2025 Final Report
Virginia ~ Chesapeake Bay
Finfish Ageing
and
Population Analysis

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Virginia Marine Resources Commission
380 Fenwick Road, Fort Monroe, VA 23651

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EXECUTIVE SUMMARY

This executive summary briefly summarizes what the Age and Growth Lab achieved in 2025 in terms of the objectives listed in the 2025 - 2026 proposal.

Objective 1: We propose to continue support of the Virginia Marine Resources Commission (VMRC) Age and Growth Laboratory, which is dedicated to providing Virginia fisheries management with reliable age estimates of marine fishes as an ongoing long-term activity. This includes yearly reports of catch-at-age of Virginia's important finfishes that are mandated by law, along with proper protocols to insure accuracy of the age estimates.

This objective is the major task the Age and Growth Lab is funded for, therefore, 15 chapters in the report are about the objective and each chapter is for one of 15 species the lab aged in 2025. We present the ageing results of 15 finfish species collected from commercial and recreational catches made in the Chesapeake Bay and Virginia waters of the Atlantic Ocean, U.S.A. in 2025. All fish were collected by the VMRC Biological Sampling Program in 2025 and aged in 2026 at the Age and Growth Laboratory of VMRC. We present age composition tables, graphs of year-class distributions, age-length keys, and measures of ageing precision for each species.

Four calcified structures (hard-parts) are used in age determination. More specifically, three calcified structures, otoliths, opercula, and pelvic spines (newly added in 2022), were used for determining fish ages of Tautog *Tautoga onitis* (n = 265). Two calcified structures, otoliths and scales, were used for determining fish ages of Menhaden *Brevoortia tyrannus* (n = 187), Striped Bass *Morone saxatilis* (n = 685), and Summer Flounder *Paralichthys dentatus* (n = 751). Comparing alternative hard-parts allowed us to assess their usefulness in determining fish age as well as the relative precision of each structure. Ages were determined from otoliths only for the following species: Atlantic Croaker *Micropogonias undulatus* (n = 279), Black Drum *Pogonias cromis* (n = 71), Bluefish *Pomatomus saltatrix* (n = 296), Cobia *Rachycentron canadum* (n = 194), Red Drum *Sciaenops ocellatus* (n = 97), Sheepshead *Archosargus probatocephalus* (n = 253), Atlantic Spadefish *Chaetodipterus faber* (n = 103), Spanish Mackerel *Scomberomorous maculates* (n = 227), Spot *Leiostomus xanthurus* (n = 195), Spotted Seatrout *Cynoscion nebulosus* (n = 227), and Weakfish *Cynoscion regalis* (n = 133). In total, we made 10,624 age readings from otoliths, scales, opercula, and spines collected during 2025. A summary of the age ranges for all species aged is presented in Table 1.

In 2025 we added Menhaden in our ageing list. We aged them using both their whole otoliths and scale acetate impressions to compare which hardpart may provide a higher precision. We are planning to continue such a comparison for the next two years, hoping that we will be able to decide which hardpart (scale vs. otolith) we eventually use for Menhaden age determination in the future.

Objective 2: VMRC will continue to develop sampling methods that are cost effective and representative of landings in the fisheries. This will produce accurate estimates of catch and effort. We have been using two-stage sampling to decide sample sizes for ageing 10 of our 15 species, which have helped to minimize costs on ageing while maximizing precision on estimates of catch-at-age.

In this report, we present sample sizes and coefficient of variation (CV) for estimates of age composition for the following species: Atlantic Croaker, Bluefish, Spadefish, Spanish Mackerel, Spot, Spotted Seatrout, Striped Bass, Summer Flounder, Tautog, and Weakfish. The sample sizes and the CVs enabled us to determine how many fish we needed to age in each length interval and to measure the precision for estimates of major age classes in each species, respectively, enhancing our efficiency and effectiveness on ageing those species. In addition, the sample size for ageing

Table 1: The minimum and maximum ages, number of fish and their hardparts collected, number of fish aged, and age readings (by both readers) for the 15 finfish species in 2025. Besides otoliths, the hardparts and age readings include scales for Menhaden (a new species was added in 2025), Striped Bass, and Summer Flounder, and both opercula and spines for Tautog. The otolith-ages are used to estimate year classes and to develop ALKs for all the species. When otolith-ages are not available, scale-ages are used for Menhaden, Striped Bass, and Summer Flounder whereas operculum-ages are used for Tautog. However, when neither otolith- nor operculum-ages are avaialbe for Tautog, spine-ages are used.

Species	Number of fish collected	Number of hard-parts	Number of fish aged	Number of read-ings	Minimum age	Maximum age
Atlantic Croaker	346	346	279	558	1	8
Black Drum	73	71	71	142	3	45
Bluefish	408	407	296	592	0	10
Cobia	197	194	194	388	3	11
Menhaden	324	470	187	682	2	7
Red Drum	98	97	97	194	0	3
Sheepshead	257	254	253	506	2	34
Spadefish	103	103	103	206	1	10
Spanish Mackerel	282	280	227	454	0	8
Spot	244	244	195	390	1	4
Spotted Seatrout	264	263	227	454	0	7
Striped Bass	821	1,063	685	1,854	2	23
Summer Flounder	774	1,201	751	2,356	0	16
Tautog	265	787	265	1,582	2	16
Weakfish	133	133	133	266	1	4
Totals	4,589	5,913	3,963	10,624		

Menhaden was estimated by ASFMC in 2025.

Objective 3: VMRC will develop routine stock assessments based on age-structured models (such as SVPA, ADAPT, Stock Synthesis, and AD Model Builder, among others where appropriate). Following several years of accumulation of aged-catch data, age-structured stock assessment models will be developed and periodically updated.

The purpose of this objective is to prepare VMRC to make contributions to stock assessment of any species along Atlantic coast when requested by Atlantic States Marine Fisheries Commission (ASMFC) and Southeast Data, Assessment and Review (SEDAR). During the past several years, the occurrence of cold stun on Spotted Seatrout were more and more frequently. Moreover, the ASMFC stock assessment has started to collect in the information on the cold stun events among the Atlantic states. In early 2026, Dr. Liao, the Lab Manager, started to develop a website-based application (Shiny app) to encourage the public to report any cold-stun event in Chesapeake Bay (possibly in Virginia waters of Atlantic Ocean). We are planning to test the app in late 2026 and/or early 2027.

Objective 4: Develop VMRC Age and Growth Laboratory web pages at VMRC web site to publish protocols, other aids such as pictures of aged otoliths for all species, and other information to assist other states and laboratories in the methods of ageing marine fishes.

Throughout the years we have continued to work on the design and content of a web page that

promotes VMRC's efforts to properly manage Virginia's marine resources through our age and growth research. In addition to educating the public on the importance of ageing fishes, the web page has been of interest to fishermen for it provides fundamental information of the life history of Virginia's fishes. We posted VMRC 2024 Ageing Lab Final Report, providing the detailed information on what the ageing lab is about, what we do in the lab, and what contributions the ageing lab makes to the coast-wide marine fisheries management.

Objective 5: We will continue developing website-based applications (apps) to enhance sharing Virginia fish and their age data with anglers and fisheries biologists in other agencies.

In 2025, we updated age-length data in VMRC four web applications (Fish Age Estimator, Fish Growth Predictor, VMRC Database App, and %MSP/%Female_SPR/%SPR Estimator). These apps help fishermen to understand the importance of knowledge on fish ages and growth, and allow fishermen and fisheries scientists to easily access and download the age and biological databases of 15 marine finfish species collected by VMRC at Chesapeake Bay and Virginia waters of Atlantic ocean from as early as 1998 to 2025 and aged by the lab.

In addition, we developed a new app VMRC Multi-Species Paired Calcified Structure Images in 2025. This app allows us to share our experience with other ageing labs on ageing Menhaden, Striped Bass, and Summer Flounder using otoliths, scales, and/or spines by comparing the images of different hardparts side by side. We will continue to add more paired images in the app while improving the quality of the current images in 2026.

In October of 2025, we sent Black Bellied Rosefish *Helicolenus dactylopterus* biological data collected by VMRC from 2004 to 2024 using different gears to Kaitlyn Tucker at University of Florida. They will use the VMRC data to develop von Bertalanff growth model with incorporation of gear selectivity. In January of 2026, we established an agreement with Dr. Thomas Nelson at George Mason University to use the VMRC Striped Bass otolith thin-section slides and age data on Dr. Nelson's research titled as "Determining how evolution, phenotypic plasticity, and eco-evolutionary interactions shape temporal dynamics of growth traits in an exploited fish population".

Objective 6: We will continue the publication of our results on accuracy and precision of ageing important marine finfish species, and their effects on stock assessments and fisheries management in scientific literature.

We continued to update the Ageing Lab Operation Protocol in 2025, revising the current method(s) and/or adding new method (s). In early April of 2026, Dr. Hank Liao, Lab Manager, participated ASMFC QAQC Ageing Workshop held at St. Petersburg, Florida, to continue learning how to age Cobia, Menhaden, Spot, Striped Bass, Tautog, and Winter Flounder *Pseudopleuronectes americanus*.

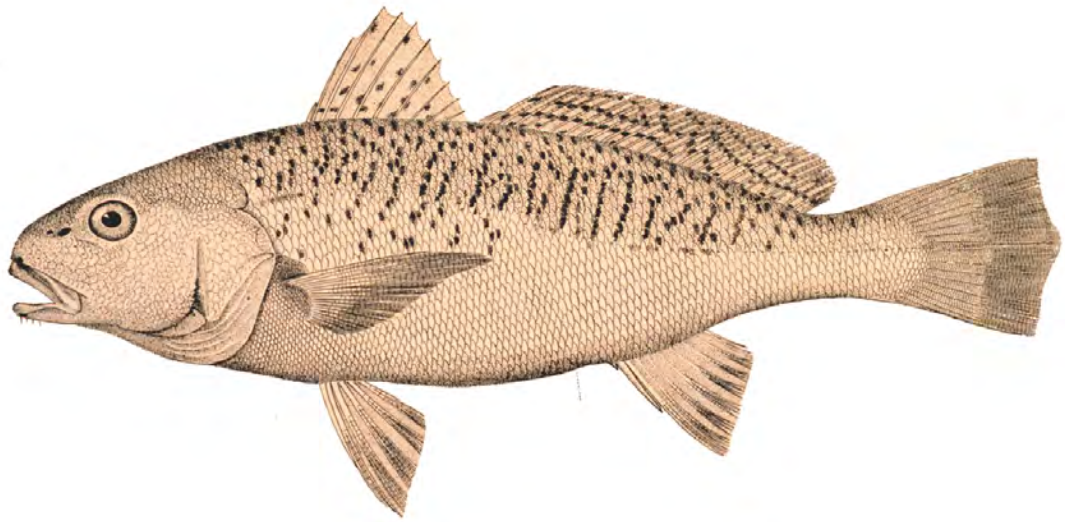
Besides above work the Age and Growth Lab did in 2025, to support environmental and wildlife agencies, and charities, we donated more than 3,486 pounds of dissected fish to the Salvation Army to feed the homeless, and Alton's Keep WildBird Rescue and Rehabilitation Center Inc., a local wildlife rescue agency which is responsible for saving injured animals found by the public.

ACKNOWLEDGMENTS

We thank Natalie Baker, Hope Dutch, Laura George, and Shannon Jones for their technical expertise in preparing otoliths, scales, opercula, and spines for age determination. They all put in long hours processing "tons" of fish in our lab. We would like also to thank the VMRC field technicians, Brandon Foor, Richard Hancock, and Chris Williams for their many efforts in this cooperative project. A special note of appreciation is extended to Ethan Simpson, VMRC Biological Sampling Program Supervisor, for his help in processing fish, collecting hardparts, and many other lab activities whenever we were short of hands in the lab.

Chapter 1

ATLANTIC CROAKER *Micropogonias undulatus*



1.1 INTRODUCTION

We aged a total of 279 Atlantic Croaker *Micropogonias undulatus*, collected by the VMRC's Biological Sampling Program for age and growth analysis in 2025. Croaker ages ranged from 1 to 8 years old with an average age of 2.3, a standard deviation of 1.5, and a standard error of 0.09. Eight age classes (1 to 8) were represented, comprising fish of the 2017 to 2024 year-classes. The sample was dominated by fish from the year-classes of 2023 and 2024 with 39.8% and 31.5%, respectively.

1.2 METHODS

1.2.1 Sample Size for Ageing

We estimated sample size for ageing Croaker in 2025 using a two-stage random sampling method (Quinn and Deriso 1999) to increase precision in estimates of age composition from fish sampled efficiently and effectively. The basic equation is:

$$A = \frac{V_a}{\theta_a^2 CV_a^2 + B_a/L} \tag{1.1}$$

where A is the sample size for ageing Croaker in 2025; θ_a stands for the proportion of Age a fish in a catch; V_a, B_a, and CV_a represent the variance components within and between length intervals, and the coefficient of variation for Age a, respectively; L is the total number of Croaker used by VMRC to estimate length distribution of the catches from 2019 to 2023. θ_a, V_a, and B_a were calculated using pooled age-length data of Croaker collected from 2019 to 2023 and using equations in Quinn and Deriso (1999). For simplicity, the equations are not listed here. The equation (1.1) indicates: 1) The more fish that are aged, the smaller the CV_a (or higher precision) that will be obtained for Age a; 2) given a sample size A, the CV_a is different for each age due to different θ_a, V_a, and B_a among different ages. Therefore, the criterion to age A (number) of fish is that A should be a number above which there is only a 1% CV_a reduction for the most abundant age in catch by ageing an additional 100 or more fish. Finally, A_l is A multiplied by the proportion of length interval l from the length distribution of the 2019 to 2023 catch. A_l is number of fish to be aged for length interval l in 2025.

1.2.2 Handling of Collections

Otoliths were received by the Age and Growth Laboratory in labeled coin envelopes, were sorted by date of capture. Their envelope labels were verified against VMRC's collection data, and each fish was assigned a unique Age and Growth Laboratory identification number. All otoliths were stored dry in their original labeled coin envelopes.

1.2.3 Preparation

Sagittal otoliths, hereafter, referred to as "otoliths", were processed for age determination following the methods described in Barbieri et al. (1993) with a few modifications. The left or right otolith was randomly selected and attached, distal side down, to a 1 x 2 inch piece of water resistant grid paper (Brand name: Write in the Rain) using hot glue. The otoliths were viewed by eye and, when necessary, under a stereo microscope to identify the location of the core, and the position of the core was marked using an ultra fine Sharpie across the otolith surface. At least one transverse cross-section (hereafter, referred to as "thin-section") was then removed from the marked core of each otolith using a Buehler IsoMet™ low-speed saw equipped with two 4-inch diameter diamond grinding wheels (hereafter, referred to as "blades"), separated by a stainless steel spacer of 0.5 mm (diameter 2.5"). Thin-sections were placed on labeled glass slides and covered with a thin layer of Flo-texx mounting medium that not only fixed the sections to the slide, but more importantly, provided enhanced contrast and greater readability by increasing light transmission through the thin-sections.

Click here to obtain the protocol at the VRMC website on how to prepare otolith thin-section using the hot glue method for ageing Atlantic Croaker.

1.2.4 Readings

The VMRC system assigns an age class to a fish based on a combination of number of annuli in a thin-section, the date of capture, and the species-specific period when the annulus is deposited. Each year, as the fish grows, its otoliths grow and leave behind markers of their age, called an annulus. Technically, an otolith annulus is the combination of both the opaque and the translucent band. In practice, only the opaque bands are counted as annuli and recorded in our ageing notation.

In 2019 a new notation method recommended by Atlantic States Marine Fisheries Commission (ASMFC) was used to assign age on Atlantic Croaker. In addition to recording the number of annulus, the margin or the growth width after the last annulus is coded from 1 to 4. The margin code "1", "2", "3", and "4" stands for no growth, the growth width less than or equal to one third of, larger than one third but less than or equal to two thirds of, and larger than two thirds of the growth width formed in the previous year, respectively.

By convention all fish in the Northern Hemisphere are assigned a birth date of January 1. In addition, each species has a specific period during which it deposits the annulus. If a fish is captured after the end of the species-specific annulus deposition period and before January 1, it is assigned an age class as the same as its annulus number without referencing its margin code. If a fish has a margin code of "1", it is assigned an age class as the same as its annulus number no matter in which month it is captured. If a fish is captured after December 31 and before its annulus deposition period, it is assigned an age class as its annulus number plus one when its margin code is "2", "3", or "4". If a fish is captured during its annulus deposition period, it is assigned an age class as the same as its annulus number when its margin code is "2" and as its annulus number plus one when its margin code is "3" or "4" (**Note: Based on the growth of Virginia species we use two criteria for Margin Code 2 to assign a fish an age class depending on its capture month, which could be different from how other states and agencies use Margin Code 2**).

For example, Atlantic Croaker otolith annulus formation occurs between April and June (Barbieri et al. 1993, 1994, and modified by CQFE/ODU). A Croaker with three visible annuli could be assigned an age of 3 or 4 depending on its capture month and margin code. When its margin code is "1", it is Age 3 no matter when it is captured. When it is captured after June and before January, it is Age 3 no matter what its margin code is. When it is captured after December and before April and its margin code is not "1", it is Age 4 ($3 + 1 = 4$). When it is captured between April and June, it is Age 3 when its margin code is "2" but Age 4 ($3 + 1 = 4$) when its margin code is "3" or "4".

When an otolith was properly sectioned, the sulcal groove came to a sharp point (Hereafter referred to as "focus") within the middle of the core (Figure 1.1). Typically the first year's annulus was found by locating the focus of the otolith section, which was characterized as a visually distinct dark, oblong region found in the center of the otolith section.

However, due to discrepancy on identification of the first annulus of Atlantic Croaker among Atlantic states, ASMFC has decided not to count the smallest annulus at the center of the thin-section as the first annulus. Following ASMFC's instruction, we didn't count the smallest annulus at the center as the first annulus in 2025.

All samples were aged by two readers in chronological order, based on collection date, without knowledge of previously estimated ages or the specimen lengths. When the readers' ages agreed, that age was assigned to the

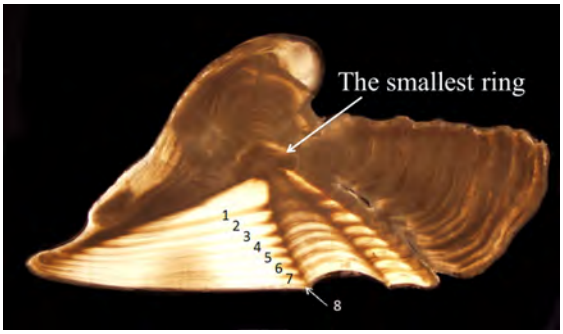


Figure 1.1: Otolith thin-sections of a 8 year-old Croaker without counting the smallest ring and with the last annulus on the edge of the thin-section

fish. When the two readers disagreed, both readers sat down together and re-aged the fish, again without any knowledge of previously estimated ages or lengths, and assigned a final age to the fish. When the readers were unable to agree on a final age, the fish was excluded from further analysis. All thin-sections were aged using a Nikon SMZ1000 stereo microscope under transmitted light and dark-field polarization at between 8 and 20 times magnification (Figure 1.1).

1.2.5 Comparison Tests

A symmetry test (Hoenig et al. 1995) and coefficient of variation (CV) analysis were used to detect any systematic difference and precision on age readings, respectively, for the following comparisons: 1) between the two readers in the current year, 2) within each reader in the current year, and 3) time-series bias between the current and previous years within each reader. The readings from the entire sample for the current year were used to examine the difference between two readers. A random sub-sample of 50 fish from the current year was selected for second readings to examine the difference within a reader. Fifty otoliths randomly selected from fish aged in 2003 were used to examine the time-series bias within each reader. A figure of 1:1 equivalence was used to illustrate those differences (Campana et al. 1995). All statistics analyses were performed in R.4.0.2 (R Core Team 2021).

1.3 RESULTS

1.3.1 Sample Size

We estimated a sample size of 410 Atlantic Croaker in 2025, ranging in length interval from 5 to 14 inches (Table 1.1). This sample size provided a range in CV for age composition approximately from the smallest CV of 7% for the major age of Age 1 to the CV of larger than 25% for the multiple minor ages (Table 1.2). In 2025, we aged 279 of 346 Croaker (The rest of fish were either without otoliths or over-collected for certain length interval(s)) collected by VMRC. We fell short in our overall collections for this optimal length-class sampling estimate by 146 fish. We were short of many fish from the major length intervals (The interval requires 10 or more fish), as a result, the precision for the estimates of major age groups would definitely be influenced significantly. Therefore, precaution should be used when developing ALK using these age data.

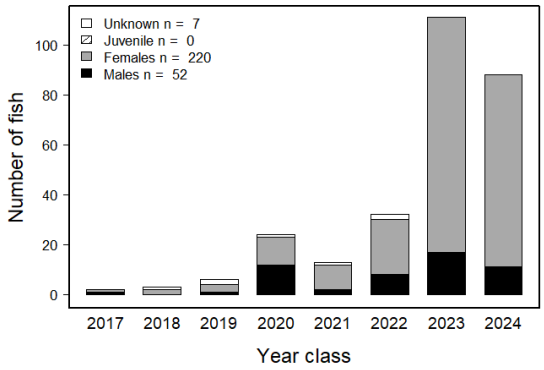


Figure 1.2: Year-class frequency distribution for Atlantic Croaker collected for ageing in 2025. Distribution is broken down by sex. 'Unknown' is for gonads that were not available for examination or were not examined for sex during sampling.

1.3.2 Reading Precision

Both readers had high self-precision. Specifically, there was no significant difference between the first and second readings for Reader 1 with an agreement of 86% and a CV of 4.48%

(test of symmetry: $\chi^2 = 5$, $df = 4$, $P = 0.2873$), and there was no significant difference between the first and second readings for Reader 2 with an agreement of 88% and a CV of 3.52% (test of symmetry: $\chi^2 = 4$, $df = 5$, $P = 0.5494$). There was no evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 89.25% and a CV of 3.23% (test of symmetry: $\chi^2 = 13.83$, $df = 10$, $P = 0.1807$) (Figure 1.3).

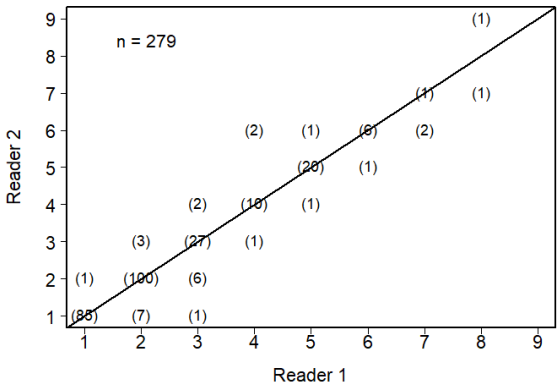


Figure 1.3: Between-reader comparison of otolith age estimates for Atlantic Croaker collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2025. The number in parentheses is number of fish.

There was no time-series bias for either reader. Reader 1 had an agreement of 98% with ages of fish aged in 2003 with a CV of 0.13% (test of symmetry: $\chi^2 = 1$, $df = 1$, $P = 0.3173$). Reader 2 also had an agreement of 100% .

1.3.3 Year Class

Of the 279 fish aged with otoliths, 8 age classes (1 to 8) were represented (Table 1.3). The average age was 2.3 years, and the standard deviation and standard error were 1.5 and 0.09, respectively. Year-class data show that the fishery was comprised of 8 year-classes: fish from the 2017 to 2024 year-classes, with fish primarily from the year classes of 2023 and 2024 with 39.8% and 31.5%, respectively. The ratio of males to females was 1:4.23 in the sample collected (Figure 1.2).

1.3.4 Age-length Key (ALK)

We developed an age-length-key (Table 1.4) that can be used in the conversion of numbers-at-length in the estimated catch to numbers-at-age using otolith ages. The table is based on VMRC's stratified sampling of landings by total length inch intervals.

Table 1.1: Number of Atlantic Croaker collected and aged in each 1-inch length interval in 2025. 'Target' represents the sample size for ageing estimated for 2025, and 'Need' represents number of fish shorted in each length interval compared to the optimum sample size for ageing and number of fish aged.

Interval	Target	Collected	Aged	Need
5 - 5.99	5	0	0	5
6 - 6.99	5	9	6	0
7 - 7.99	19	25	20	0
8 - 8.99	23	72	24	0
9 - 9.99	51	74	63	0
10 - 10.99	96	67	67	29
11 - 11.99	121	78	78	43
12 - 12.99	64	19	19	45
13 - 13.99	21	2	2	19
14 - 14.99	5	0	0	5
Totals	410	346	279	146

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Table 1.2: CV for each age estimated based on ageing the total of 410 Croaker in 2025. 'Percent' is the percentage of an age in the pooled age-length data of Croaker collected from 2019 to 2023.

Age	CV	Percent
0	0.24	2.5
1	0.07	32.64
2	0.08	28.34
3	0.11	17.48
4	0.18	6.98
5	0.2	6.2
6	>0.25	3.62
7	>0.25	1.72
8	>0.25	0.34
9	>0.25	0.09
11	>0.25	0.09

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Table 1.3: The number of Atlantic Croaker assigned to each total length-at-age category for 279 fish sampled for otolith age determination in Virginia during 2025.

	Age								
Interval	1	2	3	4	5	6	7	8	Totals
6 - 6.99	5	1	0	0	0	0	0	0	6
7 - 7.99	7	7	3	2	1	0	0	0	20
8 - 8.99	1	11	3	3	5	1	0	0	24
9 - 9.99	7	18	14	5	13	5	1	0	63
10 - 10.99	18	32	8	2	3	0	2	2	67
11 - 11.99	41	33	1	1	2	0	0	0	78
12 - 12.99	9	7	3	0	0	0	0	0	19
13 - 13.99	0	2	0	0	0	0	0	0	2
Totals	88	111	32	13	24	6	3	2	279

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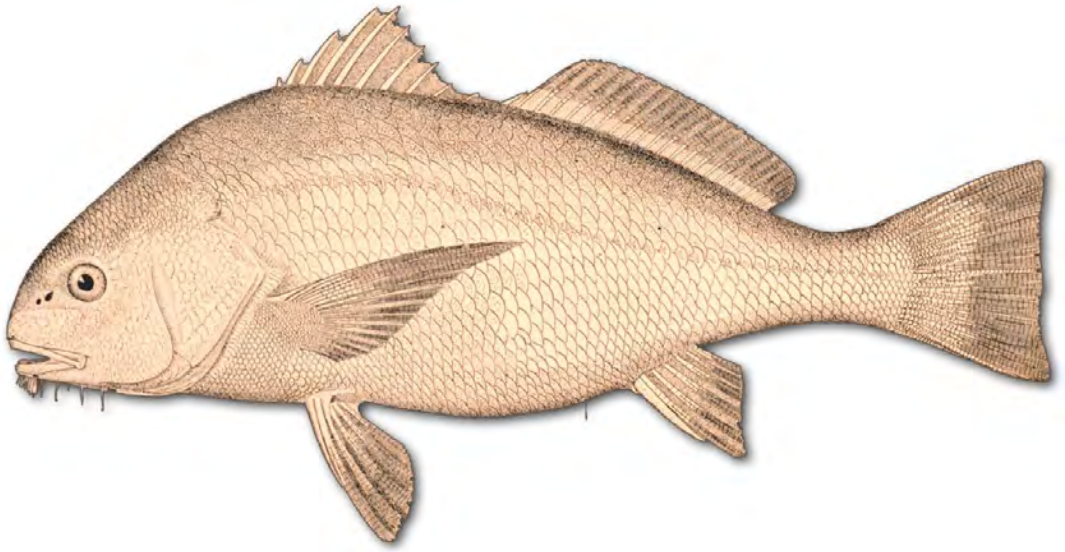
Table 1.4: Age-Length key, as proportion-at-age in each 1-inch length interval, based on otolith ages for Atlantic Croaker sampled for age determination in Virginia during 2025.

	Age							
Interval	1	2	3	4	5	6	7	8
6 - 6.99	0.83	0.17	0	0	0	0	0	0
7 - 7.99	0.35	0.35	0.15	0.1	0.05	0	0	0
8 - 8.99	0.04	0.46	0.12	0.12	0.21	0.04	0	0
9 - 9.99	0.11	0.29	0.22	0.08	0.21	0.08	0.02	0
10 - 10.99	0.27	0.48	0.12	0.03	0.04	0	0.03	0.03
11 - 11.99	0.53	0.42	0.01	0.01	0.03	0	0	0
12 - 12.99	0.47	0.37	0.16	0	0	0	0	0
13 - 13.99	0	1	0	0	0	0	0	0

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Chapter 2

BLACK DRUM *Pogonias cromis*



2.1 INTRODUCTION

We aged a total of 71 Black Drum *Pogonias cromis*, collected by the VMRC's Biological Sampling Program for age and growth analysis in 2025. Black drum ages ranged from 3 to 45 years old with an average age of 8.3, a standard deviation of 6.8, and a standard error of 0.81. Fifteen age classes (3 to 10, 13 to 14, 19 to 20, 22, 32, and 45) were represented, comprising fish of the 1980, 1993, 2003, 2005 to 2006, 2011 to 2012, and 2015 to 2022 year-classes. The sample was dominated by fish from the year-classes of 2015, 2018, 2019, 2020, 2021, and 2022 with 9.9%, 11.3%, 14.1%, 12.7%, 18.3%, and 8.4%, respectively.

2.2 METHODS

2.2.1 Handling of Collections

Sagittal otoliths, hereafter, referred to as "otoliths", were received by the Age and Growth Laboratory in labeled coin envelopes. In the lab they were sorted by date of capture, their envelope labels were verified against VMRC's collection data, and each fish was assigned a unique Age and Growth Laboratory identification number. All otoliths were stored dry in their original labeled coin envelopes.

2.2.2 Preparation

Otoliths were processed for age determination following the methods described in Bobko (1991) and Jones and Wells (1998) with a few modifications. The left or right otolith was randomly selected and attached, distal side down, to a 1 x 2 inch piece of water resistant grid paper (Brand name: Write in the Rain) using hot glue. . The otoliths were viewed by eye to identify the location of the core, and the position of the core marked using an ultra fine Sharpie across the otolith surface. At least one transverse cross-section (hereafter "thin-section") was then removed from the marked core of each otolith using a Buehler IsoMet^{TM} low-speed saw equipped with two 4-inch diameter diamond grinding wheels, separated by a stainless steel spacer of 0.5 mm (diameter 2.5"). The position of the marked core fell within the 0.5 mm space between the blades, such that the core was included in the removed thin-section. Otolith thin-sections were placed on labeled glass slides and covered with a thin layer of Flo-texx mounting medium that not only fixed the sections to the slide, but more importantly, provided enhanced contrast and greater readability by increasing light transmission through the sections.

Click here to obtain the protocol at the VMRV website on how to prepare otolith thin-section using the hot glue method for ageing Black Drum.

2.2.3 Readings

The VMRC system assigns an age class to a fish based on a combination of number of annuli in a thin-section, the date of capture, and the species-specific period when the annulus is deposited. Each year, as the fish grows, its otoliths grow and leave behind markers of their age, called an annulus. Technically, an otolith annulus is the combination of both the opaque and the translucent band. In practice, only the opaque bands are counted as annuli and recorded in our ageing notation.

In 2019 a new notation method recommended by Atlantic States Marine Fisheries Commission (ASMFC) was used to assign age on Black Drum. In addition to recording the number of annulus, the margin or the growth width after the last annulus is coded from 1 to 4. The margin code “1”, “2”, “3”, and “4” stands for no growth, the growth width less than or equal to one third of, larger than one third but less than or equal to two thirds of, and larger than two thirds of the growth width formed in the previous year, respectively.

By convention all fish in the Northern Hemisphere are assigned a birth date of January 1. In addition, each species has a specific period

during which it deposits the annulus. If a fish is captured after the end of the species-specific annulus deposition period and before January 1, it is assigned an age class as the same as its annulus number without referencing its margin code. If a fish has a margin code of "1", it is assigned an age class as the same as its annulus number no matter in which month it is captured. If a fish is captured after December 31 and before its annulus deposition period, it is assigned an age class as its annulus number plus one when its margin code is "2", "3", or "4". If a fish is captured during its annulus deposition period, it is assigned an age class as the same as its annulus number when its margin code is "2" and as its annulus number plus one when its margin code is "3" or "4" (**Note: Based on the growth of Virginia species we use two criteria for Margin Code 2 to assign a fish an age class depending on its capture month, which could be different from how other states and agencies use Margin Code 2**).

For example, Black Drum otolith annulus formation occurs between May and June (Beckman et al. 1990; Bobko 1991; Jones and Wells 1998). A Black Drum with ten visible annuli could be assigned an age of 10 or 11 depending on its capture month and margin code. When its margin code is "1", it is Age 10 no matter when it is captured. When it is captured after June and before January, it is Age 10 no matter what its margin code is. When it is captured after December and before May and its margin code is not "1", it is Age 11 (10 + 1 = 11). When it is captured between May and June, it is Age 10 when its margin code is "2" but Age 11 (10 + 1 = 11) when its margin code is "3" or "4".

When an otolith was properly sectioned, the sulcal groove came to a sharp point (Hereafter referred to as "focus") within the middle of the core (Figure 2.1). Typically the first year's annulus was found by locating the focus of the otolith section, which was characterized as a visually distinct dark, oblong region found in the center of the otolith section.

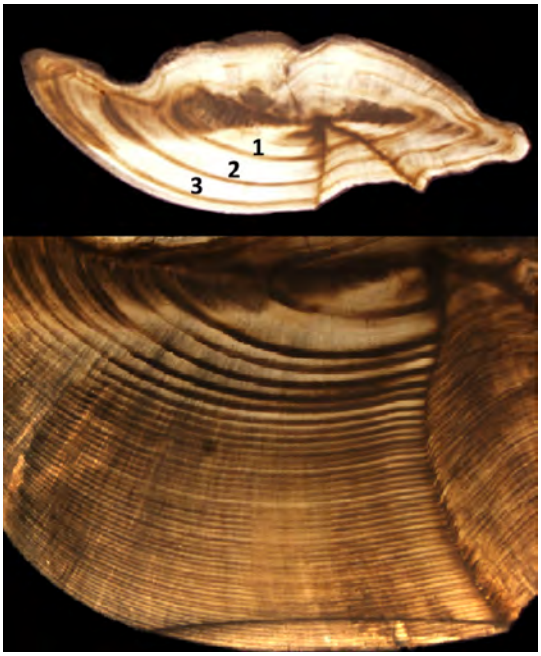


Figure 2.1: Otolith thin-sections of a 3 (Upper panel) and 47 year-old (Lower panel) Black Drum.

All samples were aged by two readers in chronological order, based on collection date, without knowledge of previously estimated ages or the specimen lengths. When the readers' ages agreed, that age was assigned to the fish. When the two readers disagreed, both readers sat down together and re-aged the fish, again without any knowledge of previously estimated ages or lengths, and assigned a final age to the fish. When the readers were unable to agree on a final age, the fish was excluded from further analysis. All thin-sections were aged using a Nikon SMZ1000 stereo microscope under transmitted light and dark-field polarization at between 8 and 20 times magnification.

2.2.4 Comparison Tests

A symmetry test (Hoenig et al. 1995) and coefficient of variation (CV) analysis were used to detect any systematic difference and precision on age readings, respectively, for the following comparisons: 1) between the two readers in the current year, 2) within each reader in the current year, and 3) time-series bias be-

tween the current and previous years within each reader. The readings from the entire sample for the current year were used to examine the difference between two readers. A random sub-sample of 50 fish from the current year was selected for second readings to examine the difference within a reader. Fifty otoliths randomly selected from fish aged in 2001 were used to examine the time-series bias within each reader. A figure of 1:1 equivalence was used to illustrate those differences (Campana et al. 1995). All statistics analyses were performed in R.4.0.2 (R Core Team 2021).

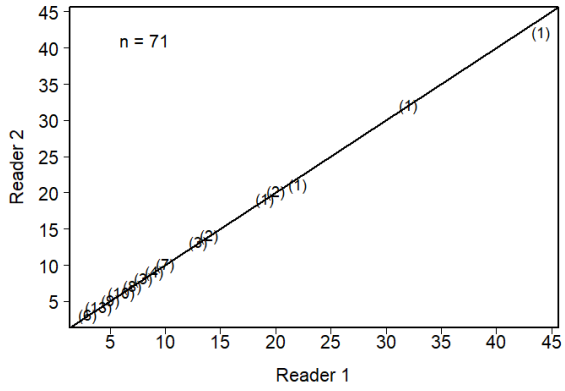


Figure 2.2: Between-reader comparison of otolith age estimates for Black Drum collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2025. The number in parentheses is number of fish.

2.3 RESULTS

2.3.1 Reading Precision

Both readers had high self-precision. Specifically, there was no significant difference between the first and second readings for Reader 1 with an agreement of 98% and a CV of 0.31% (test of symmetry: $\chi^2 = 1$, $df = 1$, $P = 0.3173$), and there was no significant difference between the first and second readings for Reader 2 with an agreement of 88% and a CV of 0.66% (test of symmetry: $\chi^2 = 6$, $df = 5$, $P = 0.3062$). There was no evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 97.18% and a CV of 0.09% (test of symmetry: $\chi^2 = 2$, $df = 2$, $P = 0.3679$) (Figure 2.2).

There was no time-series bias for either reader. Reader 1 had an agreement of 94% with ages of fish aged in 2001 with a CV of 0.13% (test of symmetry: $\chi^2 = 3$, $df = 3$, $P = 0.3916$). Reader 2 had an agreement of 80%with a CV of 3.58% (test of symmetry: $\chi^2 = 10$, $df = 8$, $P = 0.265$).

2.3.2 Year Class

We aged the otoliths of all the 71 Black Drum collected in 2025. Of the 71 fish aged, 15 age classes (3 to 10, 13 to 14, 19 to 20, 22, 32, and 45) were represented (Table 2.1). The average age was 8.3 years, and the standard deviation and standard error were 6.8 and 0.81, respectively. Year-class data show that the fishery was comprised of 15 year-classes: fish from the 1980, 1993, 2003, 2005 to 2006, 2011 to 2012, and 2015 to 2022 year-classes, with fish primarily from the year classes of 2015, 2018, 2019, 2020, 2021, and 2022 with 9.9%, 11.3%, 14.1%, 12.7%, 18.3%, and 8.4%, respectively. The ratio of males to females was 1:0.69 in the sample collected (Figure 2.3).

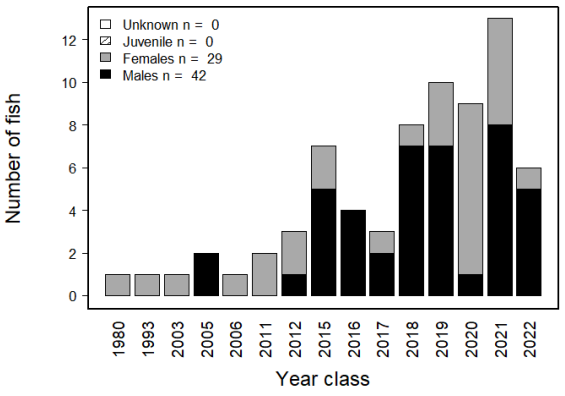


Figure 2.3: Year-class frequency distribution for Black Drum collected for ageing in 2025. Distribution is broken down by sex. 'Unknown' represents gonads that were not available for examination or were not examined for sex during sampling.

2.3.3 Age-length Key (ALK)

We developed an age-length-key (Table 2.2) that can be used in the conversion of numbers-at-length in the estimated catch to numbers-at-age using otolith ages. The table is based on VMRC's stratified sampling of landings by total length inch intervals.

Table 2.1: The number of Black Drum assigned to each total length (inch)-at-age category for 71 fish sampled for otolith age determination in Virginia during 2025.

	Age															
Interval	3	4	5	6	7	8	9	10	13	14	19	20	22	32	45	Totals
11 - 11.99	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1
12 - 12.99	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1
14 - 14.99	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1
23 - 23.99	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
24 - 24.99	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0	4
25 - 25.99	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	2
26 - 26.99	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	2
27 - 27.99	1	1	3	0	0	0	0	0	0	0	0	0	0	0	0	5
28 - 28.99	1	1	5	2	0	0	0	0	0	0	0	0	0	0	0	9
29 - 29.99	0	1	1	2	2	0	0	0	0	0	0	0	0	0	0	6
30 - 30.99	0	4	0	3	2	0	1	0	0	0	0	0	0	0	0	10
31 - 31.99	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1
32 - 32.99	0	0	0	2	1	0	1	1	0	0	0	0	0	0	0	5
33 - 33.99	0	0	0	0	1	0	2	0	0	0	0	0	0	0	0	3
34 - 34.99	0	0	0	0	0	2	0	2	0	0	0	0	0	0	0	4
35 - 35.99	0	0	0	0	0	0	0	2	2	0	0	0	0	0	0	4
36 - 36.99	0	0	0	0	1	0	0	2	1	0	0	0	0	0	0	4
37 - 37.99	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1
38 - 38.99	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	2
39 - 39.99	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1
41 - 41.99	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1
44 - 44.99	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	2
49 - 49.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
Totals	6	13	9	10	8	3	4	7	3	2	1	2	1	1	1	71

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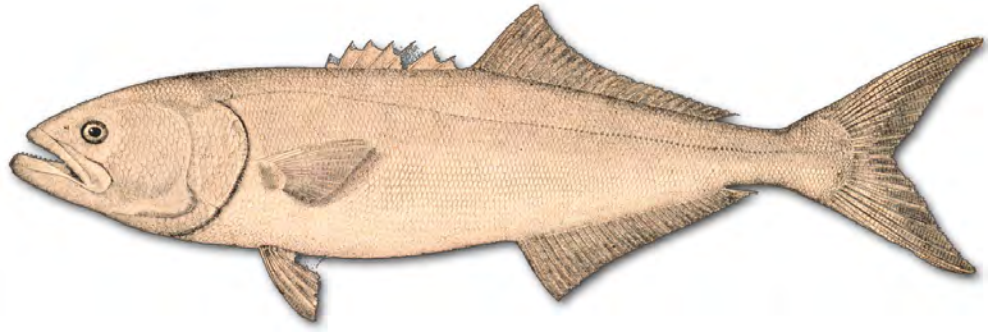
Table 2.2: Age-Length key, as proportion-at-age in each 1-inch length interval, based on otolith ages for Black Drum sampled for age determination in Virginia during 2025.

	Age														
Interval	3	4	5	6	7	8	9	10	13	14	19	20	22	32	45
11 - 11.99	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
12 - 12.99	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
14 - 14.99	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
23 - 23.99	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
24 - 24.99	0.25	0.75	0	0	0	0	0	0	0	0	0	0	0	0	0
25 - 25.99	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
26 - 26.99	0.5	0.5	0	0	0	0	0	0	0	0	0	0	0	0	0
27 - 27.99	0.2	0.2	0.6	0	0	0	0	0	0	0	0	0	0	0	0
28 - 28.99	0.11	0.11	0.56	0.22	0	0	0	0	0	0	0	0	0	0	0
29 - 29.99	0	0.17	0.17	0.33	0.33	0	0	0	0	0	0	0	0	0	0
30 - 30.99	0	0.4	0	0.3	0.2	0	0.1	0	0	0	0	0	0	0	0
31 - 31.99	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
32 - 32.99	0	0	0	0.4	0.2	0	0.2	0.2	0	0	0	0	0	0	0
33 - 33.99	0	0	0	0	0.33	0	0.67	0	0	0	0	0	0	0	0
34 - 34.99	0	0	0	0	0	0.5	0	0.5	0	0	0	0	0	0	0
35 - 35.99	0	0	0	0	0	0	0	0.5	0.5	0	0	0	0	0	0
36 - 36.99	0	0	0	0	0.25	0	0	0.5	0.25	0	0	0	0	0	0
37 - 37.99	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
38 - 38.99	0	0	0	0	0	0	0	0	0	0	0.5	0.5	0	0	0
39 - 39.99	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
41 - 41.99	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
44 - 44.99	0.5	0	0	0	0	0	0	0	0	0	0	0	0	0.5	0
49 - 49.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1

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Chapter 3

BLUEFISH *Pomatomus saltatrix*



3.1 INTRODUCTION

We aged a total of 296 Bluefish *Pomatomus saltatrix*, collected by the VMRC's Biological Sampling Program for age and growth analysis in 2025. Bluefish ages ranged from 0 to 10 years old with an average age of 1.8, a standard deviation of 1.1, and a standard error of 0.06. Seven age classes (0 to 4, and 9 to 10) were represented, comprising fish of the 2015 to 2016, and 2021 to 2025 year-classes. The sample was dominated by fish from the year-class of 2023 with 42.2%.

3.2 METHODS

3.2.1 Sample Size for Ageing

We estimated sample size for ageing Bluefish in 2025 using a two-stage random sampling method (Quinn and Deriso 1999) to increase precision in estimates of age composition from fish sampled efficiently and effectively. The basic equation is:

$$A = \frac{V_a}{\theta_a^2 CV_a^2 + B_a/L} \tag{3.1}$$

where A is the sample size for ageing Bluefish in 2025; θ_a stands for the proportion of Age a fish in a catch; V_a, B_a, and CV_a represent the variance components within and between length intervals, and the coefficient of variation for Age a, respectively; L is the total number of Bluefish used by VMRC to estimate length distribution of the catches from 2019 to 2023. θ_a, V_a, and B_a were calculated using pooled age-length data of Bluefish collected from 2019 to 2023 and using equations in Quinn and Deriso (1999). For simplicity, the equations are not listed here. The equation (1.1) indicates: 1) The more fish that are aged, the smaller the CV_a (or higher precision) that will be obtained for Age a; 2) given a sample size A, the CV_a is different for each age due to different θ_a, V_a, and B_a among different ages. Therefore, the criterion to age A (number) of fish is that A should be a number above which there is only a 1% CV_a reduction for the most abundant age in catch by ageing an additional 100 or more fish. Finally, A_l is A multiplied by the proportion of length interval l from the length distribution of the 2019 to 2023 catch. A_l is number of fish to be aged for length interval l in 2025. Based on VMRC's request in 2010, we used 1-cm length interval for Bluefish, which differed from other species (1-inch).

3.2.2 Handling of Collections

Otoliths were received by the Age and Growth Laboratory in labeled coin envelopes, and were sorted by date of capture. Their envelope labels were verified against VMRC's collection data, and each fish was assigned a unique Age and Growth Laboratory identification number. All otoliths were stored dry in their original labeled coin envelopes.

3.2.3 Preparation

We used our bake and thin-section technique to process Bluefish sagittal otoliths (hereafter, referred to as "otoliths") for age determination (Robillard et al. 2009). Otolith preparation began by randomly selecting either the right or left otolith. Each whole otolith was placed in a ceramic "Coors" spot plate well and baked in a Thermolyne 1400 furnace at 400 °C. Baking time was dependent on the otolith's size and gauged by color, with a light caramel color desired. Once a suitable color was achieved the baked otolith was embedded in epoxy resin with its distal surface orientated downwards. The otoliths were viewed under a stereo microscope to identify the location of the core. Then, the position of the core was marked using an ultra fine Sharpie across the epoxy resin surface. At least one transverse cross-section (hereafter, referred to as "thin-section") was then removed from the marked core of each otolith using a Buehler IsoMet™ low-speed saw equipped with two 4-inch diameter diamond grinding wheels (hereafter, referred to as "blades"), separated by a stainless steel spacer of 0.5 mm (diameter 2.5"). The otolith was

positioned so the blades straddled each side of the otolith core. It was crucial that this cut be perpendicular to the long axis of the otolith. Failure to do so resulted in broad and distorted winter growth zones. A proper cut resulted in annuli that were clearly defined and delineated. Once cut, thin-sections were placed on labeled glass slides and covered with a thin layer of Flo-texx mounting medium that not only fixed the sections to the slide, but more importantly, provided enhanced contrast and greater readability by increasing light transmission through the thin-section.

Click here to obtain the protocol at the VMRV website on how to prepare otolith thin-section using the epoxy resin method for ageing Bluefish.

3.2.4 Readings

The VMRC system assigns an age class to a fish based on a combination of number of annuli in a thin-section, the date of capture, and the species-specific period when the annulus is deposited. Each year, as the fish grows, its otoliths grow and leave behind markers of their age, called an annulus. Technically, an otolith annulus is the combination of both the opaque and the translucent band. In practice, only the opaque bands are counted as annuli and recorded in our ageing notation.

In 2019 a new notation method recommended by Atlantic States Marine Fisheries Commission (ASMFC) was used to assign age on Bluefish. In addition to recording the number of annulus, the margin or the growth width after the last annulus is coded from 1 to 4. The margin code “1”, “2”, “3”, and “4” stands for no growth, the growth width less than or equal to one third of, larger than one third but less than or equal to two thirds of, and larger than two thirds of the growth width formed in the previous year, respectively.

By convention all fish in the Northern Hemisphere are assigned a birth date of January 1. In addition, each species has a specific period during which it deposits the annulus. If a fish is captured after the end of the species-specific annulus deposition period and before January 1, it is assigned an age class as the same as its annulus number without referencing its margin code. If a fish has a margin code of "1", it is assigned an age class as the same as its annulus number no matter in which month it is captured. If a fish is captured after December 31 and before its annulus deposition period, it is assigned an age class as its annulus number plus one when its margin code is "2", "3", or "4". If a fish is captured during its annulus deposition period, it is assigned an age class as the same as its annulus number when its margin code is “2” and as its annulus number plus one when its margin code is “3” or “4” (**Note: Based on the growth of Virginia species we use two criteria for Margin Code 2 to assign a fish an age class depending on its capture month, which could be different from how other states and agencies use Margin Code 2**).

For example, Bluefish otolith annulus formation occurs between March and June (Robillard et al. 2009). A Bluefish with three visible annuli could be assigned an age of 3 or 4 depending on its capture month and margin code. When its margin code is "1", it is Age 3 no matter when it is captured. When it is captured after June and before January, it is Age 3 no matter what its margin code is. When it is captured after December and before March and its margin code is not "1", it is Age 4 (3 + 1 = 4). When it is captured between March and June, it is Age 3 when its margin code is "2" but Age 4 (3 + 1 = 4) when its margin code is "3" or "4".

When an otolith was properly sectioned, the sulcal groove came to a sharp point (Hereafter referred to as "focus") within the middle of the core (Figure 3.1). Typically the first year’s annulus was found by locating the focus of the otolith section, which was characterized as a visually distinct dark, oblong region found in the center of the otolith section. The first year’s annulus had the highest visibility proximal to

the focus along the edge of the sulcal groove. Once located, the first year's annulus was followed outward from the sulcal groove towards the dorsal perimeter of the otolith. Often, but not always, the first year was associated with a very distinct crenellation on the dorsal surface and a prominent protrusion on the ventral surface. Both of these landmarks had a tendency to become less prominent in older fish.

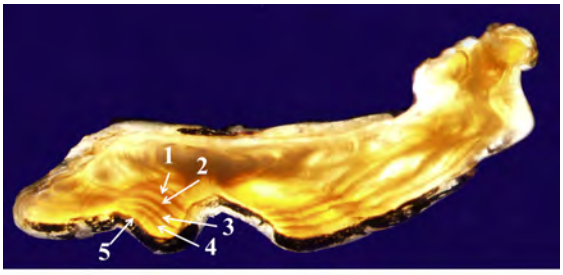


Figure 3.1: Otolith thin-section of a 5 year-old Bluefish with the last annulus on the edge of the thin-section

All thin-sections were aged by two different readers using a Nikon SMZ1000 stereo microscope under transmitted light and dark-field polarization at between 8 and 20 times magnification. Each reader aged all of the otolith samples.

Even with the bake and thin-section technique, interpretation of the growth zones from the otoliths of young Bluefish was difficult. Rapid growth within the first year of life prevents a sharp delineation between opaque and translucent zones. When the exact location of the first year was not clearly evident, and the otolith had been sectioned accurately, a combination of surface landscape (1st year crenellation) and the position of the second annuli were used to help determine the position of the first annulus.

What appeared to be "double annuli" were occasionally observed in Bluefish 4-7 years of age and older. This double-annulus formation was typically characterized by distinct and separate annuli in extremely close proximity to each other. We do not know if the formation of these double annuli were two separate annuli, or in fact only one, but they seemed to occur during times of reduced growth after maturation. "Double annuli" were considered to be one annulus when both marks joined to form a central origin (the origin being the sulcal groove and the outer peripheral edge of the otolith). If these annuli did not meet to form a central origin they were considered two distinct annuli, and were counted as such.

All samples were aged in chronological order, based on collection date, without knowledge of previously estimated ages or the specimen lengths. When the readers' ages agreed, that age was assigned to the fish. When the two readers disagreed, both readers sat down together and re-aged the fish, again without any knowledge of previously estimated ages or lengths, and assigned a final age to the fish. When the readers were unable to agree on a final age, the fish was excluded from further analysis.

3.2.5 Comparison Tests

A symmetry test (Hoenig et al. 1995) and coefficient of variation (CV) analysis were used to detect any systematic difference and precision on age readings, respectively, for the following comparisons: 1) between the two readers in the current year, 2) within each reader in the current year, and 3) time-series bias between the current and previous years within each reader. The readings from the entire sample for the current year were used to examine the difference between two readers. A random sub-sample of 50 fish from the current year was selected for second readings to examine the difference within a reader. Fifty otoliths randomly selected from fish aged in 2000 were used to examine the time-series bias within each reader. A figure of 1:1 equivalence was used to illustrate those differences (Campana et al. 1995). All statistics analyses were performed in R.4.0.2 (R Core Team 2021).

3.3 RESULTS

3.3.1 Sample Size

We estimated a sample size of 418 Bluefish in 2025, ranging in length interval from 18 to 94 centimeters (Table 3.1). This sample size provided a range in CV for age composition approximately from the smallest CV of 5% for Age 1 to the CV of larger than 25% for the multiple minor ages (Table 3.2). In 2025, we aged 296 of 407 Bluefish (The rest of fish were either without otoliths or over-collected for certain length interval(s)) collected by VMRC. We fell short in our over-all collections for this optimal length-class sampling estimate by 150 fish, as a result, the precision for the estimates of major age groups would definitely be influenced significantly. Therefore, precaution should be used when developing ALK using these age data.

3.3.2 Reading Precision

Reader 1 had moderate self-precision and Read 2 had low self-precision. Specifically, there was no significant difference between the first and second readings for Reader 1 with an agreement of 88% and a CV of 8.51% (test of symmetry: $\chi^2 = 6$, $df = 4$, $P = 0.1991$), and there was no significant difference between the first and second readings for Reader 2 with an agreement of 80% and a CV of 14.92% (test of symmetry: $\chi^2 = 3.33$, $df = 5$, $P = 0.6487$). There was an evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 81.76% and a CV of 10.06% (test of symmetry: $\chi^2 = 23.43$, $df = 10$, $P = 0.0093$) (Figure 3.2).

Reader 1 had no time series bias while Read 2 did. Reader 1 had an agreement of 92% with ages of fish aged in 2000 with a CV of 3.77% (test of symmetry: $\chi^2 = 1$, $df = 1$, $P = 0.3173$). Reader 2 had an agreement of 68% with a CV of 14.45% (test of symmetry: $\chi^2 = 16$, $df = 4$, $P = 0.003$).

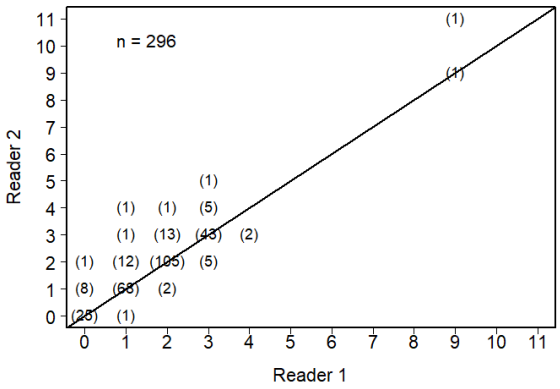


Figure 3.2: Between-reader comparison of otolith age estimates for Bluefish collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2025. The number in parentheses is number of fish.

3.3.3 Year Class

Of the 296 fish aged with otoliths, 7 age classes (0 to 4, and 9 to 10) were represented (Table 3.3). The average age was 1.8 years, and the standard deviation and standard error were 1.1 and 0.06, respectively. Year-class data show that the fishery was comprised of 7 year-classes: fish from the 2015 to 2016, and 2021 to 2025 year-classes, with fish primarily from the year class of 2023 with 42.2%. The ratio of males to females was 1:1.74 in the sample collected (Figure 3.3).

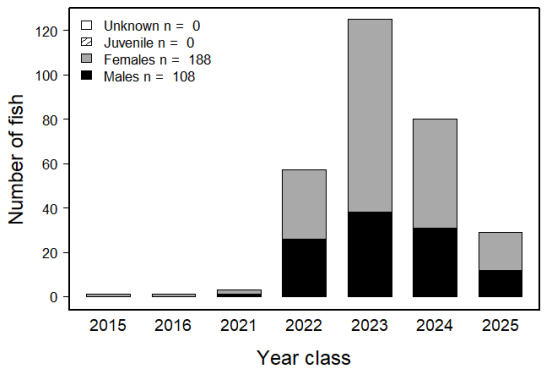


Figure 3.3: Year-class frequency distribution for Bluefish collected for ageing in 2025. Distribution is broken down by sex. 'Unknown' represents gonads that were not available for examination or were not examined for sex during sampling.

3.3.4 Age-length Key (ALK)

We developed an age-length-key (Table 3.4) that can be used in the conversion of numbers-at-length in the estimated catch to numbers-at-age using otolith ages. The table is based on VMRC's stratified sampling of landings by total length cm intervals.

Table 3.1: Number of Bluefish collected and aged in each 1-cm length interval in 2025. 'Target' represents the sample size for ageing estimated for 2025, and 'Need' represents number of fish shorted in each length interval compared to the optimum sample size for ageing and number of fish aged.

Interval	Target	Collected	Aged	Need
18 - 18.99	5	1	1	4
19 - 19.99	5	1	1	4
20 - 20.99	5	1	1	4
21 - 21.99	5	0	0	5
22 - 22.99	5	1	1	4
23 - 23.99	5	1	1	4
24 - 24.99	5	3	3	2
25 - 25.99	5	1	1	4
26 - 26.99	5	5	5	0
27 - 27.99	6	5	5	1
28 - 28.99	6	6	6	0
29 - 29.99	6	7	6	0
30 - 30.99	7	6	6	1
31 - 31.99	7	12	8	0
32 - 32.99	6	10	6	0
33 - 33.99	6	14	6	0
34 - 34.99	6	7	6	0
35 - 35.99	5	9	6	0
36 - 36.99	7	11	8	0
37 - 37.99	7	14	8	0
38 - 38.99	7	16	8	0
39 - 39.99	7	13	8	0
40 - 40.99	6	9	6	0
41 - 41.99	7	6	6	1
42 - 42.99	7	10	8	0
43 - 43.99	7	8	7	0
44 - 44.99	6	10	7	0
45 - 45.99	8	16	8	0
46 - 46.99	7	17	8	0
47 - 47.99	7	13	8	0
48 - 48.99	5	7	6	0
49 - 49.99	5	16	6	0
50 - 50.99	5	11	6	0
51 - 51.99	5	11	6	0
52 - 52.99	5	5	5	0
53 - 53.99	5	8	6	0
54 - 54.99	5	6	6	0
55 - 55.99	5	7	6	0
56 - 56.99	5	9	6	0
57 - 57.99	5	8	6	0
58 - 58.99	5	8	6	0
59 - 59.99	5	8	6	0

(To continue)

Table 3.1 (Continued)

Interval	Target	Collected	Aged	Need
60 - 60.99	5	8	6	0
61 - 61.99	5	8	6	0
62 - 62.99	5	4	4	1
63 - 63.99	5	9	9	0
64 - 64.99	5	4	4	1
65 - 65.99	5	5	5	0
66 - 66.99	5	6	6	0
67 - 67.99	5	3	3	2
68 - 68.99	5	2	2	3
69 - 69.99	5	4	4	1
70 - 70.99	5	1	1	4
71 - 71.99	5	0	0	5
72 - 72.99	5	2	2	3
73 - 73.99	5	1	1	4
74 - 74.99	5	0	0	5
75 - 75.99	5	3	3	2
76 - 76.99	5	1	1	4
77 - 77.99	5	3	3	2
78 - 78.99	5	2	2	3
79 - 79.99	5	1	1	4
80 - 80.99	5	1	1	4
81 - 81.99	5	0	0	5
82 - 82.99	5	0	0	5
83 - 83.99	5	0	0	5
84 - 84.99	5	1	1	4
85 - 85.99	5	0	0	5
86 - 86.99	5	0	0	5
87 - 87.99	5	0	0	5
88 - 88.99	5	0	0	5
89 - 89.99	5	0	0	5
90 - 90.99	5	0	0	5
91 - 91.99	5	0	0	5
92 - 92.99	5	1	1	4
93 - 93.99	5	0	0	5
94 - 94.99	5	0	0	5
Totals	418	407	296	150

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Table 3.2: CV for each age estimated based on ageing the total of 418 Bluefish in 2025. 'Percent' is the percentage of an age in the pooled age-length data of Bluefish collected from 2019 to 2023.

Age	CV	Percent
0	0.18	5.49
1	0.05	34.73
2	0.06	31.55
3	0.14	9.82
4	0.13	8.3
5	0.2	4.4
6	0.24	3.03
7	>0.25	1.52
8	>0.25	0.72
9	>0.25	0.14
10	>0.25	0.14
11	>0.25	0.07
12	>0.25	0.07

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Table 3.3: The number of Bluefish assigned to each total length (cm)-at-age category for 296 fish sampled for otolith age determination in Virginia during 2025.

	Age							
Interval	0	1	2	3	4	9	10	Totals
18 - 18.99	1	0	0	0	0	0	0	1
19 - 19.99	1	0	0	0	0	0	0	1
20 - 20.99	1	0	0	0	0	0	0	1
22 - 22.99	1	0	0	0	0	0	0	1
23 - 23.99	1	0	0	0	0	0	0	1
24 - 24.99	3	0	0	0	0	0	0	3
25 - 25.99	1	0	0	0	0	0	0	1
26 - 26.99	5	0	0	0	0	0	0	5
27 - 27.99	4	1	0	0	0	0	0	5
28 - 28.99	3	3	0	0	0	0	0	6
29 - 29.99	3	3	0	0	0	0	0	6
30 - 30.99	4	1	1	0	0	0	0	6
31 - 31.99	0	8	0	0	0	0	0	8
32 - 32.99	0	4	1	1	0	0	0	6
33 - 33.99	0	6	0	0	0	0	0	6
34 - 34.99	1	5	0	0	0	0	0	6
35 - 35.99	0	5	0	1	0	0	0	6
36 - 36.99	0	6	1	1	0	0	0	8
37 - 37.99	0	5	2	1	0	0	0	8
38 - 38.99	0	5	3	0	0	0	0	8
39 - 39.99	0	4	4	0	0	0	0	8
40 - 40.99	0	1	5	0	0	0	0	6
41 - 41.99	0	5	1	0	0	0	0	6
42 - 42.99	0	1	6	1	0	0	0	8
43 - 43.99	0	0	4	3	0	0	0	7
44 - 44.99	0	1	5	1	0	0	0	7
45 - 45.99	0	2	5	1	0	0	0	8
46 - 46.99	0	4	2	2	0	0	0	8
47 - 47.99	0	1	3	4	0	0	0	8
48 - 48.99	0	0	2	4	0	0	0	6
49 - 49.99	0	1	1	4	0	0	0	6
50 - 50.99	0	1	2	3	0	0	0	6
51 - 51.99	0	1	5	0	0	0	0	6
52 - 52.99	0	0	4	1	0	0	0	5
53 - 53.99	0	2	3	1	0	0	0	6
54 - 54.99	0	1	5	0	0	0	0	6
55 - 55.99	0	1	5	0	0	0	0	6
56 - 56.99	0	1	5	0	0	0	0	6
57 - 57.99	0	0	4	2	0	0	0	6
58 - 58.99	0	0	4	2	0	0	0	6
59 - 59.99	0	0	6	0	0	0	0	6
60 - 60.99	0	0	5	1	0	0	0	6

(To continue)

Table 3.3 (Continued)

	Age							
Interval	0	1	2	3	4	9	10	Totals
61 - 61.99	0	0	4	2	0	0	0	6
62 - 62.99	0	0	4	0	0	0	0	4
63 - 63.99	0	0	9	0	0	0	0	9
64 - 64.99	0	0	2	2	0	0	0	4
65 - 65.99	0	0	3	2	0	0	0	5
66 - 66.99	0	0	4	2	0	0	0	6
67 - 67.99	0	0	1	2	0	0	0	3
68 - 68.99	0	0	1	1	0	0	0	2
69 - 69.99	0	0	0	4	0	0	0	4
70 - 70.99	0	1	0	0	0	0	0	1
72 - 72.99	0	0	1	0	1	0	0	2
73 - 73.99	0	0	0	1	0	0	0	1
75 - 75.99	0	0	1	2	0	0	0	3
76 - 76.99	0	0	0	0	1	0	0	1
77 - 77.99	0	0	1	2	0	0	0	3
78 - 78.99	0	0	0	1	1	0	0	2
79 - 79.99	0	0	0	1	0	0	0	1
80 - 80.99	0	0	0	1	0	0	0	1
84 - 84.99	0	0	0	0	0	1	0	1
92 - 92.99	0	0	0	0	0	0	1	1
Totals	29	80	125	57	3	1	1	296

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Table 3.4: Age-Length key, as proportion-at-age in each 1-cm length interval, based on otolith ages for Bluefish sampled for age determination in Virginia during 2025.

	Age						
Interval	0	1	2	3	4	9	10
18 - 18.99	1	0	0	0	0	0	0
19 - 19.99	1	0	0	0	0	0	0
20 - 20.99	1	0	0	0	0	0	0
22 - 22.99	1	0	0	0	0	0	0
23 - 23.99	1	0	0	0	0	0	0
24 - 24.99	1	0	0	0	0	0	0
25 - 25.99	1	0	0	0	0	0	0
26 - 26.99	1	0	0	0	0	0	0
27 - 27.99	0.8	0.2	0	0	0	0	0
28 - 28.99	0.5	0.5	0	0	0	0	0
29 - 29.99	0.5	0.5	0	0	0	0	0
30 - 30.99	0.67	0.17	0.17	0	0	0	0
31 - 31.99	0	1	0	0	0	0	0
32 - 32.99	0	0.67	0.17	0.17	0	0	0
33 - 33.99	0	1	0	0	0	0	0
34 - 34.99	0.17	0.83	0	0	0	0	0
35 - 35.99	0	0.83	0	0.17	0	0	0
36 - 36.99	0	0.75	0.12	0.12	0	0	0
37 - 37.99	0	0.62	0.25	0.12	0	0	0
38 - 38.99	0	0.62	0.38	0	0	0	0
39 - 39.99	0	0.5	0.5	0	0	0	0
40 - 40.99	0	0.17	0.83	0	0	0	0
41 - 41.99	0	0.83	0.17	0	0	0	0
42 - 42.99	0	0.12	0.75	0.12	0	0	0
43 - 43.99	0	0	0.57	0.43	0	0	0
44 - 44.99	0	0.14	0.71	0.14	0	0	0
45 - 45.99	0	0.25	0.62	0.12	0	0	0
46 - 46.99	0	0.5	0.25	0.25	0	0	0
47 - 47.99	0	0.12	0.38	0.5	0	0	0
48 - 48.99	0	0	0.33	0.67	0	0	0
49 - 49.99	0	0.17	0.17	0.67	0	0	0
50 - 50.99	0	0.17	0.33	0.5	0	0	0
51 - 51.99	0	0.17	0.83	0	0	0	0
52 - 52.99	0	0	0.8	0.2	0	0	0
53 - 53.99	0	0.33	0.5	0.17	0	0	0
54 - 54.99	0	0.17	0.83	0	0	0	0
55 - 55.99	0	0.17	0.83	0	0	0	0
56 - 56.99	0	0.17	0.83	0	0	0	0
57 - 57.99	0	0	0.67	0.33	0	0	0
58 - 58.99	0	0	0.67	0.33	0	0	0
59 - 59.99	0	0	1	0	0	0	0
60 - 60.99	0	0	0.83	0.17	0	0	0

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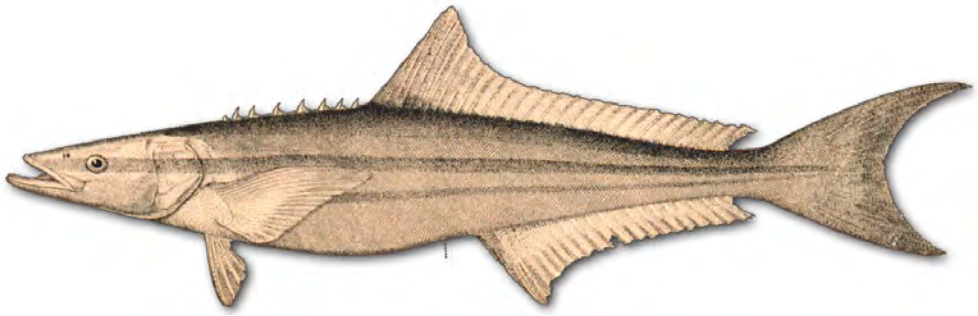
Table 3.4 (Continued)

	Age						
Interval	0	1	2	3	4	9	10
61 - 61.99	0	0	0.67	0.33	0	0	0
62 - 62.99	0	0	1	0	0	0	0
63 - 63.99	0	0	1	0	0	0	0
64 - 64.99	0	0	0.5	0.5	0	0	0
65 - 65.99	0	0	0.6	0.4	0	0	0
66 - 66.99	0	0	0.67	0.33	0	0	0
67 - 67.99	0	0	0.33	0.67	0	0	0
68 - 68.99	0	0	0.5	0.5	0	0	0
69 - 69.99	0	0	0	1	0	0	0
70 - 70.99	0	1	0	0	0	0	0
72 - 72.99	0	0	0.5	0	0.5	0	0
73 - 73.99	0	0	0	1	0	0	0
75 - 75.99	0	0	0.33	0.67	0	0	0
76 - 76.99	0	0	0	0	1	0	0
77 - 77.99	0	0	0.33	0.67	0	0	0
78 - 78.99	0	0	0	0.5	0.5	0	0
79 - 79.99	0	0	0	1	0	0	0
80 - 80.99	0	0	0	1	0	0	0
84 - 84.99	0	0	0	0	0	1	0
92 - 92.99	0	0	0	0	0	0	1

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Chapter 4

COBIA *Rachycentron canadum*



4.1 INTRODUCTION

We aged a total of 194 Cobia *Rachycentron canadum*, collected by the VMRC's Biological Sampling Program for age and growth analysis in 2025. Cobia ages ranged from 3 to 11 years old with an average age of 6.3, a standard deviation of 2.2, and a standard error of 0.16. Nine age classes (3 to 11) were represented, comprising fish of the 2014 to 2022 year-classes. The sample was dominated by fish from the year-classes of 2016, 2018, 2020, and 2021 with 16%, 17%, 19.1%, and 25.3%, respectively.

4.2 METHODS

4.2.1 Handling of Collections

Sagittal otoliths, hereafter, referred to as "otoliths", were received by the Age and Growth Laboratory in labeled coin envelopes and were sorted by date of capture, their envelope labels were verified against VMRC's collection data, and each fish was assigned a unique Age and Growth Laboratory identification number. All otoliths were stored inside of protective Axygen 2 ml micro-tubes within their original labeled coin envelopes.

4.2.2 Preparation

Otoliths were processed for age determination. The left or right otolith was randomly selected and embedded, distal side down, in epoxy resin and allowed to harden overnight. The otoliths were viewed by eye, and when necessary, under a stereo microscope to identify the location of the core, and the position of the core marked using an ultra fine Sharpie across the epoxy resin surface. At least one transverse cross-section (hereafter "thin-section") was then removed from the marked core of each otolith using a Buehler IsoMet^{TM} low-speed saw equipped with two 4-inch diameter diamond grinding Wheels, separated by a stainless steel spacer of 0.5 mm (diameter 2.5"). The position of the marked core fell within the 0.5 mm space between the blades, such that the core was included in the removed thin section. Otolith thin-sections were placed on labeled glass slides and covered with a thin layer of Flo-texx mounting medium that not only fixed the sections to the slide, but more importantly, provided enhanced contrast and greater readability by increasing light transmission through the section.

Click here to obtain the protocol at the VMRV website on how to prepare otolith thin-section using the epoxy resin method for ageing Cobia.

4.2.3 Readings

The VMRC system assigns an age class to a fish based on a combination of number of annuli in a thin-section, the date of capture, and the species-specific period when the annulus is deposited. Each year, as the fish grows, its otoliths grow and leave behind markers of their age, called an annulus. Technically, an otolith annulus is the combination of both the opaque and the translucent band. In practice, only the opaque bands are counted as annuli and recorded in our ageing notation.

In 2019 a new notation method recommended by Atlantic States Marine Fisheries Commission (ASMFC) was used to assign age on Cobia. In addition to recording the number of annulus, the margin or the growth width after the last annulus is coded from 1 to 4. The margin code “1”, “2”, “3”, and “4” stands for no growth, the growth width less than or equal to one third of, larger than one third but less than or equal to two thirds of, and larger than two thirds of the growth width formed in the previous year, respectively.

By convention all fish in the Northern Hemisphere are assigned a birth date of January 1. In addition, each species has a specific period during which it deposits the annulus. If a fish is captured after the end of the species-specific annulus deposition period and before January 1, it is assigned an age class as the same as its annulus number without referencing its mar-

gin code. If a fish has a margin code of "1", it is assigned an age class as the same as its annulus number no matter in which month it is captured. If a fish is captured after December 31 and before its annulus deposition period, it is assigned an age class as its annulus number plus one when its margin code is "2", "3", or "4". If a fish is captured during its annulus deposition period, it is assigned an age class as the same as its annulus number when its margin code is "2" and as its annulus number plus one when its margin code is "3" or "4" (**Note: Based on the growth of Virginia species we use two criteria for Margin Code 2 to assign a fish an age class depending on its capture month, which could be different from how other states and agencies use Margin Code 2**).

For example, Cobia otolith annulus formation occurs between June and July (Richards 1967 and modified by CQFE/ODU). A Cobia with five visible annuli could be assigned an age of 5 or 6 depending on its capture month and margin code. When its margin code is "1", it is Age 5 no matter when it is captured. When it is captured after July and before January, it is Age 5 no matter what its margin code is. When it is captured after December and before June and its margin code is not "1", it is Age 6 ($5 + 1 = 6$). When it is captured between June and July, it is Age 5 when its margin code is "2" but Age 6 ($5 + 1 = 6$) when its margin code is "3" or "4".

When an otolith was properly sectioned, the sulcal groove came to a sharp point (Hereafter referred to as "focus") within the middle of the core (Figure 4.1). Typically the first year's annulus was found by locating the focus of the otolith section, which was characterized as a visually distinct dark, oblong region found in the center of the otolith section.

All samples were aged by two readers in chronological order, based on collection date, without knowledge of previously estimated ages or the specimen lengths. When the readers' ages agreed, that age was assigned to the fish. When the two readers disagreed, both readers sat down together and re-aged the fish, again without any knowledge of previously estimated ages or lengths, and assigned a final age to the fish. When the readers were unable to agree on a final age, the fish was excluded from further analysis. All thin-sections were aged using a Nikon SMZ1000 stereo microscope under transmitted light and dark-field polarization at between 8 and 20 times magnification.

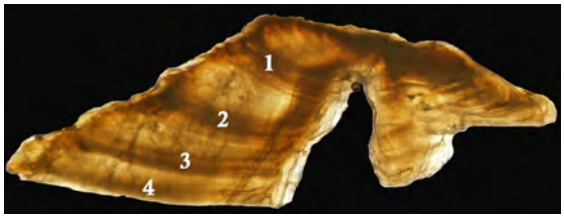


Figure 4.1: Otolith thin-section of a 4 year-old Cobia.

4.2.4 Comparison Tests

A symmetry test (Hoenig et al. 1995) and coefficient of variation (CV) analysis were used to detect any systematic difference and precision on age readings, respectively, for the following comparisons: 1) between the two readers in the current year, 2) within each reader in the current year, and 3) time-series bias between the current and previous years within each reader. The readings from the entire sample for the current year were used to examine the difference between two readers. A random sub-sample of 50 fish from the current year was selected for second readings to examine the difference within a reader. Fifty otoliths randomly selected from fish aged in 2000 were used to examine the time-series bias within each reader. A figure of 1:1 equivalence was used to illustrate those differences (Campana et al. 1995). All statistics analyses were performed in R.4.0.2 (R Core Team 2021).

4.3 RESULTS

4.3.1 Reading Precision

Both readers had high self-precision. Specifically, there was no significant difference between the first and second readings for Reader 1 with an agreement of 92% and a CV of 1.12% (test of symmetry: $\chi^2 = 4$, $df = 4$, $P = 0.406$), and there was no significant difference between the first and second readings for Reader 2 with an agreement of 94% and a CV of 0.5% (test of symmetry: $\chi^2 = 3$, $df = 3$, $P = 0.3916$). There was no evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 90.21% and a CV of 1.35% (test of symmetry: $\chi^2 = 6.33$, $df = 8$, $P = 0.6099$) (Figure 4.2).

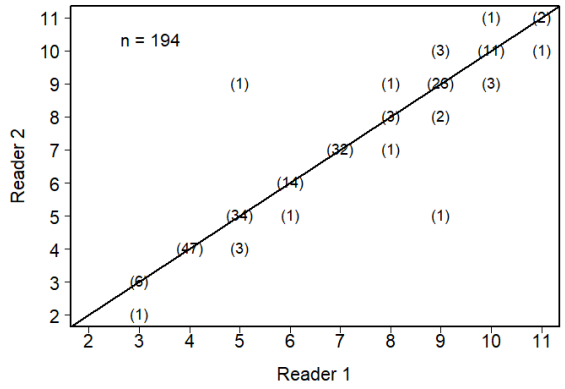


Figure 4.2: Between-reader comparison of otolith age estimates for Cobia collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2025. The number in parentheses is number of fish.

There was no time-series bias for either reader. Reader 1 had an agreement of 86% with ages of fish aged in 2000 with a CV of 1.72% (test of symmetry: $\chi^2 = 5$, $df = 6$, $P = 0.5438$). Reader 2 had an agreement of 92% with a CV of 0.53% (test of symmetry: $\chi^2 = 1.33$, $df = 2$, $P = 0.5134$).

4.3.2 Year Class

We aged the otoliths of all the 194 Cobia collected in 2025. Of the 194 fish aged, 9 age classes (3 to 11) were represented (Table 4.1). The average age was 6.3 years, and the standard deviation and standard error were 2.2 and 0.16, respectively. Year-class data show that the fishery was comprised of 9 year-classes: fish from the 2014 to 2022 year-classes, with fish primarily from the year classes of 2016, 2018, 2020, and 2021 with 16%, 17%, 19.1%, and 25.3%, respectively. The ratio of males to females was 1:1.53 in the sample collected (Figure 4.3).

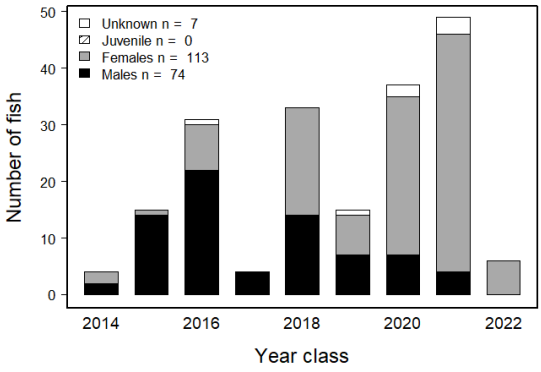


Figure 4.3: Year-class frequency distribution for Cobia collected for ageing in 2025. Distribution is broken down by sex. 'Unknown' represents gonads that were not available for examination or were not examined for sex during sampling.

4.3.3 Age-length Key (ALK)

We developed an age-length-key (Table 4.2) that can be used in the conversion of numbers-at-length in the estimated catch to numbers-at-age using otolith ages. The table is based on VMRC's stratified sampling of landings by total length inch intervals.

Table 4.1: The number of Cobia assigned to each total length (inch)-at-age category for 194 fish sampled for otolith age determination in Virginia during 2025.

	Age									
Interval	3	4	5	6	7	8	9	10	11	Totals
37 - 37.99	0	1	0	0	0	0	0	0	0	1
38 - 38.99	0	2	0	0	1	0	0	0	0	3
39 - 39.99	0	3	1	0	0	0	0	0	0	4
40 - 40.99	3	4	2	1	2	0	1	0	0	13
41 - 41.99	2	13	3	1	1	2	3	0	0	25
42 - 42.99	1	4	6	3	4	1	3	1	0	23
43 - 43.99	0	9	3	1	2	1	7	1	0	24
44 - 44.99	0	6	6	1	4	0	5	1	0	23
45 - 45.99	0	4	7	2	2	0	2	5	1	23
46 - 46.99	0	0	5	0	1	0	1	1	1	9
47 - 47.99	0	1	1	2	1	0	1	3	0	9
48 - 48.99	0	1	2	1	3	0	2	1	0	10
49 - 49.99	0	0	1	3	1	0	0	1	0	6
50 - 50.99	0	0	0	0	3	0	0	0	0	3
51 - 51.99	0	0	0	0	5	0	1	0	0	6
52 - 52.99	0	0	0	0	0	0	3	0	1	4
53 - 53.99	0	0	0	0	1	0	2	1	0	4
54 - 54.99	0	0	0	0	1	0	0	0	0	1
63 - 63.99	0	1	0	0	0	0	0	0	0	1
75 - 75.99	0	0	0	0	1	0	0	0	0	1
76 - 76.99	0	0	0	0	0	0	0	0	1	1
Totals	6	49	37	15	33	4	31	15	4	194

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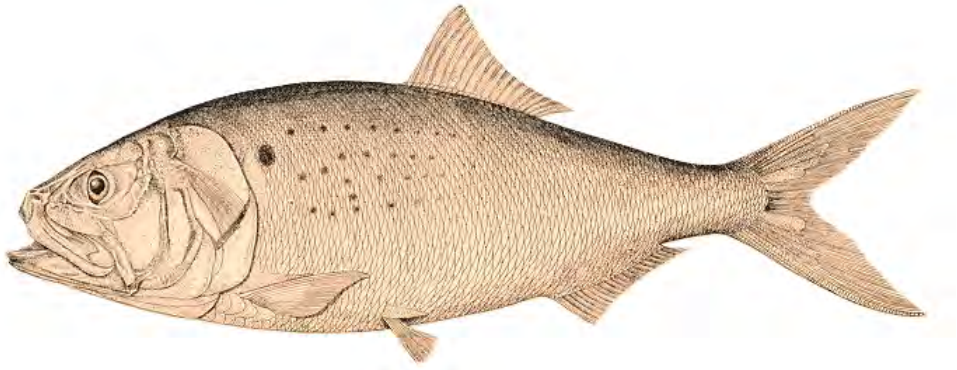
Table 4.2: Age-Length key, as proportion-at-age in each 1-inch length interval, based on otolith ages for Cobia sampled for age determination in Virginia during 2025.

	Age								
Interval	3	4	5	6	7	8	9	10	11
37 - 37.99	0	1	0	0	0	0	0	0	0
38 - 38.99	0	0.67	0	0	0.33	0	0	0	0
39 - 39.99	0	0.75	0.25	0	0	0	0	0	0
40 - 40.99	0.23	0.31	0.15	0.08	0.15	0	0.08	0	0
41 - 41.99	0.08	0.52	0.12	0.04	0.04	0.08	0.12	0	0
42 - 42.99	0.04	0.17	0.26	0.13	0.17	0.04	0.13	0.04	0
43 - 43.99	0	0.38	0.12	0.04	0.08	0.04	0.29	0.04	0
44 - 44.99	0	0.26	0.26	0.04	0.17	0	0.22	0.04	0
45 - 45.99	0	0.17	0.3	0.09	0.09	0	0.09	0.22	0.04
46 - 46.99	0	0	0.56	0	0.11	0	0.11	0.11	0.11
47 - 47.99	0	0.11	0.11	0.22	0.11	0	0.11	0.33	0
48 - 48.99	0	0.1	0.2	0.1	0.3	0	0.2	0.1	0
49 - 49.99	0	0	0.17	0.5	0.17	0	0	0.17	0
50 - 50.99	0	0	0	0	1	0	0	0	0
51 - 51.99	0	0	0	0	0.83	0	0.17	0	0
52 - 52.99	0	0	0	0	0	0	0.75	0	0.25
53 - 53.99	0	0	0	0	0.25	0	0.5	0.25	0
54 - 54.99	0	0	0	0	1	0	0	0	0
63 - 63.99	0	1	0	0	0	0	0	0	0
75 - 75.99	0	0	0	0	1	0	0	0	0
76 - 76.99	0	0	0	0	0	0	0	0	1

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Chapter 5

ATLANTIC MENHADEN *Brevoortia tyrannus*



5.1 INTRODUCTION

We aged a total of 187 Menhaden *Brevoortia tyrannus*, collected by the VMRC's Biological Sampling Program for age and growth analysis in 2025. Menhaden ages ranged from 2 to 7 years old with an average age of 4, a standard deviation of 0.9, and a standard error of 0.07. Six age classes (2 to 7) were represented, comprising fish of the 2018 to 2023 year-classes. The sample was dominated by fish from the year-classes of 2020, 2021, and 2022 with 22%, 46%, and 24%, respectively.

5.2 METHODS

5.2.1 Sample Size for Ageing

Atlantic States Marine Fisheries Commission (ASMFC) requires Virginia to collected a 10-fish per 200 mt landed for bait purpose (ASMFC 2017). Based on the ASMFC requirement, Virginia was responsible to collect 14 10-fish samples from non-purse seine in 2025, 140 fish in total.

5.2.2 Handling of Collection

Sagittal otoliths (hereafter, referred to as "otoliths") and scales were received by the Age and Growth Laboratory in labeled coin envelopes, and were sorted based on date of capture. Their envelope labels were verified against VMRC's collection data, and each fish assigned a unique Age and Growth Laboratory identification number. All otoliths and scales were stored dry within their original labeled coin envelopes; otoliths were contained inside protective Axygen 2.0 ml microtubes.

5.2.3 Preparation

5.2.3.1 *Scales*

Menhaden scales were prepared for age and growth analysis by making acetate impressions of the scale microstructure. Due to extreme variation in the size and shape of scales from individual fish, we selected only those scales that had even margins and which were of uniform size. We selected a range of six to eight preferred scales (based on overall scale size) from each fish, making sure that only non-regenerated scales were used. Scale impressions were made on extruded clear acetate sheets (25 mm x 75 mm) with a Carver Laboratory Heated Press (model "C"). The scales were pressed with the following settings:

Pressure: 15000 psi
Temperature: 77 °C (170 °F)
Time: 5 to 10 min

After pressing, the impressions were viewed with a Bell and Howell microfiche reader and checked again for regeneration and incomplete margins. Impressions that were too light, or when all scales were regenerated a new impression was made using different scales from the same fish.

Click here to refer to the protocol at the VMRC website on how to prepare scale impression using Striped Bass as an example.

5.2.3.2 *Otoliths*

We made a whole-otolith slide by putting the otolith on the microscope slide and covering the otolith with a drop of Flo-texx to fix the otolith on the slide and increase its readability.

5.2.4 Readings

The Ageing Lab system assigns an age class to a fish based on a combination of reading the information contained in otolith, the date of its capture, and the species-specific period when it deposits its annulus. Each year, as the fish grows, its otoliths grow and leave behind markers of their age, called annuli. Technically, an otolith annulus is the combination of both the opaque and the translucent bands. In practice, only the opaque bands are counted as annuli. The number of these visible dark bands replaces "x" in our notation below, and is the initial "age" assignment of the fish.

Second, the otolith is examined for translucent

growth. If no translucent growth is visible beyond the last dark annulus, the otolith is called "even" and no modification of the assigned age is made. The initial assigned age, then, is the age class of the fish. Any growth beyond the last annulus can be interpreted as either being toward the next age class or within the same age class. If translucent growth is visible beyond the last dark annulus, a "+" is added to the notation.

By convention all fish in the Northern Hemisphere are assigned a birth date of January 1, however, Menhaden birth date is assigned to March 1. In addition, each species has a specific period during which it deposits the dark band of the annulus. If a menhaden is captured after the end of the species-specific annulus deposition period and before March 1, it is assigned an age class notation of "x+x", where "x" is the number of dark bands in the otolith. If the fish is captured between March 1 and the end of the species-specific annulus deposition period, it is assigned an age class notation of "x+(x+1)". Thus, any growth beyond the last annulus, after its "birthday", but before the dark band deposition period, is interpreted as being toward the next age class.

For example, Menhaden otolith deposition occurs between March and May. A Menhaden captured between March 1 and May 31, before the end of the species' annulus formation period, with three visible annuli and some translucent growth after the last annulus, would be assigned an age class of "x+(x+1)" or 3+(3+1), noted as 3+4. This is the same age-class assigned to a fish with four visible annuli captured after the end of May 31, the period of annulus formation, which would be noted as 4+4.

Menhaden scales are also considered to have a deposition between March and May, and age class assignment using these hard-parts is conducted in the same way as otoliths.

All Menhaden samples (scale pressings and whole otoliths) were aged by two different readers in chronological order based on collection date, without knowledge of previously estimated ages or the specimen lengths. When the readers' ages agreed, that age was assigned to the fish. When the two readers disagreed, both readers sat down together and re-aged the fish again without any knowledge of previously estimated ages or lengths, then assigned a final age to the fish. When the age readers were unable to agree on a final age, the fish was excluded from further analysis.

5.2.4.1 *Scales*

We determined fish age by viewing acetate impressions of scales (Figure 5.1) using a Nikon SMZ1000 stereo microscope under transmitted light and dark-field polarization at between 8 and 20 times magnification. Annuli on Menhaden scales are identified based on two scale microstructure features, "crossing over" and circuli disruption. Primarily, "crossing over" in the lateral margins near the posterior/anterior interface of the scale is used to determine the origin of the annulus. Here compressed circuli (annulus) "cross-over" the previously deposited circuli of the previous year's growth. Typically annuli of the first three years can be observed transversing this interface as dark bands. These bands remain consistent throughout the posterior field and rejoin the posterior/anterior interface on the opposite

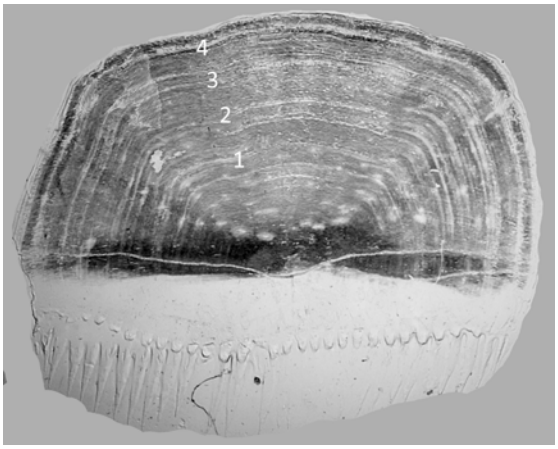


Figure 5.1: The impression of a Menhaden scale with 4 annuli. Each annulus consists of two rings very close to each other.

side of the focus.

When ageing young Menhaden, zero through age two, extreme caution must be taken as not to over age the structure. Moreover, very often one annulus may consist of two rings very close to each other, therefore, try to avoid counting the double rings as two annuli.

5.2.4.2 *Otoliths*

By convention an annulus is identified as the narrow opaque zone, or winter growth (Figure 5.2). Typically the first year's annulus can be determined by first locating the core of the otolith. The core is generally located, depending on preparation, in the center of the otolith, and is visually well defined as a dark oblong region. The first year's annulus can be located directly around the core. However, the annuli appear more visible at the anterior area than at posterior, dorsal, and ventral area.

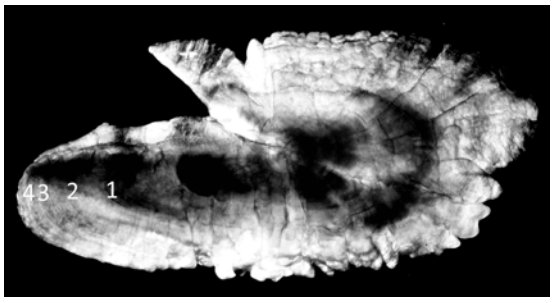


Figure 5.2: The whole otolith of a Menhaden with 4 annuli. The annuli are more visible at the anterior area.

All whole otoliths were aged by two different readers using a Nikon SMZ1000 stereo microscope under transmitted light and dark-field polarization at between 8 and 20 times magnification. Each reader aged all of the otolith samples.

5.2.5 Comparison Tests

A symmetry test (Hoenig et al. 1995) and coefficient of variation (CV) analysis were used to detect any systematic difference and precision on age readings, respectively, for following comparisons: 1) between the two readers in the current year; 2) within each reader in the current year; and 3) between scale and otoliths ages. Since we started to collect and age Menhaden in 2024 and are learning how to age them, we are not able to establish any sets of scales and otoliths for time-series analysis. The readings from the entire sample for the current year were used to examine the difference between two readers. A random sub-sample of 50 fish from the current year was selected for second readings to examine the difference within a reader. A figure of 1:1 equivalence was used to illustrate those differences (Campana et al. 1995). All statistics analyses were performed in R.4.0.2 (R Core Team 2021).

5.3 RESULTS

5.3.1 Sample Size

In 2025, the VMRC Biological Sampling Program collected 324 Menhaden in order to increase the samples of paired scales and otoliths for our comparison study on the scale- and otolith-method. As a result, we were able to age the total of 154 mehaden with paired scales and otoliths, a few more fish than estimated (140). In addition, we also aged 33 menhaden using their scales only, making the total sample size up to 187.

5.3.2 Reading Precision

5.3.2.1 *Scales*

Reader 1 and Reader 2 aged the scales of 187 Menhaden collected in 2025. Reader 1 had moderate self-precision and Read 2 had low self-precision. Specifically, there was no significant difference between the first and second readings for Reader 1 with an agreement of 54% (1 year or less agreement of 94%) and a CV of 9.7% (test of symmetry: $\chi^2 = 2.9$, $df = 5$, $P = 0.7093$), and there was no significant difference between the first and second readings for Reader 2 with an agreement of 48% (1 year or less agreement of 78%) and a CV of 14.5% (test of symmetry: $\chi^2 = 9.9$, $df = 8$, $P =$

0.2745). There was an evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 48% (1 year or less agreement of 88%) and a CV of 12.7% (test of symmetry: $\chi^2 = 40.1$, $df = 11$, $P < 0.0001$) (Figure 5.3).

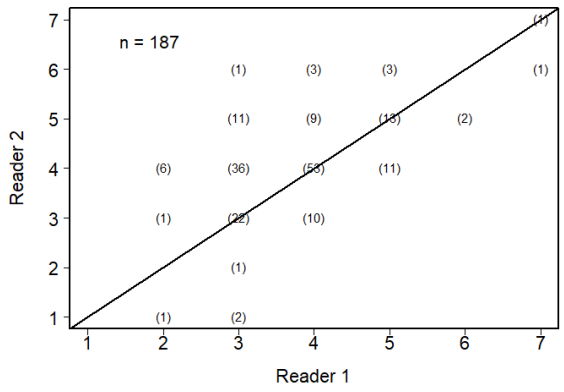


Figure 5.3: Between-reader comparison of scale age estimates for Menhaden collected in Chesapeake Bay in 2025. The number in parentheses is number of fish.

5.3.2.2 *Otoliths*

Reader 1 and Reader 2 aged the otoliths of 154 Menhaden collected in 2025. Reader 1 had moderate self-precision and Read 2 had low self-precision. Specifically, there was no significant difference between the first and second readings for Reader 1 with an agreement of 64% (1 year or less agreement of 94%) and a CV of 7.4% (test of symmetry: $\chi^2 = 4.8$, $df = 6$, $P = 0.5672$), and there was a significant difference between the first and second readings for Reader 2 with an agreement of 36% (1 year or less agreement of 80%) and a CV of 14.3% (test of symmetry: $\chi^2 = 20.3$, $df = 11$, $P = 0.041$). There was an evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 56% (1 year or less agreement of 90%) and a CV of 9.4% (test of symmetry: $\chi^2 = 32.7$, $df = 10$, $P = 0.0003$) (Figure 5.4).

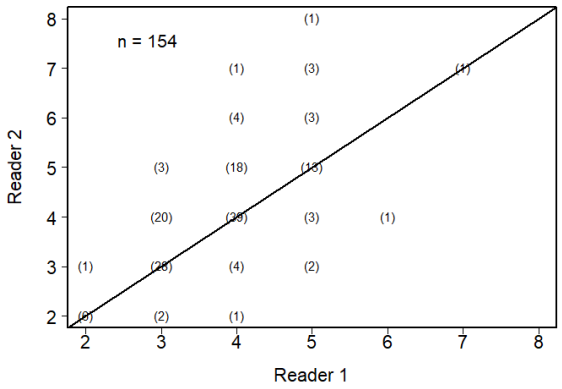


Figure 5.4: Between-reader comparison of otolith age estimates for Menhaden collected in Chesapeake Bay in 2025. The number in parentheses is number of fish.

5.3.3 Year Class

We aged the total of 187 Menhaden collected in 2025 using either their scales, otoliths, or both. Of the 187 fish aged, 6 age classes (2 to 7) were represented (Table 5.1). The average age was 4 years, and the standard deviation and standard error were 0.9 and 0.07, respectively. Year-class data show that the fishery was comprised of 6 year-classes: fish from the 2018 to 2023 year-classes, with fish primarily from the year classes of 2020, 2021, and 2022 with 22%, 46%, and 24%, respectively. The ratio of males to females was 1:1.22 in the sample collected (Figure 5.5).

5.3.4 Age-length Key (ALK)

We developed an age-length-key (Table 5.2) that can be used in the conversion of numbers-at-length in the estimated catch to numbers-at-age using both otolith and scale ages. The table is based on VMRC's stratified sampling of landings by total length inch intervals.

5.3.5 Comparison of Scale and Otolith Ages

We aged 154 pairs of Menhaden scales and otoliths. There was no evidence of systematic disagreement between otolith and scale ages (test of symmetry: $\chi^2 = 14.3$, $df = 11$, $P =$

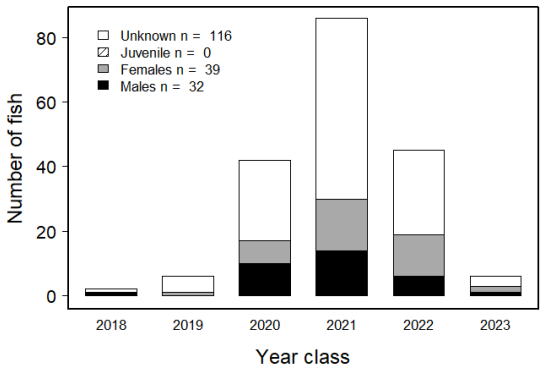


Figure 5.5: Year-class frequency distribution for Menhaden collected for ageing in 2025. Distribution is broken down by sex. 'Unknown' represents gonads that were not available for examination or were not examined for sex during sampling.

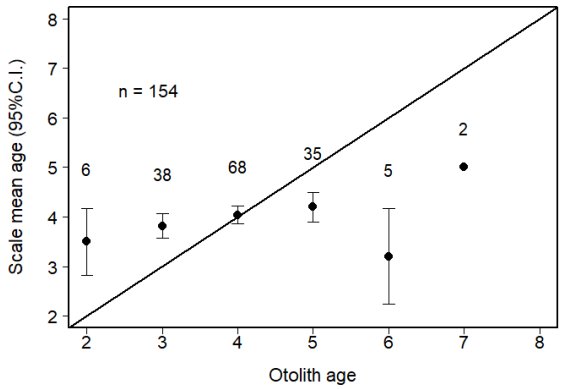


Figure 5.7: Age-bias plot for Menhaden scale and otolith age estimates in 2025. The number above the upper CI bar is number of fish.

0.2185) with an average CV of 14.2%. There was an agreement of 40% between scale and otoliths ages whereas scales were assigned a lower and higher age than otoliths for 29.2% and 30.5% of the fish, respectively (Figure 5.6). There was also little evidence of bias between otolith and scale ages using an age bias plot (Figure 5.7), with no trend of either over-ageing younger or under-ageing older fish.

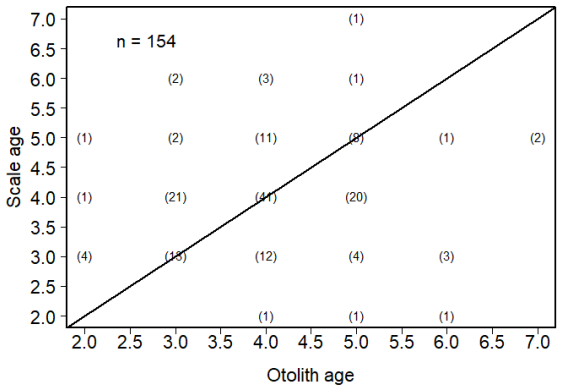


Figure 5.6: Comparison of scale and otolith age estimates for Menhaden collected in Chesapeake Bay in 2025. The number in parentheses is number of fish.

5.4 RECOMMENDATIONS

We recommend to use impressed scale slides for ageing Menhaden because it is easily to store impressed scale slides in slide boxes for a long time. In addition, we have not found any significant differences between age estimates using the scale impression and the scales between two glasses (Please see Appendix in VMRC Ageing Lab 2024 Annual Report). We also recommend whole otoliths mounted on glass slides for ageing Menhaden because it is much easier to handle, store, transport, and age whole otoliths slides than the otoliths stored in vials and aged in water. We will continue to compare the age estimates between whole otoliths and scale acetate impressions in 2026.

Table 5.1: The number of Menhaden assigned to each total length (inch)-at-age category for 187 fish sampled for both otolith and scale age determination in Virginia during 2025.

	Age						
Interval	2	3	4	5	6	7	Totals
8 - 8.99	0	0	1	0	0	0	1
9 - 9.99	0	2	1	0	0	0	3
10 - 10.99	3	6	3	1	0	0	13
11 - 11.99	1	10	21	9	2	0	43
12 - 12.99	2	20	51	19	2	1	95
13 - 13.99	0	7	9	13	2	1	32
Totals	6	45	86	42	6	2	187

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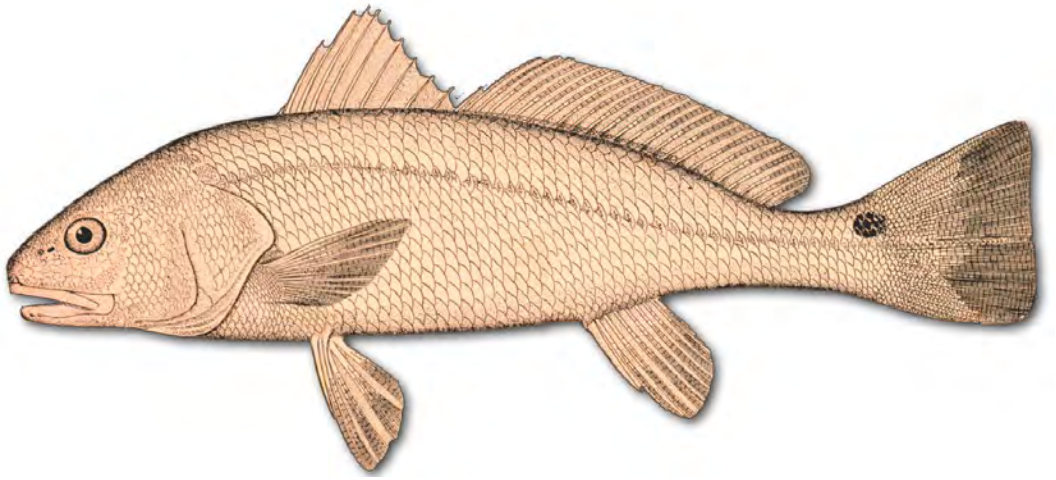
Table 5.2: Age-Length key, as proportion-at-age in each 1-inch length interval, based on both otolith and scale ages for Menhaden sampled for age determination in Virginia during 2025.

	Age					
Interval	2	3	4	5	6	7
8 - 8.99	0	0	1	0	0	0
9 - 9.99	0	0.67	0.33	0	0	0
10 - 10.99	0.23	0.46	0.23	0.08	0	0
11 - 11.99	0.02	0.23	0.49	0.21	0.05	0
12 - 12.99	0.02	0.21	0.54	0.2	0.02	0.01
13 - 13.99	0	0.22	0.28	0.41	0.06	0.03

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Chapter 6

RED DRUM *Sciaenops ocellatus*



6.1 INTRODUCTION

We aged a total of 97 Red Drum *Sciaenops ocellatus*, collected by the VMRC's Biological Sampling Program for age and growth analysis in 2025. Red drum ages ranged from 0 to 3 years old with an average age of 1.5, a standard deviation of 0.6, and a standard error of 0.06. Four age classes (0 to 3) were represented, comprising fish of the 2022 to 2025 year-classes. The sample was dominated by fish from the year-classes of 2023 and 2024 with 42.3% and 49.5%, respectively.

6.2 METHODS

6.2.1 Handling of Collections

Sagittal otoliths, hereafter, referred to as "otoliths", were received by the Age and Growth Laboratory in labeled coin envelopes, and were sorted by date of capture. Their envelope labels were verified against VMRC's collection data, and each fish was assigned a unique Age and Growth Laboratory identification number. All otoliths were stored dry in their original labeled coin envelopes.

6.2.2 Preparation

Otoliths were processed for age determination following the methods described in Ross et al. (1995) and Jones and Wells (1998) for Red Drum. The left or right sagittal otolith was randomly selected and attached, distal side down, to a 1 x 2 inch piece of water resistant grid paper (Brand name: Write in the Rain) using hot glue. The otoliths were viewed by eye, and when necessary, under a stereo microscope to identify the location of the core, and the position of the core marked using an ultra fine Sharpie across the otolith surface. At least one transverse cross-section (hereafter "thin-section") was then removed from the marked core of each otolith using a Buehler IsoMet^{TM} low-speed saw equipped with two 4-inch diameter diamond grinding Wheels, separated by a stainless steel spacer of 0.5 mm (diameter 2.5"). The position of the marked core fell within the 0.5 mm space between the blades, such that the core was included in the removed thin-section. Otolith thin-sections were placed on labeled glass slides and covered with a thin layer of Flo-texx mounting medium that not only fixed the sections to the slide, but more importantly, provided enhanced contrast and greater readability by increasing light transmission through the sections.

Click here to obtain the protocol at the VMRV website on how to prepare otolith thin-section using the hot glue method for ageing Red Drum.

6.2.3 Readings

The VMRC system assigns an age class to a fish based on a combination of number of annuli in a thin-section, the date of capture, and the species-specific period when the annulus is deposited. Each year, as the fish grows, its otoliths grow and leave behind markers of their age, called an annulus. Technically, an otolith annulus is the combination of both the opaque and the translucent band. In practice, only the opaque bands are counted as annuli and recorded in our ageing notation.

In 2019 a new notation method recommended by Atlantic States Marine Fisheries Commission (ASMFC) was used to assign age on Red Drum. In addition to recording the number of annulus, the margin or the growth width after the last annulus is coded from 1 to 4. The margin code “1”, “2”, “3”, and “4” stands for no growth, the growth width less than or equal to one third of, larger than one third but less than or equal to two thirds of, and larger than two thirds of the growth width formed in the previous year, respectively.

By convention all fish in the Northern Hemisphere are assigned a birth date of January 1. In addition, each species has a specific period during which it deposits the annulus. If a fish is captured after the end of the species-specific annulus deposition period and before January

1, it is assigned an age class as the same as its annulus number without referencing its margin code. If a fish has a margin code of "1", it is assigned an age class as the same as its annulus number no matter in which month it is captured. If a fish is captured after December 31 and before its annulus deposition period, it is assigned an age class as its annulus number plus one when its margin code is "2", "3", or "4". If a fish is captured during its annulus deposition period, it is assigned an age class as the same as its annulus number when its margin code is "2" and as its annulus number plus one when its margin code is "3" or "4" (**Note: Based on the growth of Virginia species we use two criteria for Margin Code 2 to assign a fish an age class depending on its capture month, which could be different from how other states and agencies use Margin Code 2**).

For example, Red Drum otolith annulus formation occurs between March and July (Ross et al. 1995 and modified by CQFE/ODU). A Red Drum with two visible annuli could be assigned an age of 2 or 3 depending on its capture month and margin code. When its margin code is "1", it is Age 2 no matter when it is captured. When it is captured after July and before January, it is Age 2 no matter what its margin code is. When it is captured after December and before March and its margin code is not "1", it is Age 3 (2 + 1 = 3). When it is captured between March and July, it is Age 2 when its margin code is "2" but Age 3 (2 + 1 = 3) when its margin code is "3" or "4".

When an otolith was properly sectioned, the sulcal groove came to a sharp point (Hereafter referred to as "focus") within the middle of the core (Figure 6.1). Typically the first year's annulus was found by locating the focus of the otolith section, which was characterized as a visually distinct dark, oblong region found in the center of the otolith section. However, due to discrepancy on identification of the first annulus of Red Drum among Atlantic states, ASMFC has decided not to count the smallest annulus at the center of the thin-section as the first annulus. Following ASMFC's instruction, we didn't count the smallest annulus at the center as the first annulus in 2025.

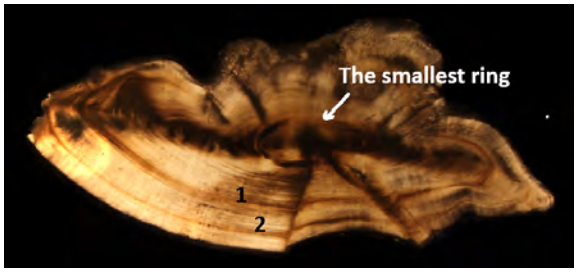


Figure 6.1: Otolith thin-sections of a red drum with 2 annuli and without counting the smallest ring.

All samples were aged by two readers in chronological order, based on collection date, without knowledge of previously estimated ages or the specimen lengths. When the readers' ages agreed, that age was assigned to the fish. When the two readers disagreed, both readers sat down together and re-aged the fish, again without any knowledge of previously estimated ages or lengths, and assigned a final age to the fish. When the readers were unable to agree on a final age, the fish was excluded from further analysis. All thin-sections were aged using a Nikon SMZ1000 stereo microscope under transmitted light and dark-field polarization at between 8 and 20 times magnification (Figure 6.1).

6.2.4 Comparison Tests

A symmetry test (Hoenig et al. 1995) and coefficient of variation (CV) analysis were used to detect any systematic difference and precision on age readings, respectively, for the following comparisons: 1) between the two readers in the current year, 2) within each reader in the current year, and 3) time-series bias between the current and previous years within each reader. The readings from the entire sample for the current year were used to examine the difference between two readers. A random sub-sample of 50 fish from the current year was selected for second readings to examine the difference within a reader. Fifty otoliths

randomly selected from fish aged in 2000 were used to examine the time-series bias within each reader. A figure of 1:1 equivalence was used to illustrate those differences (Campana et al. 1995). All statistics analyses were performed in R.4.0.2 (R Core Team 2021).

6.3 RESULTS

6.3.1 Reading Precision

Both readers had high self-precision. Specifically, there was no significant difference between the first and second readings for Reader 1 with an agreement of 94% and a CV of 2.07% (test of symmetry: $\chi^2 = 3$, $df = 2$, $P = 0.2231$), and there was no significant difference between the first and second readings for Reader 2 with an agreement of 94%and a CV of 2.45% (test of symmetry: $\chi^2 = 3$, $df = 2$, $P = 0.2231$). There was no evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 95.88% and a CV of 1.36% (test of symmetry: $\chi^2 = 4$, $df = 2$, $P = 0.1353$) (Figure 6.2).

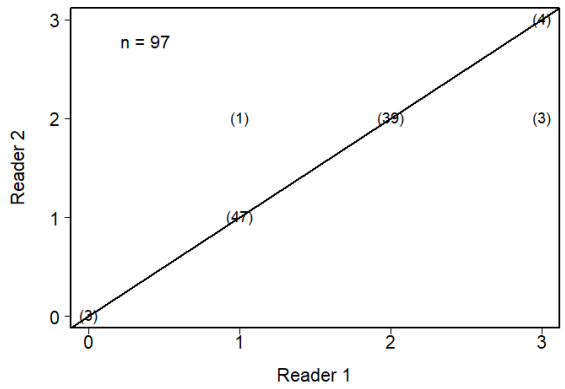


Figure 6.2: Between-reader comparison of otolith age estimates for Red Drum collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2025. The number in parentheses is number of fish.

There was no time-series bias for either reader. Reader 1 had an agreement of 94% with ages of fish aged in 2000 with a CV of 0.89% (test of symmetry: $\chi^2 = 3$, $df = 3$, $P = 0.3916$). Reader 2 had an agreement of 96% with a CV of 3.39% (test of symmetry: $\chi^2 = 2$, $df = 2$, $P = 0.3679$).

6.3.2 Year Class

We aged the otoliths of all the 97 Red Drum collected in 2025. Of the 97 fish aged, 4 age classes (0 to 3) were represented (Table 6.1). The average age was 1.5 years, and the standard deviation and standard error were 0.6 and 0.06, respectively. Year-class data show that the fishery was comprised of 4 year-classes: fish from the 2022 to 2025 year-classes, with fish primarily from the year classes of 2023 and 2024 with 42.3% and 49.5%, respectively. The ratio of males to females was 1:0.51 in the sample collected (Figure 6.3).

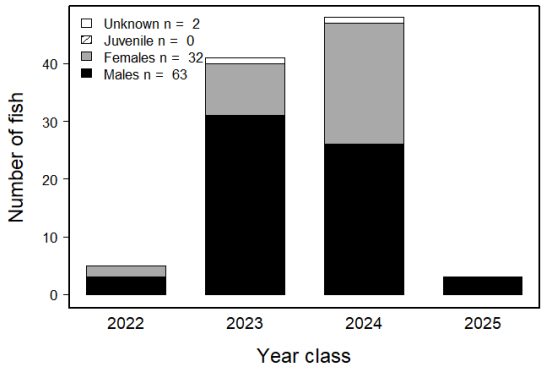


Figure 6.3: Year-class frequency distribution for Red Drum collected for ageing in 2025. Distribution is broken down by sex. 'Unknown' represents gonads that were not available for examination or were not examined for sex during sampling.

6.3.3 Age-length Key (ALK)

We developed an age-length-key (Table 6.2) that can be used in the conversion of numbers-at-length in the estimated catch to numbers-at-age using otolith ages. The table is based on VMRC's stratified sampling of landings by total length inch intervals.

Table 6.1: The number of Red Drum assigned to each total length (inch)-at-age category for 97 fish sampled for otolith age determination in Virginia during 2025.

	Age				
Interval	0	1	2	3	Totals
17 - 17.99	0	2	0	0	2
18 - 18.99	3	2	0	0	5
19 - 19.99	0	2	0	0	2
20 - 20.99	0	1	3	0	4
21 - 21.99	0	1	3	0	4
22 - 22.99	0	5	4	0	9
23 - 23.99	0	14	6	0	20
24 - 24.99	0	15	1	0	16
25 - 25.99	0	5	11	0	16
26 - 26.99	0	0	8	2	10
27 - 27.99	0	0	2	0	2
28 - 28.99	0	0	1	3	4
40 - 40.99	0	0	1	0	1
44 - 44.99	0	1	1	0	2
Totals	3	48	41	5	97

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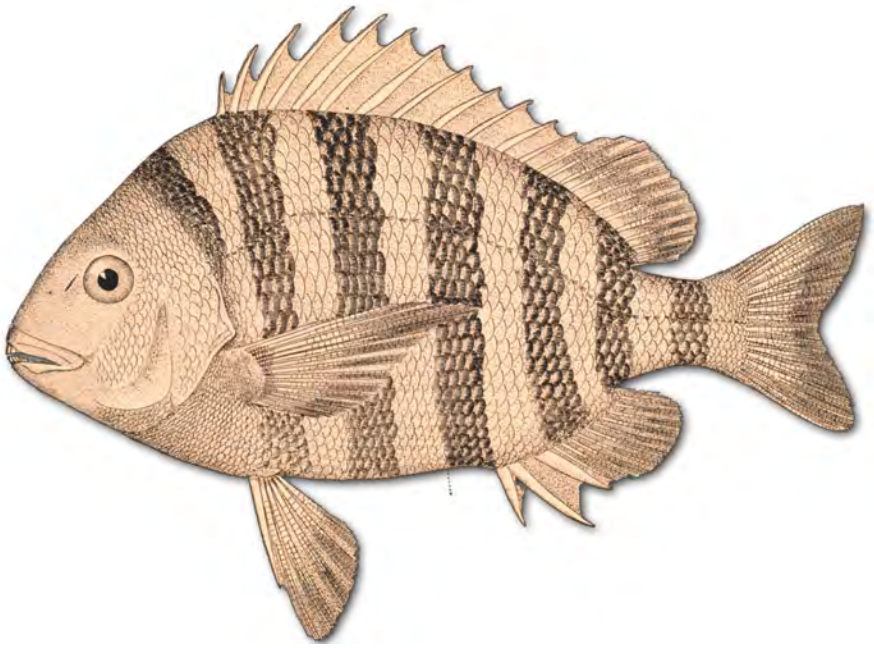
Table 6.2: Age-Length key, as proportion-at-age in each 1-inch length interval, based on otolith ages for Red Drum sampled for age determination in Virginia during 2025.

	Age			
Interval	0	1	2	3
17 - 17.99	0	1	0	0
18 - 18.99	0.6	0.4	0	0
19 - 19.99	0	1	0	0
20 - 20.99	0	0.25	0.75	0
21 - 21.99	0	0.25	0.75	0
22 - 22.99	0	0.56	0.44	0
23 - 23.99	0	0.7	0.3	0
24 - 24.99	0	0.94	0.06	0
25 - 25.99	0	0.31	0.69	0
26 - 26.99	0	0	0.8	0.2
27 - 27.99	0	0	1	0
28 - 28.99	0	0	0.25	0.75
40 - 40.99	0	0	1	0
44 - 44.99	0	0.5	0.5	0

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Chapter 7

SHEEPSHEAD *Archosargus probatocephalus*



7.1 INTRODUCTION

We aged a total of 253 Sheepshead *Archosargus probatocephalus*, collected by the VMRC's Biological Sampling Program for age and growth analysis in 2025. Sheepshead ages ranged from 2 to 34 years old with an average age of 11.5, a standard deviation of 6.8, and a standard error of 0.43. Twenty-nine age classes (2 to 15, 17 to 30, and 34) were represented, comprising fish of the 1991, 1995 to 2008, and 2010 to 2023 year-classes. The sample was dominated by fish from the year-classes of 2006, 2007, 2011, 2012, 2015, 2016, 2018, 2019, 2020, 2021, and 2022 with 4.3%, 5.9%, 11.1%, 7.1%, 6.3%, 12.2%, 4.7%, 9.5%, 4.7%, 8.3%, and 5.5%, respectively.

7.2 METHODS

7.2.1 Handling of Collections

Sagittal otoliths, hereafter, referred to as "otoliths", were received by the Age and Growth Laboratory in labeled coin envelopes,and were sorted by date of capture. Their envelope labels were verified against VMRC's collection data, and each fish was assigned a unique Age and Growth Laboratory identification number. All otoliths were stored dry in their original labeled coin envelopes.

7.2.2 Preparation

Otoliths were processed for age determination following the methods described in Ballenger (2011). The left or right otolith was randomly selected and embedded, distal side down, in epoxy resin and allowed to harden overnight. The otoliths were viewed by eye, and when necessary, under a stereo microscope to identify the location of the core, and the position of the core marked using an ultra fine Sharpie across the epoxy resin surface. At least one transverse cross-section (hereafter "thin-section") was then removed from the marked core of each otolith using a Buehler IsoMet^{TM} low-speed saw equipped with two 4-inch diameter diamond grinding Wheels, separated by a stainless steel spacer of 0.5 mm (diameter 2.5"). The position of the marked core fell within the 0.5 mm space between the blades, such that the core was included in the removed thin section. Otolith thin-sections were placed on labeled glass slides and covered with a thin layer of Flo-texx mounting medium that not only fixed the sections to the slide, but more importantly, provided enhanced contrast and greater readability by increasing light transmission through the section.

Click here to obtain the protocol at the VMRV website on how to prepare otolith thin-section using the epoxy resin method for ageing Sheepshead.

7.2.3 Readings

The VMRC system assigns an age class to a fish based on a combination of number of annuli in a thin-section, the date of capture, and the species-specific period when the annulus is deposited. Each year, as the fish grows, its otoliths grow and leave behind markers of their age, called an annulus. Technically, an otolith annulus is the combination of both the opaque and the translucent band. In practice, only the opaque bands are counted as annuli and recorded in our ageing notation.

In 2019 a new notation method recommended by Atlantic States Marine Fisheries Commission (ASMFC) was used to assign age on Sheepshead. In addition to recording the number of annulus, the margin or the growth width after the last annulus is coded from 1 to 4. The margin code “1”, “2”, “3”, and “4” stands for no growth, the growth width less than or equal to one third of, larger than one third but less than or equal to two thirds of, and larger than two thirds of the growth width formed in the previous year, respectively.

By convention all fish in the Northern Hemisphere are assigned a birth date of January 1. In addition, each species has a specific period

during which it deposits the annulus. If a fish is captured after the end of the species-specific annulus deposition period and before January 1, it is assigned an age class as the same as its annulus number without referencing its margin code. If a fish has a margin code of "1", it is assigned an age class as the same as its annulus number no matter in which month it is captured. If a fish is captured after December 31 and before its annulus deposition period, it is assigned an age class as its annulus number plus one when its margin code is "2", "3", or "4". If a fish is captured during its annulus deposition period, it is assigned an age class as the same as its annulus number when its margin code is "2" and as its annulus number plus one when its margin code is "3" or "4" (**Note: Based on the growth of Virginia species we use two criteria for Margin Code 2 to assign a fish an age class depending on its capture month, which could be different from how other states and agencies use Margin Code 2**).

For example, Sheepshead otolith annulus formation occurs between May and July (Ballenger 2011 and modified by CQFE/ODU). A Sheepshead with nine visible annuli could be assigned an age of 9 or 10 depending on its capture month and margin code. When its margin code is "1", it is Age 9 no matter when it is captured. When it is captured after July and before January, it is Age 9 no matter what its margin code is. When it is captured after December and before May and its margin code is not "1", it is Age 10 (9 + 1 = 10). When it is captured between May and July, it is Age 9 when its margin code is "2" but Age 10 (9 + 1 = 10) when its margin code is "3" or "4".

When an otolith was properly sectioned, the sulcal groove came to a sharp point (Hereafter referred to as "focus") within the middle of the core (Figure 7.1). Typically the first year's annulus was found by locating the focus of the otolith section, which was characterized as a visually distinct dark, oblong region found in the center of the otolith section.



Figure 7.1: Otolith thin-section of a 5 year-old Sheepshead

All samples were aged by two readers in chronological order, based on collection date, without knowledge of previously estimated ages or the specimen lengths. When the readers' ages agreed, that age was assigned to the fish. When the two readers disagreed, both readers sat down together and re-aged the fish, again without any knowledge of previously estimated ages or lengths, and assigned a final age to the fish. When the readers were unable to agree on a final age, the fish was excluded from further analysis. All thin-sections were aged using a Nikon SMZ1000 stereo microscope under transmitted light and dark-field polarization at between 8 and 20 times magnification.

7.2.4 Comparison Tests

A symmetry test (Hoenig et al. 1995) and coefficient of variation (CV) analysis were used to detect any systematic difference and precision on age readings, respectively, for the following comparisons: 1) between the two readers in the current year, 2) within each reader in the current year, and 3) time-series bias between the current and previous years within each reader. The readings from the entire sample for the current year were used to examine the difference between two readers. A random sub-sample of 50 fish from the current year was selected for second readings to examine the difference within a reader. Fifty otoliths randomly selected from fish aged in 2008 were used to examine the time-series bias within each reader. A figure of 1:1 equivalence was used to illustrate those differences (Campana et al. 1995). All statistics analyses were performed in R.4.0.2 (R Core Team 2021).

7.3 RESULTS

7.3.1 Reading Precision

Both readers had high self-precision. Specifically, there was no significant difference between the first and second readings for Reader 1 with an agreement of 94% and a CV of 1.42% (test of symmetry: $\chi^2 = 3$, $df = 3$, $P = 0.3916$), and there was no significant difference between the first and second readings for Reader 2 with an agreement of 76% and a CV of 3.49% (test of symmetry: $\chi^2 = 12$, $df = 10$, $P = 0.2851$). There was no evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 73.12% and a CV of 2.14% (test of symmetry: $\chi^2 = 43.6$, $df = 34$, $P = 0.1253$) (Figure 7.2).

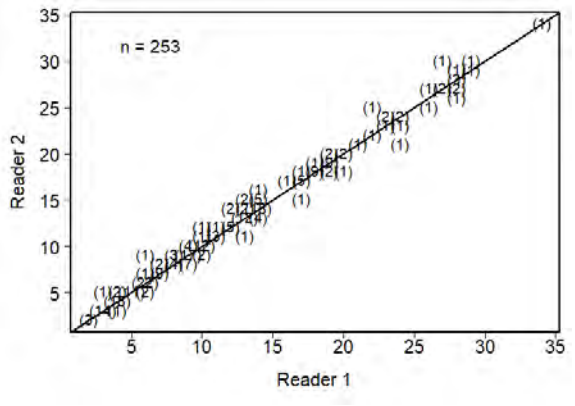


Figure 7.2: Between-reader comparison of otolith age estimates for Sheepshead collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2025. The number in parentheses is number of fish.

There was no time-series bias for either reader. Reader 1 had an agreement of 96% with ages of fish aged in 2008 with a CV of 0.35% (test of symmetry: $\chi^2 = 2$, $df = 2$, $P = 0.3679$). Reader 2 had an agreement of 96% with a CV of 0.14% (test of symmetry: $\chi^2 = 2$, $df = 2$, $P = 0.3679$).

7.3.2 Year Class

We aged the otoliths of all the 253 Sheepshead collected in 2025. Of the 253 fish aged, 29 age classes (2 to 15, 17 to 30, and 34) were represented (Table 7.1). The average age was 11.5 years, and the standard deviation and standard error were 6.8 and 0.43, respectively. Year-class data show that the fishery was comprised of 29 year-classes: fish from the 1991, 1995 to 2008, and 2010 to 2023 year-classes, with fish primarily from the year classes of 2006, 2007, 2011, 2012, 2015, 2016, 2018, 2019, 2020, 2021, and 2022 with 4.3%, 5.9%, 11.1%, 7.1%, 6.3%, 12.2%, 4.7%, 9.5%, 4.7%, 8.3%, and 5.5%, respectively. The ratio of males to females was 1:1.18 in the sample collected (Figure 7.3).

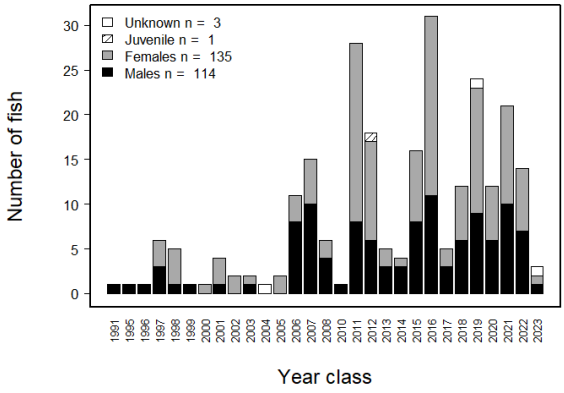


Figure 7.3: Year-class frequency distribution for Sheepshead collected for ageing in 2025. Distribution is broken down by sex. 'Unknown' represents gonads that were not available for examination or were not examined for sex during sampling.

7.3.3 Age-length Key (ALK)

We developed an age-length-key (Table 7.2) that can be used in the conversion of numbers-at-length in the estimated catch to numbers-at-age using otolith ages. The table is based on VMRC's stratified sampling of landings by total length inch intervals.

Table 7.1: The number of sheepshead assigned to each total length (inch)-at-age category for 253 fish sampled for otolith age determination in Virginia during 2025.

	Age																												
Interval	2	3	4	5	6	7	8	9	10	11	12	13	14	15	17	18	19	20	21	22	23	24	25	26	27	28	29	30	34	Totals
10 - 10.99	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
11 - 11.99	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
12 - 12.99	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
13 - 13.99	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
14 - 14.99	0	3	1	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6
15 - 15.99	1	4	4	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	12
16 - 16.99	0	4	6	4	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	15
17 - 17.99	0	1	8	3	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	16
18 - 18.99	0	0	1	1	6	0	0	3	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12
19 - 19.99	0	0	1	2	6	2	0	2	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	15
20 - 20.99	0	0	0	2	3	6	1	1	3	1	2	1	4	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	25
21 - 21.99	0	0	0	0	0	1	1	8	4	0	0	1	2	0	0	1	1	0	0	0	0	0	0	0	0	0	0	1	0	20
22 - 22.99	0	0	0	0	1	2	2	6	6	2	2	3	14	0	3	7	8	1	0	0	1	1	0	0	1	0	1	0	1	62
23 - 23.99	0	0	0	0	0	0	1	11	1	0	1	7	4	1	1	5	1	0	0	2	1	2	1	0	2	4	0	0	0	45
24 - 24.99	0	0	0	0	0	0	0	0	1	0	0	4	4	0	1	2	1	1	0	0	0	1	0	1	2	1	0	0	0	19
26 - 26.99	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	2
Totals	3	14	21	12	24	12	5	31	16	4	5	18	28	1	6	15	11	2	1	2	2	4	1	1	5	6	1	1	1	253

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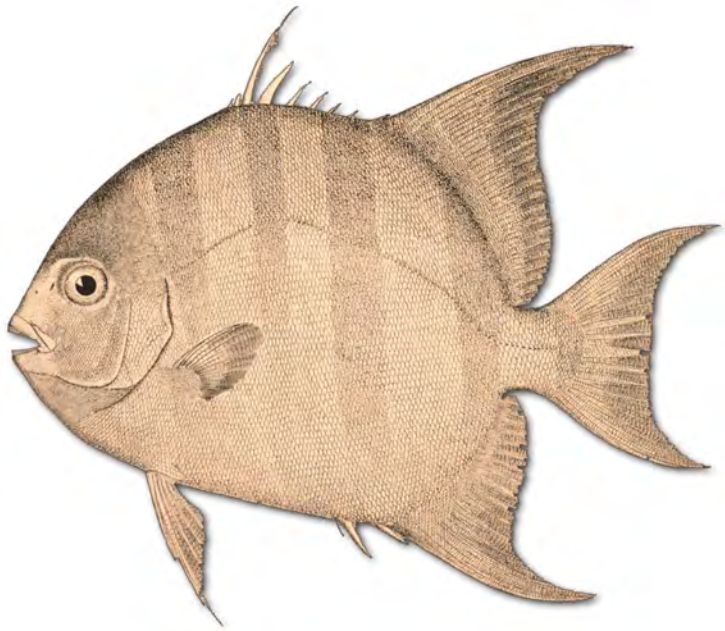
Table 7.2: Age-Length key, as proportion-at-age in each 1-inch length interval, based on otolith ages for Sheepshead sampled for age determination in Virginia during 2025.

	Age																												
Interval	2	3	4	5	6	7	8	9	10	11	12	13	14	15	17	18	19	20	21	22	23	24	25	26	27	28	29	30	34
10 - 10.99	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11 - 11.99	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12 - 12.99	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13 - 13.99	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14 - 14.99	0	0.5	0.17	0	0.33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15 - 15.99	0.08	0.33	0.33	0	0.08	0.08	0	0	0	0	0	0	0	0	0	0	0	0	0.08	0	0	0	0	0	0	0	0	0	0
16 - 16.99	0	0.27	0.4	0.27	0.07	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17 - 17.99	0	0.06	0.5	0.19	0.25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18 - 18.99	0	0	0.08	0.08	0.5	0	0	0.25	0	0	0	0.08	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19 - 19.99	0	0	0.07	0.13	0.4	0.13	0	0.13	0.07	0.07	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20 - 20.99	0	0	0	0.08	0.12	0.24	0.04	0.04	0.12	0.04	0.08	0.04	0.16	0	0.04	0	0	0	0	0	0	0	0	0	0	0	0	0	0
21 - 21.99	0	0	0	0	0	0.05	0.05	0.4	0.2	0	0	0.05	0.1	0	0	0.05	0.05	0	0	0	0	0	0	0	0	0	0	0.05	0
22 - 22.99	0	0	0	0	0.02	0.03	0.03	0.1	0.1	0.03	0.03	0.05	0.23	0	0.05	0.11	0.13	0.02	0	0	0.02	0.02	0	0	0.02	0	0.02	0	0.02
23 - 23.99	0	0	0	0	0	0	0.02	0.24	0.02	0	0.02	0.16	0.09	0.02	0.02	0.11	0.02	0	0	0.04	0.02	0.04	0.02	0	0.04	0.09	0	0	0
24 - 24.99	0	0	0	0	0	0	0	0	0.05	0	0	0.21	0.21	0	0.05	0.11	0.05	0.05	0	0	0	0.05	0	0.05	0.11	0.05	0	0	0
26 - 26.99	0	0	0	0	0	0	0	0	0	0	0	0.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0.5	0	0	0

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Chapter 8

ATLANTIC SPADEFISH
Chaetodipterus faber



8.1 INTRODUCTION

We aged a total of 103 Spadefish *Chaetodipterus faber*, collected by the VMRC's Biological Sampling Program for age and growth analysis in 2025. Spadefish ages ranged from 1 to 10 years old with an average age of 2.5, a standard deviation of 1.5, and a standard error of 0.15. Seven age classes (1 to 5, 7, and 10) were represented, comprising fish of the 2015, 2018, and 2020 to 2024 year-classes. The sample was dominated by fish from the year-class of 2023 with 58.2%.

8.2 METHODS

8.2.1 Sample Size for Ageing

We estimated sample size for ageing Spadefish in 2025 using a two-stage random sampling method (Quinn and Deriso 1999) to increase precision in estimates of age composition from fish sampled efficiently and effectively. The basic equation is:

$$A = \frac{V_a}{\theta_a^2 CV_a^2 + B_a/L} \tag{8.1}$$

where A is the sample size for ageing Spadefish in 2025; θ_a stands for the proportion of Age a fish in a catch; V_a, B_a, and CV_a represent the variance components within and between length intervals, and the coefficient of variation for Age a, respectively; L is the total number of Spadefish used by VMRC to estimate length distribution of the catches from 2019 to 2023. θ_a, V_a, and B_a were calculated using pooled age-length data of Spadefish collected from 2019 to 2023 and using equations in Quinn and Deriso (1999). For simplicity, the equations are not listed here. The equation (1.1) indicates: 1) The more fish that are aged, the smaller the CV_a (or higher precision) that will be obtained for Age a; 2) given a sample size A, the CV_a is different for each age due to different θ_a, V_a, and B_a among different ages. Therefore, the criterion to age A (number) of fish is that A should be a number above which there is only a 1% CV_a reduction for the most abundant age in catch by ageing an additional 100 or more fish. Finally, A_l is A multiplied by the proportion of length interval l from the length distribution of the 2019 to 2023 catch. A_l is number of fish to be aged for length interval l in 2025.

8.2.2 Handling of Collections

Otoliths were received by the Age and Growth Laboratory in labeled coin envelopes, and were sorted by date of capture. Their envelope labels were verified against VMRC's collection data, and each fish was assigned a unique Age and Growth Laboratory identification number. All otoliths were stored dry in their original labeled coin envelopes.

8.2.3 Preparation

We used our bake and thin-section technique to process Spadefish sagittal otoliths (hereafter, referred to as "otoliths") for age determination. Otolith preparation began by randomly selecting either the right or left otolith. Each whole otolith was placed in a ceramic "Coors" spot plate well and baked in a Thermolyne 1400 furnace at 400 °C. Baking time was dependent on the otolith's size and gauged by color, with a light caramel color desired. Once a suitable color was achieved the baked otolith was embedded in epoxy resin with its distal surface orientated downwards and allowed to harden overnight. The otoliths were viewed by eye and, when necessary, under a stereo microscope to identify the location of the core, and the position of the core was marked using an ultra fine Sharpie across the epoxy resin surface. At least one transverse cross-section (hereafter, referred to as "thin-section") was then removed from the marked core of each otolith using a Buehler IsoMet^{TM} low-speed saw equipped with two 4-inch diameter diamond grinding wheels (hereafter, referred to as "blades"), separated by a stainless steel spacer of 0.5 mm (diameter 2.5"). The otolith was positioned so the blades straddled each side of the

otolith core. It was crucial that this cut be perpendicular to the long axis of the otolith. Failure to do so resulted in broadening and distored winter growth zones. A proper cut resulted in annuli that were clearly defined and delineated. Once cut, thin-sections were placed on labeled glass slides and covered with a thin layer of Flo-texx mounting medium that not only fixed the sections to the slide, but more importantly, provided enhanced contrast and greater readability by increasing light transmission through the thin-section.

Click here to obtain the protocol at the VMRV website on how to prepare otolith thin-section using the epoxy resin method for ageing Atlantic Spadefish.

8.2.4 Readings

The VMRC system assigns an age class to a fish based on a combination of number of annuli in a thin-section, the date of capture, and the species-specific period when the annulus is deposited. Each year, as the fish grows, its otoliths grow and leave behind markers of their age, called an annulus. Technically, an otolith annulus is the combination of both the opaque and the translucent band. In practice, only the opaque bands are counted as annuli and recorded in our ageing notation.

In 2019 a new notation method recommended by Atlantic States Marine Fisheries Commission (ASMFC) was used to assign age on Spadefish. In addition to recording the number of annulus, the margin or the growth width after the last annulus is coded from 1 to 4. The margin code “1”, “2”, “3”, and “4” stands for no growth, the growth width less than or equal to one third of, larger than one third but less than or equal to two thirds of, and larger than two thirds of the growth width formed in the previous year, respectively.

By convention all fish in the Northern Hemisphere are assigned a birth date of January 1. In addition, each species has a specific period during which it deposits the annulus. If a fish is captured after the end of the species-specific annulus deposition period and before January 1, it is assigned an age class as the same as its annulus number without referencing its margin code. If a fish has a margin code of "1", it is assigned an age class as the same as its annulus number no matter in which month it is captured. If a fish is captured after December 31 and before its annulus deposition period, it is assigned an age class as its annulus number plus one when its margin code is "2", "3", or "4". If a fish is captured during its annulus deposition period, it is assigned an age class as the same as its annulus number when its margin code is “2” and as its annulus number plus one when its margin code is “3” or “4” (**Note: Based on the growth of Virginia species we use two criteria for Margin Code 2 to assign a fish an age class depending on its capture month, which could be different from how other states and agencies use Margin Code 2**).

For example, Spadefish otolith annulus formation occurs between January and July (Hayse 1987 and modified by CQFE/ODU). A Spadefish with three visible annuli could be assigned an age of 3 or 4 depending on its capture month and margin code. When its margin code is "1", it is Age 3 no matter when it is captured. When it is captured after July and before January, it is Age 3 no matter what its margin code is. When it is captured between January and July, it is Age 3 when its margin code is "2" but Age 4 (3 + 1 = 4) when its margin code is "3" or "4".

When an otolith was properly sectioned, the sulcal groove came to a sharp point (Hereafter referred to as "focus") within the middle of the core (Figure 8.1). Typically the first year’s annulus was found by locating the focus of the otolith section, which was characterized as a visually distinct dark, oblong region found in the center of the otolith section.

All samples were aged by two readers in chronological order, based on collection date, without knowledge of previously estimated

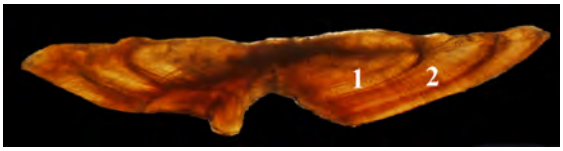


Figure 8.1: Otolith thin-section of a 2 year-old Spadefish

ages or the specimen lengths. When the readers' ages agreed, that age was assigned to the fish. When the two readers disagreed, both readers sat down together and re-aged the fish, again without any knowledge of previously estimated ages or lengths, and assigned a final age to the fish. When the readers were unable to agree on a final age, the fish was excluded from further analysis. All thin-sections were aged using a Nikon SMZ1000 stereo microscope under transmitted light and dark-field polarization at between 8 and 20 times magnification.

8.2.5 Comparison Tests

A symmetry test (Hoenig et al. 1995) and coefficient of variation (CV) analysis were used to detect any systematic difference and precision on age readings, respectively, for the following comparisons: 1) between the two readers in the current year, 2) within each reader in the current year, and 3) time-series bias between the current and previous years within each reader. The readings from the entire sample for the current year were used to examine the difference between two readers. A random sub-sample of 50 fish from the current year was selected for second readings to examine the difference within a reader. Fifty otoliths randomly selected from fish aged in 2003 were used to examine the time-series bias within each reader. A figure of 1:1 equivalence was used to illustrate those differences (Campana et al. 1995). All statistics analyses were performed in R.4.0.2 (R Core Team 2021).

8.3 RESULTS

8.3.1 Sample Size

We estimated a sample size of 343 Spadefish in 2025, ranging in length interval from 3 to 22 inches (Table 8.1). This sample size provided a range in CV for age composition approximately from the smallest CV of 8% for Age 2 to the CV of larger than 25% for the multiple minor ages (Table 8.2). In 2025, we Spadefishaged all 103 collected by VMRC. We fell short in our over-all collections for this optimal length-class sampling estimate by 240 fish. We were short of many fish from the major length intervals (The interval requires 10 or more fish), as a result, the precision for the estimates of major age groups would definitely be influenced significantly. Therefore, precaution should be used when developing ALK using these age data.

8.3.2 Reading Precision

Reader 1 had high self-precision and Read 2 had moderate self-precision. Specifically, there was no significant difference between the first and second readings for Reader 1 with an agreement of 90% and a CV of 2.41% (test of symmetry: $\chi^2 = 2$, $df = 2$, $P = 0.3679$), and there was no significant difference between the first and second readings for Reader 2 with an agreement of 84% and a CV of 5.21% (test of symmetry: $\chi^2 = 2.8$, $df = 3$, $P = 0.4235$). There was no evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 88.35% and a CV of 3.5% (test of symmetry: $\chi^2 = 8.5$, $df = 5$, $P = 0.1307$) (Figure 8.2).

There was no time-series bias for either reader. Reader 1 had an agreement of 84% with ages of fish aged in 2003 with a CV of 1.52% (test of symmetry: $\chi^2 = 8$, $df = 7$, $P = 0.3326$). Reader 2 had an agreement of 80% with a CV of 2.52% (test of symmetry: $\chi^2 = 8$, $df = 9$, $P = 0.5341$).

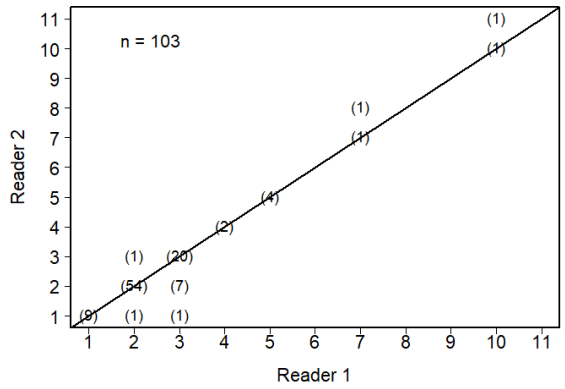


Figure 8.2: Between-reader comparison of otolith age estimates for Spadefish collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2025. The number in parentheses is number of fish.

8.3.3 Year Class

Of the 103 fish aged with otoliths, 7 age classes (1 to 5, 7, and 10) were represented (Table 8.3). The average age was 2.5 years, and the standard deviation and standard error were 1.5 and 0.15, respectively. Year-class data show that the fishery was comprised of 7 year-classes: fish from the 2015, 2018, and 2020 to 2024 year-classes, with fish primarily from the year class of 2023 with 58.2%. The ratio of males to females was 1:0.84 in the sample collected (Figure 8.3).

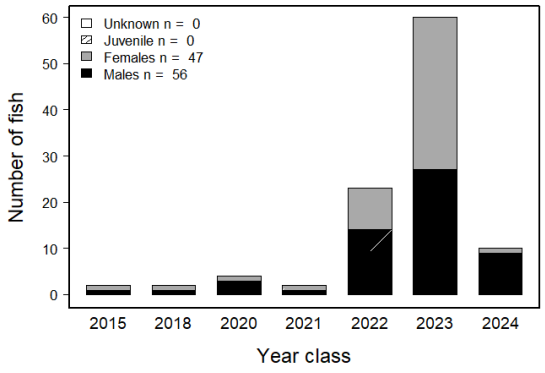


Figure 8.3: Year-class frequency distribution for Spadefish collected for ageing in 2025. Distribution is broken down by sex. 'Unknown' represents gonads that were not available for examination or were not examined for sex during sampling.

8.3.4 Age-length Key (ALK)

We developed an age-length-key (Table 8.4) that can be used in the conversion of numbers-at-length in the estimated catch to numbers-at-age using otolith ages. The table is based on VMRC's stratified sampling of landings by total length inch intervals.

Table 8.1: Number of Atlantic Spadefish collected and aged in each 1-inch length interval in 2025. 'Target' represents the sample size for ageing estimated for 2025, and 'Need' represents number of fish shorted in each length interval compared to the optimum sample size for ageing and number of fish aged.

Interval	Target	Collected	Aged	Need
3 - 3.99	5	0	0	5
4 - 4.99	7	0	0	7
5 - 5.99	25	4	4	21
6 - 6.99	41	21	21	20
7 - 7.99	41	37	37	4
8 - 8.99	28	20	20	8
9 - 9.99	18	6	6	12
10 - 10.99	18	2	2	16
11 - 11.99	17	3	3	14
12 - 12.99	20	0	0	20
13 - 13.99	21	0	0	21
14 - 14.99	15	0	0	15
15 - 15.99	16	1	1	15
16 - 16.99	14	2	2	12
17 - 17.99	20	3	3	17
18 - 18.99	14	1	1	13
19 - 19.99	8	1	1	7
20 - 20.99	5	0	0	5
21 - 21.99	5	1	1	4
22 - 22.99	5	1	1	4
Totals	343	103	103	240

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Table 8.2: CV for each age estimated based on ageing the total of 343 Spadefish in 2025. 'Percent' is the percentage of an age in the pooled age-length data of Spadefish collected from 2019 to 2023.

Age	CV	Percent
0	0.22	3.63
1	0.12	12.09
2	0.08	24.51
3	0.09	21.72
4	0.1	19.78
5	0.14	10.74
6	0.23	4.65
7	>0.25	1.94
8	>0.25	0.68
9	>0.25	0.17
10	>0.25	0.08

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Table 8.3: The number of Atlantic Spadefish assigned to each total length-at-age category for 103 fish sampled for otolith age determination in Virginia during 2025.

	Age							
Interval	1	2	3	4	5	7	10	Totals
5 - 5.99	3	1	0	0	0	0	0	4
6 - 6.99	6	15	0	0	0	0	0	21
7 - 7.99	1	31	5	0	0	0	0	37
8 - 8.99	0	9	11	0	0	0	0	20
9 - 9.99	0	1	5	0	0	0	0	6
10 - 10.99	0	2	0	0	0	0	0	2
11 - 11.99	0	1	2	0	0	0	0	3
15 - 15.99	0	0	0	1	0	0	0	1
16 - 16.99	0	0	0	0	1	1	0	2
17 - 17.99	0	0	0	1	2	0	0	3
18 - 18.99	0	0	0	0	1	0	0	1
19 - 19.99	0	0	0	0	0	1	0	1
21 - 21.99	0	0	0	0	0	0	1	1
22 - 22.99	0	0	0	0	0	0	1	1
Totals	10	60	23	2	4	2	2	103

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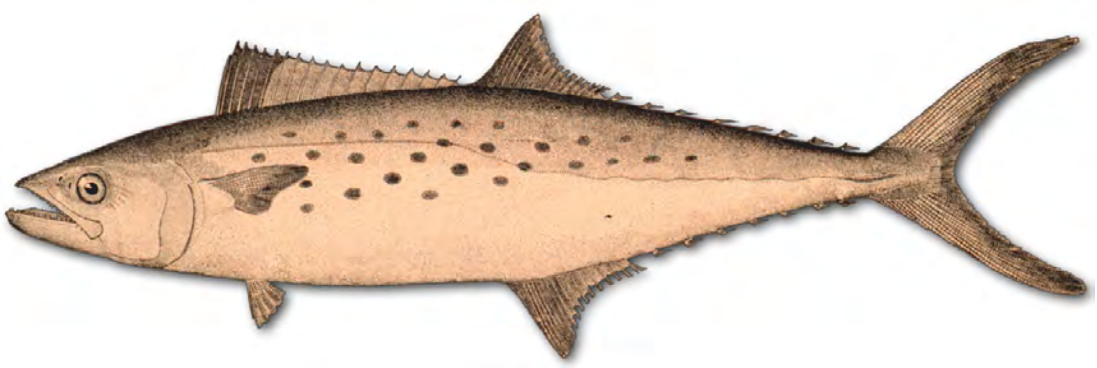
Table 8.4: Age-Length key, as proportion-at-age in each 1-inch length interval, based on otolith ages for Atlantic Spadefish sampled for age determination in Virginia during 2025.

	Age						
Interval	1	2	3	4	5	7	10
5 - 5.99	0.75	0.25	0	0	0	0	0
6 - 6.99	0.29	0.71	0	0	0	0	0
7 - 7.99	0.03	0.84	0.14	0	0	0	0
8 - 8.99	0	0.45	0.55	0	0	0	0
9 - 9.99	0	0.17	0.83	0	0	0	0
10 - 10.99	0	1	0	0	0	0	0
11 - 11.99	0	0.33	0.67	0	0	0	0
15 - 15.99	0	0	0	1	0	0	0
16 - 16.99	0	0	0	0	0.5	0.5	0
17 - 17.99	0	0	0	0.33	0.67	0	0
18 - 18.99	0	0	0	0	1	0	0
19 - 19.99	0	0	0	0	0	1	0
21 - 21.99	0	0	0	0	0	0	1
22 - 22.99	0	0	0	0	0	0	1

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Chapter 9

SPANISH MACKEREL
Scomberomorous maculatus



9.1 INTRODUCTION

We aged a total of 227 Spanish Mackerel *Scomberomorous maculatus*, collected by the VMRC's Biological Sampling Program for age and growth analysis in 2025. Spanish Mackerel ages ranged from 0 to 8 years old with an average age of 2.3, a standard deviation of 1.5, and a standard error of 0.1. Nine age classes (0 to 8) were represented, comprising fish of the 2017 to 2025 year-classes. The sample was dominated by fish from the year-classes of 2022, 2023, and 2024 with 27.8%, 26.9%, and 25.6%, respectively.

9.2 METHODS

9.2.1 Sample Size for Ageing

We estimated sample size for ageing Spanish Mackerel in 2025 using a two-stage random sampling method (Quinn and Deriso 1999) to increase precision in estimates of age composition from fish sampled efficiently and effectively. The basic equation is:

$$A = \frac{V_a}{\theta_a^2 CV_a^2 + B_a/L} \tag{9.1}$$

where A is the sample size for ageing Spanish Mackerel in 2025; θ_a stands for the proportion of Age a fish in a catch; V_a, B_a, and CV_a represent the variance components within and between length intervals, and the coefficient of variation for Age a, respectively; L is the total number of Spanish Mackerel used by VMRC to estimate length distribution of the catches from 2019 to 2023. θ_a, V_a, and B_a were calculated using pooled age-length data of Spanish Mackerel collected from 2019 to 2023 and using equations in Quinn and Deriso (1999). For simplicity, the equations are not listed here. The equation (1.1) indicates: 1) The more fish that are aged, the smaller the CV_a (or higher precision) that will be obtained for Age a; 2) given a sample size A, the CV_a is different for each age due to different θ_a, V_a, and B_a among different ages. Therefore, the criterion to age A (number) of fish is that A should be a number above which there is only a 1% CV_a reduction for the most abundant age in catch by ageing an additional 100 or more fish. Finally, A_l is A multiplied by the proportion of length interval l from the length distribution of the 2019 to 2023 catch. A_l is number of fish to be aged for length interval l in 2025.

9.2.2 Handling of Collections

Otoliths were received by the Age and Growth Laboratory in labeled coin envelopes, and were sorted by date of capture. Their envelope labels were verified against VMRC's collection data, and each fish was assigned a unique Age and Growth Laboratory identification number. All otoliths were stored dry in their original labeled coin envelopes.

9.2.3 Preparation

Sagittal otoliths, hereafter, referred to as "otolith", were processed for age determination. The left or right otolith was randomly selected and embedded, distal side down, in epoxy resin and allowed to harden overnight. The otoliths were viewed by eye, and when necessary, under a stereo microscope to identify the location of the core, and the position of the core marked using an ultra fine Sharpie across the epoxy resin surface. At least one transverse cross-section (hereafter "thin-section") was then removed from the marked core of each otolith using a Buehler IsoMet™ low-speed saw equipped with two 4-inch diameter diamond grinding Wheels, separated by a stainless steel spacer of 0.5 mm (diameter 2.5"). The position of the marked core fell within the 0.5 mm space between the blades, such that the core was included in the removed thin section. Otolith thin-sections were placed on labeled glass slides and covered with a thin layer of Flo-texx mounting medium that not only fixed the sections to the slide, but more importantly, provided enhanced contrast and greater readability by increasing light transmission through the section.

Click here to obtain the protocol at the VMRV website on how to prepare otolith thin-section using the epoxy resin method for ageing Spanish Mackerel.

9.2.4 Readings

The VMRC system assigns an age class to a fish based on a combination of number of annuli in a thin-section, the date of capture, and the species-specific period when the annulus is deposited. Each year, as the fish grows, its otoliths grow and leave behind markers of their age, called an annulus. Technically, an otolith annulus is the combination of both the opaque and the translucent band. In practice, only the opaque bands are counted as annuli and recorded in our ageing notation.

In 2019 a new notation method recommended by Atlantic States Marine Fisheries Commission (ASMFC) was used to assign age on Spanish Mackerel. In addition to recording the number of annulus, the margin or the growth width after the last annulus is coded from 1 to 4. The margin code “1”, “2”, “3”, and “4” stands for no growth, the growth width less than or equal to one third of, larger than one third but less than or equal to two thirds of, and larger than two thirds of the growth width formed in the previous year, respectively.

By convention all fish in the Northern Hemisphere are assigned a birth date of January 1. In addition, each species has a specific period during which it deposits the annulus. If a fish is captured after the end of the species-specific annulus deposition period and before January 1, it is assigned an age class as the same as its annulus number without referencing its margin code. If a fish has a margin code of "1", it is assigned an age class as the same as its annulus number no matter in which month it is captured. If a fish is captured after December 31 and before its annulus deposition period, it is assigned an age class as its annulus number plus one when its margin code is "2", "3", or "4". If a fish is captured during its annulus deposition period, it is assigned an age class as the same as its annulus number when its margin code is “2” and as its annulus number plus one when its margin code is “3” or “4” (**Note: Based on the growth of Virginia species we use two criteria for Margin Code 2 to assign a fish an age class depending on its capture month, which could be different from how other states and agencies use Margin Code 2**).

For example, Spanish Mackerel otolith annulus formation occurs between May and June (Schmidt et al. 1993). A Spanish Mackerel with two visible annuli could be assigned an age of 2 or 3 depending on its capture month and margin code. When its margin code is "1", it is Age 2 no matter when it is captured. When it is captured after June and before January, it is Age 2 no matter what its margin code is. When it is captured after December and before May and its margin code is not "1", it is Age 3 ($2 + 1 = 3$). When it is captured between May and June, it is Age 2 when its margin code is "2" but Age 3 ($2 + 1 = 3$) when its margin code is "3" or "4".

When an otolith was properly sectioned, the sulcal groove came to a sharp point (Hereafter referred to as "focus") within the middle of the core (Figure 9.1). Typically the first year’s annulus was found by locating the focus of the otolith section, which was characterized as a visually distinct dark, oblong region found in the center of the otolith section.



Figure 9.1: Otolith thin-section of a 3 year-old Spanish Mackerel with the last annulus on the edge of the thin-section

All samples were aged by two readers in chronological order, based on collection date, without knowledge of previously estimated ages or the specimen lengths. When the read-

ers' ages agreed, that age was assigned to the fish. When the two readers disagreed, both readers sat down together and re-aged the fish, again without any knowledge of previously estimated ages or lengths, and assigned a final age to the fish. When the readers were unable to agree on a final age, the fish was excluded from further analysis. All thin-sections were aged using a Nikon SMZ1000 stereo microscope under transmitted light and dark-field polarization at between 8 and 20 times magnification.

9.2.5 Comparison Tests

A symmetry test (Hoenig et al. 1995) and coefficient of variation (CV) analysis were used to detect any systematic difference and precision on age readings, respectively, for the following comparisons: 1) between the two readers in the current year, 2) within each reader in the current year, and 3) time-series bias between the current and previous years within each reader. The readings from the entire sample for the current year were used to examine the difference between two readers. A random sub-sample of 50 fish from the current year was selected for second readings to examine the difference within a reader. Fifty otoliths randomly selected from fish aged in 2003 were used to examine the time-series bias within each reader. A figure of 1:1 equivalence was used to illustrate those differences (Campana et al. 1995). All statistics analyses were performed in R.4.0.2 (R Core Team 2021).

9.3 RESULTS

9.3.1 Sample Size

We estimated a sample size of 323 Spanish Mackerel in 2025, ranging in length interval from 12 to 34 inches (Table 9.1). This sample size provided a range in CV for age composition approximately from the smallest CV of 5% for Age 1 to the CV of larger than 25% for the multiple minor ages (Table 9.2). In 2025, we aged 227 of 280 Spanish Mackerel (The rest of fish were either without otoliths or over-collected for certain length interval(s)) collected by VMRC. We fell short in our overall collections for this optimal length-class sampling estimate by 103 fish. We were short of many fish from the major length intervals (The interval requires 10 or more fish), as a result, the precision for the estimates of major age groups would definitely be influenced significantly. Therefore, precaution should be used when developing ALK using these age data.

9.3.2 Reading Precision

both readers had moderate self-precision. Specifically, there was no significant difference between the first and second readings for Reader 1 with an agreement of 88% and a CV of 7% (test of symmetry: $\chi^2 = 3$, $df = 3$, $P = 0.3916$), and there was no significant difference between the first and second readings for Reader 2 with an agreement of 78% and a CV of 9.36% (test of symmetry: $\chi^2 = 8.33$, $df = 6$, $P = 0.2147$). There was no evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 89.43% and a CV of 3.72% (test of symmetry: $\chi^2 = 8.23$, $df = 8$, $P = 0.4115$) (Figure 9.2).

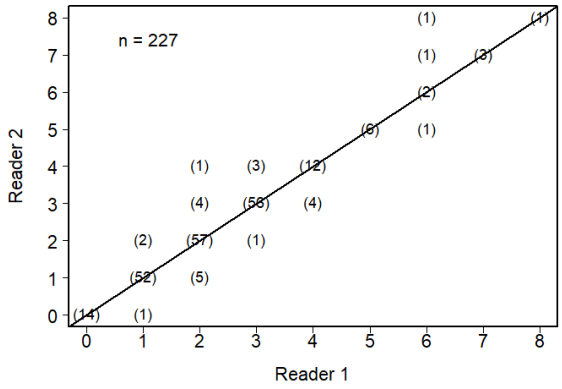


Figure 9.2: Between-reader comparison of otolith age estimates for Spanish Mackerel collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2025. The number in parentheses is number of fish.

There was no time-series bias for either reader.

Reader 1 had an agreement of 100% with fish aged in 2003. Reader 2 had an agreement of 96% with a CV of 1.89% (test of symmetry: $\chi^2 = 0$, $df = 1$, $P = 1$).

9.3.3 Year Class

Of the 227 fish aged with otoliths, 9 age classes (0 to 8) were represented (Table 9.3). The average age was 2.3 years, and the standard deviation and standard error were 1.5 and 0.1, respectively. Year-class data show that the fishery was comprised of 9 year-classes: fish from the 2017 to 2025 year-classes, with fish primarily from the year classes of 2022, 2023, and 2024 with 27.8%, 26.9%, and 25.6%, respectively. The ratio of males to females was 1:1.6 in the sample collected (Figure 9.3).

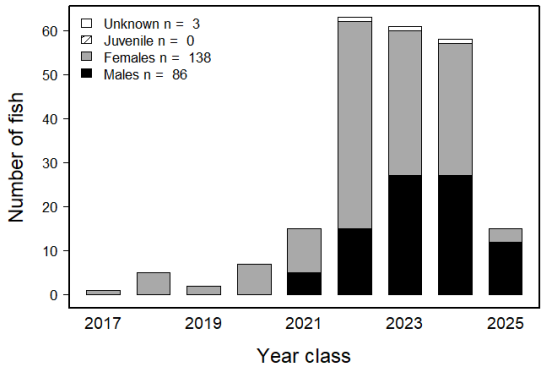


Figure 9.3: Year-class frequency distribution for Spanish Mackerel collected for ageing in 2025. Distribution is broken down by sex. 'Unknown' represents gonads that were not available for examination or were not examined for sex during sampling.

9.3.4 Age-length Key (ALK)

We developed an age-length-key (Table 9.4) that can be used in the conversion of numbers-at-length in the estimated catch to numbers-at-age using otolith ages. The table is based on VMRC's stratified sampling of landings by total length inch intervals.

Table 9.1: Number of Spanish Mackerel collected and aged in each 1-inch length interval in 2025. 'Target' represents the sample size for ageing estimated for 2025, and 'Need' represents number of fish shorted in each length interval compared to the optimum sample size for ageing and number of fish aged.

Interval	Target	Collected	Aged	Need
12 - 12.99	5	0	0	5
13 - 13.99	5	0	0	5
14 - 14.99	19	4	4	15
15 - 15.99	36	11	11	25
16 - 16.99	47	23	23	24
17 - 17.99	44	43	43	1
18 - 18.99	27	44	28	0
19 - 19.99	23	38	24	0
20 - 20.99	17	29	17	0
21 - 21.99	16	20	16	0
22 - 22.99	10	17	10	0
23 - 23.99	9	10	10	0
24 - 24.99	8	10	10	0
25 - 25.99	8	9	9	0
26 - 26.99	6	7	7	0
27 - 27.99	8	5	5	3
28 - 28.99	5	3	3	2
29 - 29.99	5	2	2	3
30 - 30.99	5	4	4	1
31 - 31.99	5	0	0	5
32 - 32.99	5	1	1	4
33 - 33.99	5	0	0	5
34 - 34.99	5	0	0	5
Totals	323	280	227	103

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Table 9.2: CV for each age estimated based on ageing the total of 323 Spanish Mackerel in 2025. 'Percent' is the percentage of an age in the pooled age-length data of Spanish Mackerel collected from 2019 to 2023.

Age	CV	Percent
0	0.22	4.83
1	0.05	42.99
2	0.09	25.59
3	0.14	11.84
4	0.16	8.93
5	0.25	3.73
6	>0.25	1.46
7	>0.25	0.55
8	>0.25	0.09

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Table 9.3: The number of Spanish Mackerel assigned to each total length-at-age category for 227 fish sampled for otolith age determination in Virginia during 2025.

	Age									
Interval	0	1	2	3	4	5	6	7	8	Totals
14 - 14.99	4	0	0	0	0	0	0	0	0	4
15 - 15.99	7	4	0	0	0	0	0	0	0	11
16 - 16.99	4	16	3	0	0	0	0	0	0	23
17 - 17.99	0	29	12	2	0	0	0	0	0	43
18 - 18.99	0	8	12	8	0	0	0	0	0	28
19 - 19.99	0	1	17	4	2	0	0	0	0	24
20 - 20.99	0	0	7	7	3	0	0	0	0	17
21 - 21.99	0	0	7	9	0	0	0	0	0	16
22 - 22.99	0	0	1	7	2	0	0	0	0	10
23 - 23.99	0	0	2	6	1	0	0	1	0	10
24 - 24.99	0	0	0	8	0	2	0	0	0	10
25 - 25.99	0	0	0	7	2	0	0	0	0	9
26 - 26.99	0	0	0	2	4	1	0	0	0	7
27 - 27.99	0	0	0	1	0	3	1	0	0	5
28 - 28.99	0	0	0	2	0	1	0	0	0	3
29 - 29.99	0	0	0	0	0	0	0	2	0	2
30 - 30.99	0	0	0	0	1	0	1	2	0	4
32 - 32.99	0	0	0	0	0	0	0	0	1	1
Totals	15	58	61	63	15	7	2	5	1	227

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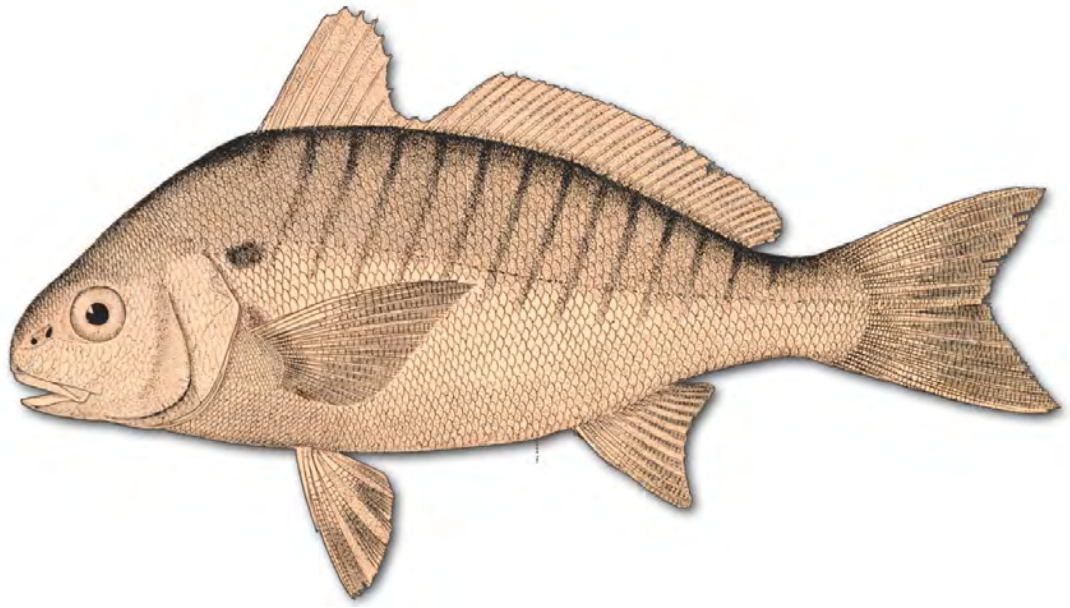
Table 9.4: Age-Length key, as proportion-at-age in each 1-inch length interval, based on otolith ages for Spanish Mackerel sampled for age determination in Virginia during 2025.

	Age								
Interval	0	1	2	3	4	5	6	7	8
14 - 14.99	1	0	0	0	0	0	0	0	0
15 - 15.99	0.64	0.36	0	0	0	0	0	0	0
16 - 16.99	0.17	0.7	0.13	0	0	0	0	0	0
17 - 17.99	0	0.67	0.28	0.05	0	0	0	0	0
18 - 18.99	0	0.29	0.43	0.29	0	0	0	0	0
19 - 19.99	0	0.04	0.71	0.17	0.08	0	0	0	0
20 - 20.99	0	0	0.41	0.41	0.18	0	0	0	0
21 - 21.99	0	0	0.44	0.56	0	0	0	0	0
22 - 22.99	0	0	0.1	0.7	0.2	0	0	0	0
23 - 23.99	0	0	0.2	0.6	0.1	0	0	0.1	0
24 - 24.99	0	0	0	0.8	0	0.2	0	0	0
25 - 25.99	0	0	0	0.78	0.22	0	0	0	0
26 - 26.99	0	0	0	0.29	0.57	0.14	0	0	0
27 - 27.99	0	0	0	0.2	0	0.6	0.2	0	0
28 - 28.99	0	0	0	0.67	0	0.33	0	0	0
29 - 29.99	0	0	0	0	0	0	0	1	0
30 - 30.99	0	0	0	0	0.25	0	0.25	0.5	0
32 - 32.99	0	0	0	0	0	0	0	0	1

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Chapter 10

SPOT *Leiostomus xanthurus*



10.1 INTRODUCTION

We aged a total of 195 Spot *Leiostomus xanthurus*, collected by the VMRC's Biological Sampling Program for age and growth analysis in 2025. Spot ages ranged from 1 to 4 years old with an average age of 1.4, a standard deviation of 0.6, and a standard error of 0.04. Four age classes (1 to 4) were represented, comprising fish of the 2021 to 2024 year-classes. The sample was dominated by fish from the year-classes of 2023 and 2024 with 35.9% and 61.5%, respectively.

10.2 METHODS

10.2.1 Sample Size for Ageing

We estimated sample size for ageing Spot in 2025 using a two-stage random sampling method (Quinn and Deriso 1999) to increase precision in estimates of age composition from fish sampled efficiently and effectively. The basic equation is:

$$A = \frac{V_a}{\theta_a^2 CV_a^2 + B_a/L} \tag{10.1}$$

where A is the sample size for ageing Spot in 2025; θ_a stands for the proportion of Age a fish in a catch; V_a, B_a, and CV_a represent the variance components within and between length intervals, and the coefficient of variation for Age a, respectively; L is the total number of Spot used by VMRC to estimate length distribution of the catches from 2019 to 2023. θ_a, V_a, and B_a were calculated using pooled age-length data of Spot collected from 2019 to 2023 and using equations in Quinn and Deriso (1999). For simplicity, the equations are not listed here. The equation (1.1) indicates: 1) The more fish that are aged, the smaller the CV_a (or higher precision) that will be obtained for Age a; 2) given a sample size A, the CV_a is different for each age due to different θ_a, V_a, and B_a among different ages. Therefore, the criterion to age A (number) of fish is that A should be a number above which there is only a 1% CV_a reduction for the most abundant age in catch by ageing an additional 100 or more fish. Finally, A_l is A multiplied by the proportion of length interval l from the length distribution of the 2019 to 2023 catch. A_l is number of fish to be aged for length interval l in 2025.

10.2.2 Handling of Collections

Otoliths were received by the Age and Growth Laboratory in labeled coin envelopes, were sorted by date of capture. Their envelope labels were verified against VMRC's collection data, and each fish was assigned a unique Age and Growth Laboratory identification number. All otoliths were stored dry in their original labeled coin envelopes.

10.2.3 Preparation

Sagittal otoliths, hereafter, referred to as "otoliths", were processed for age determination following the methods described in Barbieri et al. (1994) with a few modifications. The left or right otolith was randomly selected and embedded (distal side down) in epoxy resin and allowed to harden overnight. The otoliths were viewed by eye and, when necessary, under a stereo microscope to identify the location of the core, and the position of the core was marked using an ultra fine Sharpie across the epoxy resin surface. At least one transverse cross-section (hereafter, referred to as "thin-section") was then removed from the marked core of each otolith using a Buehler IsoMet™ low-speed saw equipped with two 4-inch diameter diamond grinding wheels (hereafter, referred to as "blades"), separated by a stainless steel spacer of 0.5 mm (diameter 2.5"). Thin-sections were placed on labeled glass slides and covered with a thin layer of Flo-texx mounting medium that not only fixed the sections to the slide, but more importantly, provided enhanced contrast and greater readability by increasing light transmission through the thin-sections.

Click here to obtain the protocol at the VMRV website on how to prepare otolith thin-section using the epoxy resin method for ageing Spot.

10.2.4 Readings

The VMRC system assigns an age class to a fish based on a combination of number of annuli in a thin-section, the date of capture, and the species-specific period when the annulus is deposited. Each year, as the fish grows, its otoliths grow and leave behind markers of their age, called an annulus. Technically, an otolith annulus is the combination of both the opaque and the translucent band. In practice, only the opaque bands are counted as annuli and recorded in our ageing notation.

In 2019 a new notation method recommended by Atlantic States Marine Fisheries Commission (ASMFC) was used to assign age on Spot. In addition to recording the number of annulus, the margin or the growth width after the last annulus is coded from 1 to 4. The margin code "1", "2", "3", and "4" stands for no growth, the growth width less than or equal to one third of, larger than one third but less than or equal to two thirds of, and larger than two thirds of the growth width formed in the previous year, respectively.

By convention all fish in the Northern Hemisphere are assigned a birth date of January 1. In addition, each species has a specific period during which it deposits the annulus. If a fish is captured after the end of the species-specific annulus deposition period and before January 1, it is assigned an age class as the same as its annulus number without referencing its margin code. If a fish has a margin code of "1", it is assigned an age class as the same as its annulus number no matter in which month it is captured. If a fish is captured after December 31 and before its annulus deposition period, it is assigned an age class as its annulus number plus one when its margin code is "2", "3", or "4". If a fish is captured during its annulus deposition period, it is assigned an age class as the same as its annulus number when its margin code is "2" and as its annulus number plus one when its margin code is "3" or "4" (**Note: Based on the growth of Virginia species we use two criteria for Margin Code 2 to assign a fish an age class depending on its capture month, which could be different from how other states and agencies use Margin Code 2**).

For example, Spot otolith annulus formation occurs between May and July (Piner and Jones 2004). A Spot with one visible annuli could be assigned an age of 1 or 2 depending on its capture month and margin code. When its margin code is "1", it is Age 1 no matter when it is captured. When it is captured after July and before January, it is Age 1 no matter what its margin code is. When it is captured after December and before May and its margin code is not "1", it is Age 2 (1 + 1 = 2). When it is captured between May and July, it is Age 1 when its margin code is "2" but Age 2 (1 + 1 = 2) when its margin code is "3" or "4".

When an otolith was properly sectioned, the sulcal groove came to a sharp point (Hereafter referred to as "focus") within the middle of the core (Figure 10.1). Typically the first year's annulus was found by locating the focus of the otolith section, which was characterized as a visually distinct dark, oblong region found in the center of the otolith section.

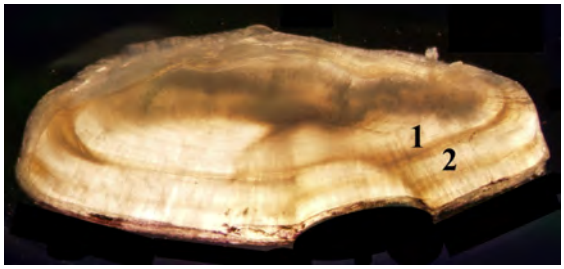


Figure 10.1: Otolith thin-section of a 2 year-old Spot

All samples were aged by two readers in chronological order, based on collection date, without knowledge of previously estimated ages or the specimen lengths. When the read-

ers' ages agreed, that age was assigned to the fish. When the two readers disagreed, both readers sat down together and re-aged the fish, again without any knowledge of previously estimated ages or lengths, and assigned a final age to the fish. When the readers were unable to agree on a final age, the fish was excluded from further analysis. All thin-sections were aged using a Nikon SMZ1000 stereo microscope under transmitted light and dark-field polarization at between 8 and 20 times magnification.

10.2.5 Comparison Tests

A symmetry test (Hoenig et al. 1995) and coefficient of variation (CV) analysis were used to detect any systematic difference and precision on age readings, respectively, for the following comparisons: 1) between the two readers in the current year, 2) within each reader in the current year, and 3) time-series bias between the current and previous years within each reader. The readings from the entire sample for the current year were used to examine the difference between two readers. A random sub-sample of 50 fish from the current year was selected for second readings to examine the difference within a reader. Fifty otoliths randomly selected from fish aged in 2000 were used to examine the time-series bias within each reader. A figure of 1:1 equivalence was used to illustrate those differences (Campana et al. 1995). All statistics analyses were performed in R.4.0.2 (R Core Team 2021).

10.3 RESULTS

10.3.1 Sample Size

We estimated a sample size of 209 Spot in 2025, ranging in length interval from 4 to 12 inches (Table 10.1). This sample size provided a range in CV for age composition approximately from the smallest CV of 3% for Age 1 to the CV of larger than 25% for the multiple minor ages (Table 10.2). In 2025, we randomly selected and aged 195 fish from 244 Spot collected by VMRC. We fell short in our over-all collections for this optimal length-class sampling estimate by 19 fish. We were not short of any fish from the major length intervals (The interval requires 10 or more fish), as a result, the precision for the estimates of major age groups would not be influenced significantly.

10.3.2 Reading Precision

Both readers had high self-precision. Specifically, there was a significant difference between the first and second readings for Reader 1 with an agreement of 90% and a CV of 4.71% (test of symmetry: $\chi^2 = 5$, $df = 1$, $P = 0.0253$), and there was no significant difference between the first and second readings for Reader 2 with an agreement of 94% and a CV of 1.91% (test of symmetry: $\chi^2 = 3$, $df = 3$, $P = 0.3916$). There was no evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 94.87% and a CV of 2.13% (test of symmetry: $\chi^2 = 0.48$, $df = 2$, $P = 0.7881$) (Figure 10.2).

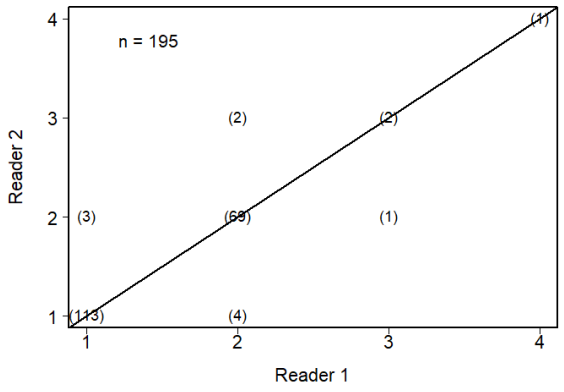


Figure 10.2: Between-reader comparison of otolith age estimates for Spot collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2025. The number in parentheses is number of fish.

There was no time-series bias for either reader. Reader 1 had an agreement of 100% with ages of fish aged in 2000. Reader 2 also had an agreement of 100% .

10.3.3 Year Class

Of the 195 fish aged with otoliths, 4 age classes (1 to 4) were represented (Table 10.3). The average age was 1.4 years, and the standard deviation and standard error were 0.6 and 0.04, respectively. Year-class data show that the fishery was comprised of 4 year-classes: fish from the 2021 to 2024 year-classes, with fish primarily from the year classes of 2023 and 2024 with 35.9% and 61.5%, respectively. The ratio of males to females was 1:6.42 in the sample collected (Figure 10.3).

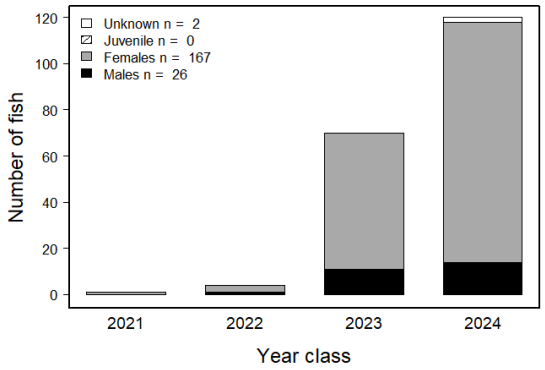


Figure 10.3: Year-class frequency distribution for Spot collected for ageing in 2025. Distribution is broken down by sex. 'Unknown' is for gonads that were not available for examination or were not examined for sex during sampling.

10.3.4 Age-length Key (ALK)

We developed an age-length-key (Table 10.4) that can be used in the conversion of numbers-at-length in the estimated catch to numbers-at-age using otolith ages. The table is based on VMRC's stratified sampling of landings by total length inch intervals.

Table 10.1: Number of Spot collected and aged in each 1-inch length interval in 2025. 'Target' represents the sample size for ageing estimated for 2025, and 'Need' represents number of fish shorted in each length interval compared to the optimum sample size for ageing and number of fish aged.

Interval	Target	Collected	Aged	Need
4 - 4.99	5	0	0	5
5 - 5.99	6	1	1	5
6 - 6.99	8	9	9	0
7 - 7.99	24	40	24	0
8 - 8.99	48	61	48	0
9 - 9.99	72	92	72	0
10 - 10.99	36	40	40	0
11 - 11.99	5	1	1	4
12 - 12.99	5	0	0	5
Totals	209	244	195	19

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Table 10.2: CV for each age estimated based on ageing the total of 209 Spot in 2025. 'Percent' is the percentage of an age in the pooled age-length data of Spot collected from 2019 to 2023.

Age	CV	Percent
0	0.2	4.51
1	0.03	82.97
2	0.19	11.38
3	>0.25	1.03
4	>0.25	0.1

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Table 10.3: The number of Spot assigned to each total length-at-age category for 195 fish sampled for otolith age determination in Virginia during 2025.

	Age				
Interval	1	2	3	4	Totals
5 - 5.99	1	0	0	0	1
6 - 6.99	9	0	0	0	9
7 - 7.99	15	9	0	0	24
8 - 8.99	17	29	2	0	48
9 - 9.99	51	18	2	1	72
10 - 10.99	27	13	0	0	40
11 - 11.99	0	1	0	0	1
Totals	120	70	4	1	195

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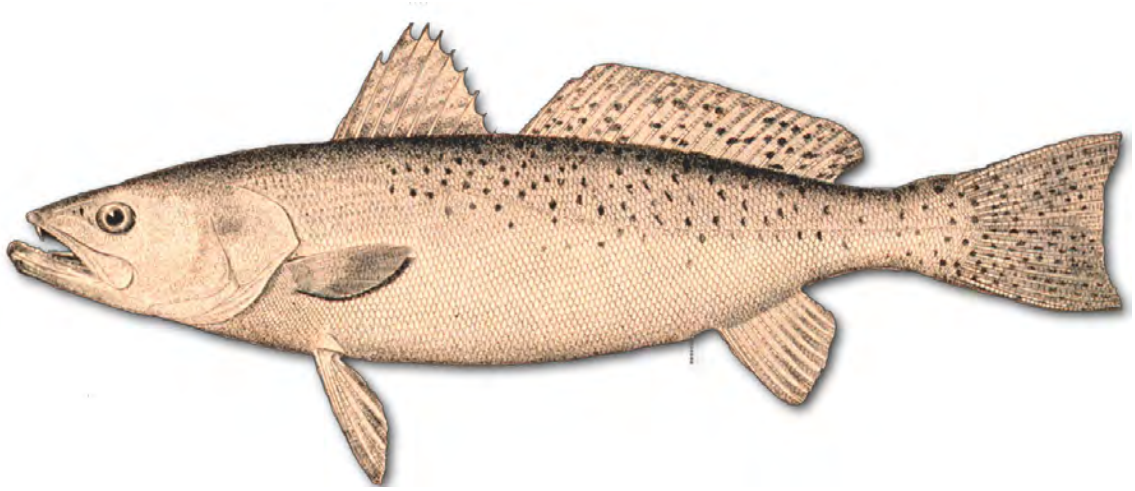
Table 10.4: Age-Length key, as proportion-at-age in each 1-inch length interval, based on otolith ages for Spot sampled for age determination in Virginia during 2025.

	Age			
Interval	1	2	3	4
5 - 5.99	1	0	0	0
6 - 6.99	1	0	0	0
7 - 7.99	0.62	0.38	0	0
8 - 8.99	0.35	0.6	0.04	0
9 - 9.99	0.71	0.25	0.03	0.01
10 - 10.99	0.68	0.32	0	0
11 - 11.99	0	1	0	0

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Chapter 11

SPOTTED SEATROUT *Cynoscion nebulosus*



11.1 INTRODUCTION

We aged a total of 227 Spotted Seatrout *Cynoscion nebulosus*, collected by the VMRC's Biological Sampling Program for age and growth analysis in 2025. Spotted seatrout ages ranged from 0 to 7 years old with an average age of 2.1, a standard deviation of 1.5, and a standard error of 0.1. Eight age classes (0 to 7) were represented, comprising fish of the 2018 to 2025 year-classes. The sample was dominated by fish from the year-classes of 2022, 2023, and 2024 with 19.4%, 30.4%, and 27.3%, respectively.

11.2 METHODS

11.2.1 Sample Size for Ageing

We estimated sample size for ageing Spotted Seatrout in 2025 using a two-stage random sampling method (Quinn and Deriso 1999) to increase precision in estimates of age composition from fish sampled efficiently and effectively. The basic equation is:

$$A = \frac{V_a}{\theta_a^2 CV_a^2 + B_a/L} \tag{11.1}$$

where A is the sample size for ageing Spotted Seatrout in 2025; θ_a stands for the proportion of Age a fish in a catch; V_a, B_a, and CV_a represent the variance components within and between length intervals, and the coefficient of variation for Age a, respectively; L is the total number of Spotted Seatrout used by VMRC to estimate length distribution of the catches from 2019 to 2023. θ_a, V_a, and B_a were calculated using pooled age-length data of Spotted Seatrout collected from 2019 to 2023 and using equations in Quinn and Deriso (1999). For simplicity, the equations are not listed here. The equation (1.1) indicates: 1) The more fish that are aged, the smaller the CV_a (or higher precision) that will be obtained for Age a; 2) given a sample size A, the CV_a is different for each age due to different θ_a, V_a, and B_a among different ages. Therefore, the criterion to age A (number) of fish is that A should be a number above which there is only a 1% CV_a reduction for the most abundant age in catch by ageing an additional 100 or more fish. Finally, A_l is A multiplied by the proportion of length interval l from the length distribution of the 2019 to 2023 catch. A_l is number of fish to be aged for length interval l in 2025.

11.2.2 Handling of Collections

Otoliths were received by the Age and Growth Laboratory in labeled coin envelopes. In the lab they were sorted by date of capture, their envelope labels were verified against VMRC's collection data, and each fish was assigned a unique Age and Growth Laboratory identification number. All otoliths were stored dry in their original labeled coin envelopes.

11.2.3 Preparation

Sagittal otoliths, hereafter, referred to as "otoliths", were processed for age determination. The left or right sagittal otolith was randomly selected and attached, distal side down, to a 1 x 2 inch piece of water resistant grid paper (Brand name: Write in the Rain) using hot glue. The otoliths were viewed by eye and, when necessary, under a stereo microscope to identify the location of the core, and the position of the core was marked using an ultra fine Sharpie across the otolith surface. At least one transverse cross-section (hereafter, referred to as "thin-section") was then removed from the marked core of each otolith using a Buehler IsoMet^{TM} low-speed saw equipped with two 4-inch diameter diamond grinding wheels (hereafter, referred to as "blades"), separated by a stainless steel spacer of 0.5 mm (diameter 2.5"). Thin-sections were placed on labeled glass slides and covered with a thin layer of Flo-texx mounting medium that not only fixed the sections to the slide, but more importantly, provided enhanced contrast and greater readability by increasing light transmission through the thin-sections.

Click here to obtain the protocol at the VMRV website on how to prepare otolith thin-section using the hot glue method for ageing Spotted Seatrout.

11.2.4 Readings

The VMRC system assigns an age class to a fish based on a combination of number of annuli in a thin-section, the date of capture, and the species-specific period when the annulus is deposited. Each year, as the fish grows, its otoliths grow and leave behind markers of their age, called an annulus. Technically, an otolith annulus is the combination of both the opaque and the translucent band. In practice, only the opaque bands are counted as annuli and recorded in our ageing notation.

In 2019 a new notation method recommended by Atlantic States Marine Fisheries Commission (ASMFC) was used to assign age on Spotted Seatrout. In addition to recording the number of annulus, the margin or the growth width after the last annulus is coded from 1 to 4. The margin code “1”, “2”, “3”, and “4” stands for no growth, the growth width less than or equal to one third of, larger than one third but less than or equal to two thirds of, and larger than two thirds of the growth width formed in the previous year, respectively.

By convention all fish in the Northern Hemisphere are assigned a birth date of January 1. In addition, each species has a specific period during which it deposits the annulus. If a fish is captured after the end of the species-specific annulus deposition period and before January 1, it is assigned an age class as the same as its annulus number without referencing its margin code. If a fish has a margin code of "1", it is assigned an age class as the same as its annulus number no matter in which month it is captured. If a fish is captured after December 31 and before its annulus deposition period, it is assigned an age class as its annulus number plus one when its margin code is "2", "3", or "4". If a fish is captured during its annulus deposition period, it is assigned an age class as the same as its annulus number when its margin code is “2” and as its annulus number plus one when its margin code is “3” or “4” (**Note: Based on the growth of Virginia species we use two criteria for Margin Code 2 to assign a fish an age class depending on its capture month, which could be different from how other states and agencies use Margin Code 2**).

For example, Spotted Seatrout otolith annulus formation occurs between March and May (Ihde and Chittenden 2003). A Spotted Seatrout with two visible annuli could be assigned an age of 2 or 3 depending on its capture month and margin code. When its margin code is "1", it is Age 2 no matter when it is captured. When it is captured after May and before January, it is Age 2 no matter what its margin code is. When it is captured after December and before March and its margin code is not "1", it is Age 3 (2 + 1 = 3). When it is captured between March and May, it is Age 2 when its margin code is "2" but Age 3 (2 + 1 = 3) when its margin code is "3" or "4".

When an otolith was properly sectioned, the sulcal groove came to a sharp point (Hereafter referred to as "focus") within the middle of the core (Figure 11.1). Typically the first year's annulus was found by locating the focus of the otolith section, which was characterized as a visually distinct dark, oblong region found in the center of the otolith section.

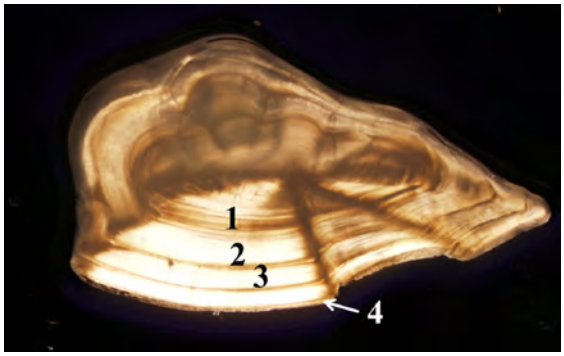


Figure 11.1: Otolith thin-section of a 4 year-old Spotted Seatrout with the last annulus on the edge of the thin-section

All samples were aged by two readers in chronological order, based on collection date, without knowledge of previously estimated ages or the specimen lengths. When the readers' ages agreed, that age was assigned to the fish. When the two readers disagreed, both readers sat down together and re-aged the fish, again without any knowledge of previously estimated ages or lengths, and assigned a final age to the fish. When the readers were unable to agree on a final age, the fish was excluded from further analysis. All thin-sections were aged using a Nikon SMZ1000 stereo microscope under transmitted light and dark-field polarization at between 8 and 20 times magnification.

11.2.5 Comparison Tests

A symmetry test (Hoenig et al. 1995) and coefficient of variation (CV) analysis were used to detect any systematic difference and precision on age readings, respectively, for the following comparisons: 1) between the two readers in the current year, 2) within each reader in the current year, and 3) time-series bias between the current and previous years within each reader. The readings from the entire sample for the current year were used to examine the difference between two readers. A random sub-sample of 50 fish from the current year was selected for second readings to examine the difference within a reader. Fifty otoliths randomly selected from fish aged in 2000 were used to examine the time-series bias within each reader. A figure of 1:1 equivalence was used to illustrate those differences (Campana et al. 1995). All statistics analyses were performed in R.4.0.2 (R Core Team 2021).

11.3 RESULTS

11.3.1 Sample Size

We estimated a sample size of 265 Spotted Seatrout in 2025, ranging in length interval from 8 to 31 inches (Table 11.1). This sample size provided a range in CV for age composition approximately from the smallest CV of 5% for Age 1 to the CV of larger than 25% for the multiple minor ages (Table 11.2). In 2025, we aged 227 of 263 Spotted Seatrout (The rest of fish were either without otoliths or over-collected for certain length interval(s)) collected by VMRC. We fell short in our overall collections for this optimal length-class sampling estimate by 51 fish. We were short of some fish from the major length intervals (The interval requires 10 or more fish), as a result, the precision for the estimates of major age groups would possibly be influenced significantly.

11.3.2 Reading Precision

Both readers had high self-precision. Specifically, there was no significant difference between the first and second readings for Reader 1 with an agreement of 100%, and there was no significant difference between the first and second readings for Reader 2 with an agreement of 100%. There was no evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 100% (Figure 11.2).

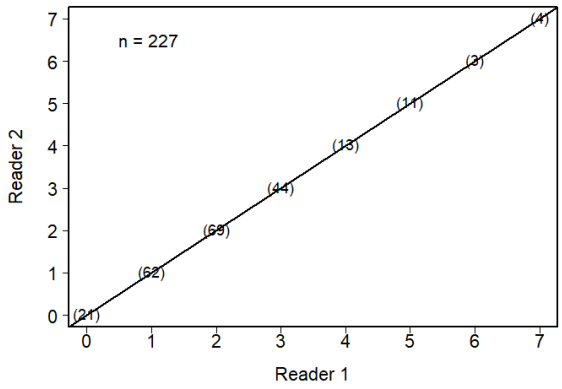


Figure 11.2: Between-reader comparison of otolith age estimates for Spotted Seatrout collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2025. The number in parentheses is number of fish.

There was no time-series bias for either reader. Reader 1 had an agreement of 98% with ages of fish aged in 2000 with a CV of 0.4% (test of

symmetry: $\chi^2 = 1$, $df = 1$, $P = 0.3173$). Reader 2 also had an agreement of 100%.

11.3.3 Year Class

Of the 227 fish aged with otoliths, 8 age classes (0 to 7) were represented (Table 11.3). The average age was 2.1 years, and the standard deviation and standard error were 1.5 and 0.1, respectively. Year-class data show that the fishery was comprised of 8 year-classes: fish from the 2018 to 2025 year-classes, with fish primarily from the year classes of 2022, 2023, and 2024 with 19.4%, 30.4%, and 27.3%, respectively. The ratio of males to females was 1:1.13 in the sample collected (Figure 11.3).

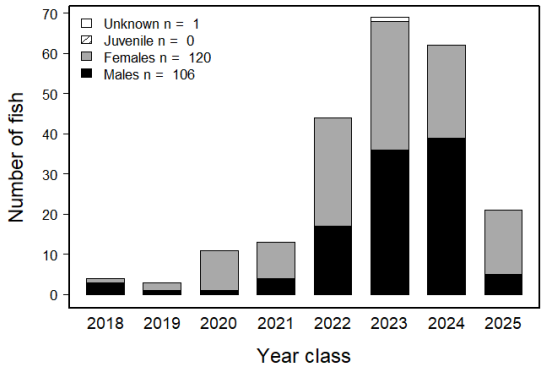


Figure 11.3: Year-class frequency distribution for Spotted Seatrout collected for ageing in 2025. Distribution is broken down by sex. 'Unknown' represents gonads that were not available for examination or were not examined for sex during sampling.

11.3.4 Age-length Key (ALK)

We developed an age-length-key (Table 11.4) that can be used in the conversion of numbers-at-length in the estimated catch to numbers-at-age using otolith ages. The table is based on VMRC's stratified sampling of landings by total length inch intervals.

Table 11.1: Number of Spotted Seatrout collected and aged in each 1-inch length interval in 2025. 'Target' represents the sample size for ageing estimated for 2025, and 'Need' represents number of fish shorted in each length interval compared to the optimum sample size for ageing and number of fish aged.

Interval	Target	Collected	Aged	Need
8 - 8.99	5	0	0	5
9 - 9.99	5	0	0	5
10 - 10.99	5	0	0	5
11 - 11.99	8	0	0	8
12 - 12.99	12	14	14	0
13 - 13.99	10	11	11	0
14 - 14.99	13	5	5	8
15 - 15.99	20	18	18	2
16 - 16.99	26	21	21	5
17 - 17.99	26	38	32	0
18 - 18.99	22	33	22	0
19 - 19.99	18	24	18	0
20 - 20.99	17	19	18	0
21 - 21.99	11	16	12	0
22 - 22.99	12	14	12	0
23 - 23.99	9	10	10	0
24 - 24.99	8	11	8	0
25 - 25.99	8	9	8	0
26 - 26.99	5	6	6	0
27 - 27.99	5	6	5	0
28 - 28.99	5	6	5	0
29 - 29.99	5	1	1	4
30 - 30.99	5	1	1	4
31 - 31.99	5	0	0	5
Totals	265	263	227	51

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Table 11.2: CV for each age estimated based on ageing the total of 265 Spotted Seatrout in 2025. 'Percent' is the percentage of an age in the pooled age-length data of Spotted Seatrout collected from 2019 to 2023.

Age	CV	Percent
0	0.11	9.79
1	0.05	46.63
2	0.09	26.47
3	0.14	11.89
4	>0.25	3.55
5	>0.25	1.67

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Table 11.3: The number of Spotted Seatrout assigned to each total length-at-age category for 227 fish sampled for otolith age determination in Virginia during 2025.

	Age								
Interval	0	1	2	3	4	5	6	7	Totals
12 - 12.99	14	0	0	0	0	0	0	0	14
13 - 13.99	6	5	0	0	0	0	0	0	11
14 - 14.99	1	4	0	0	0	0	0	0	5
15 - 15.99	0	15	3	0	0	0	0	0	18
16 - 16.99	0	14	7	0	0	0	0	0	21
17 - 17.99	0	12	19	1	0	0	0	0	32
18 - 18.99	0	9	11	2	0	0	0	0	22
19 - 19.99	0	1	11	6	0	0	0	0	18
20 - 20.99	0	2	8	7	1	0	0	0	18
21 - 21.99	0	0	3	8	1	0	0	0	12
22 - 22.99	0	0	4	7	1	0	0	0	12
23 - 23.99	0	0	2	4	3	1	0	0	10
24 - 24.99	0	0	1	4	2	0	1	0	8
25 - 25.99	0	0	0	3	4	0	0	1	8
26 - 26.99	0	0	0	2	1	1	0	2	6
27 - 27.99	0	0	0	0	0	5	0	0	5
28 - 28.99	0	0	0	0	0	3	2	0	5
29 - 29.99	0	0	0	0	0	0	0	1	1
30 - 30.99	0	0	0	0	0	1	0	0	1
Totals	21	62	69	44	13	11	3	4	227

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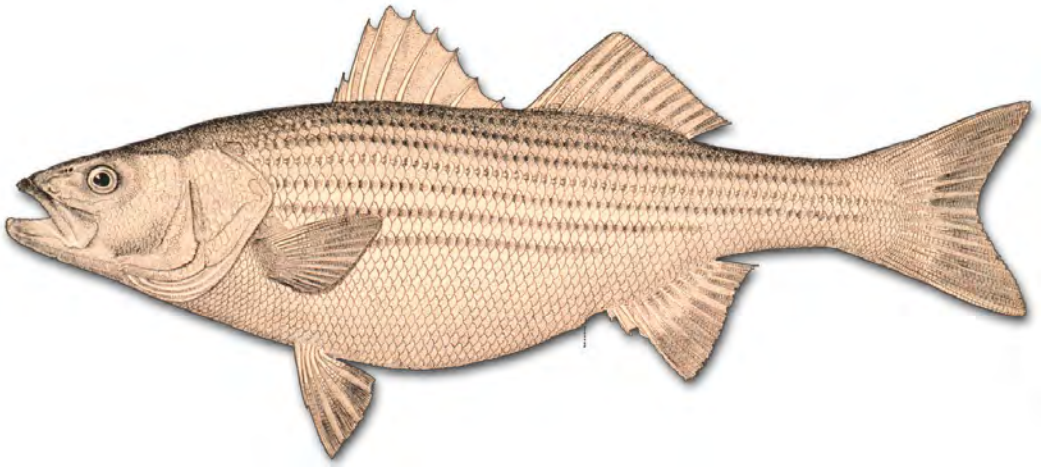
Table 11.4: Age-Length key, as proportion-at-age in each 1-inch length interval, based on otolith ages for Spotted Seatrout sampled for age determination in Virginia during 2025.

	Age							
Interval	0	1	2	3	4	5	6	7
12 - 12.99	1	0	0	0	0	0	0	0
13 - 13.99	0.55	0.45	0	0	0	0	0	0
14 - 14.99	0.2	0.8	0	0	0	0	0	0
15 - 15.99	0	0.83	0.17	0	0	0	0	0
16 - 16.99	0	0.67	0.33	0	0	0	0	0
17 - 17.99	0	0.38	0.59	0.03	0	0	0	0
18 - 18.99	0	0.41	0.5	0.09	0	0	0	0
19 - 19.99	0	0.06	0.61	0.33	0	0	0	0
20 - 20.99	0	0.11	0.44	0.39	0.06	0	0	0
21 - 21.99	0	0	0.25	0.67	0.08	0	0	0
22 - 22.99	0	0	0.33	0.58	0.08	0	0	0
23 - 23.99	0	0	0.2	0.4	0.3	0.1	0	0
24 - 24.99	0	0	0.12	0.5	0.25	0	0.12	0
25 - 25.99	0	0	0	0.38	0.5	0	0	0.12
26 - 26.99	0	0	0	0.33	0.17	0.17	0	0.33
27 - 27.99	0	0	0	0	0	1	0	0
28 - 28.99	0	0	0	0	0	0.6	0.4	0
29 - 29.99	0	0	0	0	0	0	0	1
30 - 30.99	0	0	0	0	0	1	0	0

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Chapter 12

STRIPED BASS *Morone saxatilis*



12.1 INTRODUCTION

We aged a total of 685 Striped Bass *Morone saxatilis*, collected by the VMRC's Biological Sampling Program in 2025. Of 685 aged fish, 602 and 83 fish were collected in Chesapeake Bay (bay fish) and Virginia waters of the Atlantic Ocean (ocean fish), respectively. The average bay fish age was 6.9 years with a standard deviation of 3.3 and a standard error of 0.13. Twenty-one age classes (2 to 19, and 21 to 23) were represented in the bay fish, comprising fish from the 2002 to 2004, and 2006 to 2023 year classes. The bay fish sample in 2025 was dominated by the year classes of 2014, 2015, 2018, 2019, 2020, 2021, and 2022 with 6%, 18%, 10%, 10%, 14%, 15%, and 11%, respectively. The average ocean fish age was 10.5 years with a standard deviation of 1.5 and a standard error of 0.16. Ten age classes (7 to 14, and 16 to 17) were represented in the ocean fish, comprising fish from the 2008 to 2009, and 2011 to 2018 year classes. The ocean fish sample in 2025 was dominated by the year classes of 2014 and 2015 with 22% and 54%, respectively. We also aged 242 fish using their otoliths in addition to ageing their scales. The otolith ages were compared to the scale ages to examine how close both ages were to one another (see details in Results).

12.2 METHODS

12.2.1 Sample Size for Ageing

We estimated sample sizes for ageing Striped Bass collected in both Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2025, respectively, using a two-stage random sampling method (Quinn and Deriso 1999) to increase precision in estimates of age composition from fish sampled efficiently and effectively. The basic equation is:

$$A = \frac{V_a}{\theta_a^2 CV_a^2 + B_a/L} \qquad (12.1)$$

where A is the sample size for ageing Striped Bass in 2025; θ_a stands for the proportion of Age a fish in a catch; V_a, B_a, and CV_a represent the variance components within and between length intervals, and the coefficient of variation for Age a, respectively; L is the total number of Striped Bass used by VMRC to estimate length distribution of the catches from 2019 to 2023. θ_a, V_a, and B_a were calculated using pooled age-length data of Striped Bass collected from 2019 to 2023 and using equations in Quinn and Deriso (1999). For simplicity, the equations are not listed here. The equation (1.1) indicates: 1) The more fish that are aged, the smaller the CV_a (or higher precision) that will be obtained for Age a; 2) given a sample size A, the CV_a is different for each age due to different θ_a, V_a, and B_a among different ages. Therefore, the criterion to age A (number) of fish is that A should be a number above which there is only a 1% CV_a reduction for the most abundant age in catch by ageing an additional 100 or more fish. Finally, A_l is A multiplied by the proportion of length interval l from the length distribution of the 2019 to 2023 catch. A_l is number of fish to be aged for length interval l in 2025.

12.2.2 Handling of Collection

Sagittal otoliths (hereafter, referred to as "otoliths") and scales were received by the Age and Growth Laboratory in labeled coin envelopes, and were sorted based on date of capture. Their envelope labels were verified against VMRC's collection data, and each fish assigned a unique Age and Growth Laboratory identification number. All otoliths and scales were stored dry within their original labeled coin envelopes; otoliths were contained inside protective Axygen 2.0 ml microtubes.

12.2.3 Preparation

12.2.3.1 *Otoliths*

We used our bake and thin-section technique to process Spadefish sagittal otoliths (hereafter, referred to as "otoliths") for age determination. Otolith preparation began by randomly selecting either the right or left otolith. Each

whole otolith was placed in a ceramic "Coors" spot plate well and baked in a Thermolyne 1400 furnace at 400 °C. Baking time was dependent on the otolith's size and gauged by color, with a light caramel color desired. Once a suitable color was achieved the baked otolith was embedded in epoxy resin with its distal surface orientated downwards and allowed to harden overnight. The otoliths were viewed by eye and, when necessary, under a stereo microscope to identify the location of the core, and the position of the core was marked using an ultra fine Sharpie across the epoxy resin surface. At least one transverse cross-section (hereafter, referred to as "thin-section") was then removed from the marked core of each otolith using a Buehler IsoMet^{TM} low-speed saw equipped with two 4-inch diameter diamond grinding wheels (hereafter, referred to as "blades"), separated by a stainless steel spacer of 0.5 mm (diameter 2.5"). The otolith was positioned so the blades straddled each side of the otolith core. It was crucial that this cut be perpendicular to the long axis of the otolith. Failure to do so resulted in broadening and distorted winter growth zones. A proper cut resulted in annuli that were clearly defined and delineated. Once cut, thin-sections were placed on labeled glass slides and covered with a thin layer of Flo-texx mounting medium that not only fixed the sections to the slide, but more importantly, provided enhanced contrast and greater readability by increasing light transmission through the thin-section.

Click here to obtain the protocol at the VMRV website on how to prepare otolith thin-section using the epoxy resin method for ageing Striped Bass.

12.2.3.2 *Scales*

Striped bass scales were prepared for age and growth analysis by making acetate impressions of the scale microstructure. Due to extreme variation in the size and shape of scales from individual fish, we selected only those scales that had even margins and which were of uniform size. We selected a range of four to six preferred scales (based on overall scale size) from each fish, making sure that only non-regenerated scales were used. Scale impressions were made on extruded clear acetate sheets (25 mm x 75 mm) with a Carver Laboratory Heated Press (model "C"). The scales were pressed with the following settings:

Pressure: 15000 psi
Temperature: 77 °C (170 °F)
Time: 5 to 10 min

Striped bass scales that were the size of a quarter (coin) or larger, were pressed individually for up to twenty minutes. After pressing, the impressions were viewed with a Bell and Howell microfiche reader and checked again for regeneration and incomplete margins. Impressions that were too light, or when all scales were regenerated a new impression was made using different scales from the same fish.

Click here to obtain the protocol at the VMRC website on how to prepare scale impression for ageing Striped Bass.

12.2.4 Readings

The VMRV system assigns an age class to a fish based on a combination of reading the information contained in its otolith, the date of its capture, and the species-specific period when it deposits its annulus. Each year, as the fish grows, its otoliths grow and leave behind markers of their age, called annuli. Technically, an otolith annulus is the combination of both the opaque and the translucent bands. In practice, only the opaque bands are counted as annuli. The number of these visible dark bands replaces "x" in our notation below, and is the initial "age" assignment of the fish.

Second, the otolith section is examined for translucent growth. If no translucent growth is visible beyond the last dark annulus, the otolith is called "even" and no modification of the assigned age is made. The initial assigned age, then, is the age class of the fish. Any growth beyond the last annulus can be inter-

preted as either being toward the next age class or within the same age class. If translucent growth is visible beyond the last dark annulus, a "+" is added to the notation.

By convention all fish in the Northern Hemisphere are assigned a birth date of January 1. In addition, each species has a specific period during which it deposits the dark band of the annulus. If the fish is captured after the end of the species-specific annulus deposition period and before January 1, it is assigned an age class notation of "x+x", where "x" is the number of dark bands in the otolith. If the fish is captured between January 1 and the end of the species-specific annulus deposition period, it is assigned an age class notation of "x+(x+1)". Thus, any growth beyond the last annulus, after its "birthday", but before the dark band deposition period, is interpreted as being toward the next age class.

For example, Striped Bass otolith deposition occurs between April and June (Secor et al. 1995). A Striped Bass captured between January 1 and June 30, before the end of the species' annulus formation period, with three visible annuli and some translucent growth after the last annulus, would be assigned an age class of "x+(x+1)" or 3+(3+1), noted as 3+4. This is the same age-class assigned to a fish with four visible annuli captured after the end of June 30, the period of annulus formation, which would be noted as 4+4.

Striped bass scales are also considered to have a deposition between April and June (Secor et al. 1995), and age class assignment using these hard-parts is conducted in the same way as otoliths.

All Striped Bass samples (scale pressings and sectioned otoliths) were aged by two different readers in chronological order based on collection date, without knowledge of previously estimated ages or the specimen lengths. When the readers' ages agreed, that age was assigned to the fish. When the two readers disagreed, both readers sat down together and re-aged the fish again without any knowledge of previously estimated ages or lengths, then assigned a final age to the fish. When the age readers were unable to agree on a final age, the fish was excluded from further analysis.

12.2.4.1 *Otoliths*

When an otolith was properly sectioned, the sulcal groove came to a sharp point (Hereafter referred to as "focus") within the middle of the core (Figure 12.1). By convention an annulus is identified as the narrow opaque zone, or winter growth. Typically the first year's annulus can be determined by first locating the focus of the otolith section. The focus is generally located, depending on preparation, in the center of the otolith section, and is visually well defined as a dark oblong region. The first year's annulus can be located directly below the focus, along the outer ridge of the sulcal groove on the ventral and dorsal sides of the otolith section. This insertion point along the sulcal ridge resembles a check mark (not to be confused with a false annulus). Here the annulus can be followed outwards along the ventral and dorsal surfaces where it encircles the focus. Subsequent annuli also emanate from the sulcal ridge; however, they do not encircle the focus, but rather travel outwards to the distal surface of the otolith. To be considered a true annulus, each annulus must be rooted in the sulcus and travel without interruption to the distal surface of the otolith. The annuli in Striped Bass have a tendency to split as they advance towards the distal surface. As a result, it is critical that reading path proceed in a direction down the sulcal ridge and outwards to the distal surface.

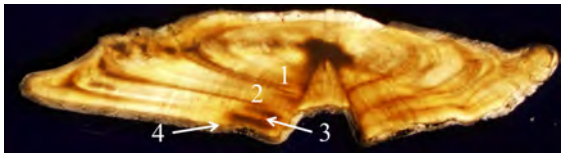


Figure 12.1: Otolith thin-section of a 4 year-old Striped Bass with the last annulus on the edge of the thin-section

All thin-sections were aged by two different readers using a Nikon SMZ1000 stereo microscope under transmitted light and dark-field polarization at between 8 and 20 times magnification. Each reader aged all of the otolith samples.

Click here to obtain the protocol at the VMRV website on how to age Striped Bass using their otolith thin-sections.

12.2.4.2 *Scales*

We determined fish age by viewing acetate impressions of scales (Figure 12.2) with a standard Bell and Howell R-735 microfiche reader equipped with 20 and 29 mm lenses. Annuli on

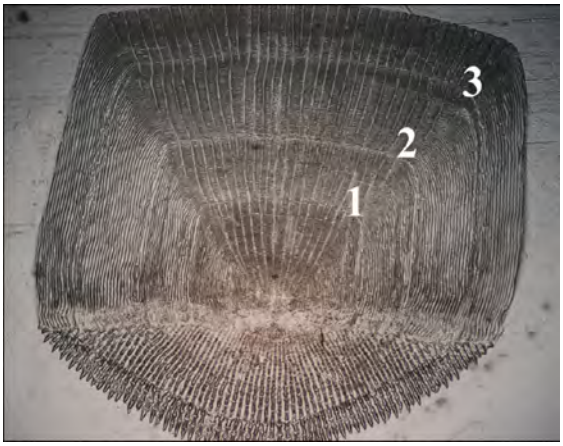


Figure 12.2: Scale impression of a 3 year-old Striped Bass.

Striped Bass scales are identified based on two scale microstructure features, "crossing over" and circuli disruption. Primarily, "crossing over" in the lateral margins near the posterior/anterior interface of the scale is used to determine the origin of the annulus. Here compressed circuli (annulus) "cross-over" the previously deposited circuli of the previous year's growth. Typically annuli of the first three years can be observed transversing this interface as dark bands. These bands remain consistent throughout the posterior field and rejoin the posterior/anterior interface on the opposite side of the focus. Annuli can also be observed in the anterior lateral field of the scale. Here the annuli typically reveal a pattern of discontinuous and suddenly breaking segmented circuli. This event can also be distinguished by the presence of concentric white lines, which are typically associated with the disruption of circuli.

Annuli can also be observed bisecting the perpendicular plain of the radial striations in the anterior field of the scale. Radii emanate out from the focus of the scale towards the outer corner margins of the anterior field. These radial striations consist mainly of segmented concave circuli. The point of intersection between radii and annuli results in a "straightening out" of the concave circuli. This straightening of the circuli should be consistent throughout the entire anterior field of the scale. This event is further amplified by the presence of concave circuli neighboring both directly above and below the annulus. The first year's annulus can be difficult to locate on some scales. It is typically best identified in the lateral field of the anterior portion of the scale. The distance from the focus to the first year's annulus is typically larger with respect to the following annuli. For the annuli two through six, summer growth generally decreases proportionally. For ages greater than six, a crowding effect of the annuli near the outer margins of the scale is observed. This crowding effect creates difficulties in edge interpretation. At this point it is best to focus on the straightening of the circuli at the anterior margins of the scale.

When ageing young Striped Bass, zero through age two, extreme caution must be taken as not to over age the structure. In young fish there is no point of reference to aid in the determination of the first year; this invariably results in over examination of the scale and such events as hatching or saltwater incursion marks (checks) may be interpreted as the first year.

12.2.5 Comparison Tests

A symmetry test (Hoenig et al. 1995) and coefficient of variation (CV) analysis were used

to detect any systematic difference and precision on age readings, respectively, for following comparisons: 1) between the two readers in the current year; 2) within each reader in the current year; 3) time-series bias between the current and previous years within each reader; and 4) between scale and otoliths ages. The readings from the entire sample for the current year were used to examine the difference between two readers. A random sub-sample of 50 fish from the current year was selected for second readings to examine the difference within a reader. Fifty otoliths and scales randomly selected from fish aged in 2000 were used to examine the time-series bias within each reader. A figure of 1:1 equivalence was used to illustrate those differences (Campana et al. 1995). All statistics analyses were performed in R.4.0.2 (R Core Team 2021).

12.3 RESULTS

12.3.1 Sample Size

The sample sizes are estimated for Striped Bass in Chesapeake Bay and Virginia waters of Atlantic Ocean, respectively. The total sample collected from each area consists of the fish with total lengths, with both otoliths and scales, otolith-only, and scale-only. The total sample aged from each area may be smaller than or equal to the total sample size.

12.3.1.1 *Chesapeake Bay*

We estimated a sample size of 563 bay Striped Bass in 2025, ranging in length interval from 10 to 55 inches (Table 12.1). This sample size provided a range in CV for age composition approximately from the smallest CV of 9% for the major age of Age 4 and 5 to the CV of larger than 25% for the multiple minor ages of the bay fish (Table 12.2). We aged all the fish with both scales and otoliths (242 fish). We randomly selected 360 fish with scale-only to age. As a result, we aged 602 of 738 fish (The rest of fish were either without any hardparts or over-collected for certain length interval(s)) collected by VMRC in Chesapeake Bay in 2025. We fell short in our over-all collections for the optimal length-class sampling estimate by 111 fish. We were short only a few fish from the major length intervals (The interval requires 10 or more fish), as a result, the precision for the estimates of major age groups would not be influenced significantly.

12.3.1.2 *Atlantic Ocean*

We estimated a sample size of 521 ocean Striped Bass in 2025, ranging in length interval from 20 to 53 inches (Table 12.3). This sample size provided a range in CV for age composition approximately from the smallest CV of 10% for the major age of Age 10, 11, and 12 to the CV of larger than 25% for the multiple minor ages of the ocean fish (Table 12.4).

We aged all of 83 fish collected by VMRC in Virginia waters of the Atlantic Ocean in 2025 using their scales (No otoliths). We fell short in our over-all collections for the optimal length-class sampling estimate by 438 fish. We were short many fish from the major length intervals (The interval requires 10 or more fish), as a result, the precision for the estimates of major age groups would definitely be influenced significantly. Therefore, precaution should be used when developing ALK using these age data.

12.3.2 Reading Precision

12.3.2.1 *Otoliths*

Reader 1 and Reader 2 aged the otoliths of 242 Striped Bass collected in 2025. Both readers had high self-precision. Specifically, there was no significant difference between the first and second readings for Reader 1 with an agreement of 92% (1 year or less agreement of 100%) and a CV of 0.7% (test of symmetry: $\chi^2 = 4$, $df = 3$, $P = 0.2615$), and there was no significant difference between the first and second readings for Reader 2 with an agreement of 90% (1 year or less agreement of 100%) and a CV of 0.9% (test of symmetry: $\chi^2 = 3$, $df = 4$, $P = 0.5578$).

There was no evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 91% (1 year or less agreement of 98%) and a CV of 0.7% (test of symmetry: χ^2 = 14.2, df = 14, P = 0.4349) (Figure 12.3).

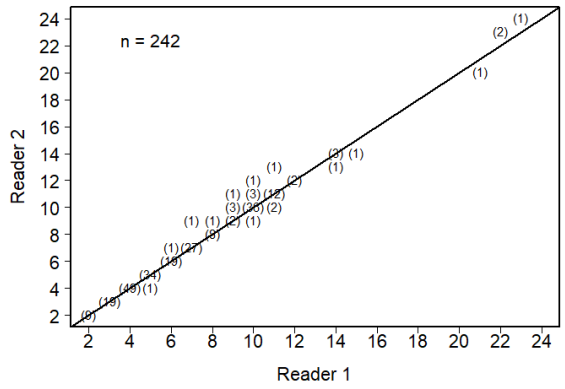


Figure 12.3: Between-reader comparison of otolith age estimates for Striped Bass collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2025. The number in parentheses is number of fish.

There was no time-series bias for either reader. Reader 1 had an agreement of 83% (1 year or less agreement of 93%) with ages of fish aged in 2000 with a CV of 2.1% (test of symmetry: χ^2 = 7.3, df = 8, P = 0.5011). Reader 2 had an agreement of 72% (1 year or less agreement of 88%) with a CV of 3.7% (test of symmetry: χ^2 = 17, df = 11, P = 0.1079).

12.3.2.2 *Scales*

Reader 1 and Reader 2 the scales of 685 Striped Bass collected in 2025. Reader 1 had high self-precision and Read 2 had moderate self-precision. Specifically, there was no significant difference between the first and second readings for Reader 1 with an agreement of 73% (1 year or less agreement of 98%) and a CV of 2.4% (test of symmetry: χ^2 = 10, df = 8, P = 0.265), and there was no significant difference between the first and second readings for Reader 2 with an agreement of 34% (1 year or less agreement of 74%) and a CV of 9.6% (test of symmetry: χ^2 = 20, df = 17, P = 0.2742).

There was an evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 64% (1 year or less agreement of 90%) and a CV of 4.7% (test of symmetry: χ^2 = 82.4, df = 42, P = 0.0002) (Figure 12.4).

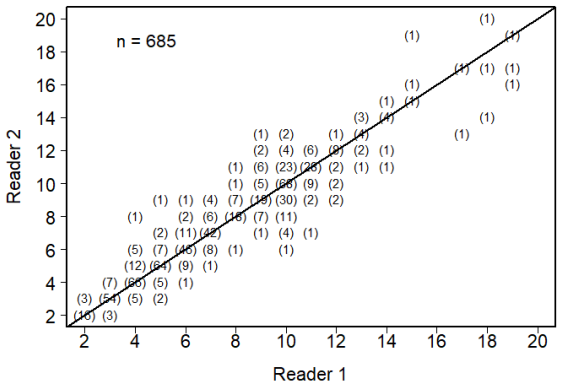


Figure 12.4: Between-reader comparison of scale age estimates for Striped Bass collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2025. The number in parentheses is number of fish.

There was no time-series bias for either reader. Reader 1 had an agreement of 57% (1 year or less agreement of 95%) with ages of fish aged in 2000 with a CV of 5.4% (test of symmetry: χ^2 = 11.3, df = 12, P = 0.5006). Reader 2 had an agreement of 82% (1 year or less agreement of 93%) with a CV of 2.4% (test of symmetry: χ^2 = 9, df = 10, P = 0.5321).

12.3.3 Year Class

The year classes were estimated using all the aged fish described in Section Sample Size for Chesapeake Bay and Virginia waters of Atlantic Ocean, respectively. The otolith ages are always the primary ages. When otolith ages are not available, the scale ages are used.

12.3.3.1 *Chesapeake Bay*

Of the 602 bay Striped Bass aged, 21 age classes (2 to 19, and 21 to 23) were represented (Table 12.5). The average age for the sample was 6.9 years. The standard deviation

and standard error were 3.3 and 0.13, respectively. Year-class data (Figure 12.5) indicates that recruitment into the fishery in Chesapeake Bay begins at age 2, which corresponds to the 2023 year-class for Striped Bass caught in 2025. Striped bass in the sample in 2025 was dominated by the year classes of 2014, 2015, 2018, 2019, 2020, 2021, and 2022 with 6%, 18%, 10%, 10%, 14%, 15%, and 11%, respectively. The sex ratio of male to female was 1:1.52 for the bay fish.

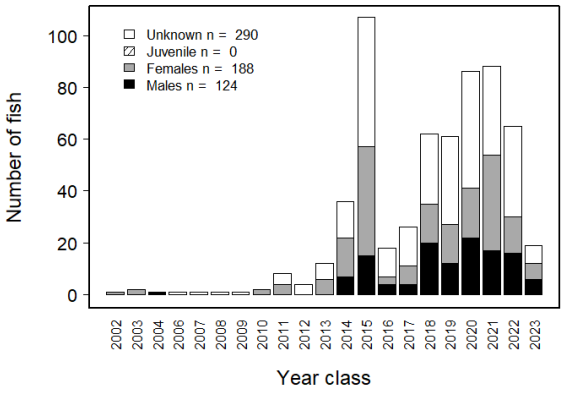


Figure 12.5: Year-class frequency distribution for Striped Bass collected in Chesapeake Bay, Virginia for ageing in 2025. Distribution is broken down by sex and estimated using scale ages. 'Unknown' represents the fish gonads that were not available for examination or were not examined for sex during sampling.

12.3.3.2 *Atlantic Ocean*

Of the 83 ocean Striped Bass aged, 10 age classes (7 to 14, and 16 to 17) were represented (Table 12.6). The average age for the sample was 10.5 years. The standard deviation and standard error were 1.5 and 0.16, respectively. Year-class data (Figure 12.6) indicates that recruitment into the fishery in Virginia waters of Atlantic ocean begins at age 7, which corresponds to the 2018 year-class for Striped Bass caught in 2025. Striped bass in the sample in 2025 was dominated by the year classes of 2014 and 2015 with 22% and 54%, respectively. The sex ratio of male to female was 1:3.67 for the ocean fish.

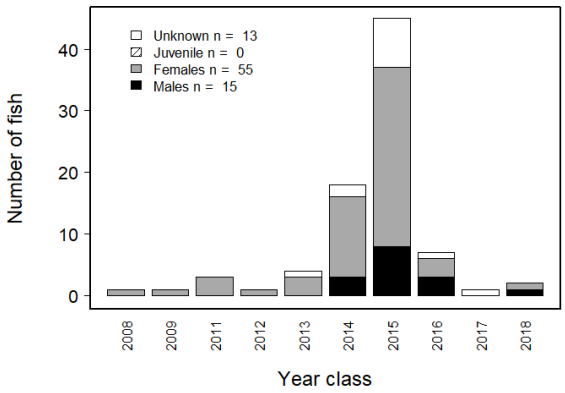


Figure 12.6: Year-class frequency distribution for Striped Bass collected in Virginia waters of the Atlantic Ocean for ageing in 2025. Distribution is broken down by sex and estimated using scale ages. 'Unknown' represents the fish gonads that were not available for examination or were not examined for sex during sampling.

12.3.4 Age-Length-Key (ALK)

We developed an age-length-key for both bay (Table 12.7) and ocean fish (Table 12.8) using scale ages, separately. The ALK can be used in the conversion of numbers-at-length in the estimated catch to numbers-at-age using scale ages. The table is based on VMRC's stratified sampling of landings by total length inch intervals.

12.3.5 Comparison of Scale and Otolith Ages

We aged 242 pairs of Striped Bass scales and otoliths. There was an evidence of systematic disagreement between otolith and scale ages (test of symmetry: $\chi^2 = 38.8$, $df = 22$, $P = 0.0148$) with an average CV of 3.5%. There was an agreement of 71% (1 year or less agreement of 93%) between scale and otoliths ages whereas scales were assigned a lower and higher age than otoliths for 21.1% and 7.9% of the fish, respectively (Figure 12.7). There was also an evidence of bias between otolith and scale ages using an age bias plot (Figure 12.8), with scale generally assigned higher ages for younger fish and lower ages for older fish than otolith age estimates.

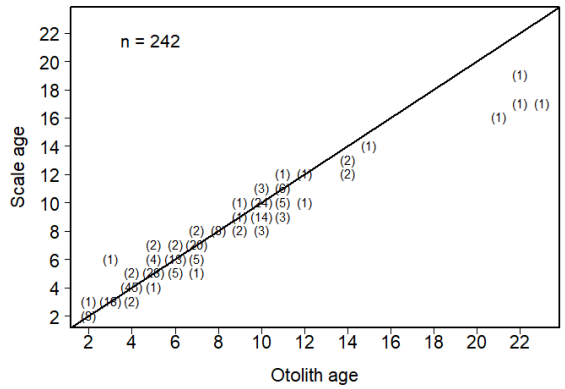


Figure 12.7: Comparison of scale and otolith age estimates for Striped Bass collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2025. The number in parentheses is number of fish.

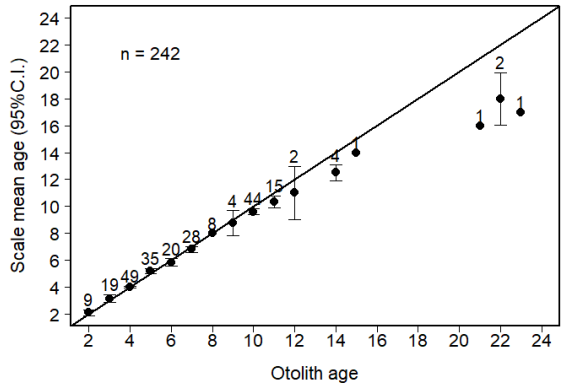


Figure 12.8: Age-bias plot for Striped Bass scale and otolith age estimates in 2025. The number above the upper CI bar is number of fish.

12.4 RECOMMENDATIONS

We recommend that VMRC and ASMFC use otoliths for ageing Striped Bass. Although preparation time is greater for otoliths compared to scales, nonetheless as the mean age of Striped Bass increases in the recovering fishery, otoliths should provide more reliable estimates of age (Secor et al. 1995; Liao et al. 2013). We will continue to compare the age estimates between otoliths and scales.

Table 12.1: Number of bay Striped Bass collected and aged in each 1-inch length interval in 2025. 'Target' represents the sample size for ageing estimated for 2025, and 'Need' represents number of fish shorted in each length interval compared to the optimum sample size for ageing and number of fish aged.

Interval	Target	Collected	Aged	Need
10 - 10.99	5	0	0	5
11 - 11.99	5	0	0	5
12 - 12.99	5	0	0	5
13 - 13.99	5	0	0	5
14 - 14.99	5	0	0	5
15 - 15.99	5	0	0	5
16 - 16.99	5	0	0	5
17 - 17.99	5	1	1	4
18 - 18.99	16	11	11	5
19 - 19.99	35	46	42	0
20 - 20.99	42	56	54	0
21 - 21.99	32	72	49	0
22 - 22.99	30	56	39	0
23 - 23.99	27	45	32	0
24 - 24.99	26	53	31	0
25 - 25.99	24	47	29	0
26 - 26.99	22	40	29	0
27 - 27.99	19	30	26	0
28 - 28.99	16	27	21	0
29 - 29.99	13	14	14	0
30 - 30.99	13	15	15	0
31 - 31.99	12	9	9	3
32 - 32.99	14	17	15	0
33 - 33.99	12	15	10	2
34 - 34.99	10	13	13	0
35 - 35.99	13	17	15	0
36 - 36.99	11	22	20	0
37 - 37.99	12	26	24	0
38 - 38.99	11	21	19	0
39 - 39.99	9	21	21	0
40 - 40.99	7	20	20	0
41 - 41.99	9	13	13	0
42 - 42.99	8	13	12	0
43 - 43.99	7	6	6	1
44 - 44.99	9	5	5	4
45 - 45.99	8	2	2	6
46 - 46.99	8	3	3	5
47 - 47.99	8	0	0	8
48 - 48.99	5	0	0	5
49 - 49.99	5	2	2	3
50 - 50.99	5	0	0	5
51 - 51.99	5	0	0	5
52 - 52.99	5	0	0	5
53 - 53.99	5	0	0	5
54 - 54.99	5	0	0	5
55 - 55.99	5	0	0	5
Totals	563	738	602	111

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Table 12.2: CV for each age estimated based on ageing the total of 563 bay Striped Bass in 2025. 'Percent' is the percentage of an age in the pooled age-length data of bay Striped Bass collected from 2019 to 2023.

Age	CV	Percent
1	>0.25	0.58
2	>0.25	0.66
3	0.15	5.91
4	0.09	15.06
5	0.09	17.24
6	0.1	13.82
7	0.14	8.38
8	0.12	9.88
9	0.14	7.77
10	0.21	3.79
11	0.23	2.95
12	>0.25	1.9
13	>0.25	0.87
14	>0.25	1.09
15	>0.25	1.49
16	0.25	2.37
17	>0.25	1.79
18	>0.25	1.82
19	>0.25	1.2
20	>0.25	0.62
21	>0.25	0.36
22	>0.25	0.26
23	>0.25	0.15
24	>0.25	0.04

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Table 12.3: Number of ocean Striped Bass collected and aged in each 1-inch length interval in 2025. 'Target' represents the sample size for ageing estimated for 2025, and 'Need' represents number of fish shorted in each length interval compared to the optimum sample size for ageing and number of fish aged.

Interval	Target	Collected	Aged	Need
20 - 20.99	5	0	0	5
21 - 21.99	5	0	0	5
22 - 22.99	5	0	0	5
23 - 23.99	5	0	0	5
24 - 24.99	5	0	0	5
25 - 25.99	5	0	0	5
26 - 26.99	5	0	0	5
27 - 27.99	5	0	0	5
28 - 28.99	5	0	0	5
29 - 29.99	5	0	0	5
30 - 30.99	5	0	0	5
31 - 31.99	5	0	0	5
32 - 32.99	5	4	4	1
33 - 33.99	8	7	7	1
34 - 34.99	13	2	2	11
35 - 35.99	21	9	9	12
36 - 36.99	40	13	13	27
37 - 37.99	54	15	15	39
38 - 38.99	70	17	17	53
39 - 39.99	59	8	8	51
40 - 40.99	48	3	3	45
41 - 41.99	38	1	1	37
42 - 42.99	26	2	2	24
43 - 43.99	18	2	2	16
44 - 44.99	13	0	0	13
45 - 45.99	5	0	0	5
46 - 46.99	8	0	0	8
47 - 47.99	5	0	0	5
48 - 48.99	5	0	0	5
49 - 49.99	5	0	0	5
50 - 50.99	5	0	0	5
51 - 51.99	5	0	0	5
52 - 52.99	5	0	0	5
53 - 53.99	5	0	0	5
Totals	521	83	83	438

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Table 12.4: CV for each age estimated based on ageing the total of 521 ocean Striped Bass in 2025. 'Percent' is the percentage of an age in the pooled age-length data of ocean Striped Bass collected from 2019 to 2023.

Age	CV	Percent
5	>0.25	0.34
6	>0.25	0.09
7	>0.25	1.02
8	0.12	10.55
9	0.11	14.38
10	0.1	17.28
11	0.1	16.6
12	0.1	16.09
13	0.19	5.02
14	0.21	4.26
15	0.2	4.26
16	>0.25	2.3
17	>0.25	2.55
18	>0.25	1.87
19	>0.25	1.36
20	>0.25	0.68
21	>0.25	0.68
22	>0.25	0.17
23	>0.25	0.17
24	>0.25	0.26
25	>0.25	0.09

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Table 12.5: The number of Striped Bass assigned to each total length-at-age category for 602 fish sampled for both otolith and scale age determination in Chesapeake Bay, Virginia during 2025.

	Age																					
Interval	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	21	22	23	Totals
17 - 17.99	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
18 - 18.99	2	5	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11
19 - 19.99	8	15	18	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	42
20 - 20.99	7	11	21	12	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	54
21 - 21.99	2	17	13	16	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	49
22 - 22.99	0	9	9	12	5	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	39
23 - 23.99	0	5	7	9	8	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	32
24 - 24.99	0	2	6	11	7	4	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	31
25 - 25.99	0	0	3	8	6	6	4	1	1	0	0	0	0	0	0	0	0	0	0	0	0	29
26 - 26.99	0	0	3	2	8	12	2	0	2	0	0	0	0	0	0	0	0	0	0	0	0	29
27 - 27.99	0	0	2	5	7	5	3	1	3	0	0	0	0	0	0	0	0	0	0	0	0	26
28 - 28.99	0	0	1	4	3	8	2	0	3	0	0	0	0	0	0	0	0	0	0	0	0	21
29 - 29.99	0	0	2	1	5	4	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	14
30 - 30.99	0	0	0	2	3	3	0	1	4	2	0	0	0	0	0	0	0	0	0	0	0	15
31 - 31.99	0	0	0	1	2	0	2	0	3	1	0	0	0	0	0	0	0	0	0	0	0	9
32 - 32.99	0	0	0	1	2	4	4	0	3	1	0	0	0	0	0	0	0	0	0	0	0	15
33 - 33.99	0	0	0	0	1	3	1	3	2	0	0	0	0	0	0	0	0	0	0	0	0	10
34 - 34.99	0	0	0	0	0	2	2	3	6	0	0	0	0	0	0	0	0	0	0	0	0	13
35 - 35.99	0	0	0	0	0	2	0	2	9	2	0	0	0	0	0	0	0	0	0	0	0	15
36 - 36.99	0	0	0	0	0	0	2	1	15	2	0	0	0	0	0	0	0	0	0	0	0	20
37 - 37.99	0	0	0	0	0	0	1	3	16	4	0	0	0	0	0	0	0	0	0	0	0	24
38 - 38.99	0	0	0	0	0	1	0	2	12	4	0	0	0	0	0	0	0	0	0	0	0	19
39 - 39.99	0	0	0	0	1	0	0	0	10	6	3	0	0	0	0	0	0	0	1	0	0	21
40 - 40.99	0	0	0	0	0	0	0	1	13	6	0	0	0	0	0	0	0	0	0	0	0	20
41 - 41.99	0	0	0	0	0	1	0	0	3	7	1	0	1	0	0	0	0	0	0	0	0	13
42 - 42.99	0	0	0	0	0	0	0	0	1	1	5	1	3	1	0	0	0	0	0	0	0	12
43 - 43.99	0	0	0	0	0	0	0	0	0	0	1	1	3	1	0	0	0	0	0	0	0	6
44 - 44.99	0	0	0	0	0	0	0	0	0	0	1	2	0	0	0	0	1	0	0	1	0	5
45 - 45.99	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	2
46 - 46.99	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	0	0	0	0	0	3
49 - 49.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	2
Totals	19	65	88	86	61	62	26	18	107	36	12	4	8	2	1	1	1	1	1	2	1	602

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Table 12.6: The number of Striped Bass assigned to each total length-at-age category for 83 fish sampled for both otolith and scale age determination in Virginia waters of Atlantic ocean during 2025.

	Age										
Interval	7	8	9	10	11	12	13	14	16	17	Totals
32 - 32.99	0	0	0	4	0	0	0	0	0	0	4
33 - 33.99	1	0	2	3	1	0	0	0	0	0	7
34 - 34.99	1	0	0	1	0	0	0	0	0	0	2
35 - 35.99	0	1	2	4	2	0	0	0	0	0	9
36 - 36.99	0	0	1	8	4	0	0	0	0	0	13
37 - 37.99	0	0	1	8	5	1	0	0	0	0	15
38 - 38.99	0	0	1	10	5	1	0	0	0	0	17
39 - 39.99	0	0	0	7	0	0	0	1	0	0	8
40 - 40.99	0	0	0	0	1	2	0	0	0	0	3
41 - 41.99	0	0	0	0	0	0	0	1	0	0	1
42 - 42.99	0	0	0	0	0	0	0	1	0	1	2
43 - 43.99	0	0	0	0	0	0	1	0	1	0	2
Totals	2	1	7	45	18	4	1	3	1	1	83

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Table 12.7: Age-Length key, as proportion-at-age in each 1-inch length interval, based on both otolith and scale ages for Striped Bass sampled in Chesapeake Bay, Virginia during 2025.

	Age																				
Interval	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	21	22	23
17 - 17.99	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18 - 18.99	0.18	0.45	0.27	0.09	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19 - 19.99	0.19	0.36	0.43	0.02	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20 - 20.99	0.13	0.2	0.39	0.22	0.04	0.02	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
21 - 21.99	0.04	0.35	0.27	0.33	0.02	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
22 - 22.99	0	0.23	0.23	0.31	0.13	0.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
23 - 23.99	0	0.16	0.22	0.28	0.25	0.06	0.03	0	0	0	0	0	0	0	0	0	0	0	0	0	0
24 - 24.99	0	0.06	0.19	0.35	0.23	0.13	0.03	0	0	0	0	0	0	0	0	0	0	0	0	0	0
25 - 25.99	0	0	0.1	0.28	0.21	0.21	0.14	0.03	0.03	0	0	0	0	0	0	0	0	0	0	0	0
26 - 26.99	0	0	0.1	0.07	0.28	0.41	0.07	0	0.07	0	0	0	0	0	0	0	0	0	0	0	0
27 - 27.99	0	0	0.08	0.19	0.27	0.19	0.12	0.04	0.12	0	0	0	0	0	0	0	0	0	0	0	0
28 - 28.99	0	0	0.05	0.19	0.14	0.38	0.1	0	0.14	0	0	0	0	0	0	0	0	0	0	0	0
29 - 29.99	0	0	0.14	0.07	0.36	0.29	0.07	0	0.07	0	0	0	0	0	0	0	0	0	0	0	0
30 - 30.99	0	0	0	0.13	0.2	0.2	0	0.07	0.27	0.13	0	0	0	0	0	0	0	0	0	0	0
31 - 31.99	0	0	0	0.11	0.22	0	0.22	0	0.33	0.11	0	0	0	0	0	0	0	0	0	0	0
32 - 32.99	0	0	0	0.07	0.13	0.27	0.27	0	0.2	0.07	0	0	0	0	0	0	0	0	0	0	0
33 - 33.99	0	0	0	0	0.1	0.3	0.1	0.3	0.2	0	0	0	0	0	0	0	0	0	0	0	0
34 - 34.99	0	0	0	0	0	0.15	0.15	0.23	0.46	0	0	0	0	0	0	0	0	0	0	0	0
35 - 35.99	0	0	0	0	0	0.13	0	0.13	0.6	0.13	0	0	0	0	0	0	0	0	0	0	0
36 - 36.99	0	0	0	0	0	0	0.1	0.05	0.75	0.1	0	0	0	0	0	0	0	0	0	0	0
37 - 37.99	0	0	0	0	0	0	0.04	0.12	0.67	0.17	0	0	0	0	0	0	0	0	0	0	0
38 - 38.99	0	0	0	0	0	0.05	0	0.11	0.63	0.21	0	0	0	0	0	0	0	0	0	0	0
39 - 39.99	0	0	0	0	0.05	0	0	0	0.48	0.29	0.14	0	0	0	0	0	0	0	0.05	0	0
40 - 40.99	0	0	0	0	0	0	0	0.05	0.65	0.3	0	0	0	0	0	0	0	0	0	0	0
41 - 41.99	0	0	0	0	0	0.08	0	0	0.23	0.54	0.08	0	0.08	0	0	0	0	0	0	0	0
42 - 42.99	0	0	0	0	0	0	0	0	0.08	0.08	0.42	0.08	0.25	0.08	0	0	0	0	0	0	0
43 - 43.99	0	0	0	0	0	0	0	0	0	0	0.17	0.17	0.5	0.17	0	0	0	0	0	0	0
44 - 44.99	0	0	0	0	0	0	0	0	0	0	0.2	0.4	0	0	0	0	0.2	0	0	0.2	0
45 - 45.99	0	0	0	0	0	0	0	0	0	0	0	0	0.5	0	0	0	0	0	0	0	0.5
46 - 46.99	0	0	0	0	0	0	0	0	0	0	0.33	0	0	0	0.33	0.33	0	0	0	0	0
49 - 49.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.5	0	0.5	0

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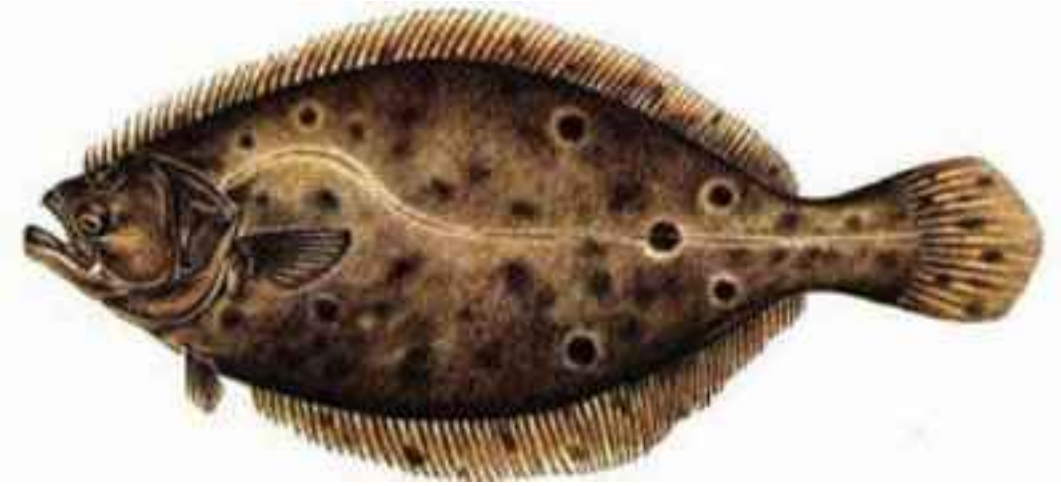
Table 12.8: Age-Length key, as proportion-at-age in each 1-inch length interval, based on both otolith and scale ages for Striped Bass sampled in Virginia waters of the Atlantic Ocean during 2025.

	Age									
Interval	7	8	9	10	11	12	13	14	16	17
32 - 32.99	0	0	0	1	0	0	0	0	0	0
33 - 33.99	0.14	0	0.29	0.43	0.14	0	0	0	0	0
34 - 34.99	0.5	0	0	0.5	0	0	0	0	0	0
35 - 35.99	0	0.11	0.22	0.44	0.22	0	0	0	0	0
36 - 36.99	0	0	0.08	0.62	0.31	0	0	0	0	0
37 - 37.99	0	0	0.07	0.53	0.33	0.07	0	0	0	0
38 - 38.99	0	0	0.06	0.59	0.29	0.06	0	0	0	0
39 - 39.99	0	0	0	0.88	0	0	0	0.12	0	0
40 - 40.99	0	0	0	0	0.33	0.67	0	0	0	0
41 - 41.99	0	0	0	0	0	0	0	1	0	0
42 - 42.99	0	0	0	0	0	0	0	0.5	0	0.5
43 - 43.99	0	0	0	0	0	0	0.5	0	0.5	0

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Chapter 13

SUMMER FLOUNDER *Paralichthys dentatus*



13.1 INTRODUCTION

We aged a total of 751 Summer Flounder *Paralichthys dentatus*, collected by the VMRC's Biological Sampling Program in 2025. Of 751 aged fish, 139 and 612 fish were collected in Chesapeake Bay (bay fish) and Virginia waters of the Atlantic Ocean (ocean fish), respectively. The average bay fish age was 2 years with a standard deviation of 1.1 and a standard error of 0.09. Seven age classes (0 to 6) were represented in the bay fish, comprising fish from the 2019 to 2025 year classes. The bay fish sample in 2025 was dominated by the year classes of 2023 and 2024 with 43% and 27%, respectively. The average ocean fish age was 5.1 years with a standard deviation of 2.2 and a standard error of 0.09. Fourteen age classes (1 to 12, 14, and 16) were represented in the ocean fish, comprising fish from the 2009, 2011, and 2013 to 2024 year classes. The ocean fish sample in 2025 was dominated by the year classes of 2018, 2019, 2020, 2021, and 2022 with 9%, 15%, 16%, 21%, and 15%, respectively. We also aged 427 fish using their otoliths in addition to ageing their scales. The otolith ages were compared to the scale ages to examine how close both ages were to one another (see details in Results).

13.2 METHODS

13.2.1 Sample Size for Ageing

We estimated sample sizes for ageing Summer Flounder collected in both Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2025, respectively, using a two-stage random sampling method (Quinn and Deriso 1999) to increase precision in estimates of age composition from fish sampled efficiently and effectively. The basic equation is:

$$A = \frac{V_a}{\theta_a^2 CV_a^2 + B_a/L} \tag{13.1}$$

where A is the sample size for ageing Summer Flounder in 2025; θ_a stands for the proportion of Age a fish in a catch; V_a, B_a, and CV_a represent the variance components within and between length intervals, and the coefficient of variation for Age a, respectively; L is the total number of Summer Flounder used by VMRC to estimate length distribution of the catches from 2019 to 2023. θ_a, V_a, and B_a were calculated using pooled age-length data of Summer Flounder collected from 2019 to 2023 and using equations in Quinn and Deriso (1999). For simplicity, the equations are not listed here. The equation (1.1) indicates: 1) The more fish that are aged, the smaller the CV_a (or higher precision) that will be obtained for Age a; 2) given a sample size A, the CV_a is different for each age due to different θ_a, V_a, and B_a among different ages. Therefore, the criterion to age A (number) of fish is that A should be a number above which there is only a 1% CV_a reduction for the most abundant age in catch by ageing an additional 100 or more fish. Finally, A_l is A multiplied by the proportion of length interval l from the length distribution of the 2019 to 2023 catch. A_l is number of fish to be aged for length interval l in 2025.

13.2.2 Handling of Collection

Sagittal otoliths (hereafter, referred to as "otoliths") and scales were received by the Age and Growth Laboratory in labeled coin envelopes, and were sorted based on date of capture, their envelope labels were verified against VMRC's collection data, and each fish assigned a unique Age and Growth Laboratory identification number. All otoliths and scales were stored dry within their original labeled coin envelopes; otoliths were contained inside protective Axygen 2.0 ml microtubes.

13.2.3 Preparation

13.2.3.1 *Otoliths*

We used our bake and thin-section technique to process Spadefish sagittal otoliths (hereafter, referred to as "otoliths") for age determination. Otolith preparation began by randomly selecting either the right or left otolith. Each whole otolith was placed in a ceramic "Coors"

spot plate well and baked in a Thermolyne 1400 furnace at 400 °C. Baking time was dependent on the otolith's size and gauged by color, with a light caramel color desired. Once a suitable color was achieved the baked otolith was embedded in epoxy resin with its distal surface orientated downwards and allowed to harden overnight. The otoliths were viewed by eye and, when necessary, under a stereo microscope to identify the location of the core, and the position of the core was marked using an ultra fine Sharpie across the epoxy resin surface. At least one transverse cross-section (hereafter, referred to as "thin-section") was then removed from the marked core of each otolith using a Buehler IsoMet^{TM} low-speed saw equipped with two 4-inch diameter diamond grinding wheels (hereafter, referred to as "blades"), separated by a stainless steel spacer of 0.5 mm (diameter 2.5"). The otolith was positioned so the blades straddled each side of the otolith core. It was crucial that this cut be perpendicular to the long axis of the otolith. Failure to do so resulted in broadening and distorted winter growth zones. A proper cut resulted in annuli that were clearly defined and delineated. Once cut, thin-sections were placed on labeled glass slides and covered with a thin layer of Flo-texx mounting medium that not only fixed the sections to the slide, but more importantly, provided enhanced contrast and greater readability by increasing light transmission through the thin-section.

Click here to obtain the protocol at the VMRV website on how to prepare otolith thin-section using the epoxy resin method for ageing Summer Flounder.

13.2.3.2 *Scales*

Summer flounder scales were prepared for age and growth analysis by making acetate impressions of the scale microstructure. Due to extreme variation in the size and shape of scales from individual fish, we selected only those scales that had even margins and which were of uniform size. We selected a range of four to six preferred scales (based on overall scale size) from each fish, making sure that only non-regenerated scales were used. Scale impressions were made on extruded clear acetate sheets (25 mm x 75 mm) with a Carver Laboratory Heated Press (model "C"). The scales were pressed with the following settings:

Pressure: 15000 psi
Temperature: 77 °C (170 °F)
Time: 5 to 10 min

Summer Flounder scales that were the size of a quarter (coin) or larger, were pressed individually for up to twenty minutes. After pressing, the impressions were viewed with a Bell and Howell microfiche reader and checked again for regeneration and incomplete margins. Impressions that were too light, or when all scales were regenerated a new impression was made using different scales from the same fish.

Click here to obtain the protocol at the VMRC website on how to prepare scale impression for ageing Summer Flounder.

13.2.4 Readings

The VMRV system assigns an age class to a fish based on a combination of reading the information contained in its otolith, the date of its capture, and the species-specific period when it deposits its annulus. Each year, as the fish grows, its otoliths grow and leave behind markers of their age, called annuli. Technically, an otolith annulus is the combination of both the opaque and the translucent bands. In practice, only the opaque bands are counted as annuli. The number of these visible dark bands replaces "x" in our notation below, and is the initial "age" assignment of the fish.

Second, the otolith section is examined for translucent growth. If no translucent growth is visible beyond the last dark annulus, the otolith is called "even" and no modification of the assigned age is made. The initial assigned age, then, is the age class of the fish. Any growth beyond the last annulus can be interpreted as either being toward the next age class

or within the same age class. If translucent growth is visible beyond the last dark annulus, a "+" is added to the notation.

By convention all fish in the Northern Hemisphere are assigned a birth date of January 1. In addition, each species has a specific period during which it deposits the dark band of the annulus. If the fish is captured after the end of the species-specific annulus deposition period and before January 1, it is assigned an age class notation of "x+x", where "x" is the number of dark bands in the otolith. If the fish is captured between January 1 and the end of the species-specific annulus deposition period, it is assigned an age class notation of "x+(x+1)". Thus, any growth beyond the last annulus, after its "birthday", but before the dark band deposition period, is interpreted as being toward the next age class.

For example, Summer Flounder otolith deposition occurs between January and April (Bolz 1999). A Summer Flounder captured between January 1 and April 30, before the end of the species' annulus formation period, with three visible annuli and some translucent growth after the last annulus, would be assigned an age class of "x+(x+1)" or 3+(3+1), noted as 3+4. This is the same age-class assigned to a fish with four visible annuli captured after the end of June 30, the period of annulus formation, which would be noted as 4+4.

Summer flounder scales are also considered to have a deposition between January and June (Bolz 1999 and modified by VMRV), and age class assignment using these hard-parts is conducted in the same way as otoliths.

All Summer Flounder samples (scale pressings and sectioned otoliths) were aged by two different readers in chronological order based on collection date, without knowledge of previously estimated ages or the specimen lengths. When the readers' ages agreed, that age was assigned to the fish. When the two readers disagreed, both readers sat down together and re-aged the fish again without any knowledge of previously estimated ages or lengths, then assigned a final age to the fish. When the age readers were unable to agree on a final age, the fish was excluded from further analysis.

13.2.4.1 *Otoliths*

When an otolith was properly sectioned, the sulcal groove came to a sharp point (Hereafter referred to as "focus") within the middle of the core (Figure 13.1). By convention an annulus is identified as the narrow opaque zone, or winter growth. Typically the first year's annulus can be determined by first locating the focus of the otolith section. The focus is generally located, depending on preparation, in the center of the otolith, and is visually well defined as a dark oblong region. The first year's annulus can be located directly below the focus, along the outer ridge of the sulcal groove on the ventral and dorsal sides of the otolith. This insertion point along the sulcal ridge resembles a check mark (not to be confused with a false annulus). Here the annulus can be followed outwards along the ventral and dorsal surfaces where it encircles the focus. Subsequent annuli also emanate from the sulcal ridge; however, they do not encircle the focus, but rather travel outwards to the distal surface of the otolith. To be considered a true annulus, each annulus must be rooted in the sulcus and travel without interruption to the distal surface of the otolith. The annuli in Summer Flounder have a tendency to split as they advance towards the distal surface. As a result, it is critical that reading path proceed in a direction down the sulcal ridge and outwards to the distal surface.

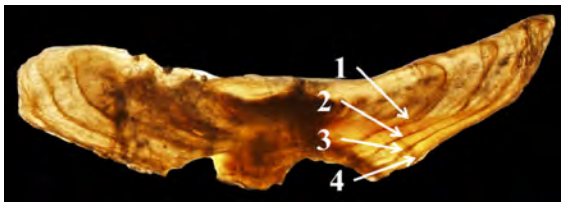


Figure 13.1: Otolith thin-section of a 4 year-old Summer Flounder with the last annulus on the edge of the thin-section

All thin-sections were aged by two different

readers using a Nikon SMZ1000 stereo microscope under transmitted light and dark-field polarization at between 8 and 20 times magnification. Each reader aged all of the otolith samples.

Click here to obtain the protocol at the VMRV website on how to age Summer Flounder using their otolith thin-sections.

13.2.4.2 *Scales*

We determined fish age by viewing acetate impressions of scales (Figure 13.2) with a standard Bell and Howell R-735 microfiche reader equipped with 20 and 29 mm lenses. Annuli

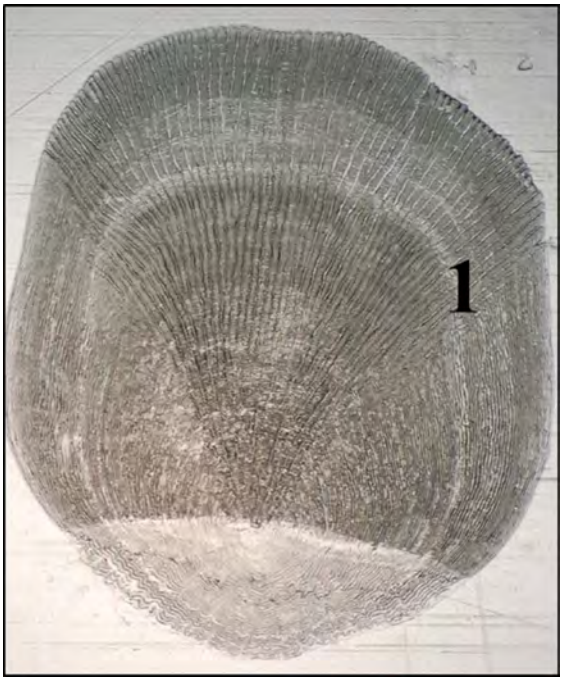


Figure 13.2: Scale impression of a 1 year-old Summer Flounder

on Summer Flounder scales are identified based on two scale microstructure features, "crossing over" and circuli disruption. Primarily, "crossing over" in the lateral margins near the posterior/anterior interface of the scale is used to determine the origin of the annulus. Here compressed circuli (annulus) "cross-over" the previously deposited circuli of the previous year's growth. Typically annuli of the first three years can be observed transversing this interface as dark bands. These bands remain consistent throughout the posterior field and rejoin the posterior/anterior interface on the opposite side of the focus. Annuli can also be observed in the anterior lateral field of the scale. Here the annuli typically reveal a pattern of discontinuous and suddenly breaking segmented circuli. This event can also be distinguished by the presence of concentric white lines, which are typically associated with the disruption of circuli.

Annuli can also be observed bisecting the perpendicular plain of the radial striations in the anterior field of the scale. Radii emanate out from the focus of the scale towards the outer corner margins of the anterior field. These radial striations consist mainly of segmented concave circuli. The point of intersection between radii and annuli results in a "straightening out" of the concave circuli. This straightening of the circuli should be consistent throughout the entire anterior field of the scale. This event is further amplified by the presence of concave circuli neighboring both directly above and below the annulus. The first year's annulus can be difficult to locate on some scales. It is typically best identified in the lateral field of the anterior portion of the scale. The distance from the focus to the first year's annulus is typically larger with respect to the following annuli. For the annuli two through six, summer growth generally decreases proportionally. For ages greater than six, a crowding effect of the annuli near the outer margins of the scale is observed. This crowding effect creates difficulties in edge interpretation. At this point it is best to focus on the straightening of the circuli at the anterior margins of the scale.

When ageing young Summer Flounder, zero through age two, extreme caution must be taken as not to over age the structure. In young fish there is no point of reference to aid in the determination of the first year; this invariably results in over examination of the scale and such events as hatching or saltwater incursion marks (checks) may be interpreted as the first year.

13.2.5 Comparison Tests

A symmetry test (Hoenig et al. 1995) and coefficient of variation (CV) analysis were used to detect any systematic difference and precision on age readings, respectively, for following comparisons: 1) between the two readers in the current year; 2) within each reader in the current year; 3) time-series bias between the current and previous years within each reader; and 4) between scale and otoliths ages. The readings from the entire sample for the current year were used to examine the difference between two readers. A random sub-sample of 50 fish from the current year was selected for second readings to examine the difference within a reader. Fifty otoliths and scales randomly selected from fish aged in 2000 were used to examine the time-series bias within each reader. A figure of 1:1 equivalence was used to illustrate those differences (Campana et al. 1995). All statistics analyses were performed in R.4.0.2 (R Core Team 2021).

13.3 RESULTS

13.3.1 Sample Size

The sample sizes are estimated for Summer Flounder in Chesapeake Bay and Virginia waters of Atlantic Ocean, respectively. The total sample collected from each area consists of the fish with total lengths, with both otoliths and scales, otolith-only, and scale-only. The total sample aged from each area may be smaller than or equal to the total sample size.

13.3.1.1 *Chesapeake Bay*

We estimated a sample size of 332 bay Summer Flounder in 2025, ranging in length interval from 8 to 30 inches (Table 13.1). This sample size provided a range in CV for age composition approximately from the smallest CV of 6% for the major age of Age 2 to the CV of larger than 25% for the multiple minor ages of the bay fish (Table 13.2). We aged all the fish with both scales and otoliths (82 fish). We aged 14 fish with otolith-only.We randomly selected 43 fish with scale-only to age. As a result, we aged all of the fish (139) collected by VMRC in Chesapeake Bay in 2025. We fell short in our over-all collections for the optimal length-class sampling estimate by 193 fish. We were short many fish from the major length intervals (The interval requires 10 or more fish), as a result, the precision for the estimates of major age groups would definitely be influenced significantly. Therefore, precaution should be used when developing ALK using these age data.

13.3.1.2 *Atlantic Ocean*

We estimated a sample size of 521 ocean Summer Flounder in 2025, ranging in length interval from 13 to 32 inches (Table 13.3). This sample size provided a range in CV for age composition approximately from the smallest CV of 9% for the major age of Age 4 to the CV of larger than 25% for the multiple minor ages of the ocean fish (Table 13.4). We aged all the fish with both scales and otoliths (345 fish). We randomly selected 267 fish with scale-only to age. As a result, we aged 612 of 635 fish (The rest of fish were either without any hardparts or over-collected for certain length interval(s)) collected by VMRC in Chesapeake Bay in 2025. We fell short in our over-all collections for the optimal length-class sampling estimate by 21 fish. We were short only a few fish from the major length intervals (The interval requires 10 or more fish), as a result, the precision for the estimates of major age groups would not be influenced significantly.

13.3.2 Reading Precision

13.3.2.1 *Otoliths*

Reader 1 and Reader 2 aged the otoliths of 441 Tautog collected in 2025. Reader 1 had high self-precision and Read 2 had low self-precision. Specifically, there was no significant difference between the first and second readings for Reader 1 with an agreement of 70% (1 year or less agreement of 94%) and a CV of 4.8% (test of symmetry: $\chi^2 = 15$, $df = 10$, P

= 0.1321), and there was no significant difference between the first and second readings for Reader 2 with an agreement of 62% (1 year or less agreement of 88%) and a CV of 11.9% (test of symmetry: $\chi^2 = 19$, $df = 12$, $P = 0.0885$). There was no evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 79% (1 year or less agreement of 98%) and a CV of 3.8% (test of symmetry: $\chi^2 = 20.9$, $df = 18$, $P = 0.2842$) (Figure 13.3).

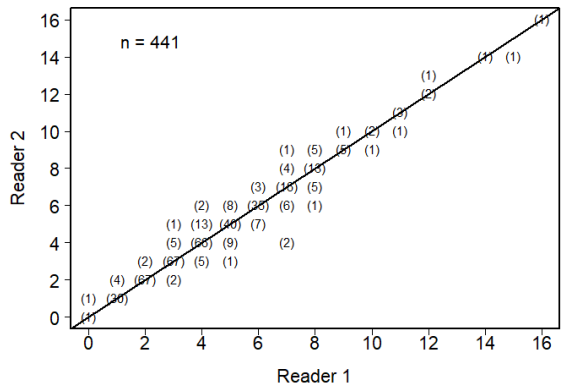


Figure 13.3: Between-reader comparison of otolith age estimates for Summer Flounder collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2025. The number in parentheses is number of fish.

There was no time-series bias for either reader. Reader 1 had an agreement of 84% (1 year or less agreement of 100%) with ages of fish aged in 2000 with a CV of 5% (test of symmetry: $\chi^2 = 4$, $df = 6$, $P = 0.6767$). Reader 2 had an agreement of 96% (1 year or less agreement of 100%) with a CV of 0.5% (test of symmetry: $\chi^2 = 2$, $df = 2$, $P = 0.3679$).

13.3.2.2 *Scales*

Reader 1 and Reader 2 aged the scales of 737 Summer Flounder collected in 2025. Reader 1 had moderate self-precision and Read 2 had low self-precision. Specifically, there was a significant difference between the first and second readings for Reader 1 with an agreement of 62% (1 year or less agreement of 92%) and a CV of 8.2% (test of symmetry: $\chi^2 = 19$, $df = 10$, $P = 0.0403$), and there was no significant difference between the first and second readings for Reader 2 with an agreement of 42% (1 year or less agreement of 84%) and a CV of 16.6% (test of symmetry: $\chi^2 = 17.2$, $df = 14$, $P = 0.2457$). There was no evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 51% (1 year or less agreement of 86%) and a CV of 11.7% (test of symmetry: $\chi^2 = 36$, $df = 38$, $P = 0.562$) (Figure 13.4).

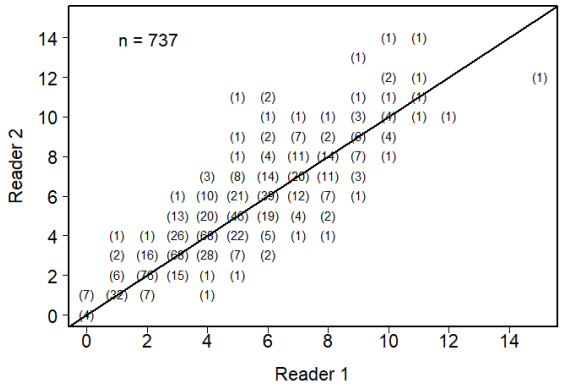


Figure 13.4: Between-reader comparison of scale age estimates for Summer Flounder collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2025. The number in parentheses is number of fish.

There was no time-series bias for either reader. Reader 1 had an agreement of 80% (1 year or less agreement of 96%) with ages of fish aged in 2000 with a CV of 4.5% (test of symmetry: $\chi^2 = 4$, $df = 6$, $P = 0.6767$). Reader 2 had an agreement of 54% (1 year or less agreement of 92%) with a CV of 10.8% (test of symmetry: $\chi^2 = 18$, $df = 12$, $P = 0.1157$).

13.3.3 Year class

The year classes were estimated using all the aged fish described in Section Sample Size for Chesapeake Bay and Virginia waters of Atlantic Ocean, respectively. When otolith ages are not available, the scale ages are used.

13.3.3.1 *Chesapeake Bay*

Of the 139 bay Summer Flounder aged, 7 age classes (0 to 6) were represented (Table 13.5). The average age for the sample was 2 years. The standard deviation and standard error were 1.1 and 0.09, respectively. Year-class data (Figure 13.5) indicates that recruitment into the fishery in Chesapeake Bay begins at age 0, which corresponds to the 2025 year-class for Summer Flounder caught in 2025. Summer flounder in the sample in 2025 was dominated by the year classes of 2023 and 2024 with 43% and 27%, respectively. The sex ratio of male to female was 1:3.79 for the bay fish.

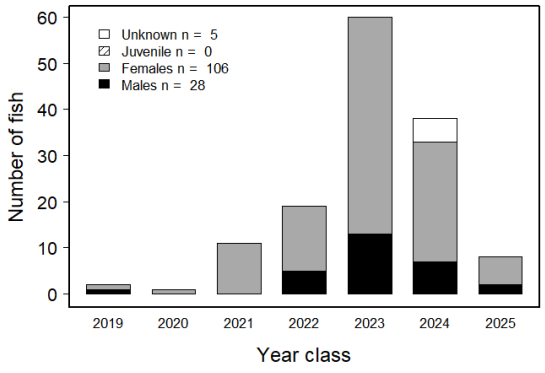


Figure 13.5: Year-class frequency distribution for Summer Flounder collected in Chesapeake Bay, Virginia for ageing in 2025. Distribution is broken down by sex. 'Unknown' represents gonads that were not available for examination or were not examined for sex during sampling.

13.3.3.2 *Atlantic Ocean*

Of the 612 ocean Summer Flounder aged, 14 age classes (1 to 12, 14, and 16) were represented (Table 13.6). The average age for the sample was 5.1 years. The standard deviation and standard error were 2.2 and 0.09, respectively. Year-class data (Figure 13.6) indicates that recruitment into the fishery in Virginia waters of Atlantic ocean begins at age 1, which corresponds to the 2024 year-class for Summer Flounder caught in 2025. Summer flounder in the sample in 2025 was dominated by the year classes of 2018, 2019, 2020, 2021, and 2022 with 9%, 15%, 16%, 21%, and 15%, respectively. The sex ratio of male to female was 1:1.76 for the ocean fish.

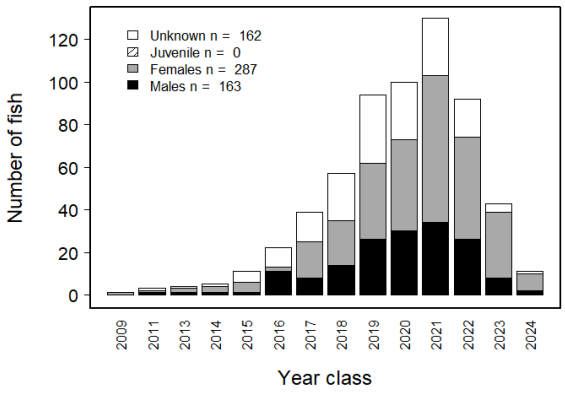


Figure 13.6: Year-class frequency distribution for Summer Flounder collected in Virginia waters of the Atlantic Ocean for ageing in 2025. Distribution is broken down by. 'Unknown' represents gonads that were not available for examination or were not examined for sex during sampling.

13.3.4 Age-Length-Key (ALK)

We developed an age-length-key for both bay (Table 13.7) and ocean fish (Table 13.8) using scale ages, separately. The ALK can be used in the conversion of numbers-at-length in the estimated catch to numbers-at-age using scale ages. The table is based on VMRC's stratified sampling of landings by total length inch intervals.

13.3.5 Comparison of Scale and Otolith Ages

We aged 427 pairs of Summer Flounder scales and otoliths. There was no evidence of systematic disagreement between otolith and scale ages (test of symmetry: $\chi^2 = 39.4$, $df = 31$, $P = 0.1444$) with an average CV of 10.8%. There was an agreement of 53% (1 year or less agreement of 86%) between scale and otoliths ages whereas scales were assigned a lower and higher age than otoliths for 29.5% and 17.6% of the fish, respectively (Figure 13.7). There was also little evidence of bias between otolith and

scale ages using an age bias plot (Figure 13.8), with no trend of either over-ageing younger or under-ageing older fish.

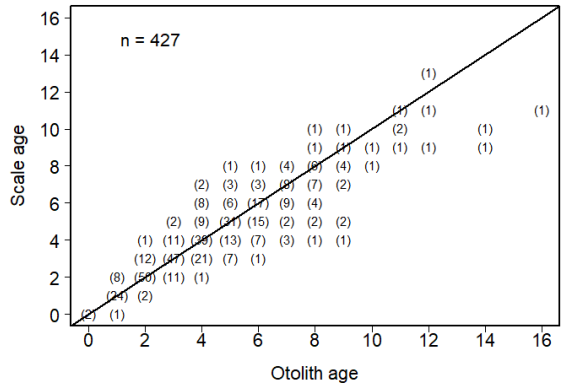


Figure 13.7: Comparison of scale and otolith age estimates for Summer Flounder collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2025. The number in parentheses is number of fish

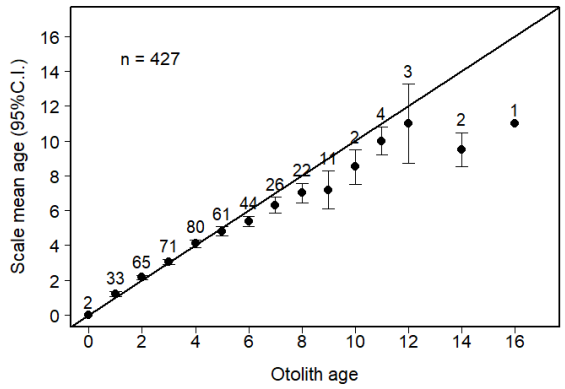


Figure 13.8: Age-bias plot for Summer Flounder scale and otolith age estimates in 2025. The number above the upper CI bar is number of fish.

13.4 RECOMMENDATIONS

Atlantic States Marine Fisheries Commission held a QAQC ageing workshop in St. Petersburg, Florida, in March of 2019 (ASMFC 2019). The workshop recommended that summer flounder should be aged using otoliths, not scales, when possible.

Table 13.1: Number of bay Summer Flounder collected and aged in each 1-inch length interval in 2025. 'Target' represents the sample size for ageing estimated for 2025, and 'Need' represents number of fish shorted in each length interval compared to the optimum sample size for ageing and number of fish aged.

Interval	Target	Collected	Aged	Need
8 - 8.99	5	2	2	3
9 - 9.99	5	1	1	4
10 - 10.99	5	1	1	4
11 - 11.99	5	0	0	5
12 - 12.99	5	1	1	4
13 - 13.99	5	5	5	0
14 - 14.99	58	24	24	34
15 - 15.99	49	25	25	24
16 - 16.99	36	22	22	14
17 - 17.99	36	16	16	20
18 - 18.99	29	13	13	16
19 - 19.99	22	7	7	15
20 - 20.99	17	9	9	8
21 - 21.99	10	8	8	2
22 - 22.99	5	5	5	0
23 - 23.99	5	0	0	5
24 - 24.99	5	0	0	5
25 - 25.99	5	0	0	5
26 - 26.99	5	0	0	5
27 - 27.99	5	0	0	5
28 - 28.99	5	0	0	5
29 - 29.99	5	0	0	5
30 - 30.99	5	0	0	5
Totals	332	139	139	193

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Table 13.2: CV for each age estimated based on ageing the total of 332 bay Summer Flounder in 2025. 'Percent' is the percentage of an age in the pooled age-length data of bay Summer Flounder collected from 2019 to 2023.

Age	CV	Percent
0	>0.25	0.06
1	0.2	7.31
2	0.06	43.9
3	0.1	26.28
4	0.14	12.96
5	0.22	5.72
6	>0.25	2.47
7	>0.25	0.94
8	>0.25	0.29
9	>0.25	0.06

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Table 13.3: Number of ocean Summer Flounder collected and aged in each 1-inch length interval in 2025. 'Target' represents the sample size for ageing estimated for 2025, and 'Need' represents number of fish shorted in each length interval compared to the optimum sample size for ageing and number of fish aged.

Interval	Target	Collected	Aged	Need
13 - 13.99	5	8	8	0
14 - 14.99	44	60	55	0
15 - 15.99	60	93	80	0
16 - 16.99	65	76	71	0
17 - 17.99	60	76	76	0
18 - 18.99	46	61	61	0
19 - 19.99	33	48	48	0
20 - 20.99	25	35	35	0
21 - 21.99	25	28	28	0
22 - 22.99	24	26	26	0
23 - 23.99	23	22	22	1
24 - 24.99	24	26	26	0
25 - 25.99	21	29	29	0
26 - 26.99	18	19	19	0
27 - 27.99	15	10	10	5
28 - 28.99	11	8	8	3
29 - 29.99	7	4	4	3
30 - 30.99	5	4	4	1
31 - 31.99	5	2	2	3
32 - 32.99	5	0	0	5
Totals	521	635	612	21

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Table 13.4: CV for each age estimated based on ageing the total of 521 ocean Summer Flounder in 2025. 'Percent' is the percentage of an age in the pooled age-length data of ocean Summer Flounder collected from 2019 to 2023.

Age	CV	Percent
1	>0.25	1.6
2	0.15	8.23
3	0.1	16.62
4	0.09	18.63
5	0.1	16.45
6	0.11	15.01
7	0.13	9.83
8	0.17	6.13
9	0.22	3.46
10	>0.25	2.26
11	>0.25	0.86
12	>0.25	0.62
13	>0.25	0.21
14	>0.25	0.08

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Table 13.5: The number of Summer Flounder assigned to each total length-at-age category for 139 fish sampled for both otolith and scale age determination in Chesapeake Bay, Virginia during 2025.

	Age							
Interval	0	1	2	3	4	5	6	Totals
8 - 8.99	2	0	0	0	0	0	0	2
9 - 9.99	1	0	0	0	0	0	0	1
10 - 10.99	0	1	0	0	0	0	0	1
12 - 12.99	0	0	1	0	0	0	0	1
13 - 13.99	2	3	0	0	0	0	0	5
14 - 14.99	2	14	7	1	0	0	0	24
15 - 15.99	1	12	12	0	0	0	0	25
16 - 16.99	0	3	18	1	0	0	0	22
17 - 17.99	0	3	8	2	3	0	0	16
18 - 18.99	0	1	8	1	2	1	0	13
19 - 19.99	0	1	2	2	2	0	0	7
20 - 20.99	0	0	3	5	1	0	0	9
21 - 21.99	0	0	1	5	1	0	1	8
22 - 22.99	0	0	0	2	2	0	1	5
Totals	8	38	60	19	11	1	2	139

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Table 13.6: The number of Summer Flounder assigned to each total length-at-age category for 612 fish sampled for both otolith and scale age determination in Virginia waters of Atlantic ocean during 2025.

	Age														
Interval	1	2	3	4	5	6	7	8	9	10	11	12	14	16	Totals
13 - 13.99	1	2	4	0	1	0	0	0	0	0	0	0	0	0	8
14 - 14.99	2	9	17	13	6	5	2	0	1	0	0	0	0	0	55
15 - 15.99	6	5	14	20	12	13	5	3	2	0	0	0	0	0	80
16 - 16.99	1	13	18	14	10	6	6	2	1	0	0	0	0	0	71
17 - 17.99	0	6	22	19	15	7	2	3	2	0	0	0	0	0	76
18 - 18.99	1	7	8	20	10	10	2	0	3	0	0	0	0	0	61
19 - 19.99	0	1	6	11	10	7	7	3	1	0	1	0	1	0	48
20 - 20.99	0	0	1	12	11	5	1	1	1	1	0	1	1	0	35
21 - 21.99	0	0	1	7	8	5	3	3	1	0	0	0	0	0	28
22 - 22.99	0	0	1	12	3	3	6	0	0	1	0	0	0	0	26
23 - 23.99	0	0	0	1	2	5	4	4	3	2	1	0	0	0	22
24 - 24.99	0	0	0	0	6	9	6	2	1	2	0	0	0	0	26
25 - 25.99	0	0	0	1	5	12	6	4	0	1	0	0	0	0	29
26 - 26.99	0	0	0	0	1	4	2	9	2	1	0	0	0	0	19
27 - 27.99	0	0	0	0	0	2	2	1	2	1	2	0	0	0	10
28 - 28.99	0	0	0	0	0	1	3	1	0	1	0	2	0	0	8
29 - 29.99	0	0	0	0	0	0	0	2	1	1	0	0	0	0	4
30 - 30.99	0	0	0	0	0	0	0	1	0	0	1	1	0	1	4
31 - 31.99	0	0	0	0	0	0	0	0	1	0	0	0	1	0	2
Totals	11	43	92	130	100	94	57	39	22	11	5	4	3	1	612

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Table 13.7: Age-Length key, as proportion-at-age in each 1-inch length interval, based on both otolith and scale ages for Summer Flounder sampled in Chesapeake Bay, Virginia during 2025.

	Age						
Interval	0	1	2	3	4	5	6
8 - 8.99	1	0	0	0	0	0	0
9 - 9.99	1	0	0	0	0	0	0
10 - 10.99	0	1	0	0	0	0	0
12 - 12.99	0	0	1	0	0	0	0
13 - 13.99	0.4	0.6	0	0	0	0	0
14 - 14.99	0.08	0.58	0.29	0.04	0	0	0
15 - 15.99	0.04	0.48	0.48	0	0	0	0
16 - 16.99	0	0.14	0.82	0.05	0	0	0
17 - 17.99	0	0.19	0.5	0.12	0.19	0	0
18 - 18.99	0	0.08	0.62	0.08	0.15	0.08	0
19 - 19.99	0	0.14	0.29	0.29	0.29	0	0
20 - 20.99	0	0	0.33	0.56	0.11	0	0
21 - 21.99	0	0	0.12	0.62	0.12	0	0.12
22 - 22.99	0	0	0	0.4	0.4	0	0.2

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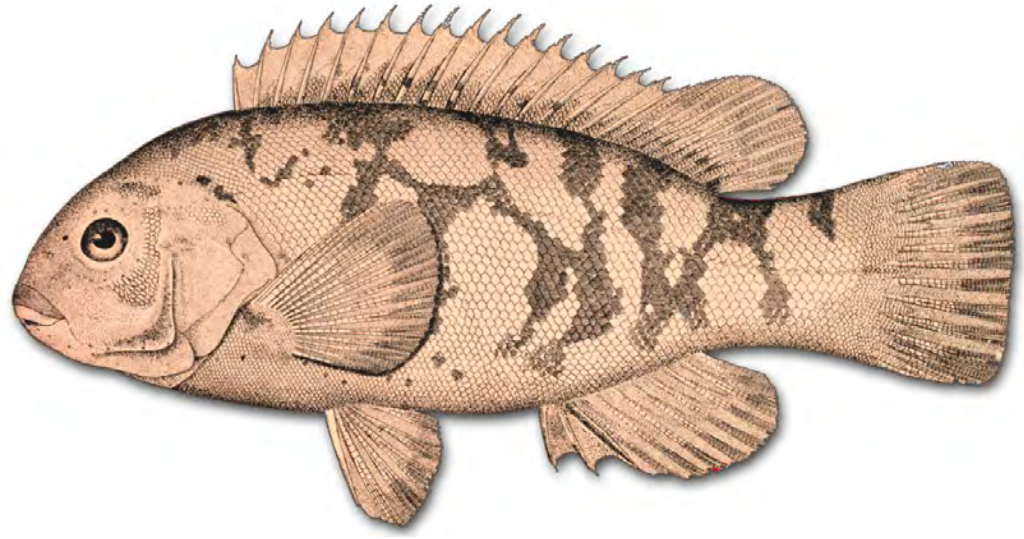
Table 13.8: Age-Length key, as proportion-at-age in each 1-inch length interval, based on both otolith and scale ages for Summer Flounder sampled in Virginia waters of the Atlantic Ocean during 2025.

	Age													
Interval	1	2	3	4	5	6	7	8	9	10	11	12	14	16
13 - 13.99	0.12	0.25	0.5	0	0.12	0	0	0	0	0	0	0	0	0
14 - 14.99	0.04	0.16	0.31	0.24	0.11	0.09	0.04	0	0.02	0	0	0	0	0
15 - 15.99	0.07	0.06	0.17	0.25	0.15	0.16	0.06	0.04	0.03	0	0	0	0	0
16 - 16.99	0.01	0.18	0.25	0.2	0.14	0.08	0.08	0.03	0.01	0	0	0	0	0
17 - 17.99	0	0.08	0.29	0.25	0.2	0.09	0.03	0.04	0.03	0	0	0	0	0
18 - 18.99	0.02	0.11	0.13	0.33	0.16	0.16	0.03	0	0.05	0	0	0	0	0
19 - 19.99	0	0.02	0.12	0.23	0.21	0.15	0.15	0.06	0.02	0	0.02	0	0.02	0
20 - 20.99	0	0	0.03	0.34	0.31	0.14	0.03	0.03	0.03	0.03	0	0.03	0.03	0
21 - 21.99	0	0	0.04	0.25	0.29	0.18	0.11	0.11	0.04	0	0	0	0	0
22 - 22.99	0	0	0.04	0.46	0.12	0.12	0.23	0	0	0.04	0	0	0	0
23 - 23.99	0	0	0	0.05	0.09	0.23	0.18	0.18	0.14	0.09	0.05	0	0	0
24 - 24.99	0	0	0	0	0.23	0.35	0.23	0.08	0.04	0.08	0	0	0	0
25 - 25.99	0	0	0	0.03	0.17	0.41	0.21	0.14	0	0.03	0	0	0	0
26 - 26.99	0	0	0	0	0.05	0.21	0.11	0.47	0.11	0.05	0	0	0	0
27 - 27.99	0	0	0	0	0	0.2	0.2	0.1	0.2	0.1	0.2	0	0	0
28 - 28.99	0	0	0	0	0	0.12	0.38	0.12	0	0.12	0	0.25	0	0
29 - 29.99	0	0	0	0	0	0	0	0.5	0.25	0.25	0	0	0	0
30 - 30.99	0	0	0	0	0	0	0	0.25	0	0	0.25	0.25	0	0.25
31 - 31.99	0	0	0	0	0	0	0	0	0.5	0	0	0	0.5	0

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Chapter 14

TAUTOG *Tautoga onitis*



14.1 INTRODUCTION

We aged a total of 265 Tautog *Tautoga onitis*, collected by the VMRC's Biological Sampling Program in 2025. Of 265 aged fish, 252 and 13 fish were collected in Chesapeake Bay (bay fish) and Virginia waters of the Atlantic Ocean (ocean fish), respectively. The average age for the bay fish was 4.5 years with a standard deviation of 1.6 and a standard error of 0.1. Ten age classes (2 to 11) were represented in the bay fish, comprising fish from the 2014 to 2023 year classes. The bay fish sample in 2025 was dominated by the year classes of 2019, 2020, 2021, and 2022 with 20%, 17%, 25%, and 25%, respectively. Only 13 ocean fish were collected, 6 to 8, 10 to 12, and 14 to 16 years old, and in the year class of 2009 to 2011, 2013 to 2015, and 2017 to 2019.

Of the 265 samples aged, 261 fish were aged with all three structures, otoliths, opercula, and spines. As a result, we were able to examine the precisions between otolith- and operculum-ages (261 pairs) and between otolith- and spine-ages (261 pairs), respectively.

14.2 METHODS

14.2.1 Sample Size for Ageing

We estimated sample sizes for ageing Tautog collected in both Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2025, respectively, using a two-stage random sampling method (Quinn and Deriso 1999) to increase precision in estimates of age composition from fish sampled efficiently and effectively. The basic equation is:

$$A = \frac{V_a}{\theta_a^2 CV_a^2 + B_a/L} \tag{14.1}$$

where A is the sample size for ageing Tautog in 2025; θ_a stands for the proportion of Age a fish in a catch; V_a, B_a, and CV_a represent the variance components within and between length intervals, and the coefficient of variation for Age a, respectively; L is the total number of Tautog used by VMRC to estimate length distribution of the catches from 2019 to 2023. θ_a, V_a, and B_a were calculated using pooled age-length data of Tautog collected from 2019 to 2023 and using equations in Quinn and Deriso (1999). For simplicity, the equations are not listed here. The equation (1.1) indicates: 1) The more fish that are aged, the smaller the CV_a (or higher precision) that will be obtained for Age a; 2) given a sample size A, the CV_a is different for each age due to different θ_a, V_a, and B_a among different ages. Therefore, the criterion to age A (number) of fish is that A should be a number above which there is only a 1% CV_a reduction for the most abundant age in catch by ageing an additional 100 or more fish. Finally, A_l is A multiplied by the proportion of length interval l from the length distribution of the 2019 to 2023 catch. A_l is number of fish to be aged for length interval l in 2025.

14.2.2 Handling of Collection

Sagittal otoliths (hereafter, referred to as "otoliths"), opercula, and pelvic spines (hereafter, referred to as "spines") were received by the Age and Growth Laboratory in labeled coin envelopes, and were sorted based on date of capture. Their envelope labels were verified against VMRC's collection data, and each fish assigned a unique Age and Growth Laboratory identification number. All otoliths, opercula, and spines were stored dry within their original labeled coin envelopes; otoliths were contained inside protective Axygen 2.0 ml microtubes.

14.2.3 Preparation

14.2.3.1 *Otoliths*

We used our bake and thin-section technique to process Tautog otoliths for age determination. Otolith preparation began by placing both whole otoliths in a ceramic "Coors" spot plate well and baked in a Thermolyne 1400 furnace at 400 °C. Baking time was dependent

on the otolith's size and gauged by color, with a light caramel color desired. Once a suitable color was achieved, the baked otoliths were embedded in epoxy resin seperatly with the distal surface orientated downwards and allowed to harden overnight. The otoliths were viewed under a stereo microscope to identify the location of the core, and the position of the core was marked using an ultra fine Sharpie across the epoxy resin surface. At least one transverse cross-section (hereafter, referred to as "thin-section") was then removed from the marked core of each otolith using a Buehler IsoMet™ low-speed saw equipped with two 4-inch diameter diamond grinding wheels (hereafter, referred to as "blades"), separated by a stainless steel spacer of 0.5 mm (diameter 2.5"). The otolith was positioned so the blades straddled each side of the otolith core. It was crucial that this cut be perpendicular to the long axis of the otolith. Failure to do so resulted in broadening and distorted winter growth zones. A proper cut resulted in annuli that were clearly defined and delineated. Once cut, thin-sections were placed on labeled glass slides and covered with a thin layer of Flo-texx mounting medium that not only fixed the sections to the slide, but more importantly, provided enhanced contrast and greater readability by increasing light transmission through the thin-section.

Click here to obtain the protocol at the VMRV website on how to prepare otolith thin-section using the epoxy resin method for ageing Tautog.

14.2.3.2 *Opercula*

Tautog opercula were boiled for several minutes to remove any attached skin and connective tissue. After boiling, opercula were inspected for damage. If there were no obvious flaws, the opercula was dried and then stored in a new, labeled envelope.

Click here to obtain the protocol at the VMRC website on how to prepare opercula for ageing Tautog.

14.2.3.3 *Spines*

Following the instructions in the ASMFC Ageing Workshop and the methods in Elzey and Trull (2016), we started to age Tautog collected in 2022 uisng their pelvic fin spines (hereafter, referred to at spines). The spines were boiled for several minutes to remove any skin and connective tissue. After boiling, spines were stored in labeled coin envelopes for at least 24 hours to ensure the spines were fully dry. Once dry, the spines were embedded in epoxy resin and allowed to cure overnight. At least three thin-sections were removed from the resin block using a Buhler Isomet low-speed saw equipped with four, 4 inch diameter diamond wafering blades each separated by a 0.75 mm stainless steel spacer. The sections were then mounted to labeled glass slides in order with the first section, closest to the body of the fish, on the right and affixed with Flo-texx mounting medium.

14.2.4 Readings

The system assigns an age class to a fish based on a combination of reading the information contained in its hardpart, the date of its capture, and the species-specific period when it deposits its annulus. Each year, as the fish grows, its hardpart grow and leave behind markers of their age, called annuli. Technically, an annulus is the combination of both the opaque and the translucent bands. In practice, only the opaque bands are counted as annuli. The number of these visible dark bands replaces "x" in our notation below, and is the initial "age" assignment of the fish.

Second, the hardpart is examined for translucent growth. If no translucent growth is visible beyond the last dark annulus, the hardpart is called "even" and no modification of the assigned age is made. The initial assigned age, then, is the age class of the fish. Any growth beyond the last annulus can be interpreted as either being toward the next age class or within the same age class. If translucent growth is visible beyond the last dark annulus, a "+" is

added to the notation.

By convention all fish in the Northern Hemisphere are assigned a birth date of January 1. In addition, each species has a specific period during which it deposits the dark band of the annulus. If the fish is captured after the end of the species-specific annulus deposition period and before January 1, it is assigned an age class notation of "x+x", where "x" is the number of dark bands in the otolith. If the fish is captured between January 1 and the end of the species-specific annulus deposition period, it is assigned an age class notation of "x+(x+1)". Thus, any growth beyond the last annulus, after its "birthday", but before the dark band deposition period, is interpreted as being toward the next age class.

For example, Tautog annulus deposition occurs between May and July (Hostetter and Munroe 1993). A Tautog captured between January 1 and July 31, before the end of the species' annulus formation period, with three visible annuli and some translucent growth after the last annulus, would be assigned an age class of "x+(x+1)" or 3+(3+1), noted as 3+4. This is the same age-class assigned to a fish with four visible annuli captured after the end of June 30, the period of annulus formation, which would be noted as 4+4.

All Tautog samples (sectioned otoliths, opercula, and sectioned spines) were aged by two different readers in chronological order based on collection date, without knowledge of the specimen lengths. When the readers' ages agreed, that age was assigned to the fish. When the two readers disagreed, both readers sat down together and re-aged the fish again without any knowledge of previously estimated ages or lengths, then assigned a final age to the fish. When the age readers were unable to agree on a final age, the fish was excluded from further analysis.

14.2.4.1 *Otoliths*

When an otolith was properly sectioned, the sulcal groove came to a sharp point (Hereafter referred to as "focus") within the middle of the core (Figure 14.1). Typically the first year's annulus was found by locating the focus of the otolith section, which was characterized as a visually distinct dark, oblong region found in the center of the otolith section.

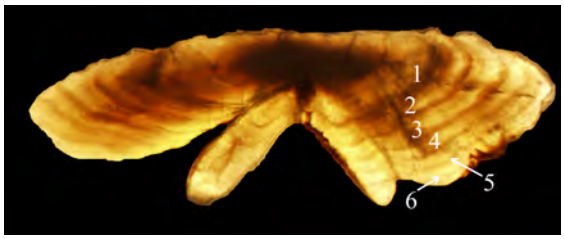


Figure 14.1: Otolith thin-section of 6 year-old Tautog

All otolith thin-sections were aged by two different readers using a Nikon SMZ1000 stereo microscope under transmitted light and dark-field polarization at between 8 and 20 times magnification.

Click here to obtain the protocol at the VMRV website on how to age Tautog using their otolith thin-sections.

14.2.4.2 *Opercula*

Opercula were aged on a light table with no magnification (Figure 14.2). Tautog opercula are also considered to have a deposition period of May through July (Hostetter and Munroe 1993), and age class assignment using these hard-parts is conducted in the same way as otoliths.

14.2.4.3 *Spines*

All spine thin-sections were aged using an Olympus BX41 compound microscope (Figure 14.3). Since there were at least three sections per slide, Reader 1 and 2 will mark their sections with a red and black dot, respectively, when they chose different sections for their age

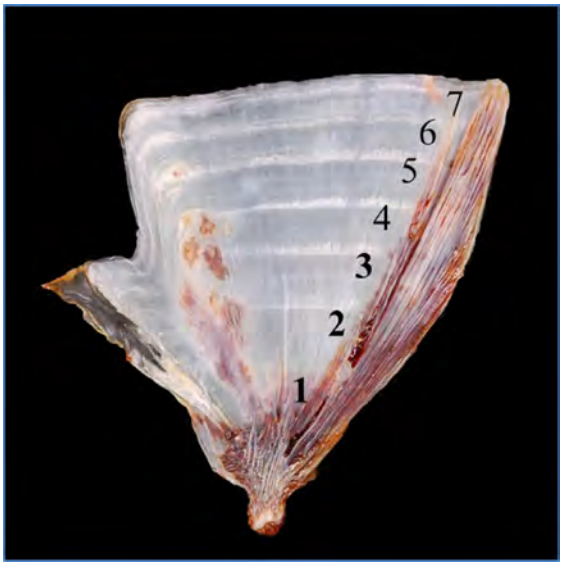


Figure 14.2: Operculum of a 7 year-old Tautog

estimates. If there was a disagreement, both marked sections were reviewed to determine a final age.

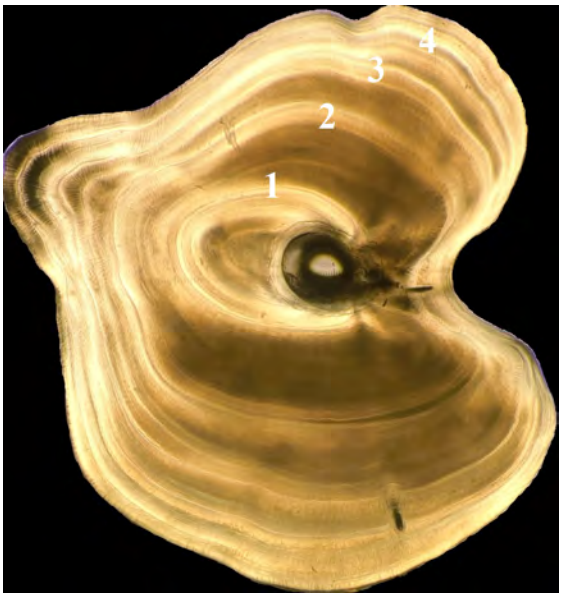


Figure 14.3: Spine of a 4 year-old Tautog

14.2.5 Comparison Tests

A symmetry test (Hoenig et al. 1995) and coefficient of variation (CV) analysis were used to detect any systematic difference and precision on age readings, respectively, for following comparisons: 1) between the two readers in the current year; 2) within each reader in the current year; 3) time-series bias between the current and previous years within each reader; 4) between operculum and otoliths ages; and 5) between spine and otoliths ages. The readings from the entire sample for the current year were used to examine the difference between two readers. A random sub-sample of 50 fish from the current year was selected for second readings to examine the difference within a reader. Fifty otoliths and opercula randomly selected from fish aged in 2000 were used to examine the time-series bias within each reader. A figure of 1:1 equivalence was used to illustrate those differences (Campana et al. 1995). All statistics analyses were performed in R.4.0.2 (R Core Team 2021).

14.3 RESULTS

14.3.1 Sample Size

The sample sizes are estimated for Tautog in Chesapeake Bay and Virginia waters of Atlantic Ocean, respectively. The total sample collected from each area consists of the fish with total lenghts and any combinations of three hardparts (Otoliths, opercula, and spines).

14.3.1.1 *Chesapeake Bay*

We estimated a sample size of 486 bay Tautog in 2025, ranging in length interval from 8 to 24 inches (Table 14.1). This sample size provided a range in CV for age composition approximately from the smallest CV of 8% for the major age of Age 4 to the CV of larger than 25% for the multiple minor ages of the bay fish (Table 14.2).

We aged all of 252 bay fish collected by VMRC in Chesapeake Bay in 2025. Of 252 aged, 248 fish were aged using their otoliths, opercula, and spines whereas 4 were aged using their opercula and spines (No otoliths). We fell short in our over-all collections for this optimal length-class sampling estimate by 245 fish. We were

short many fish from the major length intervals (The interval requires 10 or more fish), as a result, the precision for the estimates of major age groups would definitely be influenced significantly. Therefore, precaution should be used when developing ALK using these age data.

14.3.1.2 *Atlantic Ocean*

We estimated a sample size of 83 ocean Tautog in 2025, ranging in length interval from 14 to 28 inches (Table 14.3). This sample size provided a range in CV for age composition approximately from the smallest CV of 1% for the major age of Age 6 to the CV of larger than 25% for the multiple minor ages of the ocean fish (Table 14.4).

We aged all of 13 fish collected by VMRC in Virginia waters of Atlantic Ocean in 2025 using their otoliths, opercula, and spines. We fell short in our over-all collections for this optimal length-class sampling estimate by 70 fish. We were short many fish from the major length intervals (The interval requires 10 or more fish), as a result, the precision for the estimates of major age groups would definitely be influenced significantly. Therefore, precaution should be used when developing ALK using these age data.

14.3.2 Reading Precision

14.3.2.1 *Otoliths*

Reader 1 and Reader 2 aged the otoliths of 261 Tautog collected in 2025. Both readers had high self-precision. Specifically, there was no significant difference between the first and second readings for Reader 1 with an agreement of 88% (1 year or less agreement of 96%) and a CV of 1.8% (test of symmetry: $\chi^2 = 6$, $df = 6$, $P = 0.4232$), and there was no significant difference between the first and second readings for Reader 2 with an agreement of 80% (1 year or less agreement of 98%) and a CV of 2.8% (test of symmetry: $\chi^2 = 8$, $df = 8$, $P = 0.4335$).There was no evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 91% (1 year or less agreement of 99%) and a CV of 1.2% (test of symmetry: $\chi^2 = 15.8$, $df = 12$, $P = 0.199$) (Figure 14.4).

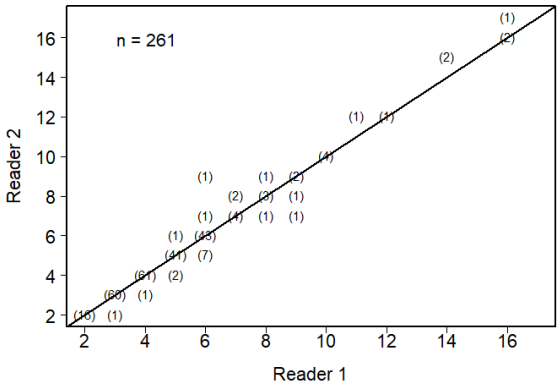


Figure 14.4: Between-reader comparison of otolith age estimates for Tautog collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2025. The number in parentheses is number of fish.

There was no time-series bias for either reader. Reader 1 had an agreement of 90% (1 year or less agreement of 100%) with ages of fish aged in 2000 with a CV of 1.2% (test of symmetry: $\chi^2 = 5$, $df = 2$, $P = 0.0821$). Reader 2 had an agreement of 88% (1 year or less agreement of 100%) with a CV of 1.3% (test of symmetry: $\chi^2 = 6$, $df = 3$, $P = 0.1116$).

14.3.2.2 *Opercula*

Reader 1 and Reader 2 aged the opercula of 265 Tautog collected in 2025. Both readers had moderate self-precision. Specifically, there was no significant difference between the first and second readings for Reader 1 with an agreement of 64% (1 year or less agreement of 98%) and a CV of 5.5% (test of symmetry: $\chi^2 = 4.3$, $df = 7$, $P = 0.7407$), and there was a significant difference between the first and second readings for Reader 2 with an agreement of 56% (1 year or less agreement of 96%) and a CV of 7.7% (test of symmetry: $\chi^2 = 19.3$, $df = 7$, $P = 0.0072$). There was no evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 55% (1 year or less agree-

ment of 94%) and a CV of 7.4% (test of symmetry: $\chi^2 = 28.7$, $df = 21$, $P = 0.1213$) (Figure 14.5).

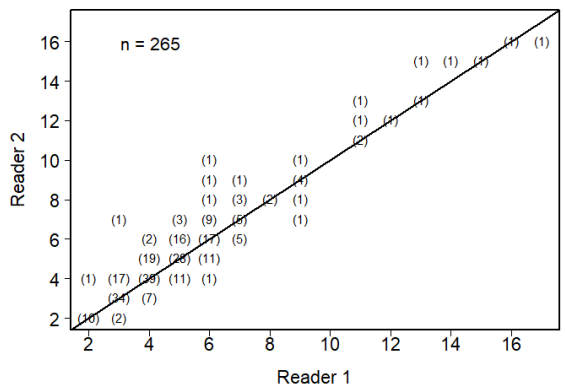


Figure 14.5: Between-reader comparison of operculum age estimates for Tautog collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2025. The number in parentheses is number of fish.

There was no time-series bias for either reader. Reader 1 had an agreement of 58% (1 year or less agreement of 98%) with ages of fish aged in 2000 with a CV of 5.6% (test of symmetry: $\chi^2 = 9$, $df = 9$, $P = 0.4373$). Reader 2 had an agreement of 40% (1 year or less agreement of 88%) with a CV of 10.1% (test of symmetry: $\chi^2 = 13$, $df = 13$, $P = 0.4478$).

14.3.2.3 *Spines*

Reader 1 and Reader 2 aged the spines of 265 Tautog collected in 2025. Both readers had moderate self-precision. Specifically, there was no significant difference between the first and second readings for Reader 1 with an agreement of 62% (1 year or less agreement of 88%) and a CV of 6.2% (test of symmetry: $\chi^2 = 9$, $df = 11$, $P = 0.6219$), and there was no significant difference between the first and second readings for Reader 2 with an agreement of 54% (1 year or less agreement of 82%) and a CV of 9.2% (test of symmetry: $\chi^2 = 14$, $df = 14$, $P = 0.4497$). There was no evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 48% (1 year or less agreement of 86%) and a CV of 9.7% (test of symmetry: $\chi^2 = 33.3$, $df = 25$, $P = 0.1239$) (Figure 14.6).

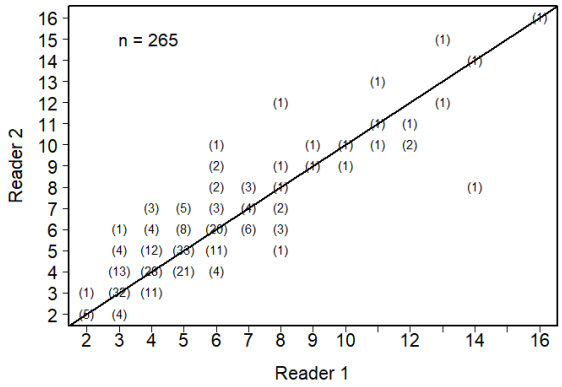


Figure 14.6: Between-reader comparison of spine age estimates for Tautog collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2025. The number in parentheses is number of fish.

We didn't conduct time-series comparison in spine ageing because we believe that we need to practice on ageing the spines for one more year before we are able to setup a set of spines for the examination of time-series precision. We will randomly select 50 spine thin-sections from our 2022 spine collection and re-age them to make a time-series set for Tautog spine ageing. We are planning to present our spine time-series precision in our 2026 annual report.

14.3.3 Year Class

Year classes were estimated using all the aged fish described in Section Sample Size for Chesapeake Bay and Virginia waters of Atlantic Ocean, respectively. The otolith ages are always the primary ages. When the otolith ages were not available, the operculum ages were used, followed by the spine ages.

14.3.3.1 *Chesapeake Bay*

Of the 252 bay Tautog aged, 10 age classes (2 to 11) were represented (Table 14.5). The average age for the sample was 4.5 years. The standard deviation and standard error were 1.6 and 0.1, respectively. Year-class data (Figure 14.7)

indicates that recruitment into the fishery in Chesapeake Bay begins at age 2, which corresponds to the 2023 year-class for Tautog caught in 2025. Tautog in the sample in 2025 was dominated by the year classes of 2019, 2020, 2021, and 2022 with 20%, 17%, 25%, and 25%, respectively. The sex ratio of male to female was 1:1.14 for the bay fish.

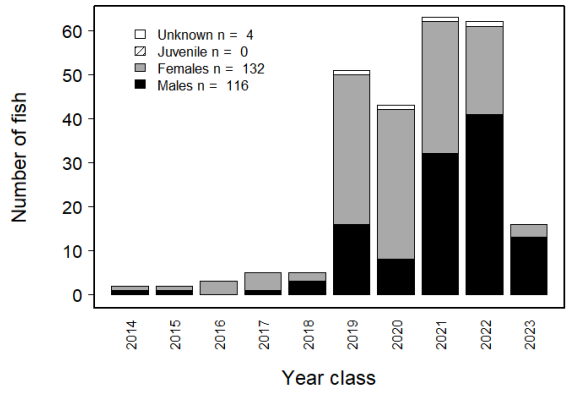


Figure 14.7: Year-class frequency distribution for Tautog collected in Chesapeake Bay, Virginia for ageing in 2025. Distribution is broken down by sex. 'Unknown' represents the fish gonads that were not available for examination or were not examined for sex during sampling.

14.3.3.2 *Atlantic Ocean*

Only 13 ocean fish was collected, 6 to 8, 10 to 12, and 14 to 16 years old, and in the year class of 2009 to 2011, 2013 to 2015, and 2017 to 2019 (Table 14.6).

14.3.4 Age-Length-Key (ALK)

We developed an age-length-key for bay fish (Table 14.7) using all the aged fish described in Section Sample Size. No ALK was developed for the ocean tautog because there was only 13 ocean fish collected and aged in 2025. The ALK can be used in the conversion of numbers-at-length in the estimated catch to numbers-at-age using operculum ages. The table is based on VMRC's stratified sampling of landings by total length inch intervals.

14.3.5 Comparisons

14.3.5.1 *Operculum vs otolith ages*

We aged 261 pairs of Tautog opercula and otoliths. There was no evidence of systematic disagreement between otolith and operculum ages (test of symmetry: $\chi^2 = 31.6$, $df = 21$, $P = 0.0641$) with an average CV of 5.6%. There was an agreement of 67% (1 year or less agreement of 93%) between operculum and otoliths ages whereas opercula were assigned a lower and higher age than otoliths for 11.5% and 21.1% of the fish, respectively (Figure 14.8). There was also little evidence of bias between otolith and operculum ages using an age bias plot(Figure 14.9), with no trend of either over-ageing younger or under-ageing older fish.

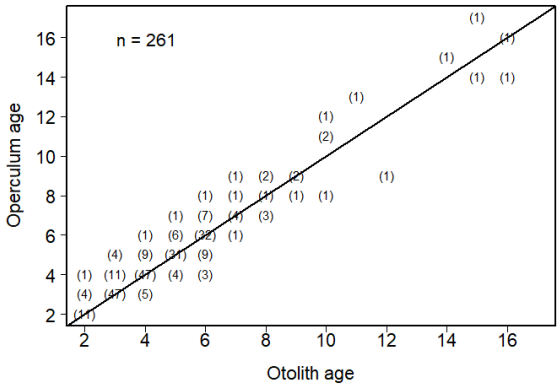


Figure 14.8: Comparison of operculum and otolith age estimates for Tautog collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2025. The number in parentheses is number of fish.

14.3.5.2 *Spine vs otolith ages*

We aged 261 pairs of Tautog spines and otoliths. There was an evidence of systematic disagreement between otolith and spine ages (test of symmetry: $\chi^2 = 55$, $df = 25$, $P = 0.0005$) with an average CV of 8.5%. There was an agreement of 57% (1 year or less agreement of 89%) between spine and otoliths ages whereas spines were assigned a lower and higher age than otoliths for 12.6% and 30.7%

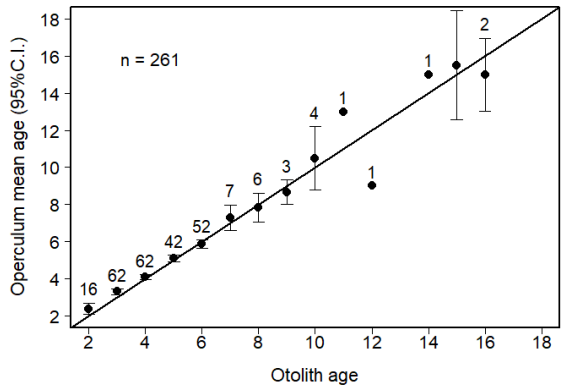


Figure 14.9: Age-bias plot for Tautog operculum and otolith age estimates in 2025. The number above the upper CI bar is number of fish.

of the fish, respectively (Figure 14.10). There was also an evidence of bias between otolith and spine ages using an age bias plot (Figure 14.11), with spine generally assigned higher ages for younger fish and lower ages for older fish than otolith age estimates.

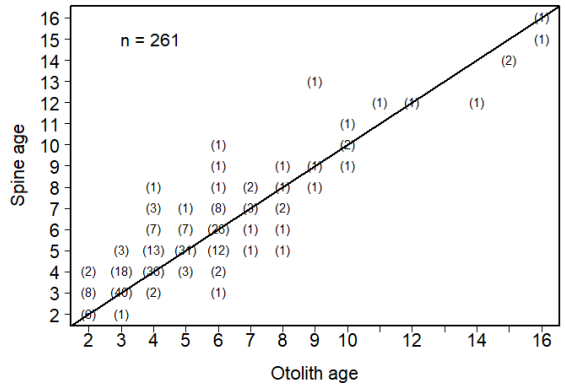


Figure 14.10: Comparison of spine and otolith age estimates for Tautog collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2025. The number in parentheses is number of fish.

14.4 RECOMMENDATIONS

Atlantic States Marine Fisheries Commission held a QAQC ageing workshop in St. Petersburg, Florida, in March of 2023. The workshop recommended that otolith ages should be used as the primary age for Tautog when possible. Our results also indicates that the precision on otolith ageing is the highest among the three hardparts we aged, supporting the ASMFC recommendation.

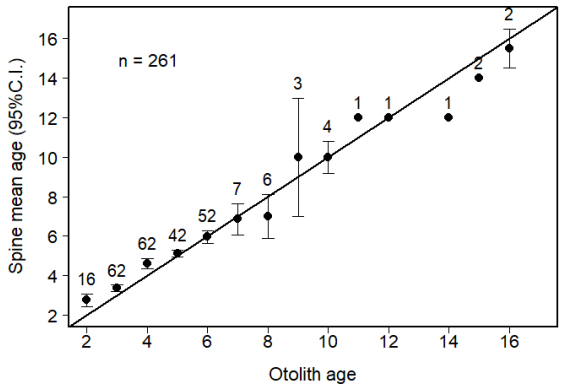


Figure 14.11: Age-bias plot for Tautog spine and otolith age estimates in 2025. The number above the upper CI bar is number of fish.

Table 14.1: Number of bay Tautog collected and aged in each 1-inch length interval in 2025. 'Target' represents the sample size for ageing estimated for 2025, and 'Need' represents number of fish shorted in each length interval compared to the optimum sample size for ageing and number of fish aged.

Interval	Target	Collected	Aged	Need
8 - 8.99	5	0	0	5
9 - 9.99	5	0	0	5
10 - 10.99	10	5	5	5
11 - 11.99	19	14	14	5
12 - 12.99	35	33	33	2
13 - 13.99	30	41	41	0
14 - 14.99	70	35	35	35
15 - 15.99	114	52	52	62
16 - 16.99	92	41	41	51
17 - 17.99	56	18	18	38
18 - 18.99	17	10	10	7
19 - 19.99	8	1	1	7
20 - 20.99	5	2	2	3
21 - 21.99	5	0	0	5
22 - 22.99	5	0	0	5
23 - 23.99	5	0	0	5
24 - 24.99	5	0	0	5
Totals	486	252	252	245

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Table 14.2: CV for each age estimated based on ageing the total of 486 bay Tautog in 2025. 'Percent' is the percentage of an age in the pooled age-length data of bay Tautog collected from 2019 to 2023.

Age	CV	Percent
1	>0.25	0.56
2	0.19	4.44
3	0.11	13.56
4	0.08	24
5	0.09	20.33
6	0.11	15.22
7	0.15	8.11
8	0.16	7
9	0.24	3.33
10	>0.25	1.67
11	>0.25	0.67
12	>0.25	0.22
13	>0.25	0.67
14	>0.25	0.11
15	>0.25	0.11

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Table 14.3: Number of ocean Tautog collected and aged in each 1-inch length interval in 2025. 'Target' represents the sample size for ageing estimated for 2025, and 'Need' represents number of fish shorted in each length interval compared to the optimum sample size for ageing and number of fish aged.

Interval	Target	Collected	Aged	Need
14 - 14.99	5	0	0	5
15 - 15.99	8	0	0	8
16 - 16.99	5	0	0	5
17 - 17.99	5	0	0	5
18 - 18.99	5	0	0	5
19 - 19.99	5	0	0	5
20 - 20.99	5	1	1	4
21 - 21.99	5	2	2	3
22 - 22.99	5	3	3	2
23 - 23.99	5	1	1	4
24 - 24.99	5	3	3	2
25 - 25.99	5	1	1	4
26 - 26.99	10	0	0	10
27 - 27.99	5	2	2	3
28 - 28.99	5	0	0	5
Totals	83	13	13	70

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Table 14.4: CV for each age estimated based on ageing the total of 83 ocean Tautog in 2025. 'Percent' is the percentage of an age in the pooled age-length data of ocean Tautog collected from 2019 to 2023.

Age	CV	Percent
4	>0.25	5.26
5	>0.25	15.79
6	0.22	21.05
10	>0.25	5.26
11	>0.25	5.26
12	>0.25	5.26
14	>0.25	5.26
16	>0.25	5.26
20	>0.25	5.26
21	>0.25	5.26

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Table 14.5: The number of Tautog assigned to each total length-at-age category for 252 fish sampled for both otolith and operculum age determination in Chesapeake Bay, Virginia during 2025.

	Age										
Interval	2	3	4	5	6	7	8	9	10	11	Totals
10 - 10.99	5	0	0	0	0	0	0	0	0	0	5
11 - 11.99	7	5	2	0	0	0	0	0	0	0	14
12 - 12.99	3	21	6	3	0	0	0	0	0	0	33
13 - 13.99	1	17	15	6	1	0	1	0	0	0	41
14 - 14.99	0	7	11	10	7	0	0	0	0	0	35
15 - 15.99	0	6	17	13	13	1	2	0	0	0	52
16 - 16.99	0	6	11	7	15	0	1	1	0	0	41
17 - 17.99	0	0	1	3	9	2	1	1	0	1	18
18 - 18.99	0	0	0	1	5	2	0	0	1	1	10
19 - 19.99	0	0	0	0	0	0	0	1	0	0	1
20 - 20.99	0	0	0	0	1	0	0	0	1	0	2
Totals	16	62	63	43	51	5	5	3	2	2	252

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Table 14.6: The number of Tautog assigned to each total length-at-age category for 13 fish sampled for both otolith and operculum age determination in Virginia waters of Atlantic ocean during 2025.

	Age									
Interval	6	7	8	10	11	12	14	15	16	Totals
20 - 20.99	0	1	0	0	0	0	0	0	0	1
21 - 21.99	0	0	1	1	0	0	0	0	0	2
22 - 22.99	1	1	0	0	0	1	0	0	0	3
23 - 23.99	0	0	0	0	0	0	0	0	1	1
24 - 24.99	0	0	0	1	1	0	1	0	0	3
25 - 25.99	0	0	0	0	0	0	0	0	1	1
27 - 27.99	0	0	0	0	0	0	0	2	0	2
Totals	1	2	1	2	1	1	1	2	2	13

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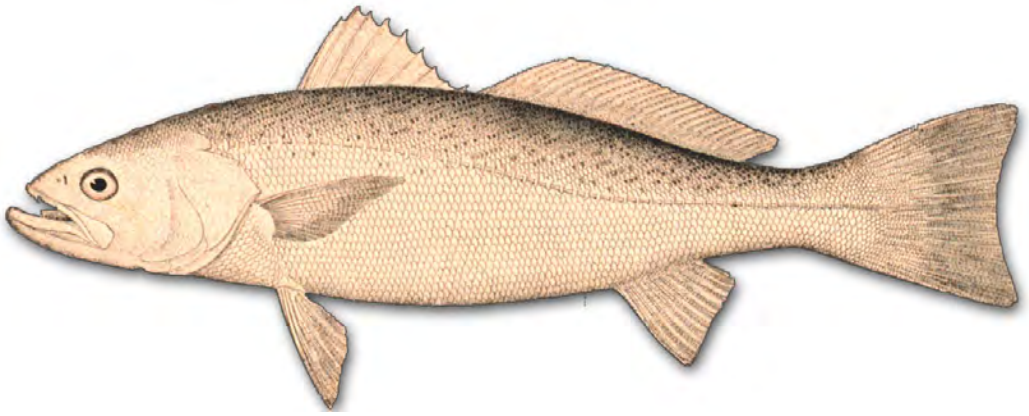
Table 14.7: Age-Length key, as proportion-at-age in each 1-inch length interval, based on both otolith and operculum ages for Tautog sampled in Chesapeake Bay, Virginia during 2025.

	Age									
Interval	2	3	4	5	6	7	8	9	10	11
10 - 10.99	1	0	0	0	0	0	0	0	0	0
11 - 11.99	0.5	0.36	0.14	0	0	0	0	0	0	0
12 - 12.99	0.09	0.64	0.18	0.09	0	0	0	0	0	0
13 - 13.99	0.02	0.41	0.37	0.15	0.02	0	0.02	0	0	0
14 - 14.99	0	0.2	0.31	0.29	0.2	0	0	0	0	0
15 - 15.99	0	0.12	0.33	0.25	0.25	0.02	0.04	0	0	0
16 - 16.99	0	0.15	0.27	0.17	0.37	0	0.02	0.02	0	0
17 - 17.99	0	0	0.06	0.17	0.5	0.11	0.06	0.06	0	0.06
18 - 18.99	0	0	0	0.1	0.5	0.2	0	0	0.1	0.1
19 - 19.99	0	0	0	0	0	0	0	1	0	0
20 - 20.99	0	0	0	0	0.5	0	0	0	0.5	0

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Chapter 15

WEAKFISH *Cynoscion regalis*



15.1 INTRODUCTION

We aged a total of 133 Weakfish *Cynoscion regalis*, collected by the VMRC's Biological Sampling Program for age and growth analysis in 2025. The Weakfish ages ranged from 1 to 4 years old with an average age of 2, a standard deviation of 0.7, and a standard error of 0.06. Four age classes (1 to 4) were represented, comprising fish of the 2021 to 2024 year-classes. The sample was dominated by fish from the year-class of 2023 with 60.1%.

15.2 METHODS

15.2.1 Sample Size for Ageing

We estimated sample size for ageing Weakfish in 2025 using a two-stage random sampling method (Quinn and Deriso 1999) to increase precision in estimates of age composition from fish sampled efficiently and effectively. The basic equation is:

$$A = \frac{V_a}{\theta_a^2 CV_a^2 + B_a/L} \tag{15.1}$$

where A is the sample size for ageing Weakfish in 2025; θ_a stands for the proportion of Age a fish in a catch; V_a, B_a, and CV_a represent the variance components within and between length intervals, and the coefficient of variation for Age a, respectively; L is the total number of Weakfish used by VMRC to estimate length distribution of the catches from 2019 to 2023. θ_a, V_a, and B_a were calculated using pooled age-length data of Weakfish collected from 2019 to 2023 and using equations in Quinn and Deriso (1999). For simplicity, the equations are not listed here. The equation (1.1) indicates: 1) The more fish that are aged, the smaller the CV_a (or higher precision) that will be obtained for Age a; 2) given a sample size A, the CV_a is different for each age due to different θ_a, V_a, and B_a among different ages. Therefore, the criterion to age A (number) of fish is that A should be a number above which there is only a 1% CV_a reduction for the most abundant age in catch by ageing an additional 100 or more fish. Finally, A_l is A multiplied by the proportion of length interval l from the length distribution of the 2019 to 2023 catch. A_l is number of fish to be aged for length interval l in 2025.

15.2.2 Handling of Collections

Otoliths were received by the Age and Growth Laboratory in labeled coin envelopes, and were sorted by date of capture. Their envelope labels were verified against VMRC's collection data, and each fish was assigned a unique Age and Growth Laboratory identification number. All otoliths were stored dry in their original labeled coin envelopes.

15.2.3 Preparation

Sagittal otoliths, hereafter, referred to as "otoliths", were processed for age determination following the methods described in Lowerre-Barbieri et al. (1994) with a few modifications. The left or right sagittal otolith was randomly selected and attached, distal side down, to a 1 x 2 inch piece of water resistant grid paper (Brand name: Write in the Rain) using hot glue. The otoliths were viewed by eye and, when necessary, under a stereo microscope to identify the location of the core, and the position of the core was marked using an ultra fine Sharpie across the otolith surface. At least one transverse cross-section (hereafter, referred to as "thin-section") was then removed from the marked core of each otolith using a Buehler IsoMet^{TM} low-speed saw equipped with two 4-inch diameter diamond grinding wheels (hereafter, referred to as "blades"), separated by a stainless steel spacer of 0.5 mm (diameter 2.5"). Thin-sections were placed on labeled glass slides and covered with a thin layer of Flo-texx mounting medium that not only fixed the sections to the slide, but more importantly, provided enhanced contrast and greater readability by increasing light transmission through the thin-sections.

Click here to obtain the protocol at the VMRV website on how to prepare otolith thin-section using the hot glue method for ageing Weakfish.

15.2.4 Readings

The VMRC system assigns an age class to a fish based on a combination of number of annuli in a thin-section, the date of capture, and the species-specific period when the annulus is deposited. Each year, as the fish grows, its otoliths grow and leave behind markers of their age, called an annulus. Technically, an otolith annulus is the combination of both the opaque and the translucent band. In practice, only the opaque bands are counted as annuli and recorded in our ageing notation.

In 2019 a new notation method recommended by Atlantic States Marine Fisheries Commission (ASMFC) was used to assign age on Weakfish. In addition to recording the number of annulus, the margin or the growth width after the last annulus is coded from 1 to 4. The margin code “1”, “2”, “3”, and “4” stands for no growth, the growth width less than or equal to one third of, larger than one third but less than or equal to two thirds of, and larger than two thirds of the growth width formed in the previous year, respectively.

By convention all fish in the Northern Hemisphere are assigned a birth date of January 1. In addition, each species has a specific period during which it deposits the annulus. If a fish is captured after the end of the species-specific annulus deposition period and before January 1, it is assigned an age class as the same as its annulus number without referencing its margin code. If a fish has a margin code of "1", it is assigned an age class as the same as its annulus number no matter in which month it is captured. If a fish is captured after December 31 and before its annulus deposition period, it is assigned an age class as its annulus number plus one when its margin code is "2", "3", or "4". If a fish is captured during its annulus deposition period, it is assigned an age class as the same as its annulus number when its margin code is “2” and as its annulus number plus one when its margin code is “3” or “4” (**Note: Based on the growth of Virginia species we use two criteria for Margin Code 2 to assign a fish an age class depending on its capture month, which could be different from how other states and agencies use Margin Code 2**).

For example, Weakfish otolith annulus formation occurs between April and June (Lowerre-Barbieri et al. 1994 and modified by CQFE/ODU). A Weakfish with two visible annuli could be assigned an age of 2 or 3 depending on its capture month and margin code. When its margin code is "1", it is Age 2 no matter when it is captured. When it is captured after June and before January, it is Age 2 no matter what its margin code is. When it is captured after December and before April and its margin code is not "1", it is Age 3 ($2 + 1 = 3$). When it is captured between April and June, it is Age 2 when its margin code is "2" but Age 3 ($2 + 1 = 3$) when its margin code is "3" or "4".

When an otolith was properly sectioned, the sulcal groove came to a sharp point (Hereafter referred to as "focus") within the middle of the core (Figure 15.1). Typically the first year’s annulus was found by locating the focus of the otolith section, which was characterized as a visually distinct dark, oblong region found in the center of the otolith section.

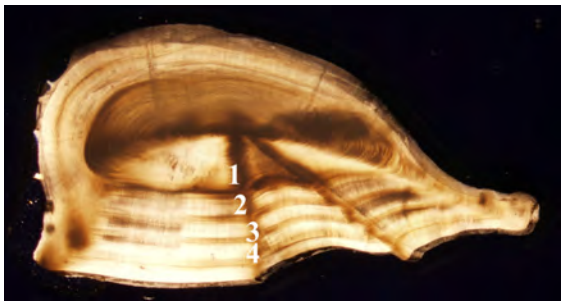


Figure 15.1: Otolith thin-section of 4 year-old Weakfish

All samples were aged by two readers in

chronological order, based on collection date, without knowledge of previously estimated ages or the specimen lengths. When the readers' ages agreed, that age was assigned to the fish. When the two readers disagreed, both readers sat down together and re-aged the fish, again without any knowledge of previously estimated ages or lengths, and assigned a final age to the fish. When the readers were unable to agree on a final age, the fish was excluded from further analysis. All thin-sections were aged using a Nikon SMZ1000 stereo microscope under transmitted light and dark-field polarization at between 8 and 20 times magnification.

15.2.5 Comparison Tests

A symmetry test (Hoenig et al. 1995) and coefficient of variation (CV) analysis were used to detect any systematic difference and precision on age readings, respectively, for the following comparisons: 1) between the two readers in the current year, 2) within each reader in the current year, and 3) time-series bias between the current and previous years within each reader. The readings from the entire sample for the current year were used to examine the difference between two readers. A random sub-sample of 50 fish from the current year was selected for second readings to examine the difference within a reader. Fifty otoliths randomly selected from fish aged in 2000 were used to examine the time-series bias within each reader. A figure of 1:1 equivalence was used to illustrate those differences (Campana et al. 1995). All statistics analyses were performed in R.4.0.2 (R Core Team 2021).

15.3 RESULTS

15.3.1 Sample Size

We estimated a sample size of 354 for ageing Weakfish in 2025, ranging in length interval from 4 to 30 inches (Table 15.1). This sample size provided a range in CV for age composition approximately from the smallest CV of 6% for Age 2 to the CV of larger than 25% for the multiple minor ages (Table 15.2). In 2025, we aged all 133 Weakfish collected by VMRC. We fell short in our over-all collections for this optimal length-class sampling estimate by 221 fish. We were short of many fish from the major length intervals (The interval requires 10 or more fish), as a result, the precision for the estimates of major age groups would definitely be influenced significantly. Therefore, precaution should be used when developing ALK using these age data.

15.3.2 Reading Precision

Both readers had high self-precision. Specifically, there was no significant difference between the first and second readings for Reader 1 with an agreement of 94% and a CV of 2.45% (test of symmetry: $\chi^2 = 3$, $df = 2$, $P = 0.2231$), and there was no significant difference between the first and second readings for Reader 2 with an agreement of 92% and a CV of 3.02% (test of symmetry: $\chi^2 = 2$, $df = 2$, $P = 0.3679$). There was no evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 99.25% and a CV of 0.35% (test of symmetry: $\chi^2 = 1$, $df = 1$, $P = 0.3173$) (Figure 15.2).

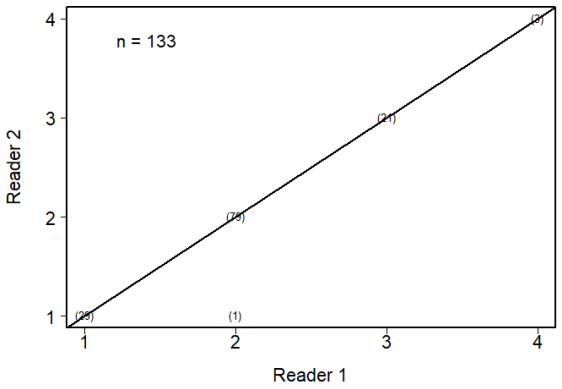


Figure 15.2: Between-reader comparison of otolith age estimates for Weakfish collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2025. The number in parentheses is number of fish.

There was no time-series bias for either reader.

Reader 1 had an agreement of 100% with ages of fish aged in 2000. Reader 2 had an agreement of 98% with a CV of 0.19% (test of symmetry: $\chi^2 = 1$, $df = 1$, $P = 0.3173$).

15.3.3 Year Class

Of the 133 fish aged with otoliths, 4 age classes (1 to 4) were represented (Table 15.3). The average age was 2 years, and the standard deviation and standard error were 0.7 and 0.06, respectively. Year-class data show that the fishery was comprised of 4 year-classes: fish from the 2021 to 2024 year-classes, with fish primarily from the year-class of 2023 with 60.1%. The ratio of males to females was 1:3.59 in the sample collected (Figure 15.3).

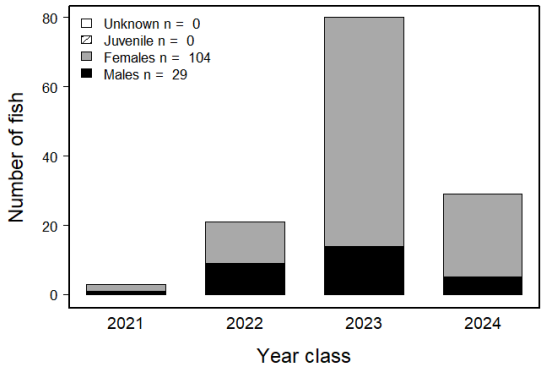


Figure 15.3: Year-class frequency distribution for Weakfish collected for ageing in 2025. Distribution is broken down by sex. 'Unknown' represents gonads that were not available for examination or were not examined for sex during sampling.

15.3.4 Age-length Key (ALK)

We developed an age-length-key (Table 15.4) that can be used in the conversion of numbers-at-length in the estimated catch to numbers-at-age using otolith ages. The table is based on VMRC's stratified sampling of landings by total length inch intervals.

Table 15.1: Number of Weakfish collected and aged in each 1-inch length interval in 2025. 'Target' represents the sample size for ageing estimated for 2025, and 'Need' represents number of fish shorted in each length interval compared to the optimum sample size for ageing and number of fish aged.

Interval	Target	Collected	Aged	Need
4 - 4.99	5	0	0	5
5 - 5.99	5	0	0	5
6 - 6.99	5	0	0	5
7 - 7.99	5	0	0	5
8 - 8.99	15	0	0	15
9 - 9.99	32	1	1	31
10 - 10.99	46	4	4	42
11 - 11.99	42	23	23	19
12 - 12.99	35	29	29	6
13 - 13.99	27	27	27	0
14 - 14.99	21	16	16	5
15 - 15.99	20	16	16	4
16 - 16.99	13	6	6	7
17 - 17.99	11	5	5	6
18 - 18.99	10	3	3	7
19 - 19.99	7	1	1	6
20 - 20.99	5	1	1	4
21 - 21.99	5	1	1	4
22 - 22.99	5	0	0	5
23 - 23.99	5	0	0	5
24 - 24.99	5	0	0	5
25 - 25.99	5	0	0	5
26 - 26.99	5	0	0	5
27 - 27.99	5	0	0	5
28 - 28.99	5	0	0	5
29 - 29.99	5	0	0	5
30 - 30.99	5	0	0	5
Totals	354	133	133	221

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Table 15.2: CV for each age estimated based on ageing the total of 354 Weakfish in 2025. 'Percent' is the percentage of an age in the pooled age-length data of Weakfish collected from 2019 to 2023.

Age	CV	Percent
0	>0.25	0.25
1	0.11	15.77
2	0.06	44.27
3	0.07	33.14
4	0.19	6.07
5	>0.25	0.51

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Table 15.3: The number of Weakfish assigned to each total length-at-age category for 133 fish sampled for otolith age determination in Virginia during 2025.

	Age				
Interval	1	2	3	4	Totals
9 - 9.99	1	0	0	0	1
10 - 10.99	2	2	0	0	4
11 - 11.99	6	15	2	0	23
12 - 12.99	8	19	2	0	29
13 - 13.99	6	18	3	0	27
14 - 14.99	4	8	4	0	16
15 - 15.99	2	7	6	1	16
16 - 16.99	0	4	1	1	6
17 - 17.99	0	4	1	0	5
18 - 18.99	0	2	1	0	3
19 - 19.99	0	0	0	1	1
20 - 20.99	0	1	0	0	1
21 - 21.99	0	0	1	0	1
Totals	29	80	21	3	133

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Table 15.4: Age-Length key, as proportion-at-age in each 1-inch length interval, based on otolith ages for Weakfish sampled for age determination in Virginia during 2025.

	Age			
Interval	1	2	3	4
9 - 9.99	1	0	0	0
10 - 10.99	0.5	0.5	0	0
11 - 11.99	0.26	0.65	0.09	0
12 - 12.99	0.28	0.66	0.07	0
13 - 13.99	0.22	0.67	0.11	0
14 - 14.99	0.25	0.5	0.25	0
15 - 15.99	0.12	0.44	0.38	0.06
16 - 16.99	0	0.67	0.17	0.17
17 - 17.99	0	0.8	0.2	0
18 - 18.99	0	0.67	0.33	0
19 - 19.99	0	0	0	1
20 - 20.99	0	1	0	0
21 - 21.99	0	0	1	0

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