil, marked with a straight line through the core. B) The line is wrapped around to the dorsal side since this side faces upward while being mounted. For longer otoliths, the posterior end (in the red box) needs to be clipped so it does not interfere with the alignment in the previous and subsequent rows.

12.4.2 Tray Preparation and Mounting

The entire inner surface area of the trays (two blocks per tray) are first coated with bowling wax then a thin layer of a mold release agent. Allow 20 minutes to dry, pour a thin layer of the resin mixture on the bottom, and let it harden overnight.

Place the tray, with a unique block identifier, in the viewing station vice underneath the video camera. Mix a small batch of the resin mixture to act as the 'glue' to hold the otoliths in place while they are being mounted. Pour a very thin layer of 'glue' over the bottom of the block. Note, too much may cause the otoliths to drift, especially the small ones. It is important to always check the alignment of the otoliths even after they have been mounted (Figure 12.4.3).

Align the groove on the side of the tray to the line on the screen. Place the otoliths on the block so the nucleus mark is underneath the line on the screen. Each tray groove indicates where the row is on the block.

Allow the glue to harden the otoliths in place for least one hour. Make sure to periodically check and adjust any otoliths that have drifted.

Once the otoliths are set, make a larger resin mixture and carefully pour over the set otoliths. The resin needs to cure overnight and is ready to be processed the following day.

12.4.3 Sectioning the Blocks

Once the resin has hardened, the molds need to be scored before disassembling. A scoring tool is used in the grooves to mark where the nuclei are mounted. Only then are the blocks removed from the trays. The blocks are secured in the saw vice of the L250 and are ready to be sectioned (Figure 12.4.4).

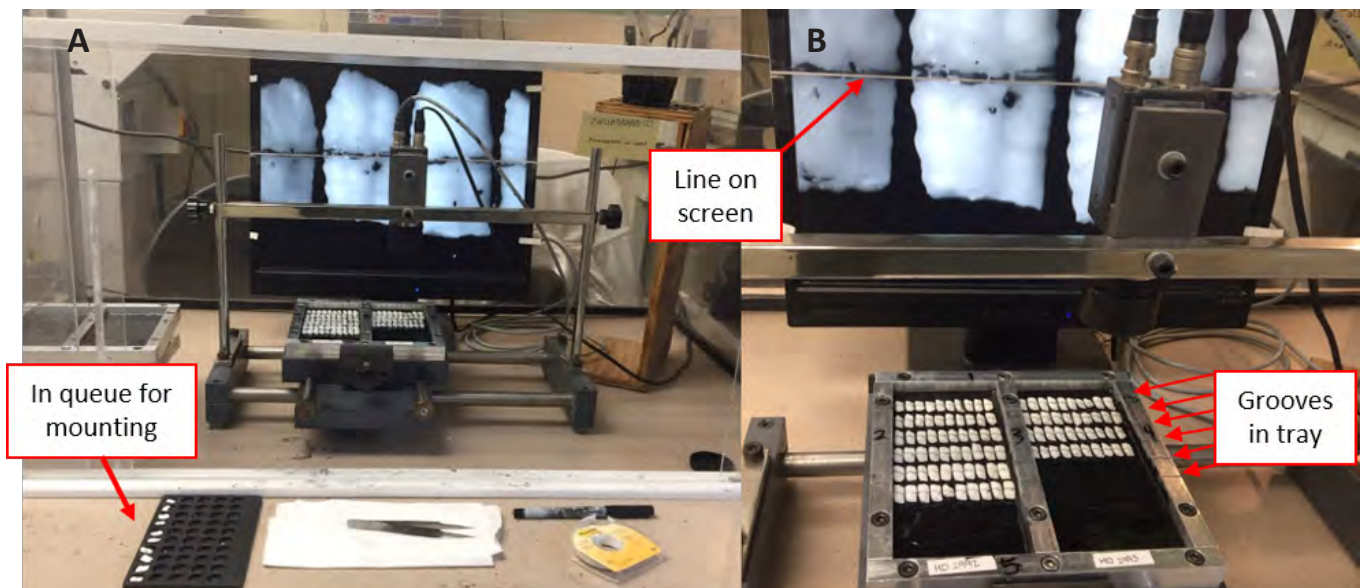


Figure 12.4.3 A) Otoliths are lined up in a row and ready to be mounted on the tray which is secured in a vice underneath a video camera. Each block receives a unique label which stays with the block during all stages of mounting, sectioning and labeling. B) There are six grooves on each of the three vertical sides of the tray. These grooves are aligned to the line on the screen, and the otoliths are mounted so the nucleus line matches that on the screen. Once the 'glue' has set the otoliths in place, they are covered with more resin and left to cure overnight.

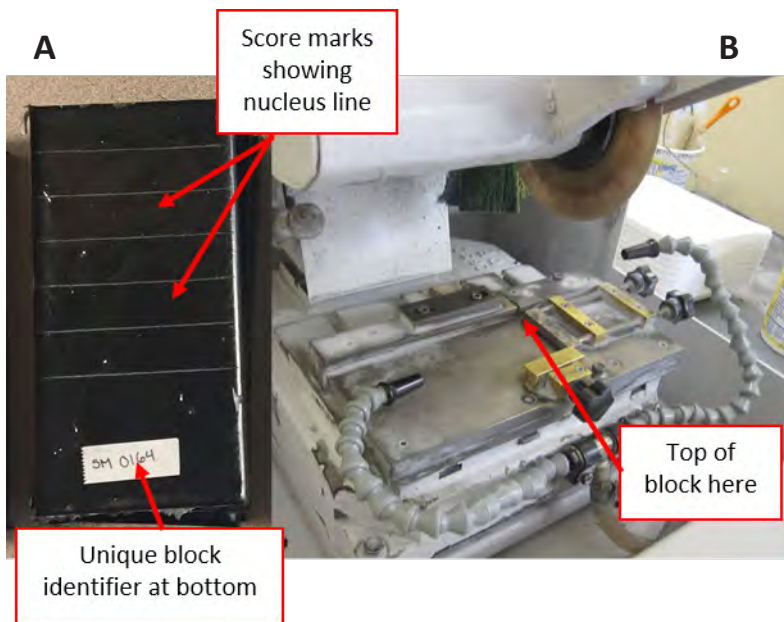


Figure 12.4.4 A) Once the blocks are cured, a scoring tool is placed in the tray grooves, and a line, corresponding to the nuclei embedded below, is etched onto the block. The unique block label can be seen at the bottom of the block. B) When ready to section, the block is secured on the cutting platform and is sectioned from top to bottom.

Once the block is secured in the saw vice, the coolant is turned on and the first cut is made. There will be one thin strip of otolith sections per row. The first cut through the block is just above the first score mark and results in a thick section that is kept. The spacer is put into place where the end piece was removed and the block is pushed flush against the spacer and secured in place. The second cut is just below the first score mark and results in a thin strip of sectioned otoliths for the first row (Figure 12.4.5).

The cuts alternate from thick to thin sections until all six rows of the block are sectioned. A block with six rows of otoliths requires thirteen cuts to produce six thin sections and seven corresponding thick sections. Each section is wiped clean as it is removed from the saw to remove any coolant before it is labeled and mounted on a large slide (Figure 12.4.6).

12.4.4 Mounting Thin Sections

The thin and thick sections are labeled with white-out tape. All the thin sections from the same block are then mounted on a single large slide using a crafting spray glue. The large slide is labeled and a thin layer of glue is sprayed over the surface. The strips are pressed down onto the slide and its appropriate barcode is placed on the bottom. The thick sections are bagged and kept with the large slides (Figure 12.4.6). Once the spray glue is dried, the sections are ready to be aged.

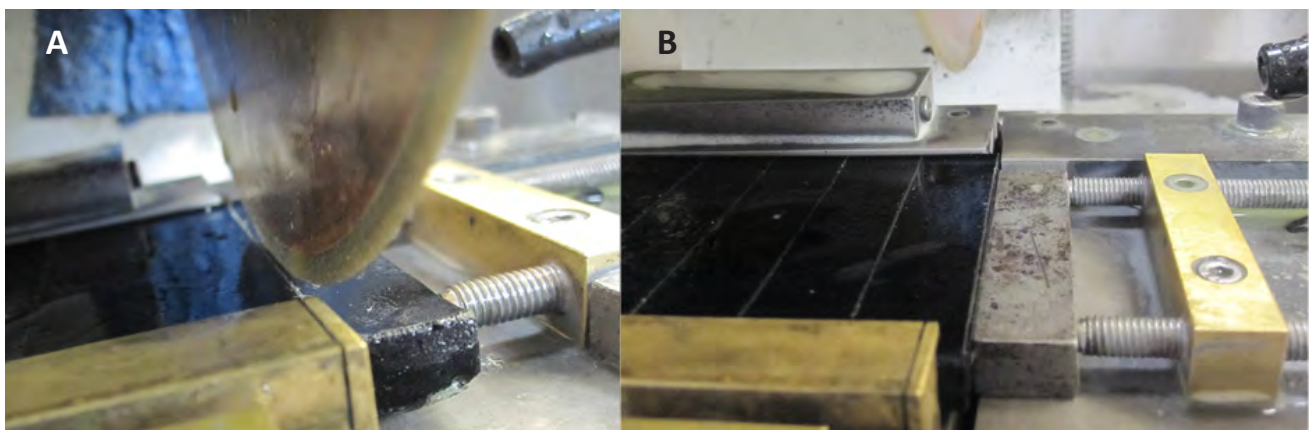


Figure 12.4.5 A) The first cut is made right above the top score mark and produces a thick section. This will be the secondary view, if needed, for ageing. B) To make the thin section, the spacer is then placed between the block and adjusting screws, and the cut will land just below the score mark. This 0.5mm section will be the primary view used for ageing. The process repeats with the third cut right above the second score mark and the fourth cut with the spacer between the block and adjusting screws.

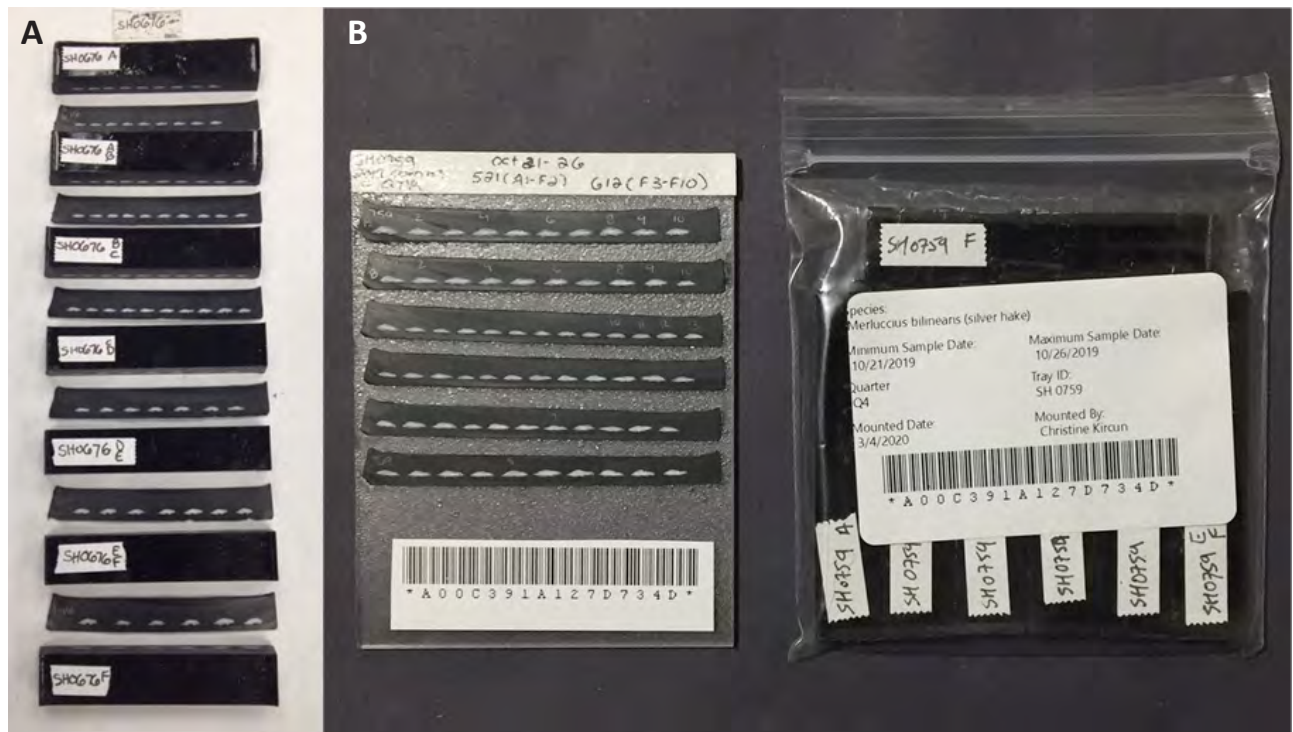


Figure 12.4.6 A) This block is completely sectioned and labeled. The rows are identified using the letters A-F. Each row of sectioned otoliths has three views for ageing: the thin section (primary) and the thick sections (secondary) directly before and after. B) The thin sections are glued onto a larger labeled tray. Majority of the samples come to the Woods Hole lab already barcoded. This requires both the thin and thick sections to be labeled with their unique barcode identification.

12.0 Appendices

12.5 Production Ageing

Species under production ageing protocols by various agencies and labs. Methodology utilized in each species include SC = scales, OT = otoliths, WOT = whole otoliths, OP = opercula, SN = spines, RY = fin rays, and VRT = vertebrae. Video or website assistance for specific methods is provided where applicable.

| Species | Common Name | ITIS-code | Agency/Lab | Methodology | Website Support |
|------------------------------------|-------------------|-----------|---|-------------|---|
| <i>Albula vulpes</i> | Bonefish | 161121 | FLFWC | OT | FWC Ageing Website |
| <i>Alosa aestivalis</i> | Blueback Herring | 161703 | MADMF, NJDFW | WOT, SC | |
| | | | SCDNR, MDDNR, CTDEEP | SC | |
| <i>Alosa pseudoharengus</i> | Alewife | 161706 | MADMF, NJDFW | WOT | |
| | | | CTDEEP, MADMF | SC | |
| <i>Alosa sapidissima</i> | Shad | 161702 | CTDEEP, MADMF, NCDMF, SCDNR, MDDNR, NJDFW | SC | |
| | | | MADMF | WOT | |
| <i>Anguilla rostrata</i> | American Eel | 161127 | FLFWC, SCDNR, MDDNR, NJDFW, LDWF | OT | FWC Ageing Website |
| <i>Archosargus probatocephalus</i> | Sheepshead | 169189 | FLFWC, SCDNR, LDWF, AMRD, VMRC, USM/GCRL | OT | OT Preparation |
| | | | | | CQFE/ODU |
| | | | | | FWC Ageing Website |
| <i>Balistes caprisus</i> | Gray Triggerfish | 173138 | FLFWC, SCDNR, LDWF, AMRD | SN | FWC Ageing Website |
| <i>Brevoortia patronus</i> | Gulf Menhaden | 161734 | USM/GCRL, AMRD | SC, WOT | |
| <i>Brevoortia tyrannus</i> | Atlantic Menhaden | 161732 | CTDEEP, VIMS, RIDEM, NCDMF, MDDNR, NJDFW | SC | |
| | | | VIMS, RIDEM | OT | VIMS Multispecies Research Group Age and Growth |
| | | | VIMS | WOT | |
| <i>Caulolatilus microps</i> | Blueline Tilefish | 168543 | SCDNR | OT | |
| <i>Centropomus undecimalis</i> | Common Snook | 167648 | FLFWC | OT | FWC Ageing Website |

| Species | Common Name | ITIS-code | Agency/Lab | Methodology | Website Support |
|-------------------------------------|---------------------|-----------|--|-------------|---|
| <i>Centropristis striata</i> | Black Sea Bass | 167687 | FLFWC, SCDNR, VIMS, MADMF, RIDEM | OT | VIMS Multispecies Research Group Age and Growth FWC Ageing Website |
| | | | MADMF, NEFSC, SCDNR, NJDFW | WOT | http://www.nefsc.noaa.gov/fbp/age-man.html |
| | | | MADMF, NEFSC, RIDEM | SC | http://www.nefsc.noaa.gov/fbp/age-man.html |
| <i>Chaetodipterus faber</i> | Atlantic Spadefish | 169539 | VMRC | OT | OT Preparation |
| <i>Clupea harengus</i> | Atlantic Herring | 161722 | NEFSC | WOT | http://www.nefsc.noaa.gov/fbp/age-man.html |
| <i>Cynoscion arenarius</i> | Sand seatrout | 169243 | AMRD, USM/GCRL | OT | |
| <i>Cynoscion nebulosus</i> | Spotted Seatrout | 169239 | FLFWC, SCDNR, LDWF, GADNR, AMRD, VMRC, NCDMF, USM/GCRL | OT | OT Preparation |
| | | | | | FWC Ageing Website |
| <i>Cynoscion regalis</i> | Weakfish | 169241 | CTDEEP, VMRC, RIDEM, NCDMF, VIMS, SCDNR, MDDNR, NJDFW | OT | VIMS Multispecies Research Group Age and Growth OT Preparation |
| | | | CTDEEP, VMRC | SC | |
| <i>Epinephelus itajara</i> | Goliath Grouper | 167695 | FLFWC | OT | FWC Ageing Website |
| <i>Epinephelus morio</i> | Red Grouper | 167702 | FLFWC, SCDNR | OT | FWC Ageing Website |
| | | | FLFWC | SN | FWC Ageing Website |
| <i>Gadus morhua</i> | Atlantic Cod | 164712 | NEFSC | OT | http://www.nefsc.noaa.gov/fbp/age-man.html |
| <i>Glyptocephalus cynoglossus</i> | Witch Flounder | 172873 | NEFSC | OT | http://www.nefsc.noaa.gov/fbp/age-man.html |
| <i>Haemulon plumieri</i> | White Grunt | 613026 | SCDNR | OT | |
| <i>Heliocolenus dactylopterus</i> | Blackbelly Rosefish | 166787 | SCDNR | OT | |
| <i>Hippoglossoides platessoides</i> | American Plaice | 172877 | NEFSC | OT | http://www.nefsc.noaa.gov/fbp/age-man.html |
| <i>HyporthVMRCs niveatus</i> | Snowy Grouper | 167705 | SCDNR | OT | |
| <i>Lachnolaimus maximus</i> | Hogfish | 170566 | FLFWC, SCDNR | OT | FWC Ageing Website |
| <i>Leiostomus xanthurus</i> | Spot | 169267 | VIMS, VMRC, NCDMF, SCDNR, MDDNR | OT | OT Preparation |
| <i>Limanda ferruginea</i> | Yellowtail Flounder | 172909 | NEFSC | SC | http://www.nefsc.noaa.gov/fbp/age-man.html |

| Species | Common Name | ITIS-code | Agency/Lab | Methodology | Website Support |
|--------------------------------------|-------------------|-----------|--|-------------|---|
| <i>Lopholatilus chamaeleonticeps</i> | Golden Tilefish | 168546 | NEFSC, SCDNR | OT | |
| <i>Lutjanus analis</i> | Mutton Snapper | 168849 | FLFWC, SCDNR | OT | FWC Ageing Website |
| | | | FLFWC | SN | FWC Ageing Website |
| <i>Lutjanus campechanus</i> | Red Snapper | 168853 | FLFWC, SCDNR, LDWF, AMRD, USM/GCRL | OT | FWC Ageing Website |
| | | | FLFWC | SN | FWC Ageing Website |
| <i>Lutjanus griseus</i> | Gray Snapper | 168848 | FLFWC, SCDNR, LDWF, AMRD | OT | FWC Ageing Website |
| | | | FLFWC | SN | FWC Ageing Website |
| <i>Lutjanus synagris</i> | Lane Snapper | 168860 | FLFWC, SCDNR, AMRD, USM/GCRL | OT | FWC Ageing Website |
| | | | FLFWC | SN | FWC Ageing Website |
| <i>Melanogrammus aeglefinus</i> | Haddock | 164744 | NEFSC | OT | http://www.nefsc.noaa.gov/fbp/age-man.html |
| <i>Menticirrhus americanus</i> | Southern Kingfish | 169274 | AMRD, SCDNR, USM/GCRL | OT | |
| <i>Menticirrhus littoralis</i> | Gulf Kingfish | 169275 | AMRD | OT | |
| <i>Merluccius bilinearis</i> | Silver Hake | 164791 | NEFSC | OT | http://www.nefsc.noaa.gov/fbp/age-man.html |
| <i>Micropogonias undulatus</i> | Atlantic Croaker | 169283 | VIMS, AMRD, VMRC, NCDMF, SCDNR, MDDNR, NJDFW, USM/GCRL | OT | VIMS Multispecies Research Group Age and Growth |
| | | | CTDEEP | SC | OT Preparation |
| <i>Morone americana</i> | White Perch | 167678 | VIMS, MDDNR | OT | VIMS Multispecies Research Group Age and Growth |
| | | | VIMS, MADMF, VMRC, RIDEM, NCDMF, NJDFW | OT | VIMS Multispecies Research Group Age and Growth |
| <i>Morone saxatilis</i> | Striped Bass | 167680 | MADMF, VMRC, RIDEM, MDDNR | SC | SC Preparation |
| | | | FLFWC, LDWF, AMRD, SCDNR, USM/GCRL | OT | OT Preparation |
| <i>Mugil cephalus</i> | Striped Mullet | 170335 | FLFWC, LDWF, AMRD, SCDNR, USM/GCRL | OT | FWC Ageing Website |
| <i>Mugil curema</i> | White mullet | 170336 | AMRD | OT | |
| <i>Mycteroperca bonaci</i> | Black Grouper | 167760 | FLFWC, SCDNR | OT | FWC Ageing Website |
| | | | FLFWC | SN | FWC Ageing Website |
| <i>Mycteroperca microlepis</i> | Gag Grouper | 167759 | FLFWC, SCDNR, AMRD | OT | FWC Ageing Website |
| | | | FLFWC | SN | FWC Ageing Website |

| Species | Common Name | ITIS-code | Agency/Lab | Methodology | Website Support |
|--------------------------------------|--------------------|-----------|---|-------------|---|
| <i>Mycteroperca phenax</i> | Scamp | 167763 | FLFWC, SCDNR | OT | FWC Ageing Website |
| | | | FLFWC | SN | FWC Ageing Website |
| <i>Ocyurus chrysurus</i> | Yellowtail Snapper | 168907 | FLFWC | OT, SN | FWC Ageing Website |
| <i>Osmerus mordax</i> | Rainbow Smelt | 162041 | MADMF | SC | |
| <i>Pagrus pagrus</i> | Red Porgy | 169207 | FLFWC, SCDNR | OT | FWC Ageing Website |
| <i>Paralichthys albigutta</i> | Gulf Flounder | 172736 | AMRD | OT | |
| <i>Paralichthys dentatus</i> | Summer Flounder | 172735 | VIMS, NEFSC, VMRC, RIDEM, NCDMF, NJDFW | OT | VIMS Multispecies Research Group Age and Growth |
| | | | | | http://www.nefsc.noaa.gov/fbp/age-man.html |
| | | | CTDEEP, NEFSC, VMRC, RIDEM | SC | http://www.nefsc.noaa.gov/fbp/age-man.html |
| | | | | | SC Preparation |
| <i>Paralichthys lethostigma</i> | Southern Flounder | 172738 | FLFWC, LDWF, AMRD, SCDNR, USM/GCRL | OT | FWC Ageing Website |
| | | | | | |
| <i>Peprilus triacanthus</i> | Butterfish | 172567 | VIMS, NEFSC | WOT | VIMS Multispecies Research Group Age and Growth |
| | | | | | http://www.nefsc.noaa.gov/fbp/age-man.html |
| <i>Pogonias cromis</i> | Black Drum | 169288 | FLFWC, LDWF, VIMS, AMRD, VMRC, NCDMF, SCDNR, USM/GCRL | OT | OT Preparation |
| | | | | | FWC Ageing Website |
| <i>Pollachius virens</i> | Pollock | 164727 | NEFSC | OT | http://www.nefsc.noaa.gov/fbp/age-man.html |
| <i>Pomatomus saltatrix</i> | Bluefish | 168559 | FLFWC, CTDEEP, VIMS, MADMF, NEFSC, VMRC, RIDEM, NCDMF, SCDNR, NJDFW | OT | VIMS Multispecies Research Group Age and Growth |
| | | | | | OT Preparation |
| | | | CTDEEP, NEFSC | SC | FWC Ageing Website |
| <i>Pseudopleuronectes americanus</i> | Winter Flounder | 172905 | CTDEEP, VIMS, MADMF, NEFSC, RIDEM, NJDFW | OT | VIMS Multispecies Research Group Age and Growth |
| | | | CTDEEP, MADMF, NJDFW | WOT | |
| | | | NEFSC, RIDEM | SC | |

| Species | Common Name | ITIS-code | Agency/Lab | Methodology | Website Support |
|--------------------------------|-------------------|-----------|--|-------------|--|
| <i>Rachycentron canadum</i> | Cobia | 168566 | VMRC, SCDNR, LDWF | OT | OT Preparation |
| <i>Rhomboplites aurorubens</i> | Vermilion Snapper | 168909 | FLFWC, SCDNR, LDWF, AMRD, USM/GCRL | OT | FWC Ageing Website |
| | | | FLFWC | SN | FWC Ageing Website |
| <i>Sciaenops ocellatus</i> | Red Drum | 169290 | FLFWC, SCDNR, LDWF, GADNR, VIMS, AMRD, VMRC, NCDMF, USM/GCRL | OT | OT Preparation FWC Ageing Website |
| | | | FLFWC | SN | FWC Ageing Website |
| <i>Scomber scombrus</i> | Atlantic Mackerel | 172414 | NEFSC | WOT | http://www.nefsc.noaa.gov/fbp/age-man.html |
| <i>Scomberomorus cavalla</i> | King Mackerel | 172435 | FLFWC, SCDNR, LDWF, AMRD, NCDMF | OT | FWC Ageing Website |
| <i>Scomberomorus maculatus</i> | Spanish Mackerel | 172436 | AMRD, VMRC, NCDMF, SCDNR | OT | OT Preparation |
| <i>Scophthalmus aquosus</i> | Windowpane | 172746 | NEFSC, VIMS | OT | VIMS Multispecies Research Group Age and Growth |
| <i>Sebastes fasciatus</i> | Acadian Redfish | 166774 | NEFSC | OT | http://www.nefsc.noaa.gov/fbp/age-man.html |
| <i>Seriola dumerili</i> | Greater Amberjack | 168689 | FLFWC, SCDNR, LDWF | OT | FWC Ageing Website |
| <i>Stenotomus chrysops</i> | Scup | 169182 | VIMS, NEFSC, RIDEM | OT | VIMS Multispecies Research Group Age and Growth http://www.nefsc.noaa.gov/fbp/age-man.html |
| | | | CTDEEP, MAMDF, NEFSC, RIDEM | SC | http://www.nefsc.noaa.gov/fbp/age-man.html |
| <i>Tautoga onitis</i> | Tautog | 170479 | CTDEEP, MADMF, VMRC, RIDEM, VIMS | OT | OT Preparation |
| | | | CTDEEP, MADMF, VMRC, RIDEM, VIMS, MDDNR, NJDFW | OPC | OPC Preparation |
| | | | MADMF, VIMS | SN | |
| <i>Urophycis chuss</i> | Red Hake | 164730 | NEFSC | OT | http://www.nefsc.noaa.gov/fbp/age-man.html |
| <i>Urophycis tenuis</i> | White Hake | 164732 | NEFSC | OT | |

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12.0 Appendices

12.6 Nonproduction Ageing

Species which have been aged by various agencies and labs, their numbers (when available), and the methodologies used. Methodology utilized in each species include SC = scales, OT = otoliths, WOT = whole otoliths, OP = opercula, SN = spines, RY = fin rays, and VRT = vertebrae. More information on these individual species can be found by contacting the agency or lab indicated.

| Species | Common Name | ITIS | Total Aged | Agency/Lab | Methodology |
|------------------------------------|---------------------|--------|------------|---------------|-------------|
| <i>Acanthocybium solandri</i> | Wahoo | 172451 | | FLFWC, LDWF | |
| <i>Acipenser oxyrinchus</i> | Atlantic Sturgeon | 553269 | | SCDNR, CTDEEP | 2nd RY |
| <i>Acipenser brevirostrum</i> | Shortnose Sturgeon | 161069 | | SCDNR, CTDEEP | 2nd RY |
| <i>Alectis ciliaris</i> | African Pompano | 168602 | | FLFWC | |
| <i>Alosa aestivalis</i> | Blueback Herring | 161703 | 2,174 | VIMS, MDDNR | WOT |
| <i>Alosa pseudoharengus</i> | Alewife | 161706 | 2,832 | VIMS, MDDNR | WOT |
| <i>Alosa sapidissima</i> | American Shad | 161702 | 2,442 | VIMS | WOT |
| <i>Aluterus monoceros</i> | Unicorn Filefish | 173133 | | FLFWC | |
| <i>Ameiurus catus</i> | White Catfish | 164037 | | FLFWC | |
| <i>Anarhichas lupus</i> | Atlantic Wolffish | 171341 | | MADMF | |
| <i>Anchoa mitchilli</i> | Bay Anchovy | 161839 | 200 | GCRL | OT |
| <i>Ancylopsetta quadrocellata</i> | Ocellated Flounder | 172757 | | FLFWC | |
| <i>Anisotremus virginicus</i> | Porkfish | 169086 | | FLFWC | |
| <i>Archosargus probatocephalus</i> | Sheepshead | 169189 | 1,461 | VIMS | OT |
| | | | | GADNR | |
| <i>Ariopsis felis</i> | Hardhead Catfish | 680665 | | SCDNR | OT |
| <i>Bagre marinus</i> | Gafftopsail Catfish | 164159 | | FLFWC | |
| <i>Bairdiella chrysoura</i> | Silver Perch | 169259 | 406 | VIMS | OT |
| <i>Bairdiella chrysoura</i> | Silver Perch | 169259 | | FLFWC | |
| <i>Balistes caprisicus</i> | Gray Triggerfish | 173138 | | MSDMR | SP |
| <i>Brevoortia patronus</i> | Gulf Menhaden | 161734 | | MSDMR | SC |
| <i>Brevoortia tyrannus</i> | Atlantic Menhaden | 161732 | | MDDNR | WOT |
| <i>Brotula barbata</i> | Bearded Brotula | 164818 | | FLFWC | |
| <i>Calamus arctifrons</i> | Grass Porgy | 169196 | | FLFWC | |
| <i>Calamus bajonado</i> | Jolthead Porgy | 169197 | | FLFWC | |
| <i>Calamus nodosus</i> | Knobbed Porgy | 169201 | | FLFWC | |
| <i>Calamus proridens</i> | Littlehead Porgy | 169203 | | FLFWC | |
| <i>Calmus penna</i> | Sheepshead Porgy | 169205 | | FLFWC | |
| <i>Caranx crysos</i> | Blue Runner | 168612 | | FLFWC | |
| <i>Caranx hippos</i> | Crevalle Jack | 168609 | | FLFWC | |

| Species | Common Name | ITIS | Total Aged | Agency/Lab | Methodology |
|-----------------------------------|----------------------|--------|------------|--------------|-------------|
| <i>Caranx latus</i> | Horse-eye Jack | 168610 | | FLFWC | |
| <i>Carcharhinus isodon</i> | Finetooth Shark | 160409 | 776 | GCRL | VRT |
| <i>Caulolatilus cyanops</i> | Blackline Tilefish | 168541 | | FLFWC | |
| <i>Caulolatilus microps</i> | Blueline Tilefish | 168543 | | FLFWC, VMRC | OT |
| <i>Centropomus ensiferus</i> | Swordspine Snook | 167645 | | FLFWC | |
| <i>Centropomus mexicanus</i> | Largescale Fat Snook | 553302 | | FLFWC | |
| <i>Centropomus parallelus</i> | Fat Snook | 167646 | | FLFWC | |
| <i>Centropomus pectinatus</i> | Tarpon Snook | 167647 | | FLFWC | |
| <i>Centropomus viridis</i> | White Snook | 645617 | | FLFWC | |
| <i>Centropristis ocyurus</i> | Bank Sea Bass | 167690 | | FLFWC, SCDNR | OT |
| <i>Centropristis philadelphia</i> | Rock Sea Bass | 167691 | | FLFWC | |
| <i>Cephalopholis cruentata</i> | Graysby | 181220 | | FLFWC | |
| <i>Chaetodipterus faber</i> | Atlantic Spadefish | 169539 | | FLFWC | |
| <i>Clupea harengus</i> | Atlantic Herring | 161722 | 947 | VIMS | OT |
| <i>Coryphaena hippurus</i> | Dolphin | 168791 | | FLFWC | |
| <i>Cynoscion arenarius</i> | Sand Seatrout | 169243 | | FLFWC, MSDMR | OT |
| <i>Cynoscion nebulosus</i> | Spotted Seatrout | 169239 | 69 | VIMS | OT |
| <i>Cynoscion nothus</i> | Silver Seatrout | 169240 | | FLFWC | |
| <i>Cynoscion regalis</i> | Weakfish | 169241 | | FLFWC, GADNR | |
| <i>Cynoscion spp.</i> | Seatrout sp. | 169238 | | FLFWC | |
| <i>Dasyatis americana</i> | Southern Stingray | 160951 | 178 | VIMS | VRT |
| <i>Dasyatis centroura</i> | Roughtail Stingray | 160952 | 25 | VIMS | VRT |
| <i>Dasyatis sabina</i> | Atlantic Stingray | 160953 | 86 | VIMS | VRT |
| <i>Dasyatis sayi</i> | Bluntnose Stingray | 160954 | 546 | VIMS | VRT |
| <i>Diplectrum bivittatum</i> | Dwarf Sand Perch | 167796 | | FLFWC | |
| <i>Diplectrum formosum</i> | Sand Perch | 167793 | | FLFWC | |
| <i>Elagatis bipinnulata</i> | Rainbow Runner | 168738 | | FLFWC | |
| <i>Elops saurus</i> | Ladyfish | 161111 | | FLFWC | |
| <i>Epinephelus adscensionis</i> | Rock Hind | 167696 | | FLFWC, SCDNR | OT |
| <i>Epinephelus drummondhayi</i> | Speckled Hind | 167698 | | FLFWC, SCDNR | OT |
| <i>Epinephelus flavolimbatus</i> | Yellowedge Grouper | 167699 | | FLFWC, SCDNR | OT |
| <i>Epinephelus nigritus</i> | Warsaw Grouper | 167704 | | FLFWC, SCDNR | OT |
| <i>Epinephelus niveatus</i> | Snowy Grouper | 167705 | | FLFWC, SCDNR | OT |
| <i>Epinephelus striatus</i> | Nassau Grouper | 167706 | | FLFWC | |
| <i>Eugerres plumieri</i> | Striped Mojarra | 645433 | | FLFWC | |
| <i>Euthynnus alletteratus</i> | Little Tunny | 172402 | | FLFWC | |

| Species | Common Name | ITIS | Total Aged | Agency/Lab | Methodology |
|--|----------------------|--------|------------|-------------------------------|-------------|
| <i>Gadus morhua</i> | Atlantic Cod | 164712 | | MADMF | |
| <i>Gymnura altavela</i> | Spiny Butterfly Ray | 160961 | 336 | VIMS | VRT |
| <i>Gymnura micrura</i> | Smooth Butterfly Ray | 160962 | 246 | VIMS | VRT |
| <i>Haemulon album</i> | Margate | 169060 | | FLFWC | |
| <i>Haemulon aurolineatum</i> | Tomtate | 169058 | | FLFWC, SCDNR | OT |
| <i>Haemulon plumierii</i> | White Grunt | 613026 | | FLFWC | |
| <i>Halieutichthys aculeatus</i> | Pancake Batfish | 164594 | | FLFWC | |
| <i>Helicolenus dactylopterus dactylopterus</i> | Blackbelly rosefish | 166787 | | VMRC | OT |
| <i>Hippocampus erectus</i> | Spotted Seahorse | 166488 | | FLFWC | |
| <i>Hippoglossus hippoglossus</i> | Atlantic Halibut | 172933 | | MADMF | OT |
| <i>Holacanthus bermudensis</i> | Blue Angelfish | 169626 | | FLFWC | |
| <i>Holocentrus adscensionis</i> | Squirrelfish | 166172 | | FLFWC, SCDNR | OT |
| <i>Hyperoglyphe perciformis</i> | Barrelfish | 172512 | | FLFWC | |
| <i>Ictalurus punctatus</i> | Channel Catfish | 163998 | | LDWF | OT |
| <i>Lagodon rhomboides</i> | Pinfish | 169187 | | FLFWC, SCDNR | |
| <i>Leiostomus xanthurus</i> | Spot | 169267 | | FLFWC | |
| <i>Leucoraja erinacea</i> | Little Skate | 564130 | 4,975 | VIMS | VRT |
| <i>Leucoraja ocellata</i> | Winter Skate | 564145 | 4,031 | VIMS | VRT |
| <i>Limanda ferruginea</i> | Yellowtail Flounder | 172909 | 97 | VIMS | OT |
| <i>Lobotes surinamensis</i> | Tripletail | 169007 | | FLFWC, GADNR, MSDMR, USM/GCRL | |
| <i>Lophius americanus</i> | Monkfish | 164499 | 186 | VIMS | VRT |
| <i>Lopholatilus chamaeleonticeps</i> | Golden Tilefish | 168546 | | VMRC | OT |
| <i>Lutjanus apVMRCs</i> | Schoolmaster | 168850 | | FLFWC | |
| <i>Lutjanus cyanopterus</i> | Cubera Snapper | 168847 | | FLFWC | |
| <i>Lutjanus griseus</i> | Gray Snapper | 168848 | | MSDMR, USM/GCRL | OT |
| <i>Lutjanus spp.</i> | Snapper sp. | 168846 | | FLFWC | |
| <i>Lutjanus vivanus</i> | Silk Snapper | 168861 | | FLFWC | |
| <i>Malacanthus plumieri</i> | Sand Tilefish | 168548 | | FLFWC | |
| <i>Megalops atlanticus</i> | Tarpon | 161116 | | GCRL | WOT |
| | | | | FLFWC, USM/GCRL | OT |
| <i>Melanogrammus aeglefinus</i> | Haddock | 164744 | 59 | VIMS | OT |
| <i>Menticirrhus americanus</i> | Southern Kingfish | 169274 | | FLFWC, MSDMR, GADNR, USM/GCRL | OT |
| <i>Menticirrhus littoralis</i> | Gulf Kingfish | 169275 | 54 | USM/GCRL | OT |
| | | | | FLFWC | |

| Species | Common Name | ITIS | Total Aged | Agency/Lab | Methodology |
|------------------------------------|-------------------------|--------|------------|----------------------------|-------------|
| <i>Menticirrhus saxatilis</i> | Northern Kingfish | 169276 | | USM/GCRL | OT |
| | | | | FLFWC | |
| <i>Menticirrhus spp.</i> | Kingfish spp. | | 1,516 | VIMS | OT |
| <i>Merluccius bilinearis</i> | Silver Hake | 164791 | 4,491 | VIMS | OT |
| <i>Micropogonias undulatus</i> | Atlantic Croaker | 169283 | | FLFWC, MSDMR, GADNR | OT |
| <i>Micropterus salmoides</i> | Largemouth Bass | 168160 | | FLFWC | |
| <i>Morone americana</i> | White Perch | 167678 | | MADMF | |
| <i>Morone saxatilis</i> | Striped Bass | 167680 | | FLFWC | |
| <i>Mugil curema</i> | White Mullet | 170336 | | FLFWC | |
| <i>Mustelus canis</i> | Smooth Dogfish | 160230 | 3,669 | VIMS | VRT |
| <i>Mycteroperca interstitialis</i> | Yellowmouth Grouper | 167762 | | FLFWC | |
| <i>Myliobatis freminvillei</i> | Bullnose Ray | 564391 | 334 | VIMS | VRT |
| <i>Neomerinthe hemingwayi</i> | Spinycheek Scorpionfish | 166794 | | FLFWC | |
| <i>Opsanus tau</i> | Oyster Toadfish | 164423 | | FLFWC | |
| <i>Orthopristis chrysoptera</i> | Pigfish | 169077 | | FLFWC | |
| <i>Paralichthys albigutta</i> | Gulf Flounder | 172736 | | FLFWC | |
| <i>Paralichthys dentatus</i> | Summer Flounder | 172735 | | FLFWC, GADNR, MADMF | |
| <i>Paralichthys lethostigma</i> | Southern Flounder | 172738 | | GADNR | |
| <i>Pareques umbrosus</i> | Cubbyu | 169319 | | FLFWC, SCDNR | OT |
| <i>Pogonias cromis</i> | Black Drum | 169288 | | GADNR, MSDMR | OT |
| <i>Pomacanthus arcuatus</i> | Gray Angelfish | 169632 | | FLFWC | |
| <i>Pristipomoides aquilonaris</i> | Wenchman | 168913 | | FLFWC | |
| <i>Pterois volitans</i> | Red Lionfish | 166883 | | FLFWC, SCDNR, USM/ GCRL | OT |
| <i>Rachycentron canadum</i> | Cobia | 168566 | | FLFWC, USM/GCRL | |
| <i>Raja eglanteria</i> | Clearnose Skate | 160855 | 5,840 | VIMS | VRT |
| <i>Rhinoptera bonasus</i> | Cownose Ray | 160985 | 374 | VIMS, USM/GCRL | VRT |
| <i>Rhinoptera brasiliensis</i> | Brazilian Cownose Ray | 160986 | 58 | GCRL | VRT |
| <i>Rhomboplites aurorubens</i> | Vermilion Snapper | 168909 | | MSDMR | OT |
| <i>Sarotherodon melanotheron</i> | Blackchin Tilapia | 553311 | | FLFWC | |
| <i>Scomber colias</i> | Atlantic Chub Mackerel | 172413 | 460 | GCRL | WOT |
| <i>Scomber scombrus</i> | Atlantic Mackerel | 172414 | 237 | VIMS | WOT |
| <i>Scomberomorus maculatus</i> | Spanish Mackerel | 172436 | 53 | VIMS | WOT |
| <i>Scomberomorus maculatus</i> | Spanish Mackerel | 172436 | | FLFWC, MSDMR | OT |
| <i>Scomberomorus regalis</i> | Cero | 172437 | | FLFWC | |
| <i>Scophthalmus aquosus</i> | Windowpane Flounder | 172746 | 2,795 | VIMS | OT |
| <i>Selene vomer</i> | Lookdown | 168680 | | FLFWC | |

| Species | Common Name | ITIS | Total Aged | Agency/Lab | Methodology |
|-------------------------------|----------------------|--------|------------|-------------|-------------|
| <i>Seriola dumerili</i> | Greater Amberjack | 168689 | | MSDMR | OT |
| <i>Seriola fasciata</i> | Lesser Amberjack | 168690 | | FLFWC | |
| <i>Seriola rivoliana</i> | Almaco Jack | 168691 | | FLFWC | |
| <i>Seriola spp.</i> | Amberjack spp. | 168688 | | FLFWC | |
| <i>Seriola zonata</i> | Banded Rudderfish | 168693 | | FLFWC | |
| <i>Sphyræna barracuda</i> | Great Barracuda | 170429 | | FLFWC | |
| <i>Squalus acanthias</i> | Spiny Dogfish | 160617 | 2,490 | VIMS | SN |
| <i>Syacium papillosum</i> | Dusky Flounder | 172793 | | FLFWC | |
| <i>SynVMRCs foetens</i> | Inshore Lizardfish | 162376 | | FLFWC | |
| <i>Tautoga onitis</i> | Tautog | 170479 | 276 | VIMS | OT, OP |
| <i>Tautoga onitis</i> | Tautog | 170479 | | MDDNR | SN |
| <i>Tautoglabrus adspersus</i> | Cunner | 170481 | | MADMF | |
| <i>Thunnus albacares</i> | Yellowfin Tuna | 172423 | | LDWF | |
| <i>Thunnus atlanticus</i> | Blackfin Tuna | 172427 | | FLFWC, LDWF | |
| <i>Trachinocephalus myops</i> | Snakefish | 162420 | | FLFWC | |
| <i>Trachinotus carolinus</i> | Florida Pompano | 168708 | | FLFWC | |
| <i>Trachinotus falcatus</i> | Permit | 168709 | | FLFWC | |
| <i>Trichiurus lepturus</i> | Atlantic Cutlassfish | 172385 | | FLFWC | |
| <i>Umbrina coroides</i> | Sand Drum | 169298 | | FLFWC | |
| <i>Xiphias gladius</i> | Swordfish | 172482 | | LDWF | |
| | | | | USM/GCRL | SN |
| <i>Mycteroperca tigris</i> | Tiger Grouper | 167767 | 90 | SCDNR | OT |
| <i>Gymnothorax moringa</i> | Spotted Moray | 161188 | | SCDNR | OT |
| <i>Gymnothorax vicinus</i> | Purplemouth Moray | 161193 | | SCDNR | OT |
| <i>Gymnothorax polygonius</i> | Polygon Moray | 161191 | | SCDNR | OT |
| <i>Gymnothorax saxicola</i> | Ocellated Moray | 161192 | | SCDNR | OT |
| <i>Muraena robusta</i> | Stout Moray | 161242 | | SCDNR | OT |
| <i>Muraena retifera</i> | Reticulate Moray | 161240 | | SCDNR | OT |

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