2023 Final Report
Virginia ~ Chesapeake Bay Finfish Ageing and Population Analysis

Hongsheng Liao, Gabriel Salomon, Jessica L. Branscome, and Alicia Nelson

June 28, 2024

Fisheries Management Division
Virginia Marine Resources Commission
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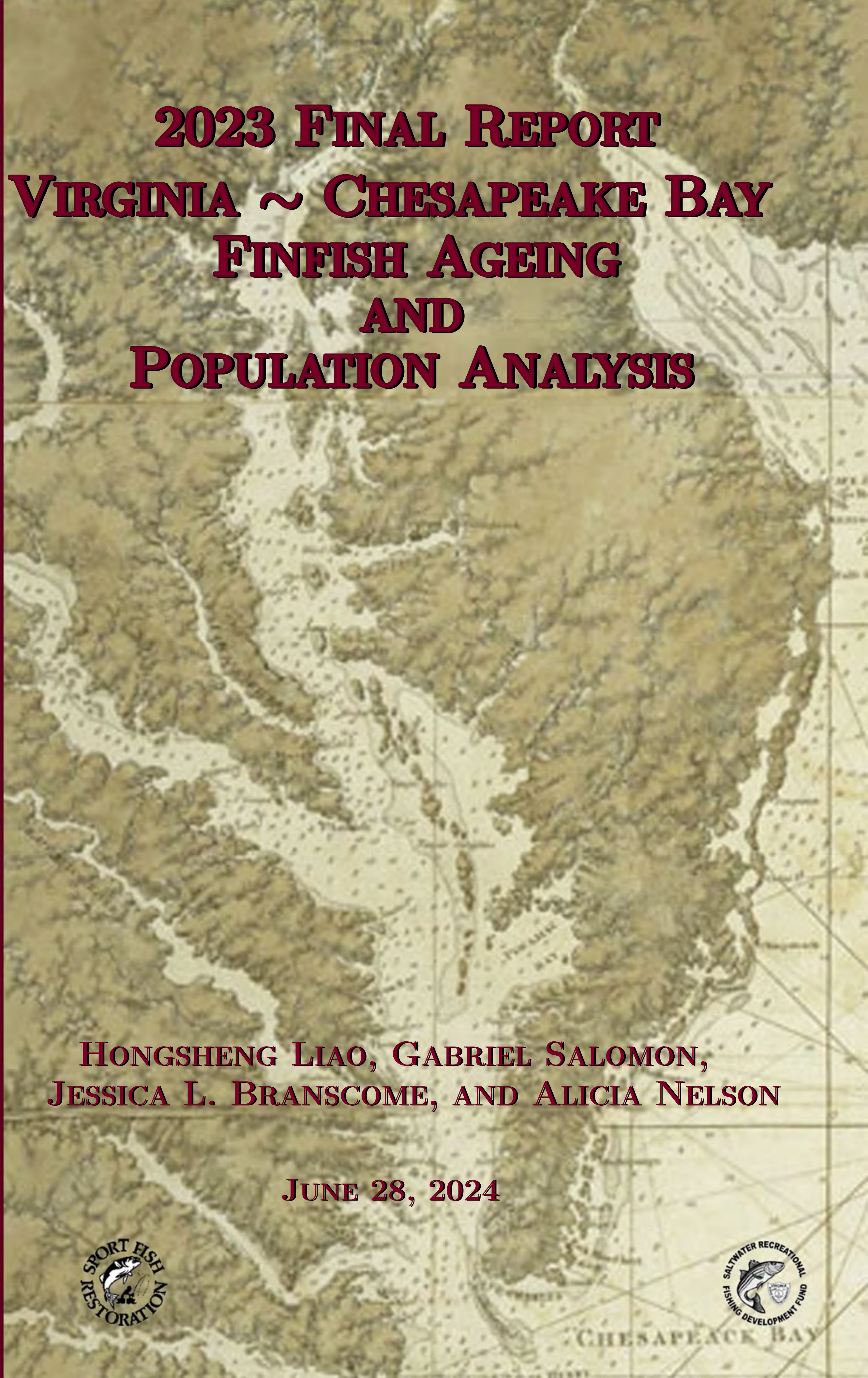

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EXECUTIVE SUMMARY

This executive summary briefly summarizes what the Age and Growth Lab achieved in 2023 in terms of the objectives listed in the 2023 - 2024 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, 14 chapters in the report are about the objective and each chapter is for one of 14 species the lab aged in 2023. We present the ageing results of 14 finfish species collected from commercial and recreational catches made in the Chesapeake Bay and Virginia waters of the Atlantic Ocean, U.S.A. in 2023. All fish were collected by the VMRC Biological Sampling Program in 2023 and aged in 2024 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 = 248). Two calcified structures, otoliths and scales, were used for determining fish ages of Striped Bass *Morone saxatilis* (n = 840) and Summer Flounder *Paralichthys dentatus* (n = 825). 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 = 270), Black Drum *Pogonias cromis* (n = 82), Bluefish *Pomatomus saltatrix* (n = 286), Cobia *Rachycentron canadum* (n = 311), Red Drum *Sciaenops ocellatus* (n = 65), Sheepshead *Archosargus probatocephalus* (n = 333), Atlantic Spadefish *Chaetodipterus faber* (n = 220), Spanish Mackerel *Scomberomorous maculates* (n = 280), Spot *Leiostomus xanthurus* (n = 168), Spotted Seatrout *Cynoscion nebulosus* (n = 259), and Weakfish *Cynoscion regalis* (n = 282). In total, we made 9,208 age readings from otoliths, scales, opercula, and spines collected during 2023. A summary of the age ranges for all species aged is presented in Table 1.

In 2023 we aged not only their opercula and otoliths as prior to 2022, we also aged their spines as well for the second consecutive year. This allowed us to compare the precision in ageing between these three hardparts for Tautog.

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 14 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.

Objective 3: VMRC will develop routine stock assessments based on age-structured models (such

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 14 finfish species in 2023. Besides otoliths, the hardparts and age readings include scales for Striped Bass and Summer Flounder, and both opercula and spines for Tautog. The otolith-ages are reported for all the species. When otolith-ages are not available, scale-ages are reported for Striped Bass and Summer Flounder whereas operculum-ages are reported for Tautog. However, when neither otolith- nor operculum-ages are avaialbe for Tautog, spine-ages are reported.

Species	Number of fish collected	Number of hard-parts	Number of fish aged	Number of readings	Minimum age	Maximum age
Atlantic Croaker	449	449	270	540	0	5
Black Drum	82	82	82	164	1	28
Bluefish	465	464	286	572	0	12
Cobia	313	311	311	622	2	13
Red Drum	65	65	65	130	0	2
Sheepshead	336	333	333	333	1	31
Spadefish	249	249	220	440	1	8
Spanish Mackerel	356	356	280	560	0	8
Spot	256	255	168	336	0	2
Spotted Seatrout	359	359	259	518	0	5
Striped Bass	1,143	1,395	840	1,345	3	34
Summer Flounder	920	1,392	825	1,771	1	16
Tautog	248	732	248	980	2	16
Weakfish	296	296	282	564	1	5
Totals	5,537	6,738	4,469	9,208		

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). In March of 2024, we provided the scale slides of 106 Menhaden collected in 2023 to the NOAA Ageing Lab at Beaufort, NC, for the Menhaden Stock Assessment. In order to start to age Menhaden in-house, we not only provided Menhaden scale slides (glass slides) but also made two scale slides (glass vs. acetate) and two otolith slides (thin-section vs. whole otolith) from each fish of 45 Menhaden collected in 2023. For the purpose of practicing, we aged all four slides of each fish, and examined the precisions on each of the four slides within each reader and between two readers. The results are presented in APPENDIX (Chapter 15).

In November of 2023, Dr. Hongsheng Liao, Lab Manager, participated the ASMFC Menhaden Ageing Workshop at Beaufort, NC. In June of 2024, Gabriel Salamon (Chief Technician) and Jem Baldisimo (Fellow from the Commonwealth of Virginia Engineering & Science policy Fellowship) visited the NOAA Beaufort Ageing Lab and aged the VMRC's menhaden scale and otolith slides with the staff in the Beaufort Ageing Lab. This colloration work will not only enhance the quality on ageing Menhaden in-house but also help us to identify which hardpart and which technique method will provide the most precise age estimates for Menhaden.

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 2022 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. In 2023, we replaced the species-specific protocols with the hardpart-specific protocols on how to process their calcified structures. As a result, we posted four protocols on processing otolith thin-sections, scales, opercula, and spines, respecitively. In addition, we posted a new video on how to make acetate impressions of fish scales, and revised the Weakfish Otolith Removal video.

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 2023, We updated age-length data in VMRC four web applications (Fish Age Estimator, Fish Growth Predictor, VMRC/CQFE 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 14 marine finfish species collected by VMRC at Chesapeake Bay and Virginia waters of Atlantic ocean from as early as 1998 to 2023 and aged by the lab.

In 2023, we provided otolith thin-section slides of Atlantic Croaker (13), Bluefish (21), Black Drum (9), Cobia (2), and Spot (11), pairded otolith thin-section and scale slides of Striped Bass (17) and Summer Flounder (20), and paired otolith thin-section slides, opercula, and spine thin-section slides of Tautog (3) to the ASMFC QAQC Ageing Workshop (The number in parentheses is the sample size for each species.). In March of 2023, the Chief Technician Mr. Gabriel Salomon participated the workshop at St. Petersburg, FL.

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 2023. Each time we revise an old processing method or add a new method, we update the protocol.

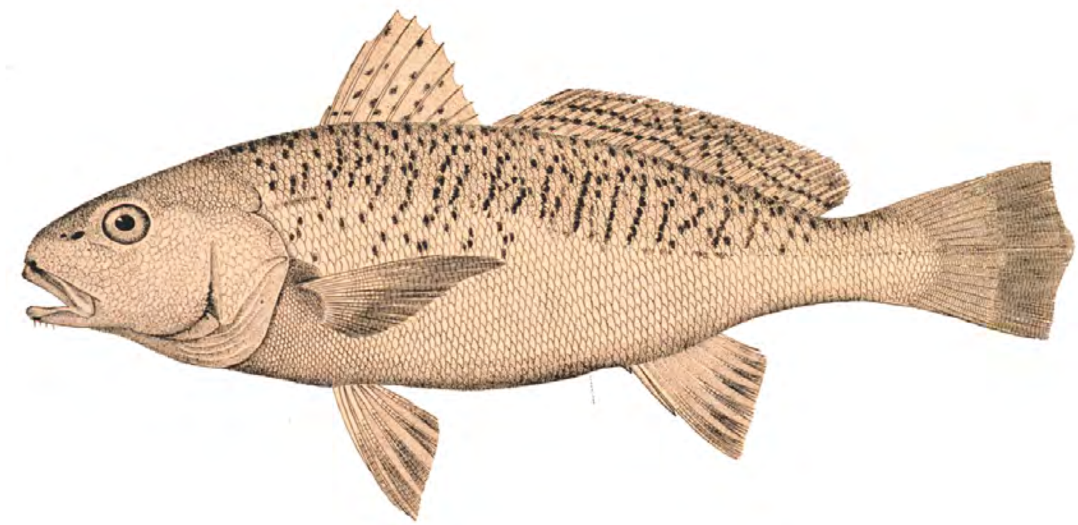
Besides the above work, to support environmental and wildlife agencies, and charities, In 2023 we donated more than 2,699 pounds of dissected fish to the Salvation Army to feed the homeless, and Alton's Keep WildBird Rescue and Rehabilitation Center Inc., a local wildlife rescue agency which is responsible for saving injured animals found by the public.

ACKNOWLEDGMENTS

We thank Marben Abutin and Natalie Baker 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, Richard Hancock, Myra Thompson, 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 270 Atlantic Croaker *Micropogonias undulatus*, collected by the VMRC's Biological Sampling Program for age and growth analysis in 2023. Atlantic Croaker ages ranged from 0 to 5 years old with an average age of 1.8, a standard deviation of 0.9, and a standard error of 0.05. Six age classes (0 to 5) 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 21.1%, 21.9%, and 51.9%, respectively.

1.2 METHODS

1.2.1 Sample Size for Ageing

We estimated sample size for ageing Atlantic Croaker in 2023 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 Atlantic Croaker in 2023; 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 Atlantic Croaker used by VMRC to estimate length distribution of the catches from 2017 to 2021. θ_a, V_a, and B_a were calculated using pooled age-length data of Atlantic Croaker collected from 2017 to 2021 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 2017 to 2021 catch. A_l is number of fish to be aged for length interval l in 2023.

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 VMRC Ageing Lab website on how to prepare otolith thin-section for ageing Atlantic Croaker using the Glue Method.

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). An Atlantic 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 2023.

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-

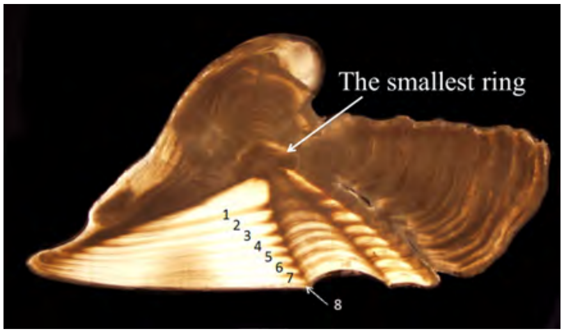


Figure 1.1: Otolith thin-sections of a 8 year-old Atlantic Croaker without counting the smallest ring and with the last annulus on the edge of the thin-section

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 (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 446 Atlantic Croaker in 2023, ranging in length interval from 4 to 16 inches (Table 1.1). This sample size provided a range in *CV* for age composition approximately from the smallest *CV* of 9% for the major age of Age 5 to the *CV* of larger than 25% for the multiple minor ages (Table 1.2). In 2023, we randomly selected and aged 270 fish from 449 Atlantic Croaker collected by VMRC. We fell short in our over-all collections for this optimal length-class sampling estimate by 190 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.

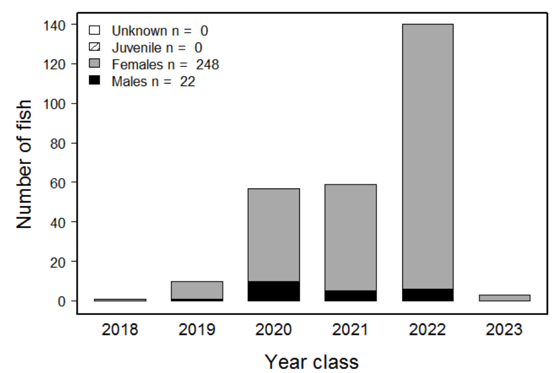


Figure 1.2: Year-class frequency distribution for Atlantic Croaker collected for ageing in 2023. Distribution is broken down by sex. 'Unknown' is for gonads that were not available for examination or were not examined for sex during sampling.

1.3.2 Year Class

Of the 270 fish aged with otoliths, 6 age classes (0 to 5) were represented (Table 1.3). The average age was 1.8 years, and the standard deviation and standard error were 0.9 and 0.05, 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 21.1%, 21.9%, and 51.9%, respectively. The ratio of males to females was 1:11.27 in the sample collected (Figure 1.2).

1.3.3 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.

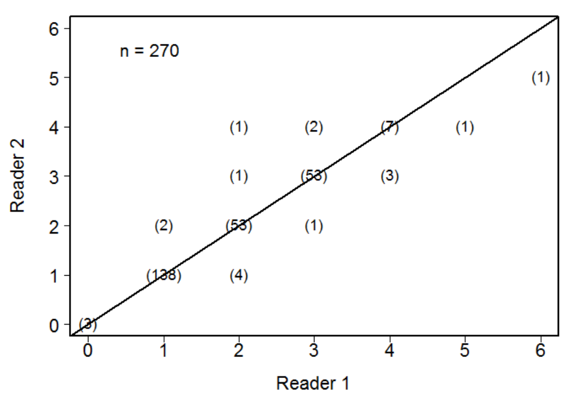


Figure 1.3: Between-reader comparison of otolith age estimates for Atlantic Croaker collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2023. The number in parentheses is number of fish.

1.3.4 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 90% and a *CV* of 3.17% (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 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 94.07% and a *CV* of 1.91% (test of symmetry: $\chi^2 = 3.87$, $df = 6$, $P = 0.6947$) (Figure 1.3).

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% .

Table 1.1: Number of Atlantic Croaker collected and aged in each 1-inch length interval in 2023. 'Target' represents the sample size for ageing estimated for 2023, 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	1	1	4
6 - 6.99	5	1	1	4
7 - 7.99	19	21	21	0
8 - 8.99	15	113	16	0
9 - 9.99	41	124	42	0
10 - 10.99	72	82	82	0
11 - 11.99	116	79	79	37
12 - 12.99	103	26	26	77
13 - 13.99	44	2	2	42
14 - 14.99	11	0	0	11
15 - 15.99	5	0	0	5
16 - 16.99	5	0	0	5
Totals	446	449	270	190

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Table 1.2: CV for each age estimated based on ageing the total of 446 Atlantic Croaker in 2023. 'Percent' is the percentage of an age in the pooled age-length data of Atlantic Croaker collected from 2017 to 2021.

Age	CV	Percent
0	0.2	2.88
1	0.11	16.61
2	0.11	17.65
3	0.13	11.82
4	0.12	13.9
5	0.09	21.09
6	0.14	10.7
7	>0.25	2.96
8	>0.25	1.76
9	>0.25	0.56
11	>0.25	0.08

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Table 1.3: The number of Atlantic Croaker assigned to each total length-at-age category for 270 fish sampled for otolith age determination in Virginia during 2023.

	Age						
Interval	0	1	2	3	4	5	Totals
5 - 5.99	1	0	0	0	0	0	1
6 - 6.99	1	0	0	0	0	0	1
7 - 7.99	1	10	7	3	0	0	21
8 - 8.99	0	6	2	7	1	0	16
9 - 9.99	0	12	10	15	5	0	42
10 - 10.99	0	36	24	19	3	0	82
11 - 11.99	0	59	11	7	1	1	79
12 - 12.99	0	15	5	6	0	0	26
13 - 13.99	0	2	0	0	0	0	2
Totals	3	140	59	57	10	1	270

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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 2023.

	Age					
Interval	0	1	2	3	4	5
5 - 5.99	1	0	0	0	0	0
6 - 6.99	1	0	0	0	0	0
7 - 7.99	0.05	0.48	0.33	0.14	0	0
8 - 8.99	0	0.38	0.12	0.44	0.06	0
9 - 9.99	0	0.29	0.24	0.36	0.12	0
10 - 10.99	0	0.44	0.29	0.23	0.04	0
11 - 11.99	0	0.75	0.14	0.09	0.01	0.01
12 - 12.99	0	0.58	0.19	0.23	0	0
13 - 13.99	0	1	0	0	0	0

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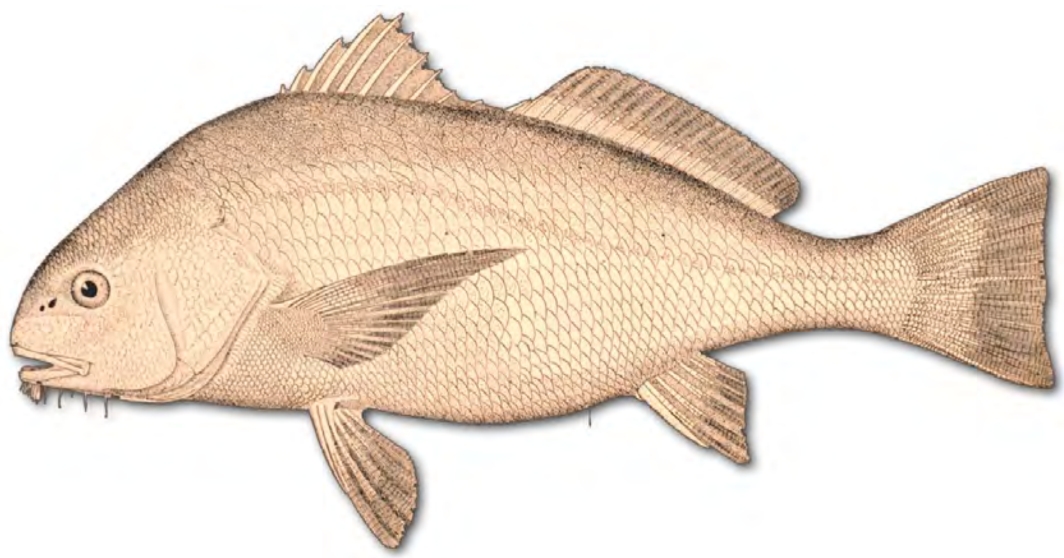
Chapter 2

BLACK DRUM *Pogonias cromis*



2.1 INTRODUCTION

We aged a total of 82 Black Drum *Pogonias cromis*, collected by the VMRC's Biological Sampling Program for age and growth analysis in 2023. Black drum ages ranged from 1 to 28 years old with an average age of 12.4, a standard deviation of 8.4, and a standard error of 0.93. Twenty age classes (1 to 9, 11 to 12, 14, 16 to 18, 20, 22, 24 to 25, and 28) were represented, comprising fish of the 1995, 1998 to 1999, 2001, 2003, 2005 to 2007, 2009, 2011 to 2012, and 2014 to 2022 year-classes. The sample was dominated by fish from the year-classes of 1995, 2001, 2006, 2007, 2018, 2019, and 2021 with 6.1%, 15.8%, 6.1%, 8.5%, 9.8%, 14.6%, and 8.5%, 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 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 "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 sections.

Click here to obtain the protocol at the VMRC Ageing Lab website on how to prepare otolith thin-section for ageing Black Drum using the Glue Method.

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.

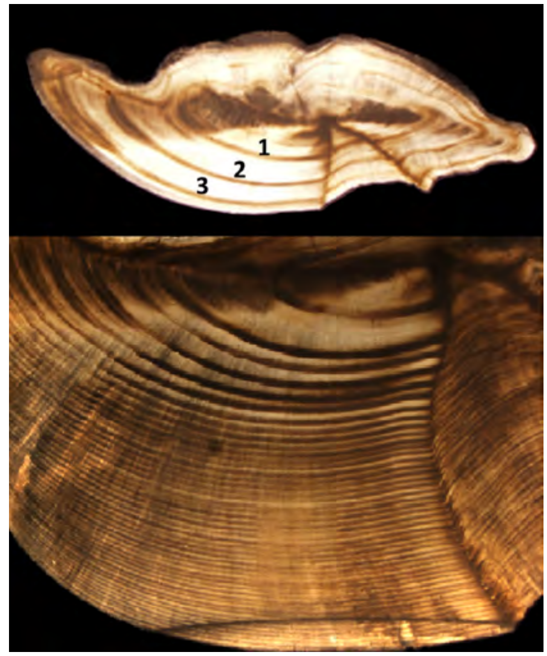


Figure 2.1: Otolith thin-sections of a 3 (Upper panel) and 47 year-old (Lower panel) Black Drum.

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 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 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).

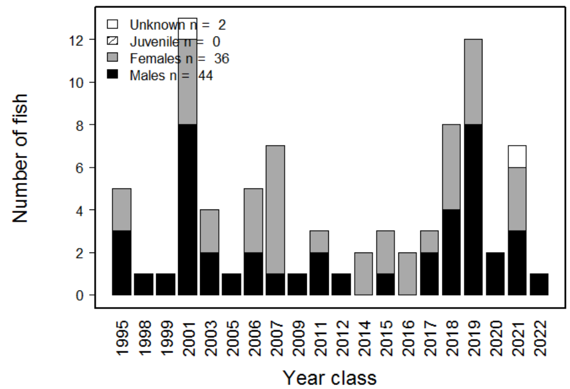


Figure 2.2: Year-class frequency distribution for Black Drum collected for ageing in 2023. Distribution is broken down by sex. 'Unknown' represents gonads that were not available for examination or were not examined for sex during sampling.

2.3 RESULTS

2.3.1 Year Class

We aged the otoliths of all the 82 Black Drum collected in 2023. Of the 82 fish aged, 20 age classes (1 to 9, 11 to 12, 14, 16 to 18, 20, 22, 24 to 25, and 28) were represented (Table 2.1). The average age was 12.4 years, and the standard deviation and standard error were 8.4 and 0.93, respectively. Year-class data show that the fishery was comprised of 20 year-classes: fish from the 1995, 1998 to 1999, 2001, 2003, 2005 to 2007, 2009, 2011 to 2012, and 2014 to 2022 year-classes, with fish primarily from the year classes of 1995, 2001, 2006, 2007, 2018, 2019, and 2021 with 6.1%, 15.8%, 6.1%, 8.5%, 9.8%, 14.6%, and 8.5%, respectively. The ratio of males to females was 1:0.82 in the sample collected (Figure 2.2).

2.3.2 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.

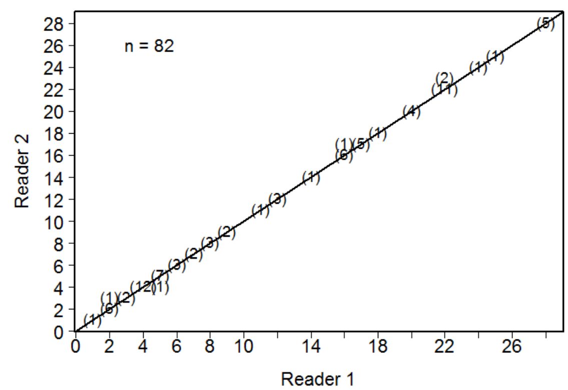


Figure 2.3: Between-reader comparison of otolith age estimates for Black Drum collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2023. The number in parentheses is number of fish.

2.3.3 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 96% and a CV of 0.63% (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

93.9% and a CV of 0.67% (test of symmetry: $\chi^2 = 5$, $df = 4$, $P = 0.2873$) (Figure 2.3).

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 0.14% (test of symmetry: $\chi^2 = 3$, $df = 3$, $P = 0.3916$). Reader 2 had an agreement of 90%with a CV of 0.68% (test of symmetry: $\chi^2 = 5$, $df = 5$, $P = 0.4159$).

Table 2.1: The number of Black Drum assigned to each total length (inch)-at-age category for 82 fish sampled for otolith age determination in Virginia during 2023.

	Age																			
Interval	1	2	3	4	5	6	7	8	9	11	12	14	16	17	18	20	22	24	25	28	Totals
18 - 18.99	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
19 - 19.99	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
20 - 20.99	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
21 - 21.99	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
22 - 22.99	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
23 - 23.99	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
24 - 24.99	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
25 - 25.99	0	1	1	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6
26 - 26.99	0	1	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5
27 - 27.99	0	0	0	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
28 - 28.99	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
29 - 29.99	0	0	0	0	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
30 - 30.99	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	3
31 - 31.99	0	0	0	0	0	1	0	2	0	0	0	0	0	0	0	0	0	0	0	0	3
32 - 32.99	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	2
33 - 33.99	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1
34 - 34.99	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1
35 - 35.99	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1
36 - 36.99	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1
37 - 37.99	0	0	0	0	0	0	0	0	0	0	1	0	2	0	0	0	0	0	0	0	3
38 - 38.99	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	2	0	0	0	4
39 - 39.99	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	2
40 - 40.99	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	2	1	0	0	0	6
41 - 41.99	0	0	0	0	0	0	0	0	0	0	0	0	3	2	0	1	2	0	0	0	8
42 - 42.99	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	2	0	0	1	4
43 - 43.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	1	0	0	5
44 - 44.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	3	5
45 - 45.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	2
51 - 51.99	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Totals	1	7	2	12	8	3	2	3	2	1	3	1	7	5	1	4	13	1	1	5	82

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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 2023.

	Age																			
Interval	1	2	3	4	5	6	7	8	9	11	12	14	16	17	18	20	22	24	25	28
18 - 18.99	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19 - 19.99	0	0.5	0	0	0.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20 - 20.99	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
21 - 21.99	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
22 - 22.99	0.5	0	0	0.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
23 - 23.99	0	0	0.5	0.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
24 - 24.99	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
25 - 25.99	0	0.17	0.17	0.33	0.33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
26 - 26.99	0	0.2	0	0.8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
27 - 27.99	0	0	0	0.33	0.67	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
28 - 28.99	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
29 - 29.99	0	0	0	0	0.67	0.33	0	0	0	0	0	0	0	0	0	0	0	0	0	0
30 - 30.99	0	0	0	0.33	0	0.33	0.33	0	0	0	0	0	0	0	0	0	0	0	0	0
31 - 31.99	0	0	0	0	0	0.33	0	0.67	0	0	0	0	0	0	0	0	0	0	0	0
32 - 32.99	0	0	0	0	0	0	0	0.5	0.5	0	0	0	0	0	0	0	0	0	0	0
33 - 33.99	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
34 - 34.99	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
35 - 35.99	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
36 - 36.99	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
37 - 37.99	0	0	0	0	0	0	0	0	0	0	0.33	0	0.67	0	0	0	0	0	0	0
38 - 38.99	0	0	0	0	0	0	0	0	0	0	0	0.25	0	0	0.25	0	0.5	0	0	0
39 - 39.99	0	0	0	0	0	0	0	0	0	0	0	0	0.5	0	0	0.5	0	0	0	0
40 - 40.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0.5	0	0.33	0.17	0	0	0
41 - 41.99	0	0	0	0	0	0	0	0	0	0	0	0	0.38	0.25	0	0.12	0.25	0	0	0
42 - 42.99	0	0	0	0	0	0	0	0	0	0	0	0	0.25	0	0	0	0.5	0	0	0.25
43 - 43.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.8	0.2	0	0
44 - 44.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.2	0	0.2	0.6
45 - 45.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.5	0	0	0.5
51 - 51.99	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0

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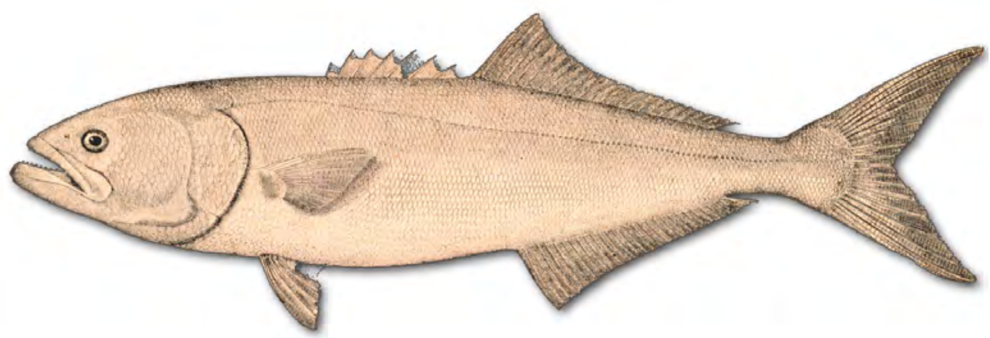
Chapter 3

BLUEFISH *Pomatomus saltatrix*



3.1 INTRODUCTION

We aged a total of 286 Bluefish *Pomatomus saltatrix*, collected by the VMRC's Biological Sampling Program for age and growth analysis in 2023. Bluefish ages ranged from 0 to 12 years old with an average age of 2, a standard deviation of 1.3, and a standard error of 0.08. Eight age classes (0 to 6, and 12) were represented, comprising fish of the 2011, and 2017 to 2023 year-classes. The sample was dominated by fish from the year-classes of 2021 and 2022 with 43% and 32.9%, respectively.

3.2 METHODS

3.2.1 Sample Size for Ageing

We estimated sample size for ageing Bluefish in 2023 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 2023; 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 2017 to 2021. θ_a, V_a, and B_a were calculated using pooled age-length data of Bluefish collected from 2017 to 2021 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 2017 to 2021 catch. A_l is number of fish to be aged for length interval l in 2023. 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 inside of protective Axygen 2 ml micro-tubes within 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 VMRC Ageing Lab website on how to prepare otolith thin-section for ageing Bluefish using the Epoxy Resin Method.

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.

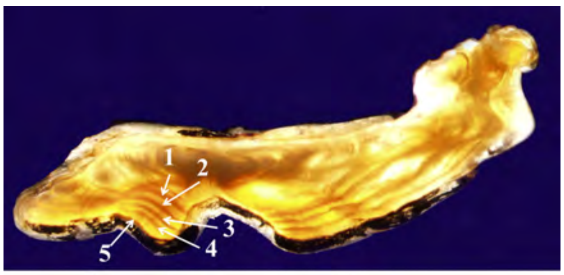


Figure 3.1: Otolith thin-section of a 5 year-old Bluefish with the last annulus on the edge of the thin-section

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 458 Bluefish in 2023, ranging in length interval from 14 to 121 centimeters (Table 3.1). This sample size provided a range in *CV* for age composition approximately from the smallest *CV* of 6% for Age 1 and 2 to the *CV* of larger than 25% for the multiple minor ages (Table 3.2). In 2023, we randomly selected and aged 286 fish from 464 Bluefish collected by VMRC. We fell short in our over-all collections for this optimal length-class sampling estimate by 187 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.

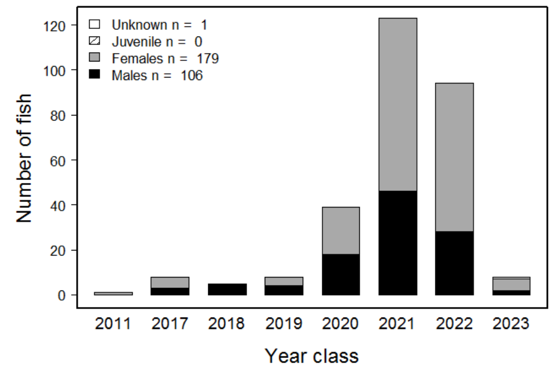


Figure 3.2: Year-class frequency distribution for Bluefish collected for ageing in 2023. Distribution is broken down by sex. 'Unknown' represents gonads that were not available for examination or were not examined for sex during sampling.

3.3.2 Year Class

Of the 286 fish aged with otoliths, 8 age classes (0 to 6, and 12) were represented (Table 3.3). The average age was 2 years, and the standard deviation and standard error were 1.3 and 0.08, respectively. Year-class data show that the fishery was comprised of 8 year-classes: fish from the 2011, and 2017 to 2023 year-classes, with fish primarily from the year classes of 2021 and 2022 with 43% and 32.9%, respectively. The ratio of males to females was 1:1.69 in the sample collected (Figure 3.2).

3.3.3 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.

3.3.4 Reading Precision

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 92% and a *CV* of 2.86% (test of symmetry: $\chi^2 = 2$, $df = 3$, $P = 0.5724$), and there was no significant difference between the first and second readings for Reader 2 with an agreement of 74% and a *CV* of 11.16% (test of symmetry: $\chi^2 = 5$, $df = 6$, $P = 0.5438$). There was no evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 77.62% and a *CV* of 9.38% (test of symmetry: $\chi^2 = 18.97$, $df = 11$, $P = 0.0616$) (Figure 3.3).

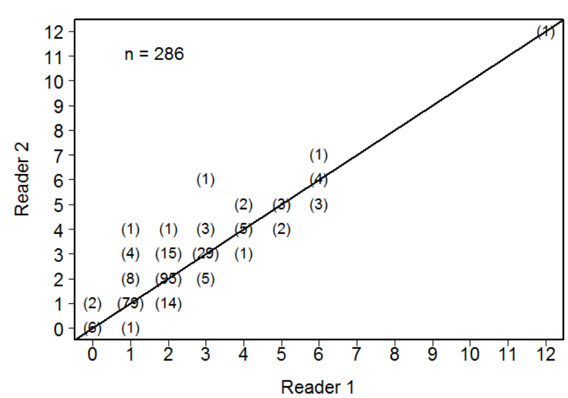


Figure 3.3: Between-reader comparison of otolith age estimates for Bluefish collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2023. The number in parentheses is number of fish.

There was no time-series bias for either reader. Reader 1 had an agreement of 92% with ages of fish aged in 2000 with a CV of 3.23% (test of symmetry: $\chi^2 = 1.33$, $df = 2$, $P = 0.5134$). Reader 2 had an agreement of 82% with a CV of 17.56% (test of symmetry: $\chi^2 = 9$, $df = 4$, $P = 0.0611$).

Table 3.1: Number of Bluefish collected and aged in each 1-cm length interval in 2023. 'Target' represents the sample size for ageing estimated for 2023, 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
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	0	0	5
22 - 22.99	5	0	0	5
23 - 23.99	5	0	0	5
24 - 24.99	5	1	1	4
25 - 25.99	5	3	3	2
26 - 26.99	6	3	3	3
27 - 27.99	6	7	7	0
28 - 28.99	6	11	6	0
29 - 29.99	6	10	6	0
30 - 30.99	7	13	8	0
31 - 31.99	7	17	8	0
32 - 32.99	6	12	6	0
33 - 33.99	6	12	6	0
34 - 34.99	6	9	6	0
35 - 35.99	6	13	6	0
36 - 36.99	8	9	8	0
37 - 37.99	7	9	8	0
38 - 38.99	8	7	7	1
39 - 39.99	8	21	8	0
40 - 40.99	7	20	8	0
41 - 41.99	8	17	8	0
42 - 42.99	8	16	8	0
43 - 43.99	8	14	8	0
44 - 44.99	6	26	6	0
45 - 45.99	9	17	10	0
46 - 46.99	7	19	8	0
47 - 47.99	8	17	8	0
48 - 48.99	6	22	6	0
49 - 49.99	5	9	6	0
50 - 50.99	5	15	6	0
51 - 51.99	6	7	6	0
52 - 52.99	5	6	6	0
53 - 53.99	5	10	6	0
54 - 54.99	6	5	5	1
55 - 55.99	6	7	6	0
56 - 56.99	5	4	4	1
57 - 57.99	5	8	6	0

(To continue)

Table 3.1 (Continued)

Interval	Target	Collected	Aged	Need
58 - 58.99	5	6	6	0
59 - 59.99	5	5	5	0
60 - 60.99	5	5	5	0
61 - 61.99	5	4	4	1
62 - 62.99	5	4	4	1
63 - 63.99	5	5	5	0
64 - 64.99	5	7	7	0
65 - 65.99	5	1	1	4
66 - 66.99	5	1	1	4
67 - 67.99	5	2	2	3
68 - 68.99	5	2	2	3
69 - 69.99	5	1	1	4
70 - 70.99	5	0	0	5
71 - 71.99	5	1	1	4
72 - 72.99	5	0	0	5
73 - 73.99	5	1	1	4
74 - 74.99	5	1	1	4
75 - 75.99	5	2	2	3
76 - 76.99	5	4	4	1
77 - 77.99	5	4	4	1
78 - 78.99	5	1	1	4
79 - 79.99	5	3	3	2
80 - 80.99	5	1	1	4
81 - 81.99	5	3	3	2
82 - 82.99	5	1	1	4
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	1	1	4
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	0	0	5
93 - 93.99	5	0	0	5
94 - 94.99	5	0	0	5
95 - 95.99	5	0	0	5
96 - 96.99	5	0	0	5
121 - 121.99	5	0	0	5
Totals	458	464	286	187

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Table 3.2: CV for each age estimated based on ageing the total of 458 Bluefish in 2023. 'Percent' is the percentage of an age in the pooled age-length data of Bluefish collected from 2017 to 2021.

Age	CV	Percent
0	0.14	6.52
1	0.06	30.13
2	0.06	29.41
3	0.14	8.94
4	0.12	7.79
5	0.19	4.47
6	0.19	4.29
7	0.2	3.86
8	>0.25	2.6
9	>0.25	1.21
10	>0.25	0.6
11	>0.25	0.12
12	>0.25	0.06

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Table 3.3: The number of Bluefish assigned to each total length (cm)-at-age category for 286 fish sampled for otolith age determination in Virginia during 2023.

	Age								
Interval	0	1	2	3	4	5	6	12	Totals
20 - 20.99	1	0	0	0	0	0	0	0	1
24 - 24.99	0	1	0	0	0	0	0	0	1
25 - 25.99	1	1	1	0	0	0	0	0	3
26 - 26.99	0	2	1	0	0	0	0	0	3
27 - 27.99	0	6	1	0	0	0	0	0	7
28 - 28.99	0	5	1	0	0	0	0	0	6
29 - 29.99	1	5	0	0	0	0	0	0	6
30 - 30.99	4	4	0	0	0	0	0	0	8
31 - 31.99	0	8	0	0	0	0	0	0	8
32 - 32.99	0	6	0	0	0	0	0	0	6
33 - 33.99	0	6	0	0	0	0	0	0	6
34 - 34.99	0	6	0	0	0	0	0	0	6
35 - 35.99	0	4	2	0	0	0	0	0	6
36 - 36.99	0	5	1	2	0	0	0	0	8
37 - 37.99	0	7	1	0	0	0	0	0	8
38 - 38.99	1	4	1	1	0	0	0	0	7
39 - 39.99	0	4	4	0	0	0	0	0	8
40 - 40.99	0	3	4	1	0	0	0	0	8
41 - 41.99	0	1	5	2	0	0	0	0	8
42 - 42.99	0	2	5	0	1	0	0	0	8
43 - 43.99	0	1	6	1	0	0	0	0	8
44 - 44.99	0	1	3	2	0	0	0	0	6
45 - 45.99	0	2	6	2	0	0	0	0	10
46 - 46.99	0	1	6	1	0	0	0	0	8
47 - 47.99	0	1	6	1	0	0	0	0	8
48 - 48.99	0	0	6	0	0	0	0	0	6
49 - 49.99	0	0	6	0	0	0	0	0	6
50 - 50.99	0	1	5	0	0	0	0	0	6
51 - 51.99	0	0	6	0	0	0	0	0	6
52 - 52.99	0	2	3	0	1	0	0	0	6
53 - 53.99	0	2	3	1	0	0	0	0	6
54 - 54.99	0	2	2	1	0	0	0	0	5
55 - 55.99	0	1	4	1	0	0	0	0	6
56 - 56.99	0	0	3	1	0	0	0	0	4
57 - 57.99	0	0	4	1	1	0	0	0	6
58 - 58.99	0	0	5	1	0	0	0	0	6
59 - 59.99	0	0	5	0	0	0	0	0	5
60 - 60.99	0	0	4	1	0	0	0	0	5
61 - 61.99	0	0	3	1	0	0	0	0	4
62 - 62.99	0	0	2	2	0	0	0	0	4
63 - 63.99	0	0	2	3	0	0	0	0	5
64 - 64.99	0	0	2	5	0	0	0	0	7

(To continue)

Table 3.3 (Continued)

	Age								
Interval	0	1	2	3	4	5	6	12	Totals
65 - 65.99	0	0	0	1	0	0	0	0	1
66 - 66.99	0	0	0	0	1	0	0	0	1
67 - 67.99	0	0	1	1	0	0	0	0	2
68 - 68.99	0	0	0	1	1	0	0	0	2
69 - 69.99	0	0	0	0	1	0	0	0	1
71 - 71.99	0	0	1	0	0	0	0	0	1
73 - 73.99	0	0	0	1	0	0	0	0	1
74 - 74.99	0	0	0	0	0	1	0	0	1
75 - 75.99	0	0	0	1	1	0	0	0	2
76 - 76.99	0	0	1	0	0	1	2	0	4
77 - 77.99	0	0	1	1	0	2	0	0	4
78 - 78.99	0	0	0	1	0	0	0	0	1
79 - 79.99	0	0	0	1	0	1	1	0	3
80 - 80.99	0	0	0	0	0	0	1	0	1
81 - 81.99	0	0	0	0	1	0	2	0	3
82 - 82.99	0	0	0	0	0	0	1	0	1
84 - 84.99	0	0	0	0	0	0	1	0	1
88 - 88.99	0	0	0	0	0	0	0	1	1
Totals	8	94	123	39	8	5	8	1	286

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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 2023.

	Age							
Interval	0	1	2	3	4	5	6	12
20 - 20.99	1	0	0	0	0	0	0	0
24 - 24.99	0	1	0	0	0	0	0	0
25 - 25.99	0.33	0.33	0.33	0	0	0	0	0
26 - 26.99	0	0.67	0.33	0	0	0	0	0
27 - 27.99	0	0.86	0.14	0	0	0	0	0
28 - 28.99	0	0.83	0.17	0	0	0	0	0
29 - 29.99	0.17	0.83	0	0	0	0	0	0
30 - 30.99	0.5	0.5	0	0	0	0	0	0
31 - 31.99	0	1	0	0	0	0	0	0
32 - 32.99	0	1	0	0	0	0	0	0
33 - 33.99	0	1	0	0	0	0	0	0
34 - 34.99	0	1	0	0	0	0	0	0
35 - 35.99	0	0.67	0.33	0	0	0	0	0
36 - 36.99	0	0.62	0.12	0.25	0	0	0	0
37 - 37.99	0	0.88	0.12	0	0	0	0	0
38 - 38.99	0.14	0.57	0.14	0.14	0	0	0	0
39 - 39.99	0	0.5	0.5	0	0	0	0	0
40 - 40.99	0	0.38	0.5	0.12	0	0	0	0
41 - 41.99	0	0.12	0.62	0.25	0	0	0	0
42 - 42.99	0	0.25	0.62	0	0.12	0	0	0
43 - 43.99	0	0.12	0.75	0.12	0	0	0	0
44 - 44.99	0	0.17	0.5	0.33	0	0	0	0
45 - 45.99	0	0.2	0.6	0.2	0	0	0	0
46 - 46.99	0	0.12	0.75	0.12	0	0	0	0
47 - 47.99	0	0.12	0.75	0.12	0	0	0	0
48 - 48.99	0	0	1	0	0	0	0	0
49 - 49.99	0	0	1	0	0	0	0	0
50 - 50.99	0	0.17	0.83	0	0	0	0	0
51 - 51.99	0	0	1	0	0	0	0	0
52 - 52.99	0	0.33	0.5	0	0.17	0	0	0
53 - 53.99	0	0.33	0.5	0.17	0	0	0	0
54 - 54.99	0	0.4	0.4	0.2	0	0	0	0
55 - 55.99	0	0.17	0.67	0.17	0	0	0	0
56 - 56.99	0	0	0.75	0.25	0	0	0	0
57 - 57.99	0	0	0.67	0.17	0.17	0	0	0
58 - 58.99	0	0	0.83	0.17	0	0	0	0
59 - 59.99	0	0	1	0	0	0	0	0
60 - 60.99	0	0	0.8	0.2	0	0	0	0
61 - 61.99	0	0	0.75	0.25	0	0	0	0
62 - 62.99	0	0	0.5	0.5	0	0	0	0
63 - 63.99	0	0	0.4	0.6	0	0	0	0
64 - 64.99	0	0	0.29	0.71	0	0	0	0

(To continue)

Table 3.4 (Continued)

	Age							
Interval	0	1	2	3	4	5	6	12
65 - 65.99	0	0	0	1	0	0	0	0
66 - 66.99	0	0	0	0	1	0	0	0
67 - 67.99	0	0	0.5	0.5	0	0	0	0
68 - 68.99	0	0	0	0.5	0.5	0	0	0
69 - 69.99	0	0	0	0	1	0	0	0
71 - 71.99	0	0	1	0	0	0	0	0
73 - 73.99	0	0	0	1	0	0	0	0
74 - 74.99	0	0	0	0	0	1	0	0
75 - 75.99	0	0	0	0.5	0.5	0	0	0
76 - 76.99	0	0	0.25	0	0	0.25	0.5	0
77 - 77.99	0	0	0.25	0.25	0	0.5	0	0
78 - 78.99	0	0	0	1	0	0	0	0
79 - 79.99	0	0	0	0.33	0	0.33	0.33	0
80 - 80.99	0	0	0	0	0	0	1	0
81 - 81.99	0	0	0	0	0.33	0	0.67	0
82 - 82.99	0	0	0	0	0	0	1	0
84 - 84.99	0	0	0	0	0	0	1	0
88 - 88.99	0	0	0	0	0	0	0	1

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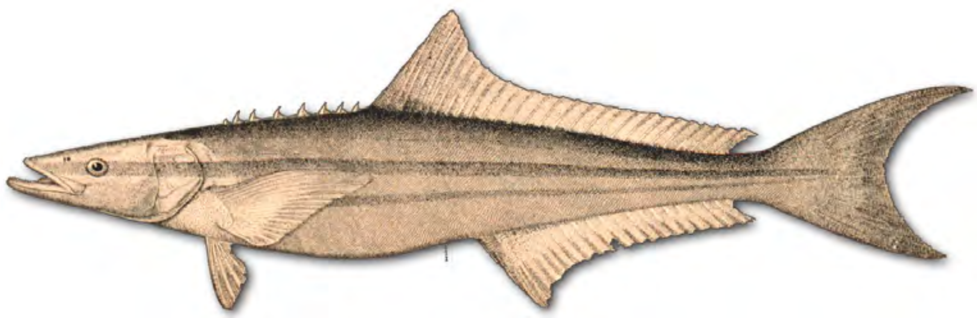
Chapter 4

COBIA *Rachycentron canadum*



4.1 INTRODUCTION

We aged a total of 311 Cobia *Rachycentron canadum*, collected by the VMRC's Biological Sampling Program for age and growth analysis in 2023. Cobia ages ranged from 2 to 13 years old with an average age of 5.5, a standard deviation of 1.9, and a standard error of 0.11. Ten age classes (2 to 9, 11, and 13) were represented, comprising fish of the 2010, 2012, and 2014 to 2021 year-classes. The sample was dominated by fish from the year-classes of 2016, 2018, 2019, and 2020 with 23.1%, 26.4%, 17%, and 15.1%, 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™ 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 VMRC Ageing Lab website on how to prepare otolith thin-section for ageing Cobia using the Epoxy Resin Method.

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 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, 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.

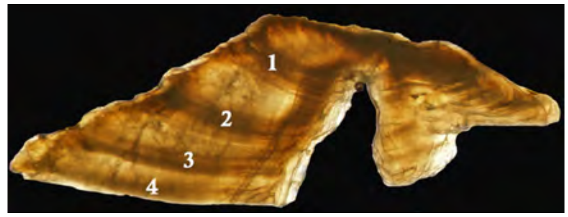


Figure 4.1: Otolith thin-section of a 4 year-old Cobia.

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 Year Class

We aged the otoliths of all the 311 Cobia collected in 2023. Of the 311 fish aged, 10 age classes (2 to 9, 11, and 13) were represented (Table 4.1). The average age was 5.5 years, and the standard deviation and standard error were 1.9 and 0.11, respectively. Year-class data show that the fishery was comprised of 10 year-classes: fish from the 2010, 2012, and 2014 to 2021 year-classes, with fish primarily from the year classes of 2016, 2018, 2019, and 2020 with 23.1%, 26.4%, 17%, and 15.1%, respectively. The ratio of males to females was 1:1.86 in the sample collected (Figure 4.2).

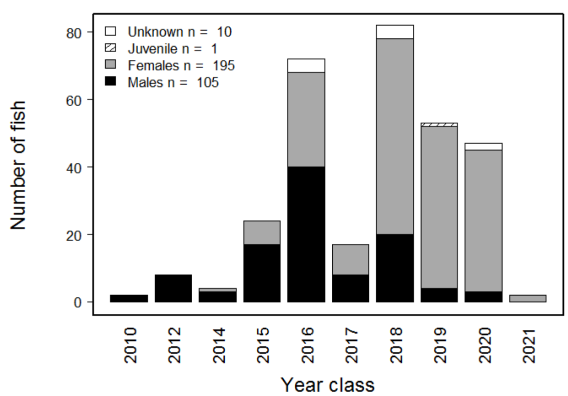


Figure 4.2: Year-class frequency distribution for Cobia collected for ageing in 2023. Distribution is broken down by sex. 'Unknown' represents gonads that were not available for examination or were not examined for sex during sampling.

4.3.2 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.

4.3.3 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 96% and a CV of 0.72% (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 88% and a CV of 2.17% (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 91.96% and a CV of 1.29% (test of symmetry: $\chi^2 = 11.83$, $df = 8$, $P = 0.1588$) (Figure 4.3).

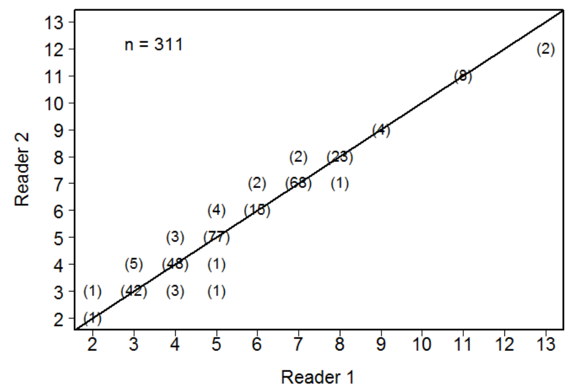


Figure 4.3: Between-reader comparison of otolith age estimates for Cobia collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2023. The number in parentheses is number of fish.

There was no time-series bias for either reader. Reader 1 had an agreement of 82% with ages of fish aged in 2000 with a CV of 1.69% (test of symmetry: $\chi^2 = 6.33$, $df = 7$, $P = 0.5014$). Reader 2 had an agreement of 74% with a CV of 3.48% (test of symmetry: $\chi^2 = 8.33$, $df = 7$, $P = 0.3041$).

Table 4.1: The number of Cobia assigned to each total length (inch)-at-age category for 311 fish sampled for otolith age determination in Virginia during 2023.

	Age										
Interval	2	3	4	5	6	7	8	9	11	13	Totals
34 - 34.99	1	0	0	0	0	0	0	0	0	0	1
35 - 35.99	0	1	1	0	0	0	0	0	0	0	2
37 - 37.99	0	8	0	0	0	0	0	0	0	0	8
38 - 38.99	0	7	1	3	1	1	0	1	0	0	14
39 - 39.99	0	10	4	8	0	4	0	0	0	0	26
40 - 40.99	1	10	8	6	1	5	1	0	0	0	32
41 - 41.99	0	8	7	3	2	7	3	0	0	0	30
42 - 42.99	0	1	8	5	3	14	2	1	0	0	34
43 - 43.99	0	0	8	6	1	4	0	1	0	0	20
44 - 44.99	0	2	5	7	1	5	7	0	0	0	27
45 - 45.99	0	0	8	13	1	1	3	0	1	1	28
46 - 46.99	0	0	1	11	1	1	0	0	5	0	19
47 - 47.99	0	0	1	7	1	1	2	0	0	0	12
48 - 48.99	0	0	1	7	2	8	0	0	1	0	19
49 - 49.99	0	0	0	2	0	3	0	0	1	1	7
50 - 50.99	0	0	0	2	1	6	2	1	0	0	12
51 - 51.99	0	0	0	1	0	3	0	0	0	0	4
52 - 52.99	0	0	0	0	0	2	1	0	0	0	3
53 - 53.99	0	0	0	1	0	2	0	0	0	0	3
54 - 54.99	0	0	0	0	0	3	1	0	0	0	4
55 - 55.99	0	0	0	0	0	2	0	0	0	0	2
56 - 56.99	0	0	0	0	2	0	0	0	0	0	2
57 - 57.99	0	0	0	0	0	0	1	0	0	0	1
62 - 62.99	0	0	0	0	0	0	1	0	0	0	1
Totals	2	47	53	82	17	72	24	4	8	2	311

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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 2023.

	Age									
Interval	2	3	4	5	6	7	8	9	11	13
34 - 34.99	1	0	0	0	0	0	0	0	0	0
35 - 35.99	0	0.5	0.5	0	0	0	0	0	0	0
37 - 37.99	0	1	0	0	0	0	0	0	0	0
38 - 38.99	0	0.5	0.07	0.21	0.07	0.07	0	0.07	0	0
39 - 39.99	0	0.38	0.15	0.31	0	0.15	0	0	0	0
40 - 40.99	0.03	0.31	0.25	0.19	0.03	0.16	0.03	0	0	0
41 - 41.99	0	0.27	0.23	0.1	0.07	0.23	0.1	0	0	0
42 - 42.99	0	0.03	0.24	0.15	0.09	0.41	0.06	0.03	0	0
43 - 43.99	0	0	0.4	0.3	0.05	0.2	0	0.05	0	0
44 - 44.99	0	0.07	0.19	0.26	0.04	0.19	0.26	0	0	0
45 - 45.99	0	0	0.29	0.46	0.04	0.04	0.11	0	0.04	0.04
46 - 46.99	0	0	0.05	0.58	0.05	0.05	0	0	0.26	0
47 - 47.99	0	0	0.08	0.58	0.08	0.08	0.17	0	0	0
48 - 48.99	0	0	0.05	0.37	0.11	0.42	0	0	0.05	0
49 - 49.99	0	0	0	0.29	0	0.43	0	0	0.14	0.14
50 - 50.99	0	0	0	0.17	0.08	0.5	0.17	0.08	0	0
51 - 51.99	0	0	0	0.25	0	0.75	0	0	0	0
52 - 52.99	0	0	0	0	0	0.67	0.33	0	0	0
53 - 53.99	0	0	0	0.33	0	0.67	0	0	0	0
54 - 54.99	0	0	0	0	0	0.75	0.25	0	0	0
55 - 55.99	0	0	0	0	0	1	0	0	0	0
56 - 56.99	0	0	0	0	1	0	0	0	0	0
57 - 57.99	0	0	0	0	0	0	1	0	0	0
62 - 62.99	0	0	0	0	0	0	1	0	0	0

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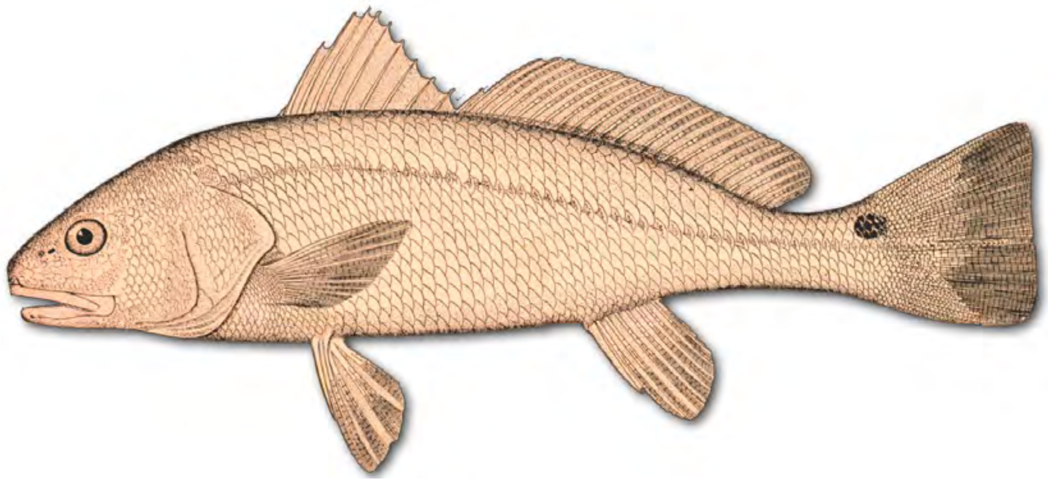
Chapter 5

RED DRUM *Sciaenops ocellatus*



5.1 INTRODUCTION

We aged a total of 65 Red Drum *Sciaenops ocellatus*, collected by the VMRC's Biological Sampling Program for age and growth analysis in 2023. Red drum ages ranged from 0 to 2 years old with an average age of 1, a standard deviation of 0.4, and a standard error of 0.05. Three age classes (0 to 2) were represented, comprising fish of the 2021 to 2023 year-classes. The sample was dominated by fish from the year-class of 2022 with 83.1%.

5.2 METHODS

5.2.1 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.

5.2.2 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 VMRC Ageing Lab website on how to prepare otolith thin-section for ageing Red Drum using the Glue Method.

5.2.3 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 5.1). 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 2023.

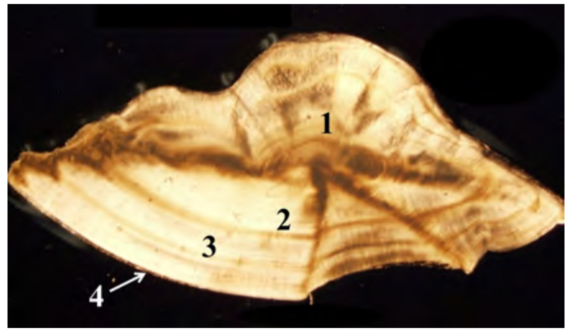


Figure 5.1: Otolith thin-section of a 3 year-old Red Drum with the last annulus on the edge of the thin-section

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 (Figure 4.1).

5.2.4 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).

5.3 RESULTS

5.3.1 Year Class

We aged the otoliths of all the 65 Red Drum collected in 2023. Of the 65 fish aged, 3 age classes (0 to 2) were represented (Table 5.1). The average age was 1 year, and the standard deviation and standard error were 0.4 and 0.05, respectively. Year-class data show that the fishery was comprised of 3 year-classes: fish from the 2021 to 2023 year-classes, with fish primarily from the year class of 2022 with 83.1%. The ratio of males to females was 1:0.34 in the sample collected (Figure 5.2).

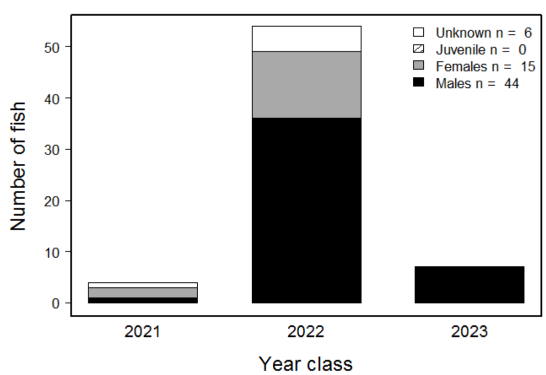


Figure 5.2: Year-class frequency distribution for Red Drum collected for ageing in 2023. Distribution is broken down by sex. 'Unknown' represents gonads that were not available for examination or were not examined for sex during sampling.

5.3.2 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 otolith ages. The table is based on VMRC's stratified sampling of landings by total length inch intervals.

5.3.3 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 0.94% (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 100%. There was no evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 100% (Figure 5.3).

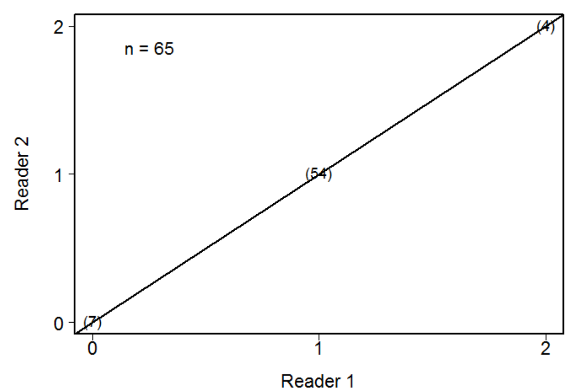


Figure 5.3: Between-reader comparison of otolith age estimates for Red Drum collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2023. The number in parentheses is number of fish.

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% .

Table 5.1: The number of Red Drum assigned to each total length (inch)-at-age category for 65 fish sampled for otolith age determination in Virginia during 2023.

	Age			
Interval	0	1	2	Totals
16 - 16.99	0	1	0	1
17 - 17.99	0	2	0	2
18 - 18.99	4	7	0	11
19 - 19.99	0	8	0	8
20 - 20.99	1	7	0	8
21 - 21.99	0	7	0	7
22 - 22.99	1	1	0	2
23 - 23.99	1	7	0	8
24 - 24.99	0	9	3	12
25 - 25.99	0	3	1	4
26 - 26.99	0	2	0	2
Totals	7	54	4	65

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Table 5.2: 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 2023.

	Age		
Interval	0	1	2
16 - 16.99	0	1	0
17 - 17.99	0	1	0
18 - 18.99	0.36	0.64	0
19 - 19.99	0	1	0
20 - 20.99	0.12	0.88	0
21 - 21.99	0	1	0
22 - 22.99	0.5	0.5	0
23 - 23.99	0.12	0.88	0
24 - 24.99	0	0.75	0.25
25 - 25.99	0	0.75	0.25
26 - 26.99	0	1	0

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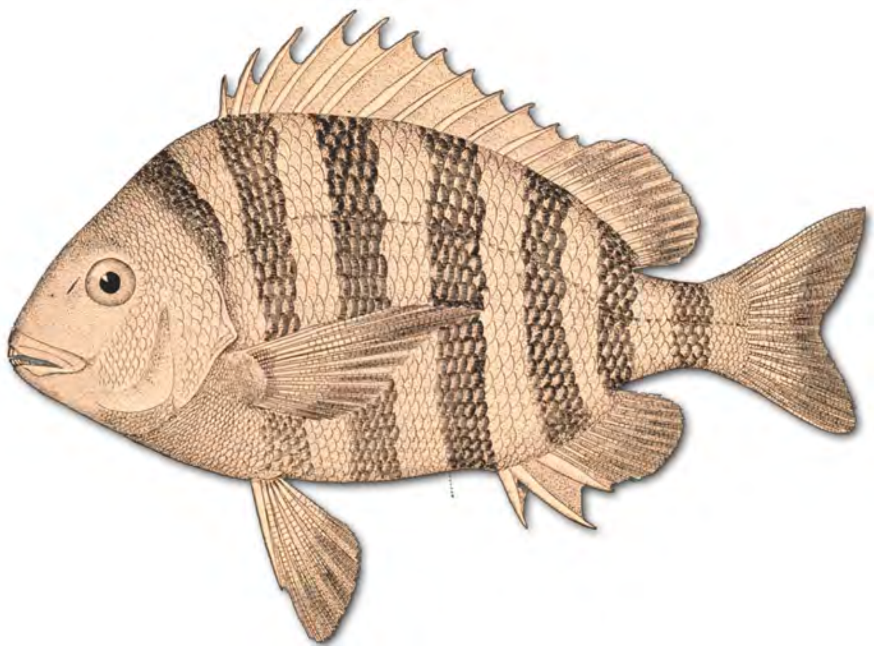
Chapter 6

SHEEPSHEAD *Archosargus probatocephalus*



6.1 INTRODUCTION

We aged a total of 333 Sheepshead *Archosargus probatocephalus*, collected by the VMRC's Biological Sampling Program for age and growth analysis in 2023. Sheepshead ages ranged from 1 to 31 years old with an average age of 10.1, a standard deviation of 7, and a standard error of 0.38. Twenty-six age classes (1 to 13, 15 to 20, 22, 24, 26 to 27, and 29 to 31) were represented, comprising fish of the 1992 to 1994, 1996 to 1997, 1999, 2001, 2003 to 2008, and 2010 to 2022 year-classes. The sample was dominated by fish from the year-classes of 2007, 2011, 2012, 2015, 2016, 2018, 2019, and 2021 with 6%, 14.1%, 3.9%, 9.6%, 11.4%, 4.8%, 14.1%, and 6.3%, 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 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™ 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 VMRC Ageing Lab website on how to prepare otolith thin-section for ageing Sheepshead using the Epoxy Resin Method.

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 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 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.

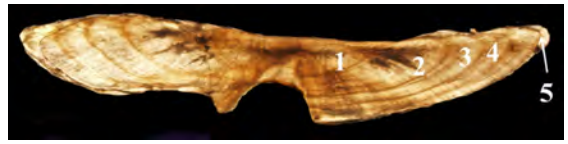


Figure 6.1: Otolith thin-section of a 5 year-old Sheepshead

All samples were aged by Reader 1 in chronological order, based on collection date, without knowledge of previously estimated ages or the specimen lengths. Reader 2 had no enough training on ageing Sheepshead otoliths, therefore, the age estimates from Reader 1 were used as the final ages. Reader 2 will have enough training during 2024 and will be ready to age Sheepshead otoliths collected in 2024. 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.

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) within Reader 1 in the current year and 2) time-series bias between the current and previous years within Reader 1. A random sub-sample of 50 fish from the current year was selected for second readings to examine the difference within Reader 1. Fifty otoliths randomly selected from fish aged in 2008 were used to examine the time-series bias within Reader 1. 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 Year Class

Reader 1 aged the otoliths of all the 333 Sheepshead collected in 2023. Of the 333 fish aged, 26 age classes (1 to 13, 15 to 20, 22, 24, 26 to 27, and 29 to 31) were represented (Table 6.1). The average age was 10.1 years, and the standard deviation and standard error were 7 and 0.38, respectively. Year-class data show that the fishery was comprised of 26 year-classes: fish from the 1992 to 1994, 1996 to 1997, 1999, 2001, 2003 to 2008, and 2010 to 2022 year-classes, with fish primarily from the year classes of 2007, 2011, 2012, 2015, 2016, 2018, 2019, and 2021 with 6%, 14.1%, 3.9%, 9.6%, 11.4%, 4.8%, 14.1%, and 6.3%, respectively. The ratio of males to females was 1:1.29 in the sample collected (Figure 6.2).

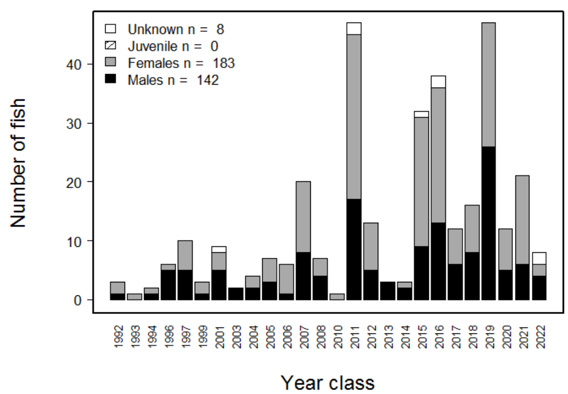


Figure 6.2: Year-class frequency distribution for Sheepshead collected for ageing in 2023. Distribution is broken down by sex. 'Unknown' represents gonads that were not available for examination or were not examined for sex during sampling.

6.3.2 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.

6.3.3 Reading Precision

There was no significant difference between the first and second readings for Reader 1 with an agreement of 86% and a *CV* of 1.62% (test of symmetry: $\chi^2 = 5$, $df = 6$, $P = 0.5438$).

Reader 1 had an agreement of 96% with ages of fish aged in 2008 and a *CV* of 0.3% (test of symmetry: $\chi^2 = 2$, $df = 2$, $P = 0.3679$).

Table 6.1: The number of sheepshead assigned to each total length (inch)-at-age category for 333 fish sampled for otolith age determination in Virginia during 2023.

	Age																										
Interval	1	2	3	4	5	6	7	8	9	10	11	12	13	15	16	17	18	19	20	22	24	26	27	29	30	31	Totals
8 - 8.99	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	0	2
9 - 9.99	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	1
10 - 10.99	3	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	4
11 - 11.99	2	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5
12 - 12.99	0	13	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	15
13 - 13.99	0	2	2	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8
14 - 14.99	0	2	5	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11
15 - 15.99	0	0	3	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11
16 - 16.99	0	0	0	13	2	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	19
17 - 17.99	0	0	0	11	4	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	18
18 - 18.99	0	0	0	5	5	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12
19 - 19.99	0	0	0	2	4	0	5	3	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	15
20 - 20.99	0	0	0	0	1	4	12	4	0	0	1	2	0	0	0	0	0	0	1	0	0	0	0	0	0	0	25
21 - 21.99	0	0	0	0	0	3	7	13	1	2	5	16	1	1	1	0	4	0	1	1	0	2	0	1	0	0	59
22 - 22.99	0	0	0	0	0	1	10	10	1	1	6	18	0	2	5	3	0	3	0	4	1	4	2	0	0	1	72
23 - 23.99	0	0	0	0	0	0	0	1	1	0	1	9	0	4	10	3	1	1	0	3	0	3	3	0	0	2	42
24 - 24.99	0	0	0	0	0	0	0	0	0	0	0	1	0	0	4	0	1	0	0	1	2	0	1	1	1	0	12
25 - 25.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	2
Totals	8	21	12	47	16	12	38	32	3	3	13	47	1	7	20	6	7	4	2	9	3	10	6	2	1	3	333

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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 Sheepshead sampled for age determination in Virginia during 2023.

	Age																									
Interval	1	2	3	4	5	6	7	8	9	10	11	12	13	15	16	17	18	19	20	22	24	26	27	29	30	31
8 - 8.99	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
9 - 9.99	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
10 - 10.99	0.75	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
11 - 11.99	0.4	0.6	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	0.87	0.13	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	0.25	0.25	0.5	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	0.18	0.45	0.36	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	0	0.27	0.73	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16 - 16.99	0	0	0	0.68	0.11	0.05	0.16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17 - 17.99	0	0	0	0.61	0.22	0.11	0.06	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	0.42	0.42	0.08	0	0.08	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19 - 19.99	0	0	0	0.13	0.27	0	0.33	0.2	0	0	0	0.07	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20 - 20.99	0	0	0	0	0.04	0.16	0.48	0.16	0	0	0.04	0.08	0	0	0	0	0	0	0.04	0	0	0	0	0	0	0
21 - 21.99	0	0	0	0	0	0.05	0.12	0.22	0.02	0.03	0.08	0.27	0.02	0.02	0.02	0	0.07	0	0.02	0.02	0	0.03	0	0.02	0	0
22 - 22.99	0	0	0	0	0	0.01	0.14	0.14	0.01	0.01	0.08	0.25	0	0.03	0.07	0.04	0	0.04	0	0.06	0.01	0.06	0.03	0	0	0.01
23 - 23.99	0	0	0	0	0	0	0	0.02	0.02	0	0.02	0.21	0	0.1	0.24	0.07	0.02	0.02	0	0.07	0	0.07	0.07	0	0	0.05
24 - 24.99	0	0	0	0	0	0	0	0	0	0	0	0.08	0	0	0.33	0	0.08	0	0	0.08	0.17	0	0.08	0.08	0.08	0
25 - 25.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.5	0	0	0	0	0.5	0	0	0	0

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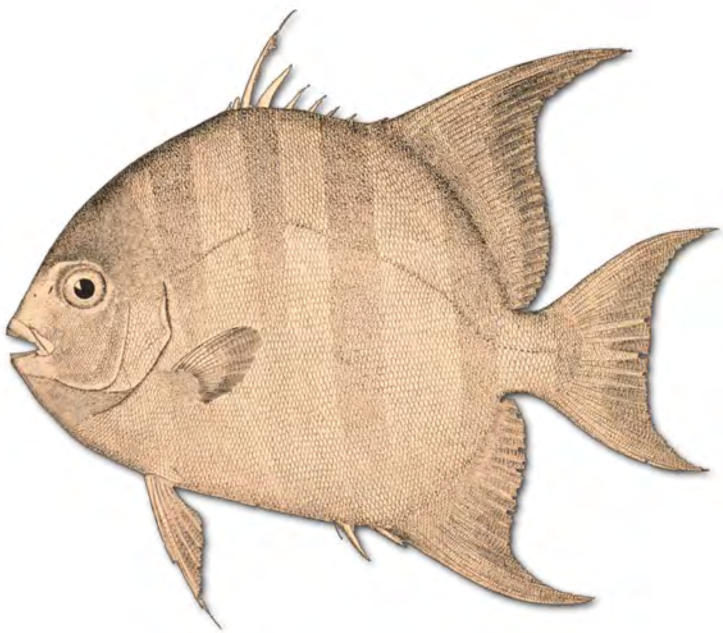
Chapter 7

ATLANTIC SPADEFISH
Chaetodipterus faber



7.1 INTRODUCTION

We aged a total of 220 Spadefish *Chaetodipterus faber*, collected by the VMRC's Biological Sampling Program for age and growth analysis in 2023. Spadefish ages ranged from 1 to 8 years old with an average age of 2.7, a standard deviation of 1.5, and a standard error of 0.1. Eight age classes (1 to 8) were represented, comprising fish of the 2015 to 2022 year-classes. The sample was dominated by fish from the year-classes of 2020, 2021, and 2022 with 27.7%, 32.3%, and 21.4%, respectively.

7.2 METHODS

7.2.1 Sample Size for Ageing

We estimated sample size for ageing Spadefish in 2023 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:

$$A = \frac{V_a}{\theta_a^2 CV_a^2 + B_a/L} \tag{7.1}$$

where A is the sample size for ageing Spadefish in 2023; 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 2017 to 2021. θ_a, V_a, and B_a were calculated using pooled age-length data of Spadefish collected from 2017 to 2021 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 2017 to 2021 catch. A_l is number of fish to be aged for length interval l in 2023.

7.2.2 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 inside of protective Axygen 2 ml micro-tubes within their original labeled coin envelopes.

7.2.3 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™ 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 VMRC Ageing Lab website on how to prepare otolith thin-section for ageing Spadefish using the Epoxy Resin Method.

7.2.4 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 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.

All samples were aged by Reader 1 in chrono-

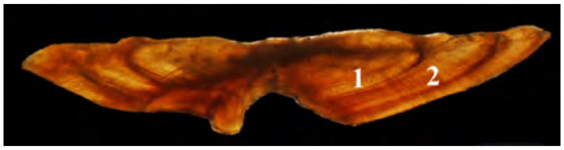


Figure 7.1: Otolith thin-section of a 2 year-old Spadefish

logical order, based on collection date, without knowledge of previously estimated ages or the specimen lengths. Reader 2 had no enough training on ageing Spadefish otoliths, therefore, the age estimates from Reader 1 were used as the final ages. Reader 2 will have enough training during 2024 and will be ready to age Spadefish otoliths collected in 2024. 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.

7.2.5 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) within Reader 1 in the current year and 2) time-series bias between the current and previous years within Reader 1. A random sub-sample of 50 fish from the current year was selected for second readings to examine the difference within Reader 1. Fifty otoliths randomly selected from fish aged in 2003 were used to examine the time-series bias within Reader 1. 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 Sample Size

We estimated a sample size of 294 Spadefish in 2023, ranging in length interval from 3 to 21 inches (Table 7.1). This sample size provided a range in CV for age composition approximately from the smallest CV of 7% for Age 2 to the CV of larger than 25% for the multiple minor ages (Table 7.2). In 2023, we aged 220 of 249 Spadefish (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 87 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.

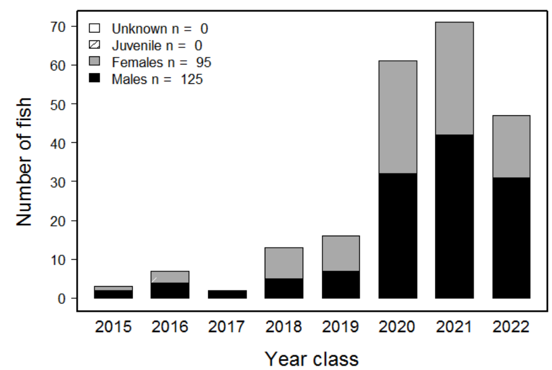


Figure 7.2: Year-class frequency distribution for Spadefish collected for ageing in 2023. Distribution is broken down by sex. 'Unknown' represents gonads that were not available for examination or were not examined for sex during sampling.

7.3.2 Year Class

Of the 220 fish aged with otoliths, 8 age classes (1 to 8) were represented (Table 7.3). The average age was 2.7 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 2015 to 2022 year-classes, with fish primarily from the year classes of 2020, 2021, and 2022 with 27.7%, 32.3%, and 21.4%, respectively. The ratio of males to females was 1:0.76 in the sample collected (Figure 7.2).

7.3.3 Age-length Key (ALK)

We developed an age-length-key (Table 7.4) 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.

7.3.4 Reading Precision

There was no significant difference between the first and second readings for Reader 1 with an agreement of 92% and a *CV* of 3.09% (test of symmetry: $\chi^2 = 1.33$, $df = 2$, $P = 0.5134$).

Reader 1 had an agreement of 80% with ages of fish aged in 2003 with a *CV* of 2.67% (test of symmetry: $\chi^2 = 10$, $df = 8$, $P = 0.265$).

Table 7.1: Number of Atlantic Spadefish collected and aged in each 1-inch length interval in 2023. 'Target' represents the sample size for ageing estimated for 2023, 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	1	1	6
5 - 5.99	14	20	20	0
6 - 6.99	34	48	34	0
7 - 7.99	37	51	39	0
8 - 8.99	26	29	26	0
9 - 9.99	17	17	17	0
10 - 10.99	14	17	17	0
11 - 11.99	13	15	15	0
12 - 12.99	21	13	13	8
13 - 13.99	18	5	5	13
14 - 14.99	15	5	5	10
15 - 15.99	13	4	4	9
16 - 16.99	12	9	9	3
17 - 17.99	19	4	4	15
18 - 18.99	12	4	4	8
19 - 19.99	7	4	4	3
20 - 20.99	5	1	1	4
21 - 21.99	5	2	2	3
Totals	294	249	220	87

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Table 7.2: CV for each age estimated based on ageing the total of 294 Spadefish in 2023. 'Percent' is the percentage of an age in the pooled age-length data of Spadefish collected from 2017 to 2021.

Age	CV	Percent
0	>0.25	3.05
1	0.18	7.56
2	0.07	29.38
3	0.1	23.49
4	0.13	15.42
5	0.14	12.8
6	0.22	5.67
7	>0.25	1.6
8	>0.25	0.73
9	>0.25	0.22
10	>0.25	0.07

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Table 7.3: The number of Atlantic Spadefish assigned to each total length-at-age category for 220 fish sampled for otolith age determination in Virginia during 2023.

	Age								
Interval	1	2	3	4	5	6	7	8	Totals
4 - 4.99	1	0	0	0	0	0	0	0	1
5 - 5.99	19	1	0	0	0	0	0	0	20
6 - 6.99	18	15	1	0	0	0	0	0	34
7 - 7.99	7	23	9	0	0	0	0	0	39
8 - 8.99	2	14	10	0	0	0	0	0	26
9 - 9.99	0	9	8	0	0	0	0	0	17
10 - 10.99	0	7	7	2	1	0	0	0	17
11 - 11.99	0	1	12	2	0	0	0	0	15
12 - 12.99	0	1	8	4	0	0	0	0	13
13 - 13.99	0	0	3	1	1	0	0	0	5
14 - 14.99	0	0	3	2	0	0	0	0	5
15 - 15.99	0	0	0	2	2	0	0	0	4
16 - 16.99	0	0	0	2	4	0	3	0	9
17 - 17.99	0	0	0	0	3	0	1	0	4
18 - 18.99	0	0	0	0	2	2	0	0	4
19 - 19.99	0	0	0	1	0	0	3	0	4
20 - 20.99	0	0	0	0	0	0	0	1	1
21 - 21.99	0	0	0	0	0	0	0	2	2
Totals	47	71	61	16	13	2	7	3	220

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Table 7.4: 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 2023.

	Age							
Interval	1	2	3	4	5	6	7	8
4 - 4.99	1	0	0	0	0	0	0	0
5 - 5.99	0.95	0.05	0	0	0	0	0	0
6 - 6.99	0.53	0.44	0.03	0	0	0	0	0
7 - 7.99	0.18	0.59	0.23	0	0	0	0	0
8 - 8.99	0.08	0.54	0.38	0	0	0	0	0
9 - 9.99	0	0.53	0.47	0	0	0	0	0
10 - 10.99	0	0.41	0.41	0.12	0.06	0	0	0
11 - 11.99	0	0.07	0.8	0.13	0	0	0	0
12 - 12.99	0	0.08	0.62	0.31	0	0	0	0
13 - 13.99	0	0	0.6	0.2	0.2	0	0	0
14 - 14.99	0	0	0.6	0.4	0	0	0	0
15 - 15.99	0	0	0	0.5	0.5	0	0	0
16 - 16.99	0	0	0	0.22	0.44	0	0.33	0
17 - 17.99	0	0	0	0	0.75	0	0.25	0
18 - 18.99	0	0	0	0	0.5	0.5	0	0
19 - 19.99	0	0	0	0.25	0	0	0.75	0
20 - 20.99	0	0	0	0	0	0	0	1
21 - 21.99	0	0	0	0	0	0	0	1

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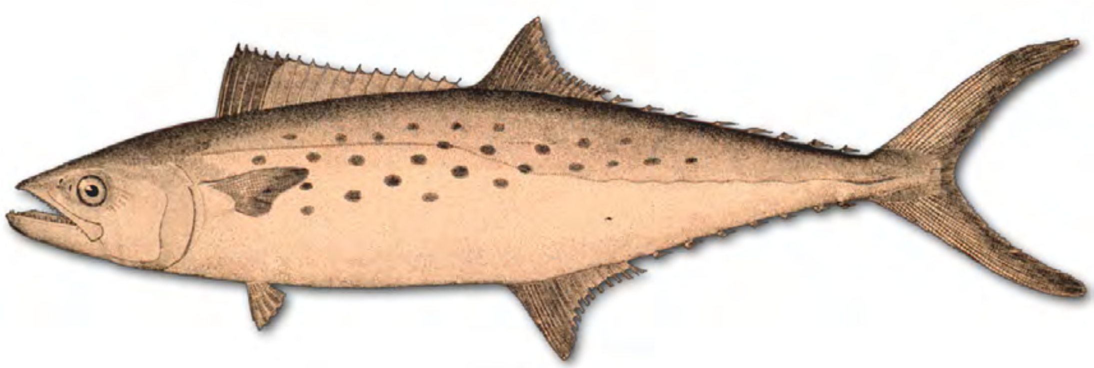
Chapter 8

SPANISH MACKEREL
Scomberomorous maculatus



8.1 INTRODUCTION

We aged a total of 280 Spanish Mackerel *Scomberomorous maculatus*, collected by the VMRC's Biological Sampling Program for age and growth analysis in 2023. Spanish Mackerel ages ranged from 0 to 8 years old with an average age of 1.9, a standard deviation of 1.3, and a standard error of 0.08. Eight age classes (0 to 6, and 8) were represented, comprising fish of the 2015, and 2017 to 2023 year-classes. The sample was dominated by fish from the year-classes of 2021 and 2022 with 21.8% and 53.2%, respectively.

8.2 METHODS

8.2.1 Sample Size for Ageing

We estimated sample size for ageing Spanish Mackerel in 2023 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 Spanish Mackerel in 2023; 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 2017 to 2021. θ_a, V_a, and B_a were calculated using pooled age-length data of Spanish Mackerel collected from 2017 to 2021 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 2017 to 2021 catch. A_l is number of fish to be aged for length interval l in 2023.

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 inside of protective Axygen 2 ml micro-tubes within their original labeled coin envelopes.

8.2.3 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 VMRC Ageing Lab website on how to prepare otolith thin-section for ageing Spanish Mackerel using the Epoxy Resin Method.

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 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 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.

Figure 8.1: 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 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 296 Spanish Mackerel in 2023, ranging in length interval from 12 to 32 inches (Table 8.1). 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 8.2). In 2023, we randomly selected and aged 280 fish from 356 Spanish Mackerel collected by VMRC. We fell short in our over-all collections for this optimal length-class sampling estimate by 39 fish. We were short of 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.

8.3.2 Year Class

Of the 280 fish aged with otoliths, 8 age classes (0 to 6, and 8) were represented (Table 8.3). The average age was 1.9 years, and the standard deviation and standard error were 1.3 and 0.08, respectively. Year-class data show that the fishery was comprised of 8 year-classes: fish from the 2015, and 2017 to 2023 year-classes, with fish primarily from the year classes of 2021 and 2022 with 21.8% and 53.2%, respectively. The ratio of males to females was 1:3.59 in the sample collected (Figure 8.2).

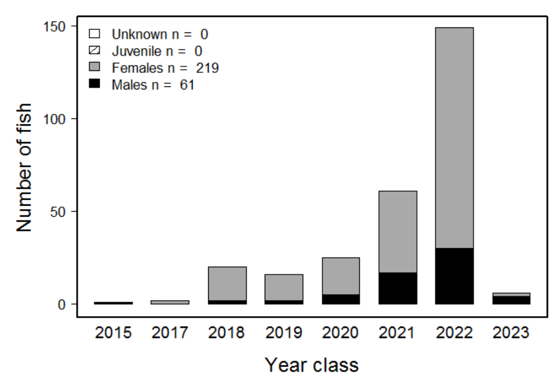


Figure 8.2: Year-class frequency distribution for Spanish Mackerel collected for ageing in 2023. Distribution is broken down by sex. 'Unknown' represents gonads that were not available for examination or were not examined for sex during sampling.

8.3.3 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.

8.3.4 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 92% and a CV of 3.61% (test of symmetry: $\chi^2 = 4$, $df = 4$, $P = 0.406$). There was no evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 88.21% and a CV of 4.56% (test of symmetry: $\chi^2 = 4.43$, $df = 8$, $P = 0.8165$) (Figure 8.3).

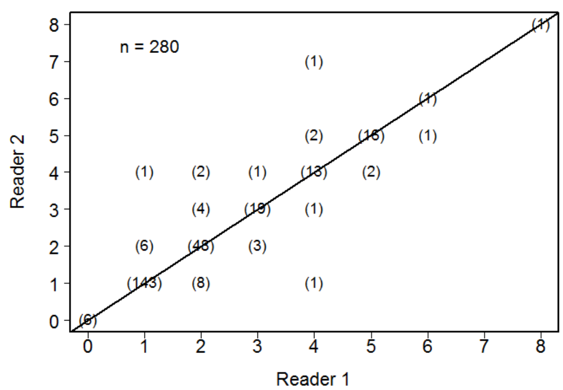


Figure 8.3: Between-reader comparison of otolith age estimates for Spanish Mackerel collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2023. The number in parentheses is number of fish.

There was no time-series bias for either reader. Reader 1 had an agreement of 98% with fish aged in 2003 with a CV of 0.4% (test of symmetry: $\chi^2 = 1$, $df = 1$, $P = 0.3173$). Reader 2 had an agreement of 84% with a CV of 6.29% (test of symmetry: $\chi^2 = 4.67$, $df = 2$, $P = 0.097$).

Table 8.1: Number of Spanish Mackerel collected and aged in each 1-inch length interval in 2023. 'Target' represents the sample size for ageing estimated for 2023, 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	1	1	4
13 - 13.99	5	3	3	2
14 - 14.99	22	12	12	10
15 - 15.99	36	35	35	1
16 - 16.99	42	58	53	0
17 - 17.99	41	55	42	0
18 - 18.99	25	40	26	0
19 - 19.99	21	34	22	0
20 - 20.99	16	23	16	0
21 - 21.99	15	23	16	0
22 - 22.99	9	24	10	0
23 - 23.99	8	11	9	0
24 - 24.99	8	6	6	2
25 - 25.99	7	7	7	0
26 - 26.99	5	9	7	0
27 - 27.99	6	10	10	0
28 - 28.99	5	3	3	2
29 - 29.99	5	2	2	3
30 - 30.99	5	0	0	5
31 - 31.99	5	0	0	5
32 - 32.99	5	0	0	5
Totals	296	356	280	39

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Table 8.2: CV for each age estimated based on ageing the total of 296 Spanish Mackerel in 2023. 'Percent' is the percentage of an age in the pooled age-length data of Spanish Mackerel collected from 2017 to 2021.

Age	CV	Percent
0	>0.25	2.01
1	0.05	43.24
2	0.08	31.64
3	0.13	14.38
4	0.22	5.27
5	>0.25	1.82
6	>0.25	1.05
7	>0.25	0.58

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Table 8.3: The number of Spanish Mackerel assigned to each total length-at-age category for 280 fish sampled for otolith age determination in Virginia during 2023.

	Age								
Interval	0	1	2	3	4	5	6	8	Totals
12 - 12.99	1	0	0	0	0	0	0	0	1
13 - 13.99	2	1	0	0	0	0	0	0	3
14 - 14.99	1	11	0	0	0	0	0	0	12
15 - 15.99	2	31	2	0	0	0	0	0	35
16 - 16.99	0	51	2	0	0	0	0	0	53
17 - 17.99	0	38	4	0	0	0	0	0	42
18 - 18.99	0	10	12	3	1	0	0	0	26
19 - 19.99	0	5	14	3	0	0	0	0	22
20 - 20.99	0	0	13	1	1	1	0	0	16
21 - 21.99	0	1	9	5	1	0	0	0	16
22 - 22.99	0	0	3	3	3	1	0	0	10
23 - 23.99	0	1	1	4	1	2	0	0	9
24 - 24.99	0	0	0	3	0	2	1	0	6
25 - 25.99	0	0	1	0	2	3	1	0	7
26 - 26.99	0	0	0	0	4	2	0	1	7
27 - 27.99	0	0	0	2	2	6	0	0	10
28 - 28.99	0	0	0	1	1	1	0	0	3
29 - 29.99	0	0	0	0	0	2	0	0	2
Totals	6	149	61	25	16	20	2	1	280

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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 Spanish Mackerel sampled for age determination in Virginia during 2023.

	Age							
Interval	0	1	2	3	4	5	6	8
12 - 12.99	1	0	0	0	0	0	0	0
13 - 13.99	0.67	0.33	0	0	0	0	0	0
14 - 14.99	0.08	0.92	0	0	0	0	0	0
15 - 15.99	0.06	0.89	0.06	0	0	0	0	0
16 - 16.99	0	0.96	0.04	0	0	0	0	0
17 - 17.99	0	0.9	0.1	0	0	0	0	0
18 - 18.99	0	0.38	0.46	0.12	0.04	0	0	0
19 - 19.99	0	0.23	0.64	0.14	0	0	0	0
20 - 20.99	0	0	0.81	0.06	0.06	0.06	0	0
21 - 21.99	0	0.06	0.56	0.31	0.06	0	0	0
22 - 22.99	0	0	0.3	0.3	0.3	0.1	0	0
23 - 23.99	0	0.11	0.11	0.44	0.11	0.22	0	0
24 - 24.99	0	0	0	0.5	0	0.33	0.17	0
25 - 25.99	0	0	0.14	0	0.29	0.43	0.14	0
26 - 26.99	0	0	0	0	0.57	0.29	0	0.14
27 - 27.99	0	0	0	0.2	0.2	0.6	0	0
28 - 28.99	0	0	0	0.33	0.33	0.33	0	0
29 - 29.99	0	0	0	0	0	1	0	0

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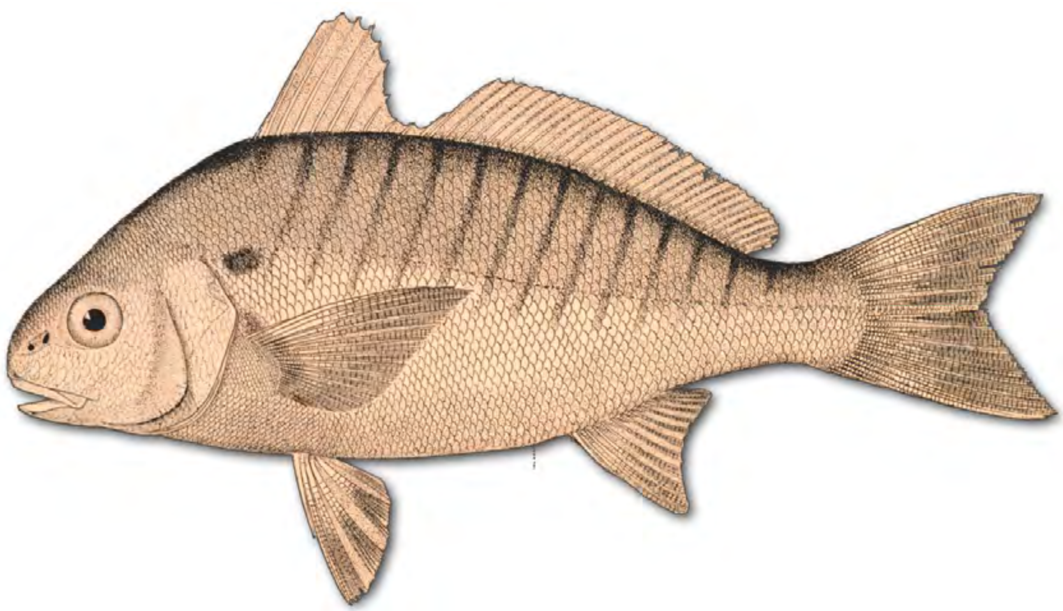
Chapter 9

SPOT *Leiostomus xanthurus*



9.1 INTRODUCTION

We aged a total of 168 Spot *Leiostomus xanthurus*, collected by the VMRC's Biological Sampling Program for age and growth analysis in 2023. Spot ages ranged from 0 to 2 years old with an average age of 1, a standard deviation of 0.4, and a standard error of 0.03. Three age classes (0 to 2) were represented, comprising fish of the 2021 to 2023 year-classes. The sample was dominated by fish from the year-class of 2022 with 86.9%.

9.2 METHODS

9.2.1 Sample Size for Ageing

We estimated sample size for ageing Spot in 2023 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 Spot in 2023; 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 2017 to 2021. θ_a, V_a, and B_a were calculated using pooled age-length data of Spot collected from 2017 to 2021 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 2017 to 2021 catch. A_l is number of fish to be aged for length interval l in 2023.

9.2.2 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 inside of protective Axygen 2 ml micro-tubes within their original labeled coin envelopes.

9.2.3 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 VMRC Ageing Lab website on how to prepare otolith thin-section for ageing Spot using the Epoxy Resin Method.

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 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 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.

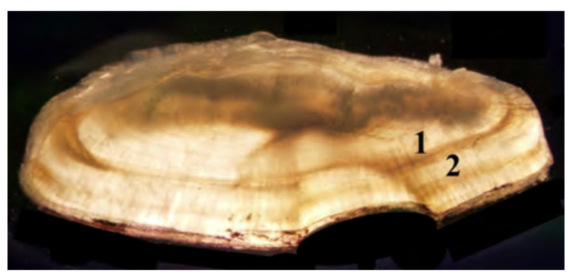


Figure 9.1: Otolith thin-section of a 2 year-old Spot

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 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.

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 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).

9.3 RESULTS

9.3.1 Sample Size

We estimated a sample size of 177 Spot in 2023, ranging in length interval from 4 to 12 inches (Table 9.1). This sample size provided a range in CV for age composition approximately from the smallest CV of 4% for Age 1 to the CV of larger than 25% for the multiple minor ages (Table 9.2). In 2023, we randomly selected and aged 168 fish from 255 Spot collected by VMRC. We fell short in our over-all collections for this optimal length-class sampling estimate by 36 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.

9.3.2 Year Class

Of the 168 fish aged with otoliths, 3 age classes (0 to 2) were represented (Table 9.3). The average age was 1 years, and the standard deviation and standard error were 0.4 and 0.03, respectively. Year-class data show that the fishery was comprised of 3 year-classes: fish from the 2021 to 2023 year-classes, with fish primarily from the year class of 2022 with 86.9%. The ratio of males to females was 1:4.96 in the sample collected (Figure 9.2).

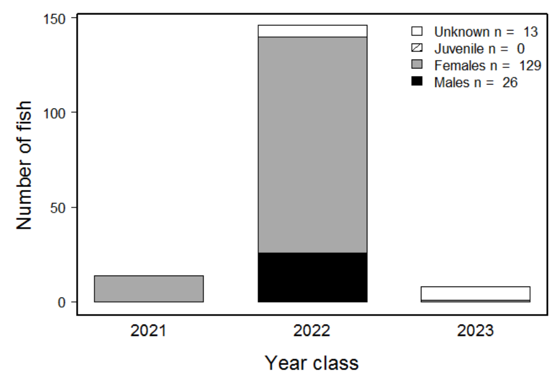


Figure 9.2: Year-class frequency distribution for Spot collected for ageing in 2023. Distribution is broken down by sex. 'Unknown' is for gonads that were not available for examination or were not examined for sex during sampling.

9.3.3 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.

9.3.4 Reading Precision

both readers had low self-precision. Specifically, there was a difference between the first and second readings for Reader 1 with an agreement of 82% and a *CV* of 17.91% (test of symmetry: $\chi^2 = 9$, $df = 2$, $P = 0.0111$), and there was no significant difference between the first and second readings for Reader 2 with an agreement of 90% and a *CV* of 10.37% (test of symmetry: $\chi^2 = 0.33$, $df = 2$, $P = 0.8465$). There was an evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 95.24% and a *CV* of 5.61% (test of symmetry: $\chi^2 = 6$, $df = 2$, $P = 0.0498$) (Figure 9.3).

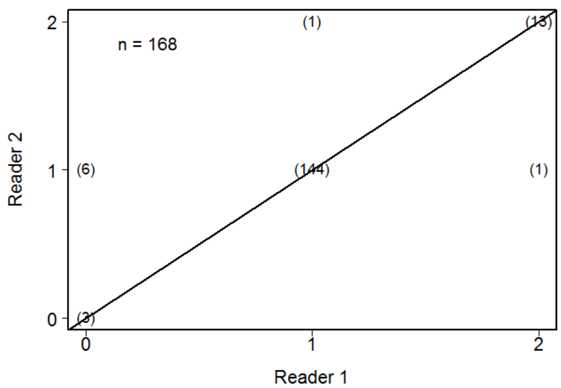


Figure 9.3: Between-reader comparison of otolith age estimates for Spot collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2023. The number in parentheses is number of fish.

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 2.83% (test of symmetry: $\chi^2 = 1$, $df = 1$, $P = 0.3173$). Reader 2 had an agreement of 96% with a *CV* of 5.66% (test of symmetry: $\chi^2 = 2$, $df = 1$, $P = 0.1573$).

Table 9.1: Number of Spot collected and aged in each 1-inch length interval in 2023. 'Target' represents the sample size for ageing estimated for 2023, 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	10	10	0
6 - 6.99	5	14	6	0
7 - 7.99	20	64	20	0
8 - 8.99	38	89	54	0
9 - 9.99	57	62	62	0
10 - 10.99	37	16	16	21
11 - 11.99	5	0	0	5
12 - 12.99	5	0	0	5
Totals	177	255	168	36

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Table 9.2: CV for each age estimated based on ageing the total of 177 Spot in 2023. 'Percent' is the percentage of an age in the pooled age-length data of Spot collected from 2017 to 2021.

Age	CV	Percent
0	0.19	5.14
1	0.04	75.9
2	0.16	17.24
3	>0.25	1.24
4	>0.25	0.19
5	>0.25	0.29

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Table 9.3: The number of Spot assigned to each total length-at-age category for 168 fish sampled for otolith age determination in Virginia during 2023.

	Age			
Interval	0	1	2	Totals
5 - 5.99	8	2	0	10
6 - 6.99	0	6	0	6
7 - 7.99	0	16	4	20
8 - 8.99	0	51	3	54
9 - 9.99	0	59	3	62
10 - 10.99	0	12	4	16
Totals	8	146	14	168

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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 Spot sampled for age determination in Virginia during 2023.

	Age		
Interval	0	1	2
5 - 5.99	0.8	0.2	0
6 - 6.99	0	1	0
7 - 7.99	0	0.8	0.2
8 - 8.99	0	0.94	0.06
9 - 9.99	0	0.95	0.05
10 - 10.99	0	0.75	0.25

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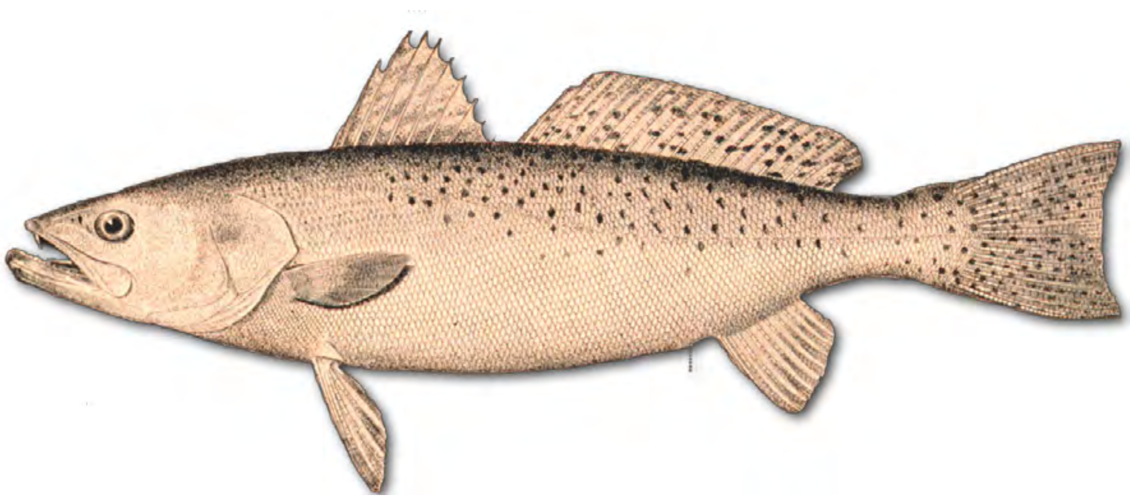
Chapter 10

SPOTTED SEATROUT *Cynoscion nebulosus*



10.1 INTRODUCTION

We aged a total of 259 Spotted Seatrout *Cynoscion nebulosus*, collected by the VMRC's Biological Sampling Program for age and growth analysis in 2023. Spotted seatrout ages ranged from 0 to 5 years old with an average age of 1.8, a standard deviation of 1.2, and a standard error of 0.07. Six age classes (0 to 5) were represented, comprising fish of the 2018 to 2023 year-classes. The sample was dominated by fish from the year-class of 2022 with 50.2%.

10.2 METHODS

10.2.1 Sample Size for Ageing

We estimated sample size for ageing Spotted Seatrout in 2023 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 Spotted Seatrout in 2023; 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 2017 to 2021. θ_a, V_a, and B_a were calculated using pooled age-length data of Spotted Seatrout collected from 2017 to 2021 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 2017 to 2021 catch. A_l is number of fish to be aged for length interval l in 2023.

10.2.2 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.

10.2.3 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™ 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 VMRC Ageing Lab website on how to prepare otolith thin-section for ageing Spotted Seatrout using the Glue Method.

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 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 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.

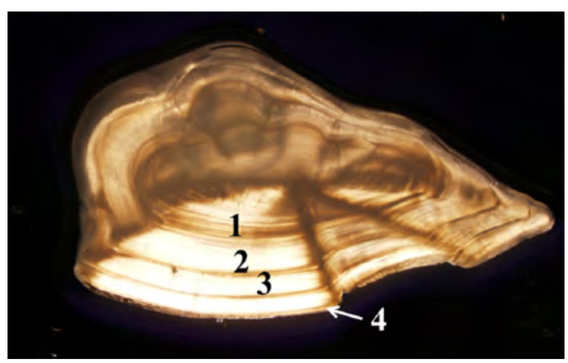


Figure 10.1: Otolith thin-section of a 4 year-old Spotted Seatrout with the last annulus on the edge of the thin-section

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.

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 303 Spotted Seatrout in 2023, ranging in length interval from 7 to 31 inches (Table 10.1). 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 10.2). In 2023, we randomly selected and aged 259 fish from 359 Spotted Seatrout collected by VMRC. We fell short in our over-all collections for this optimal length-class sampling estimate by 58 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.

10.3.2 Year Class

Of the 259 fish aged with otoliths, 6 age classes (0 to 5) were represented (Table 10.3). The average age was 1.8 years, and the standard deviation and standard error were 1.2 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 class of 2022 with 50.2%. The ratio of males to females was 1:1.24 in the sample collected (Figure 10.2).

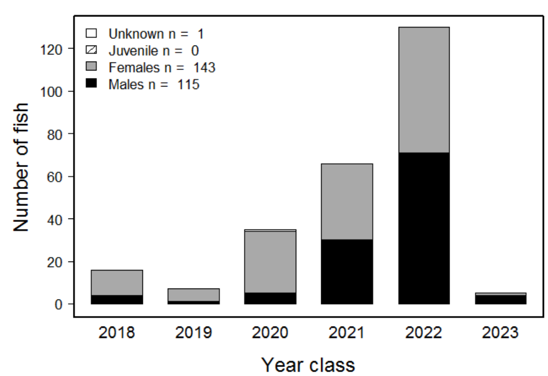


Figure 10.2: Year-class frequency distribution for Spotted Seatrout collected for ageing in 2023. Distribution is broken down by sex. 'Unknown' represents gonads that were not available for examination or were not examined for sex during sampling.

10.3.3 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.

10.3.4 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 96% and a *CV* of 0.88% (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 98.07% and a *CV* of 0.57% (test of symmetry: $\chi^2 = 5$, $df = 3$, $P = 0.1718$) (Figure 10.3).

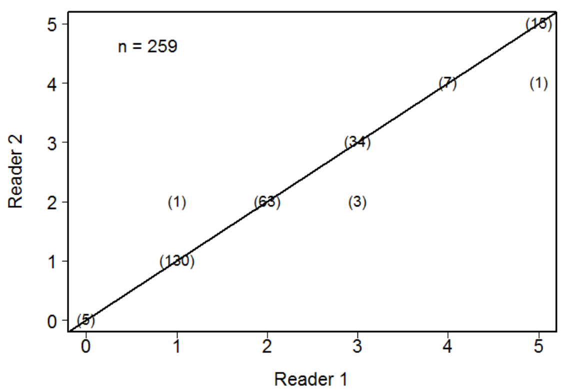


Figure 10.3: Between-reader comparison of otolith age estimates for Spotted Seatrout collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2023. The number in parentheses is number of fish.

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 0.22% (test of symmetry: $\chi^2 = 1$, $df = 1$, $P = 0.3173$). Reader 2 had an agreement of 98% with a *CV* of 0.22% (test of symmetry: $\chi^2 = 1$, $df = 1$, $P = 0.3173$).

Table 10.1: Number of Spotted Seatrout collected and aged in each 1-inch length interval in 2023. 'Target' represents the sample size for ageing estimated for 2023, 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
7 - 7.99	5	0	0	5
8 - 8.99	5	0	0	5
9 - 9.99	5	0	0	5
10 - 10.99	6	0	0	6
11 - 11.99	9	5	5	4
12 - 12.99	17	5	5	12
13 - 13.99	12	13	13	0
14 - 14.99	15	9	9	6
15 - 15.99	22	33	23	0
16 - 16.99	29	45	30	0
17 - 17.99	29	47	30	0
18 - 18.99	25	45	28	0
19 - 19.99	21	32	23	0
20 - 20.99	20	25	20	0
21 - 21.99	12	18	12	0
22 - 22.99	13	21	14	0
23 - 23.99	10	18	10	0
24 - 24.99	10	11	10	0
25 - 25.99	8	12	9	0
26 - 26.99	5	8	6	0
27 - 27.99	5	6	6	0
28 - 28.99	5	6	6	0
29 - 29.99	5	0	0	5
30 - 30.99	5	0	0	5
31 - 31.99	5	0	0	5
Totals	303	359	259	58

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Table 10.2: CV for each age estimated based on ageing the total of 303 Spotted Seatrout in 2023. 'Percent' is the percentage of an age in the pooled age-length data of Spotted Seatrout collected from 2017 to 2021.

Age	CV	Percent
0	0.13	9.48
1	0.05	46.83
2	0.08	31.58
3	0.14	10.19
4	>0.25	1.5
5	>0.25	0.43

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Table 10.3: The number of Spotted Seatrout assigned to each total length-at-age category for 259 fish sampled for otolith age determination in Virginia during 2023.

	Age						
Interval	0	1	2	3	4	5	Totals
11 - 11.99	3	2	0	0	0	0	5
12 - 12.99	1	4	0	0	0	0	5
13 - 13.99	1	12	0	0	0	0	13
14 - 14.99	0	9	0	0	0	0	9
15 - 15.99	0	20	3	0	0	0	23
16 - 16.99	0	23	7	0	0	0	30
17 - 17.99	0	24	6	0	0	0	30
18 - 18.99	0	20	8	0	0	0	28
19 - 19.99	0	12	10	1	0	0	23
20 - 20.99	0	4	12	4	0	0	20
21 - 21.99	0	0	6	4	1	1	12
22 - 22.99	0	0	6	6	0	2	14
23 - 23.99	0	0	4	4	0	2	10
24 - 24.99	0	0	3	6	0	1	10
25 - 25.99	0	0	1	4	3	1	9
26 - 26.99	0	0	0	4	2	0	6
27 - 27.99	0	0	0	1	1	4	6
28 - 28.99	0	0	0	1	0	5	6
Totals	5	130	66	35	7	16	259

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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 Spotted Seatrout sampled for age determination in Virginia during 2023.

	Age					
Interval	0	1	2	3	4	5
11 - 11.99	0.6	0.4	0	0	0	0
12 - 12.99	0.2	0.8	0	0	0	0
13 - 13.99	0.08	0.92	0	0	0	0
14 - 14.99	0	1	0	0	0	0
15 - 15.99	0	0.87	0.13	0	0	0
16 - 16.99	0	0.77	0.23	0	0	0
17 - 17.99	0	0.8	0.2	0	0	0
18 - 18.99	0	0.71	0.29	0	0	0
19 - 19.99	0	0.52	0.43	0.04	0	0
20 - 20.99	0	0.2	0.6	0.2	0	0
21 - 21.99	0	0	0.5	0.33	0.08	0.08
22 - 22.99	0	0	0.43	0.43	0	0.14
23 - 23.99	0	0	0.4	0.4	0	0.2
24 - 24.99	0	0	0.3	0.6	0	0.1
25 - 25.99	0	0	0.11	0.44	0.33	0.11
26 - 26.99	0	0	0	0.67	0.33	0
27 - 27.99	0	0	0	0.17	0.17	0.67
28 - 28.99	0	0	0	0.17	0	0.83

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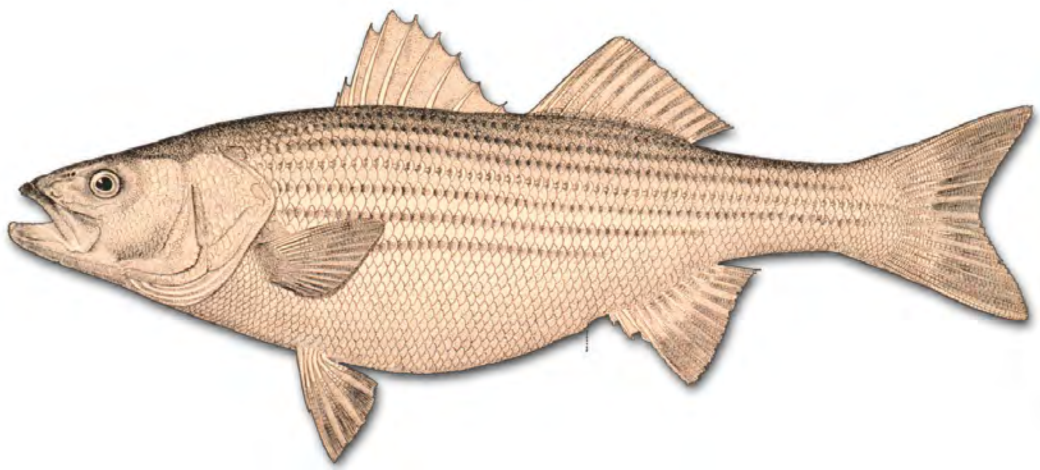
Chapter 11

STRIPED BASS *Morone saxatilis*



11.1 INTRODUCTION

We aged a total of 840 Striped Bass *Morone saxatilis*, collected by the VMRC's Biological Sampling Program in 2023. Of 840 aged fish, 490 and 350 fish were collected in Chesapeake Bay (bay fish) and Virginia waters of the Atlantic Ocean (ocean fish), respectively. The average bay fish age was 7.9 years with a standard deviation of 5 and a standard error of 0.23. Twenty-five age classes (3 to 27) were represented in the bay fish, comprising fish from the 1996 to 2020 year classes. The bay fish sample in 2023 was dominated by the year classes of 2014, 2015, 2017, 2018, 2019, and 2020 with 9%, 18%, 6%, 20%, 17%, and 6%, respectively. The average ocean fish age was 11.7 years with a standard deviation of 3.4 and a standard error of 0.18. Eighteen age classes (8 to 22, 25, 27, and 34) were represented in the ocean fish, comprising fish from the 1989, 1996, 1998, and 2001 to 2015 year classes. The ocean fish sample in 2023 was dominated by the year classes of 2010, 2011, 2012, 2013, 2014, and 2015 with 7%, 25%, 15%, 9%, 22%, and 8%, respectively. We also aged 252 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).

11.2 METHODS

11.2.1 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 2023, 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:

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

where A is the sample size for ageing Striped Bass in 2023; 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 2017 to 2021. θ_a, V_a, and B_a were calculated using pooled age-length data of Striped Bass collected from 2017 to 2021 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 2017 to 2021 catch. A_l is number of fish to be aged for length interval l in 2023.

11.2.2 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.

11.2.3 Preparation

11.2.3.1 *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™ 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 VMRC Ageing Lab website on how to prepare otolith thin-section for ageing Striped Bass using the Epoxy Resin Method.

11.2.3.2 *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.

11.2.4 Readings

The CQFE 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, 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 of 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.

However, because Reader 2 had no experience on ageing Striped bass scales, Reader 2 didn't age Striped bass scales collected in 2023, instead, the scale ages estimated by Reader 1 were used as the final ages.

11.2.4.1 *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 11.1). 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.

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 mag-

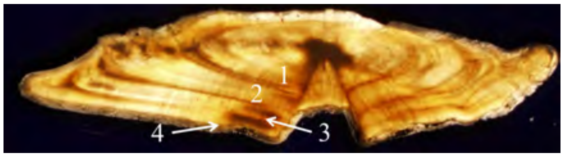


Figure 11.1: Otolith thin-section of a 4 year-old Striped Bass with the last annulus on the edge of the thin-section

nification. Each reader aged all of the otolith samples.

11.2.4.2 *Scales*

Reader 1 determined fish age by viewing acetate impressions of scales (Figure 11.2) with a standard Bell and Howell R-735 microfiche reader equipped with 20 and 29 mm lenses. Reader 2 has no experience on ageing Striped Bass scales, will be trained in 2024, and will be able to age the scales collected in 2024 during early 2025.

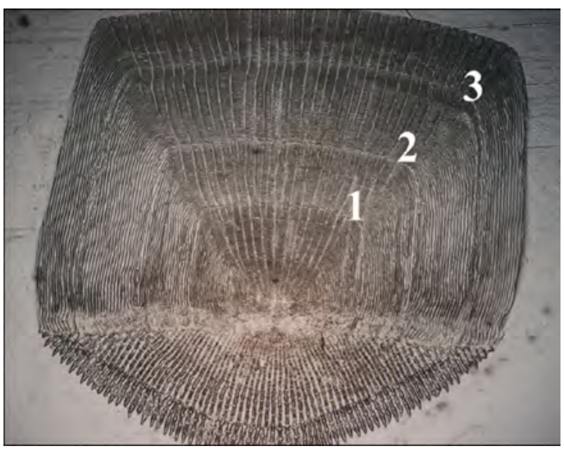


Figure 11.2: Scale impression of a 3 year-old Striped Bass.

Annuli on 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.

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 following comparisons: 1) on the otolith ages between the two readers in the current year; 2) on the otolith ages within each reader in the current year; 3) time-series bias on the otolith ages between the current and previous years within each reader; 4) on the scale ages within Reader 1 in the current year; 5) time-series bias on the scale ages within Reader 1; and 6) between Reader 1's scale ages and the final otoliths ages. The readings on the otolith thin-sections from the entire sample for the current year were used to examine the difference between two readers. A random sub-sample of the scale and otolith slides from 50 fish from the current year was selected for second readings to examine the difference within a reader (Reader 1 read both scale and otolith sub-samples whereas Reader 2 read only the otolith sub-samples). Fifty otoliths and scales randomly selected from fish aged in 2000 were used to examine the time-series bias within each reader (Reader 1 read both scale and otolith random samples whereas Reader 2 read only the otolith random samples). 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

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.

11.3.1.1 *Chesapeake Bay*

We estimated a sample size of 477 bay Striped Bass in 2023, ranging in length interval from 10 to 55 inches (Table 11.1). This sample size provided a range in CV for age composition approximately from the smallest CV of 10% for the major age of Age 6 to the CV of larger than 25% for the multiple minor ages of the bay fish (Table 11.2). We aged all the fish with both scales and otoliths (216 fish). We aged 1 fish with otolith-only. We randomly selected 273 fish with scale-only to age. As a result, we aged 490 of 746 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 2023. We fell short in our over-all collections for the optimal length-class sampling estimate by 61 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.

11.3.1.2 *Atlantic Ocean*

We estimated a sample size of 474 ocean Striped Bass in 2023, ranging in length interval from 20 to 53 inches (Table 11.3). This sample size provided a range in CV for age composition approximately from the smallest CV of 9% for the major age of Age 10 to the CV of larger than 25% for the multiple minor ages of the ocean fish (Table 11.4). We aged all the fish with both scales and otoliths (36 fish). We randomly selected 314 fish with scale-only to age. As a result, we aged 350 of 397 fish (The rest of fish were either without any hardparts or over-collected for certain length interval(s)) collected by VMRC in Virginia waters of the Atlantic Ocean in 2023. We fell short in our over-all collections for the optimal length-class sampling estimate by 147 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.

11.3.2 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.

11.3.2.1 *Chesapeake Bay*

Of the 490 bay Striped Bass aged, 25 age classes (3 to 27) were represented (Table 11.5). The average age for the sample was 7.9 years. The standard deviation and standard error were 5 and 0.23, respectively. Year-class data (Figure 11.3) indicates that recruitment into the fishery in Chesapeake Bay begins at age 3, which corresponds to the 2020 year-class for Striped Bass caught in 2023. Striped bass in the sample in 2023 was dominated by the year classes of 2014, 2015, 2017, 2018, 2019, and 2020 with 9%, 18%, 6%, 20%, 17%, and 6%, respectively. The sex ratio of male to female was 1:1.4 for the bay fish.

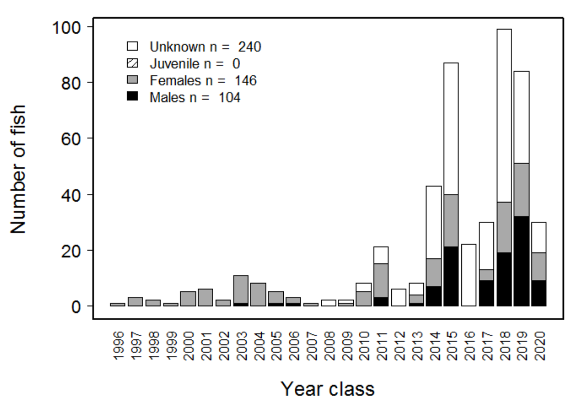


Figure 11.3: Year-class frequency distribution for Striped Bass collected in Chesapeake Bay, Virginia for ageing in 2023. Distribution is broken down by sex and estimated using scale ages. 'Unknown' represents the fish gonads that were not available for examination or were not examined for sex during sampling.

11.3.2.2 *Atlantic Ocean*

Of the 350 ocean Striped Bass aged, 18 age classes (8 to 22, 25, 27, and 34) were represented (Table 11.6). The average age for the sample was 11.7 years. The standard deviation and standard error were 3.4 and 0.18, respectively. Year-class data (Figure 11.4) indicates that recruitment into the fishery in Virginia waters of Atlantic ocean begins at age 8, which corresponds to the 2015 year-class for Striped Bass caught in 2023. Striped bass in the sample in 2023 was dominated by the year classes of 2010, 2011, 2012, 2013, 2014, and 2015 with 7%, 25%, 15%, 9%, 22%, and 8%, respectively. The sex ratio of male to female was 1:5.79 for the ocean fish.

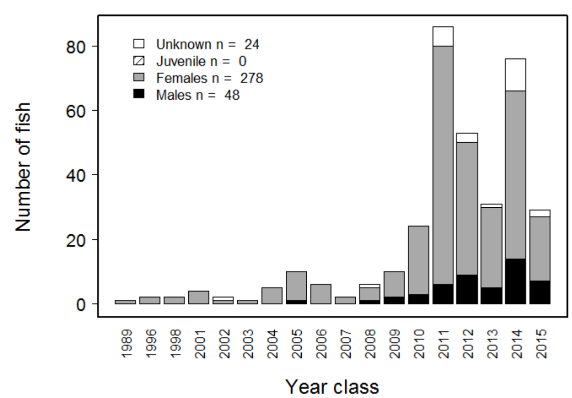


Figure 11.4: Year-class frequency distribution for Striped Bass collected in Virginia waters of the Atlantic Ocean for ageing in 2023. Distribution is broken down by sex and estimated using scale ages. 'Unknown' represents the fish gonads that were not available for examination or were not examined for sex during sampling.

11.3.3 Age-Length-Key (ALK)

We developed an age-length-key for both bay (Table 11.7) and ocean fish (Table 11.8) 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.

11.3.4 Reading Precision

11.3.4.1 *Otoliths*

Reader 1 and Reader 2 aged the otoliths of 253 Striped Bass collected in 2023. 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 0.4% (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 84% and a *CV* of 1.1% (test of symmetry: $\chi^2 = 8$, $df = 7$, $P = 0.3326$). There was an evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 83% (1 year or less agreement of 99%) and a *CV* of 1% (test of symmetry: $\chi^2 = 33.3$, $df = 21$, $P = 0.0426$) (Figure 11.5).

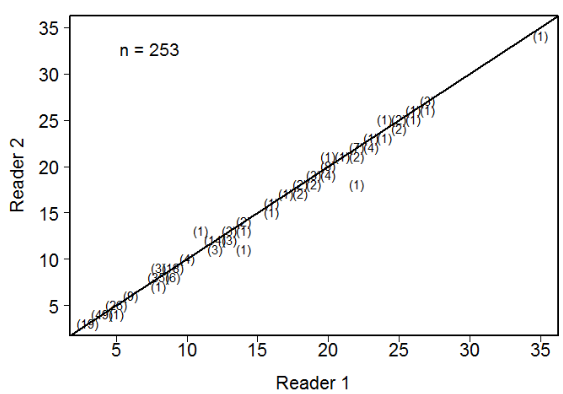


Figure 11.5: Between-reader comparison of otolith age estimates for Striped Bass collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2023. The number in parentheses is number of fish.

There was no time-series bias for either reader. Reader 1 had an agreement of 82% with ages of fish aged in 2000 with a *CV* of 1.8% (test of symmetry: $\chi^2 = 11$, $df = 8$, $P = 0.2017$). Reader 2 had an agreement of 68% with a *CV* of 3.7% (test of symmetry: $\chi^2 = 13.7$, $df = 11$, $P = 0.252$).

11.3.4.2 *Scales*

Reader 1 aged the scales of 839 Striped Bass collected in 2023. There was no significant difference between the first and second readings for Reader 1 with an agreement of 74% (1 year or less agreement of 94%) and a *CV* of 3% (test of symmetry: $\chi^2 = 10$, $df = 10$, $P = 0.4405$)

Reader 1 had no time series bias, having an agreement of 43% with ages of fish aged in 2000 and a *CV* of 7.2% (test of symmetry: $\chi^2 = 21.3$, $df = 14$, $P = 0.0934$).

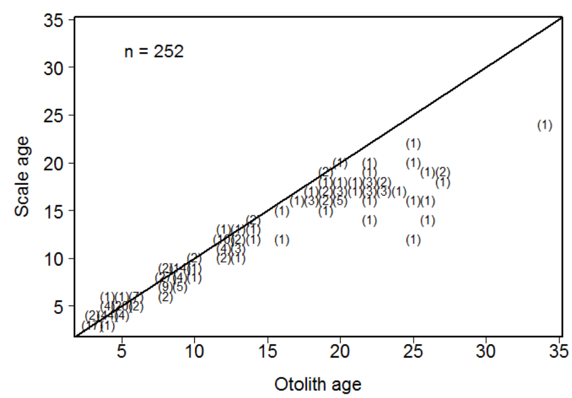


Figure 11.6: Comparison of scale and otolith age estimates for Striped Bass collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2023. The number in parentheses is number of fish.

11.3.5 Comparison of Scale and Otolith Ages

Reader 1 aged 252 pairs of Striped Bass scales and otoliths (One fish with otoliths only was excluded from this comparison.). There was an evidence of systematic disagreement between otolith and scale ages (test of symmetry: $\chi^2 = 83.7$, $df = 50$, $P = 0.002$) with an average *CV* of 6.1%. There was an agreement of 58% between scale and otoliths ages whereas scales were assigned a lower and higher age than otoliths for 37.3% and 4.4% of the fish, respectively (Figure 11.6). There was also an evidence of bias between otolith and scale ages using an age bias plot (Figure 11.7), with scale generally assigned higher ages for younger fish and lower ages for older fish than otolith age estimates.

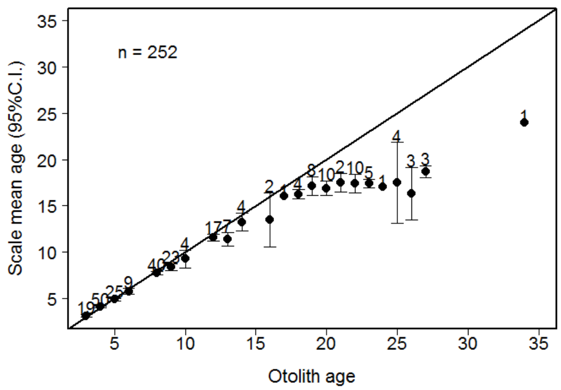


Figure 11.7: Age-bias plot for Striped Bass scale and otolith age estimates in 2023. The number above the upper CI bar is number of fish.

11.4 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 11.1: Number of bay Striped Bass collected and aged in each 1-inch length interval in 2023. 'Target' represents the sample size for ageing estimated for 2023, 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	11	19	13	0
19 - 19.99	25	45	30	0
20 - 20.99	32	79	38	0
21 - 21.99	25	74	33	0
22 - 22.99	23	56	35	0
23 - 23.99	23	37	24	0
24 - 24.99	22	47	24	0
25 - 25.99	20	41	23	0
26 - 26.99	19	29	20	0
27 - 27.99	16	18	16	0
28 - 28.99	15	15	15	0
29 - 29.99	13	15	14	0
30 - 30.99	12	11	11	1
31 - 31.99	11	13	12	0
32 - 32.99	14	17	15	0
33 - 33.99	12	16	12	0
34 - 34.99	10	9	9	1
35 - 35.99	11	17	13	0
36 - 36.99	11	16	12	0
37 - 37.99	13	21	15	0
38 - 38.99	11	13	12	0
39 - 39.99	7	21	10	0
40 - 40.99	6	25	7	0
41 - 41.99	6	16	8	0
42 - 42.99	7	13	11	0
43 - 43.99	5	8	7	0
44 - 44.99	7	11	9	0
45 - 45.99	7	9	8	0
46 - 46.99	7	9	9	0
47 - 47.99	6	13	12	0
48 - 48.99	5	7	7	0
49 - 49.99	5	4	4	1
50 - 50.99	5	1	1	4
51 - 51.99	5	0	0	5
52 - 52.99	5	0	0	5
55 - 55.99	5	0	0	5
Totals	477	746	490	61

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Table 11.2: CV for each age estimated based on ageing the total of 477 bay Striped Bass in 2023. 'Percent' is the percentage of an age in the pooled age-length data of bay Striped Bass collected from 2017 to 2021.

Age	CV	Percent
1	>0.25	0.55
2	>0.25	0.48
3	0.18	5.09
4	0.11	13.5
5	0.11	14.82
6	0.1	17
7	0.14	8.9
8	0.16	7.48
9	0.16	7.34
10	0.19	4.92
11	0.25	3.15
12	>0.25	2.7
13	>0.25	2.18
14	>0.25	2.32
15	>0.25	1.63
16	>0.25	2.08
17	>0.25	1.21
18	>0.25	1.56
19	>0.25	1.14
20	>0.25	0.69
21	>0.25	0.59
22	>0.25	0.42
23	>0.25	0.17
24	>0.25	0.07

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Table 11.3: Number of ocean Striped Bass collected and aged in each 1-inch length interval in 2023. 'Target' represents the sample size for ageing estimated for 2023, 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
22 - 22.99	5	0	0	5
25 - 25.99	5	1	1	4
26 - 26.99	5	1	1	4
27 - 27.99	5	0	0	5
28 - 28.99	5	0	0	5
29 - 29.99	5	1	1	4
30 - 30.99	5	0	0	5
31 - 31.99	5	2	2	3
32 - 32.99	8	2	2	6
33 - 33.99	13	1	1	12
34 - 34.99	22	6	6	16
35 - 35.99	33	13	13	20
36 - 36.99	46	26	26	20
37 - 37.99	54	43	43	11
38 - 38.99	57	62	62	0
39 - 39.99	43	50	50	0
40 - 40.99	38	55	38	0
41 - 41.99	30	49	30	0
42 - 42.99	20	31	20	0
43 - 43.99	13	17	17	0
44 - 44.99	9	13	13	0
45 - 45.99	5	7	7	0
46 - 46.99	7	8	8	0
47 - 47.99	6	5	5	1
48 - 48.99	5	3	3	2
49 - 49.99	5	1	1	4
50 - 50.99	5	0	0	5
51 - 51.99	5	0	0	5
53 - 53.99	5	0	0	5
Totals	474	397	350	147

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Table 11.4: CV for each age estimated based on ageing the total of 474 ocean Striped Bass in 2023. 'Percent' is the percentage of an age in the pooled age-length data of ocean Striped Bass collected from 2017 to 2021.

Age	CV	Percent
5	>0.25	0.34
6	>0.25	0.26
7	>0.25	2.22
8	0.13	10.06
9	0.12	12.53
10	0.09	21.23
11	0.13	12.36
12	0.15	9.63
13	0.17	7.08
14	0.16	8.35
15	0.2	4.94
16	0.25	3.07
17	>0.25	2.9
18	>0.25	1.36
19	>0.25	1.62
20	>0.25	0.68
21	>0.25	0.68
22	>0.25	0.34
23	>0.25	0.17
25	>0.25	0.09

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Table 11.5: The number of Striped Bass assigned to each total length-at-age category for 490 fish sampled for both otolith and scale age determination in Chesapeake Bay, Virginia during 2023.

	Age																								
Interval	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	Totals
17 - 17.99	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	1
18 - 18.99	6	6	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	13
19 - 19.99	8	14	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	30
20 - 20.99	3	24	11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	38
21 - 21.99	6	16	11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	33
22 - 22.99	4	9	16	5	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	35
23 - 23.99	0	6	9	4	3	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	24
24 - 24.99	2	2	7	4	4	4	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	24
25 - 25.99	0	2	10	3	5	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	23
26 - 26.99	0	1	4	4	2	8	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20
27 - 27.99	0	1	3	2	0	6	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	16
28 - 28.99	0	1	4	2	1	5	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	15
29 - 29.99	0	2	3	3	1	3	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	14
30 - 30.99	0	0	9	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11
31 - 31.99	0	0	3	0	0	8	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12
32 - 32.99	0	0	0	1	1	11	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	15
33 - 33.99	0	0	0	1	1	9	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12
34 - 34.99	0	0	0	0	0	5	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9
35 - 35.99	0	0	0	0	1	10	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	13
36 - 36.99	0	0	0	0	1	6	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12
37 - 37.99	0	0	0	0	1	5	7	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	15
38 - 38.99	0	0	0	0	0	4	4	2	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12
39 - 39.99	0	0	0	0	0	0	3	1	1	4	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10
40 - 40.99	0	0	0	0	0	0	0	0	2	1	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	7
41 - 41.99	0	0	0	0	0	0	0	0	0	7	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8
42 - 42.99	0	0	0	0	0	0	1	3	0	5	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	11
43 - 43.99	0	0	0	0	0	0	0	0	1	2	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	7
44 - 44.99	0	0	0	0	0	0	0	0	1	0	1	0	0	1	1	0	1	2	0	0	1	0	1	0	0	9
45 - 45.99	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	1	0	0	3	0	1	0	0	8
46 - 46.99	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	2	2	1	1	0	0	0	1	0	9
47 - 47.99	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	3	3	1	2	0	1	0	0	0	12
48 - 48.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	2	0	0	1	0	0	0	1	7
49 - 49.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	3	0	0	0	0	0	4
50 - 50.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
Totals	30	84	99	30	22	87	43	8	6	21	8	2	2	1	3	5	8	11	2	6	5	1	2	3	1	490

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Table 11.6: The number of Striped Bass assigned to each total length-at-age category for 350 fish sampled for both otolith and scale age determination in Virginia waters of Atlantic ocean during 2023.

	Age																	
Interval	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	25	27	34	Totals
25 - 25.99	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
26 - 26.99	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
29 - 29.99	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
31 - 31.99	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
32 - 32.99	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
33 - 33.99	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
34 - 34.99	2	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6
35 - 35.99	1	10	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	13
36 - 36.99	7	15	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	26
37 - 37.99	8	18	5	5	6	1	0	0	0	0	0	0	0	0	0	0	0	0	43
38 - 38.99	7	17	8	15	12	2	0	1	0	0	0	0	0	0	0	0	0	0	62
39 - 39.99	1	6	7	13	17	5	1	0	0	0	0	0	0	0	0	0	0	0	50
40 - 40.99	0	0	6	9	17	2	3	0	0	0	1	0	0	0	0	0	0	0	38
41 - 41.99	0	1	1	5	15	6	1	1	0	0	0	0	0	0	0	0	0	0	30
42 - 42.99	0	0	0	4	11	1	2	0	0	0	1	0	1	0	0	0	0	0	20
43 - 43.99	0	0	0	0	5	6	1	1	0	0	1	1	0	0	1	0	1	0	17
44 - 44.99	0	0	0	0	3	1	1	2	0	2	1	1	0	0	1	1	0	0	13
45 - 45.99	0	0	0	0	0	0	1	0	1	1	3	1	0	0	0	0	0	0	7
46 - 46.99	0	0	0	0	0	0	0	1	1	1	2	1	0	1	0	0	0	1	8
47 - 47.99	0	0	0	0	0	0	0	0	0	2	1	0	0	0	0	1	1	0	5
48 - 48.99	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	0	0	0	3
49 - 49.99	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1
Totals	29	76	31	53	86	24	10	6	2	6	10	5	1	2	4	2	2	1	350

(Go back to text)

Table 11.7: 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 2023.

	Age																							
Interval	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
17 - 17.99	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
18 - 18.99	0.46	0.46	0.08	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19 - 19.99	0.27	0.47	0.27	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20 - 20.99	0.08	0.63	0.29	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
21 - 21.99	0.18	0.48	0.33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
22 - 22.99	0.11	0.26	0.46	0.14	0.03	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
23 - 23.99	0	0.25	0.38	0.17	0.12	0.08	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
24 - 24.99	0.08	0.08	0.29	0.17	0.17	0.17	0.04	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
25 - 25.99	0	0.09	0.43	0.13	0.22	0.04	0.09	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
26 - 26.99	0	0.05	0.2	0.2	0.1	0.4	0.05	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
27 - 27.99	0	0.06	0.19	0.12	0	0.38	0.25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
28 - 28.99	0	0.07	0.27	0.13	0.07	0.33	0.13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
29 - 29.99	0	0.14	0.21	0.21	0.07	0.21	0.14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
30 - 30.99	0	0	0.82	0.09	0	0	0.09	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
31 - 31.99	0	0	0.25	0	0	0.67	0.08	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
32 - 32.99	0	0	0	0.07	0.07	0.73	0.13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
33 - 33.99	0	0	0	0.08	0.08	0.75	0.08	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
34 - 34.99	0	0	0	0	0	0.56	0.44	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
35 - 35.99	0	0	0	0	0.08	0.77	0.15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
36 - 36.99	0	0	0	0	0.08	0.5	0.42	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
37 - 37.99	0	0	0	0	0.07	0.33	0.47	0.13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
38 - 38.99	0	0	0	0	0	0.33	0.33	0.17	0.08	0.08	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
39 - 39.99	0	0	0	0	0	0	0.3	0.1	0.1	0.4	0.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
40 - 40.99	0	0	0	0	0	0	0	0	0.29	0.14	0.43	0.14	0	0	0	0	0	0	0	0	0	0	0	0	0
41 - 41.99	0	0	0	0	0	0	0	0	0	0.88	0.12	0	0	0	0	0	0	0	0	0	0	0	0	0	0
42 - 42.99	0	0	0	0	0	0	0.09	0.27	0	0.45	0	0	0	0	0	0.09	0	0	0	0	0	0	0	0.09	0
43 - 43.99	0	0	0	0	0	0	0	0	0.14	0.29	0.14	0.14	0	0	0.14	0.14	0	0	0	0	0	0	0	0	0
44 - 44.99	0	0	0	0	0	0	0	0	0.11	0	0.11	0	0	0.11	0.11	0	0.11	0.22	0	0	0.11	0	0.11	0	0
45 - 45.99	0	0	0	0	0	0	0	0	0	0	0.12	0	0.12	0	0.12	0	0	0.12	0	0	0.38	0	0.12	0	0
46 - 46.99	0	0	0	0	0	0	0	0	0	0	0	0	0.11	0	0	0.11	0.22	0.22	0.11	0.11	0	0	0	0.11	0
47 - 47.99	0	0	0	0	0	0	0	0	0	0.08	0	0	0	0	0	0.08	0.25	0.25	0.08	0.17	0	0.08	0	0	0
48 - 48.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.14	0.29	0.29	0	0	0.14	0	0	0	0.14
49 - 49.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.25	0	0.75	0	0	0	0	0
50 - 50.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0

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Table 11.8: 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 2023.

	Age																	
Interval	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	25	27	34
25 - 25.99	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
26 - 26.99	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
29 - 29.99	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
31 - 31.99	0.5	0.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
32 - 32.99	0.5	0.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
33 - 33.99	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
34 - 34.99	0.33	0.67	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
35 - 35.99	0.08	0.77	0.08	0.08	0	0	0	0	0	0	0	0	0	0	0	0	0	0
36 - 36.99	0.27	0.58	0.12	0.04	0	0	0	0	0	0	0	0	0	0	0	0	0	0
37 - 37.99	0.19	0.42	0.12	0.12	0.14	0.02	0	0	0	0	0	0	0	0	0	0	0	0
38 - 38.99	0.11	0.27	0.13	0.24	0.19	0.03	0	0.02	0	0	0	0	0	0	0	0	0	0
39 - 39.99	0.02	0.12	0.14	0.26	0.34	0.1	0.02	0	0	0	0	0	0	0	0	0	0	0
40 - 40.99	0	0	0.16	0.24	0.45	0.05	0.08	0	0	0	0.03	0	0	0	0	0	0	0
41 - 41.99	0	0.03	0.03	0.17	0.5	0.2	0.03	0.03	0	0	0	0	0	0	0	0	0	0
42 - 42.99	0	0	0	0.2	0.55	0.05	0.1	0	0	0	0.05	0	0.05	0	0	0	0	0
43 - 43.99	0	0	0	0	0.29	0.35	0.06	0.06	0	0	0.06	0.06	0	0	0.06	0	0.06	0
44 - 44.99	0	0	0	0	0.23	0.08	0.08	0.15	0	0.15	0.08	0.08	0	0	0.08	0.08	0	0
45 - 45.99	0	0	0	0	0	0	0.14	0	0.14	0.14	0.43	0.14	0	0	0	0	0	0
46 - 46.99	0	0	0	0	0	0	0	0.12	0.12	0.12	0.25	0.12	0	0.12	0	0	0	0.12
47 - 47.99	0	0	0	0	0	0	0	0	0	0.4	0.2	0	0	0	0	0.2	0.2	0
48 - 48.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0.33	0.67	0	0	0
49 - 49.99	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0

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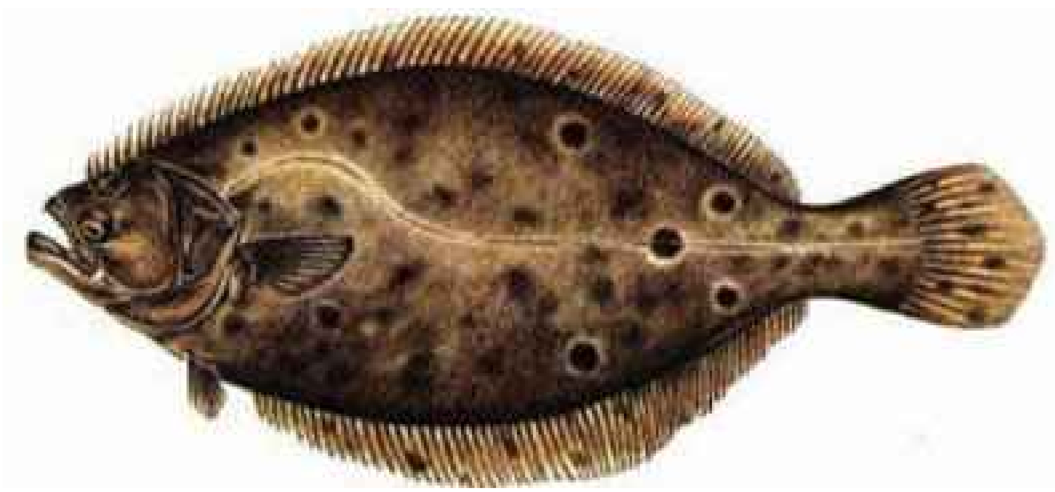
Chapter 12

SUMMER FLOUNDER *Paralichthys dentatus*



12.1 INTRODUCTION

We aged a total of 825 Summer Flounder *Paralichthys dentatus*, collected by the VMRC's Biological Sampling Program in 2023. Of 825 aged fish, 364 and 461 fish were collected in Chesapeake Bay (bay fish) and Virginia waters of the Atlantic Ocean (ocean fish), respectively. The average bay fish age was 2.7 years with a standard deviation of 1 and a standard error of 0.05. Seven age classes (1 to 7) were represented in the bay fish, comprising fish from the 2016 to 2022 year classes. The bay fish sample in 2023 was dominated by the year classes of 2020 and 2021 with 35% and 46%, respectively. The average ocean fish age was 5.6 years with a standard deviation of 2.5 and a standard error of 0.12. Fifteen age classes (2 to 16) were represented in the ocean fish, comprising fish from the 2007 to 2021 year classes. The ocean fish sample in 2023 was dominated by the year classes of 2016, 2017, 2018, 2019, and 2020 with 13%, 14%, 16%, 18%, and 13%, respectively. We also aged 473 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 Summer Flounder collected in both Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2023, 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 Summer Flounder in 2023; 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 2017 to 2021. θ_a, V_a, and B_a were calculated using pooled age-length data of Summer Flounder collected from 2017 to 2021 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 2017 to 2021 catch. A_l is number of fish to be aged for length interval l in 2023.

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 VMRC Ageing Lab website on how to prepare otolith thin-section for ageing Summer Flounder using the Epoxy Resin Method.

12.2.3.2 *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.

12.2.4 Readings

The CQFE 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 CQFE), and age class assignment using these hard-parts is conducted in the same way as otoliths.

All Summer Flounder samples of the 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, 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.

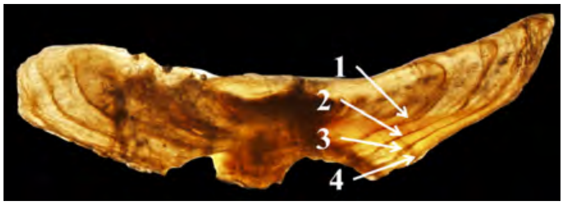


Figure 12.1: Otolith thin-section of a 4 year-old Summer Flounder with the last annulus on the edge of the thin-section

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.

12.2.4.2 *Scales*

Reader 1 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. Reader 2 has no experience on ageing Summer Flounder scales, will be trained in 2024, and will be able to age the scales collected in 2024 during early 2025.

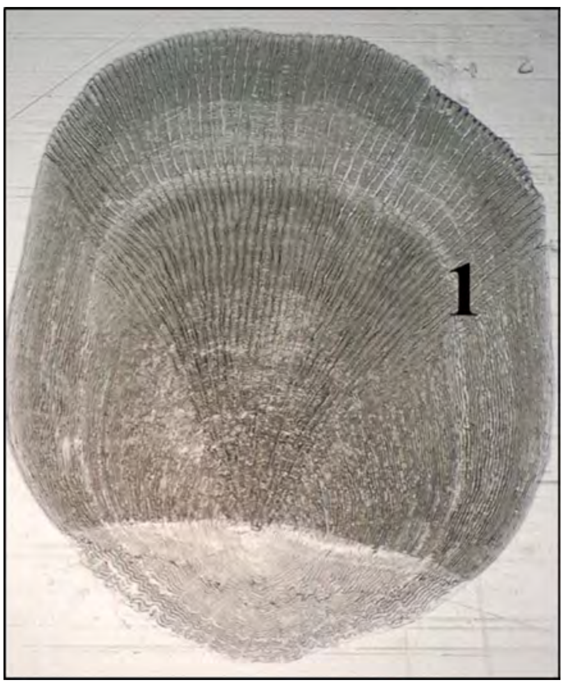


Figure 12.2: Scale impression of a 1 year-old Summer Flounder

Annuli 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.

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) on the otolith ages between the two readers in the current year; 2) on the otolith ages within each reader in the current year; 3) time-series bias on the otolith ages between the current and previous years within each reader; 4) on the scale ages within Reader 1 in the current year; 5) time-series bias on the scale ages within Reader 1; and 6) between Reader 1's scale ages and the final otoliths ages. The readings on the otolith thin-section from the entire sample for the current year were used to examine the difference between two readers. A random sub-sample of the scale and otolith slides from 50 fish from the current year was selected for second readings to examine the difference within a reader (Reader 1 read both scale and otolith sub-samples whereas Reader 2 read only the otolith sub-samples). Fifty otoliths and scales randomly selected from fish aged in 2000 were used to examine the time-series bias within each reader (Reader 1 read both scale and otolith random samples whereas Reader 2 read only the otolith random samples). 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 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.

12.3.1.1 *Chesapeake Bay*

We estimated a sample size of 359 bay Summer Flounder in 2023, ranging in length interval from 8 to 27 inches (Table 12.1). 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 12.2). We aged all the fish with both scales and otoliths (144 fish). We randomly selected 220 fish with scale-only to age. As a result, we aged 364 of 430 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 2023. We fell short in our over-all collections for the optimal length-class sampling estimate by 25 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 476 ocean Summer Flounder in 2023, ranging in length interval from 13 to 32 inches (Table 12.3). 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 12.4). We aged all the fish with both scales and otoliths (329 fish). We randomly selected 132 fish with scale-only to age. As a result, we aged 461 of 489 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 2023. We fell short in our over-all collections for the optimal length-class sampling estimate by 48 fish. We were short 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.

12.3.2 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.

12.3.2.1 *Chesapeake Bay*

Of the 364 bay Summer Flounder aged, 7 age classes (1 to 7) were represented (Table 12.5). The average age for the sample was 2.7 years. The standard deviation and standard error were 1 and 0.05, respectively. Year-class data (Figure 12.3) indicates that recruitment into the fishery in Chesapeake Bay begins at age 1, which corresponds to the 2022 year-class for Summer Flounder caught in 2023. Summer flounder in the sample in 2023 was dominated by the year classes of 2020 and 2021 with 35% and 46%, respectively. The sex ratio of male to female was 1:143 for the bay fish.

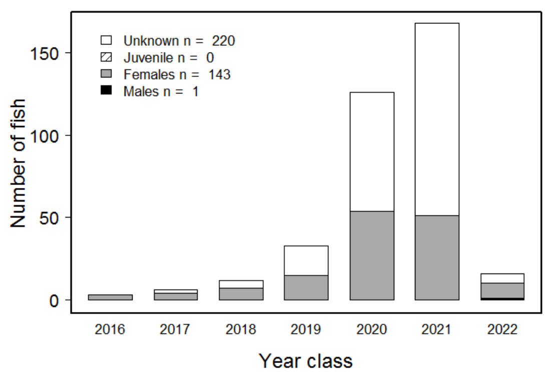


Figure 12.3: Year-class frequency distribution for Summer Flounder collected in Chesapeake Bay, Virginia for ageing in 2023. Distribution is broken down by sex. 'Unknown' represents gonads that were not available for examination or were not examined for sex during sampling.

12.3.2.2 *Atlantic Ocean*

Of the 461 ocean Summer Flounder aged, 15 age classes (2 to 16) were represented (Table 12.6). The average age for the sample was 5.6 years. The standard deviation and standard error were 2.5 and 0.12, respectively. Year-class data (Figure 12.4) indicates that recruitment into the fishery in Virginia waters of Atlantic ocean begins at age 2, which corresponds to the 2021 year-class for Summer Flounder caught in 2023. Summer flounder in the sample in 2023 was dominated by the year classes of 2016, 2017, 2018, 2019, and 2020 with 13%, 14%, 16%, 18%, and 13%, respectively. The sex ratio of male to female was 1:0.75 for the ocean fish.

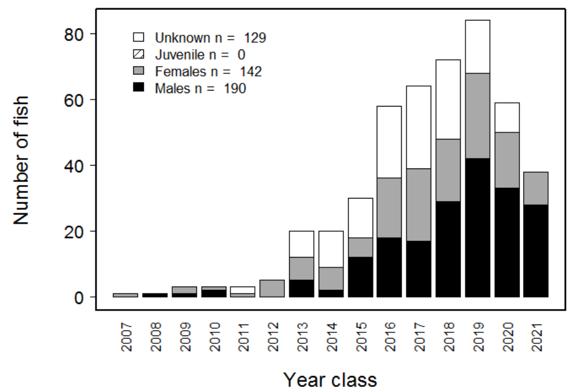


Figure 12.4: Year-class frequency distribution for Summer Flounder collected in Virginia waters of the Atlantic Ocean for ageing in 2023. Distribution is broken down by. 'Unknown' represents gonads that were not available for examination or were not examined for sex during sampling.

12.3.3 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.

12.3.4 Reading Precision

12.3.4.1 *Otoliths*

Reader 1 and Reader 2 aged the otoliths of 473 Summer Flounder collected in 2023. 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 82% and a *CV* of 3.9% (test of symmetry: $\chi^2 = 5$, $df = 6$, $P = 0.5438$), and there was no significant difference between the first and second readings for Reader 2 with an agreement of 74% and a *CV* of 3.5% (test of symmetry: $\chi^2 = 9$, $df = 10$, $P = 0.5321$). There was no evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 84% (1 year or less agreement of 98%) and a *CV* of 3% (test of symmetry: $\chi^2 = 23.2$, $df = 16$, $P = 0.1091$) (Figure 12.5).

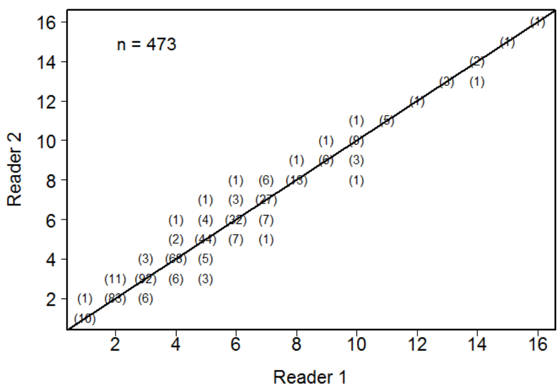


Figure 12.5: Between-reader comparison of otolith age estimates for Summer Flounder collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2023. The number in parentheses is number of fish.

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 3.6% (test of symmetry: $\chi^2 = 2.3$, $df = 4$, $P = 0.6747$). Reader 2 had an agreement of 94% with a *CV* of 1.4% (test of symmetry: $\chi^2 = 1$, $df = 2$, $P = 0.6065$).

12.3.4.2 *Scales*

Reader 1 aged the scales of 825 Summer Flounder collected in 2023. 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 92%) and a *CV* of 7% (test of symmetry: $\chi^2 = 13.3$, $df = 10$, $P = 0.2056$)

Reader 1 had no time series bias, having an agreement of 78% with ages of fish aged in 2000 and a *CV* of 5.4% (test of symmetry: $\chi^2 = 7.8$, $df = 5$, $P = 0.1676$).

12.3.5 Comparison of Scale and Otolith Ages

Reader 1 aged 473 pairs of Summer Flounder scales and otoliths. There was no evidence of systematic disagreement between otolith and scale ages (test of symmetry: $\chi^2 = 31.1$, $df = 33$, $P = 0.5598$) with an average *CV* of 9.6%. There was an agreement of 55% between scale and otoliths ages whereas scales were assigned a lower and higher age than otoliths for 26% and 18.8% of the fish, respectively (Figure 12.6). There was also little evidence of bias between otolith and scale ages using an age bias plot (Figure 12.7), with no trend of either over-ageing younger or under-ageing older fish.

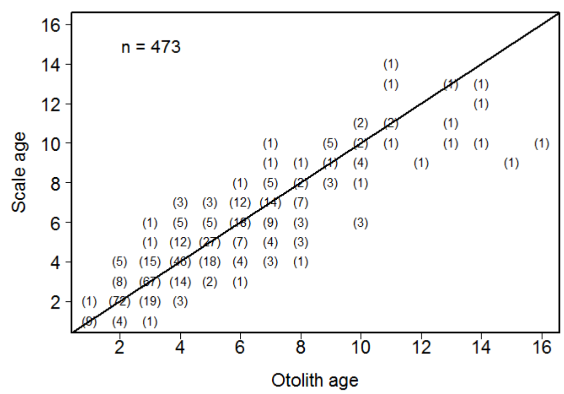


Figure 12.6: Comparison of scale and otolith age estimates for Summer Flounder collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2023. The number in parentheses is number of fish.

12.4 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 sum-

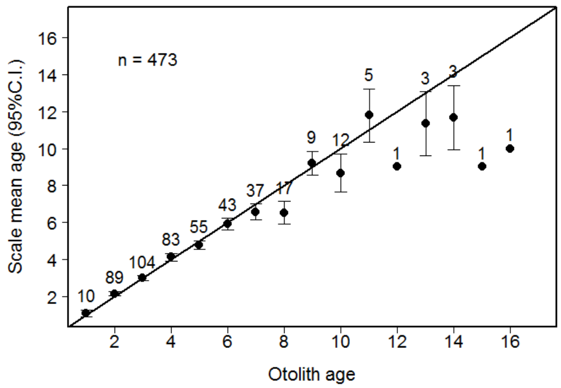


Figure 12.7: Age-bias plot for Summer Flounder scale and otolith age estimates in 2023. The number above the upper CI bar is number of fish.

mer flounder should be aged using otoliths, not scales, when possible.

Table 12.1: Number of bay Summer Flounder collected and aged in each 1-inch length interval in 2023. 'Target' represents the sample size for ageing estimated for 2023, 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
13 - 13.99	5	1	1	4
14 - 14.99	73	82	77	0
15 - 15.99	59	94	67	0
16 - 16.99	44	70	45	0
17 - 17.99	44	53	46	0
18 - 18.99	35	39	37	0
19 - 19.99	26	32	32	0
20 - 20.99	24	21	21	3
21 - 21.99	13	14	14	0
22 - 22.99	6	10	10	0
23 - 23.99	5	7	7	0
24 - 24.99	5	5	5	0
25 - 25.99	5	1	1	4
26 - 26.99	5	0	0	5
27 - 27.99	5	1	1	4
Totals	359	430	364	25

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Table 12.2: CV for each age estimated based on ageing the total of 359 bay Summer Flounder in 2023. 'Percent' is the percentage of an age in the pooled age-length data of bay Summer Flounder collected from 2017 to 2021.

Age	CV	Percent
0	>0.25	0.12
1	0.16	9.46
2	0.06	39.62
3	0.09	25.31
4	0.14	12.89
5	0.17	8.1
6	>0.25	3.25
7	>0.25	0.95
8	>0.25	0.24
9	>0.25	0.06

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Table 12.3: Number of ocean Summer Flounder collected and aged in each 1-inch length interval in 2023. 'Target' represents the sample size for ageing estimated for 2023, 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	5	5	0
14 - 14.99	37	60	51	0
15 - 15.99	56	80	65	0
16 - 16.99	59	66	62	0
17 - 17.99	53	49	49	4
18 - 18.99	41	31	31	10
19 - 19.99	30	23	23	7
20 - 20.99	24	22	22	2
21 - 21.99	23	22	22	1
22 - 22.99	23	16	16	7
23 - 23.99	23	18	18	5
24 - 24.99	22	23	23	0
25 - 25.99	19	19	19	0
26 - 26.99	16	15	15	1
27 - 27.99	14	11	11	3
28 - 28.99	10	8	8	2
29 - 29.99	6	9	9	0
30 - 30.99	5	8	8	0
31 - 31.99	5	3	3	2
32 - 32.99	5	1	1	4
Totals	476	489	461	48

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Table 12.4: CV for each age estimated based on ageing the total of 476 ocean Summer Flounder in 2023. 'Percent' is the percentage of an age in the pooled age-length data of ocean Summer Flounder collected from 2017 to 2021.

Age	CV	Percent
0	>0.25	0.04
1	>0.25	2.17
2	0.15	8.17
3	0.1	17.42
4	0.09	20.08
5	0.1	17.02
6	0.12	13.84
7	0.14	9.26
8	0.18	5.75
9	0.25	3.1
10	>0.25	1.57
11	>0.25	1.01
12	>0.25	0.44
13	>0.25	0.08
14	>0.25	0.04

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Table 12.5: The number of Summer Flounder assigned to each total length-at-age category for 364 fish sampled for both otolith and scale age determination in Chesapeake Bay, Virginia during 2023.

	Age							
Interval	1	2	3	4	5	6	7	Totals
13 - 13.99	1	0	0	0	0	0	0	1
14 - 14.99	8	49	19	1	0	0	0	77
15 - 15.99	4	52	10	1	0	0	0	67
16 - 16.99	3	28	13	1	0	0	0	45
17 - 17.99	0	26	17	2	1	0	0	46
18 - 18.99	0	4	23	9	1	0	0	37
19 - 19.99	0	5	21	5	1	0	0	32
20 - 20.99	0	2	12	5	1	1	0	21
21 - 21.99	0	2	4	2	3	3	0	14
22 - 22.99	0	0	3	3	1	0	3	10
23 - 23.99	0	0	3	2	1	1	0	7
24 - 24.99	0	0	1	2	1	1	0	5
25 - 25.99	0	0	0	0	1	0	0	1
27 - 27.99	0	0	0	0	1	0	0	1
Totals	16	168	126	33	12	6	3	364

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Table 12.6: The number of Summer Flounder assigned to each total length-at-age category for 461 fish sampled for both otolith and scale age determination in Virginia waters of Atlantic ocean during 2023.

	Age															
Interval	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Totals
13 - 13.99	1	2	1	0	1	0	0	0	0	0	0	0	0	0	0	5
14 - 14.99	13	9	11	8	5	3	2	0	0	0	0	0	0	0	0	51
15 - 15.99	14	17	15	8	3	5	1	0	2	0	0	0	0	0	0	65
16 - 16.99	7	11	16	12	7	5	3	0	1	0	0	0	0	0	0	62
17 - 17.99	3	10	10	7	6	5	5	0	2	0	0	1	0	0	0	49
18 - 18.99	0	4	10	4	4	6	0	1	0	0	0	1	0	1	0	31
19 - 19.99	0	1	9	8	1	1	3	0	0	0	0	0	0	0	0	23
20 - 20.99	0	3	2	6	5	3	0	2	0	0	0	0	1	0	0	22
21 - 21.99	0	2	4	5	7	4	0	0	0	0	0	0	0	0	0	22
22 - 22.99	0	0	2	2	5	6	1	0	0	0	0	0	0	0	0	16
23 - 23.99	0	0	1	5	5	3	4	0	0	0	0	0	0	0	0	18
24 - 24.99	0	0	2	5	6	4	2	2	2	0	0	0	0	0	0	23
25 - 25.99	0	0	1	1	5	4	2	3	2	1	0	0	0	0	0	19
26 - 26.99	0	0	0	1	1	5	0	5	1	2	0	0	0	0	0	15
27 - 27.99	0	0	0	0	2	3	2	0	1	1	1	0	1	0	0	11
28 - 28.99	0	0	0	0	1	1	4	1	1	0	0	0	0	0	0	8
29 - 29.99	0	0	0	0	0	0	0	2	5	1	1	0	0	0	0	9
30 - 30.99	0	0	0	0	0	0	1	4	1	0	1	1	0	0	0	8
31 - 31.99	0	0	0	0	0	0	0	0	1	0	0	0	1	0	1	3
32 - 32.99	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1
Totals	38	59	84	72	64	58	30	20	20	5	3	3	3	1	1	461

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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 Summer Flounder sampled in Chesapeake Bay, Virginia during 2023.

	Age						
Interval	1	2	3	4	5	6	7
13 - 13.99	1	0	0	0	0	0	0
14 - 14.99	0.1	0.64	0.25	0.01	0	0	0
15 - 15.99	0.06	0.78	0.15	0.01	0	0	0
16 - 16.99	0.07	0.62	0.29	0.02	0	0	0
17 - 17.99	0	0.57	0.37	0.04	0.02	0	0
18 - 18.99	0	0.11	0.62	0.24	0.03	0	0
19 - 19.99	0	0.16	0.66	0.16	0.03	0	0
20 - 20.99	0	0.1	0.57	0.24	0.05	0.05	0
21 - 21.99	0	0.14	0.29	0.14	0.21	0.21	0
22 - 22.99	0	0	0.3	0.3	0.1	0	0.3
23 - 23.99	0	0	0.43	0.29	0.14	0.14	0
24 - 24.99	0	0	0.2	0.4	0.2	0.2	0
25 - 25.99	0	0	0	0	1	0	0
27 - 27.99	0	0	0	0	1	0	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 Summer Flounder sampled in Virginia waters of the Atlantic Ocean during 2023.

	Age														
Interval	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
13 - 13.99	0.2	0.4	0.2	0	0.2	0	0	0	0	0	0	0	0	0	0
14 - 14.99	0.25	0.18	0.22	0.16	0.1	0.06	0.04	0	0	0	0	0	0	0	0
15 - 15.99	0.22	0.26	0.23	0.12	0.05	0.08	0.02	0	0.03	0	0	0	0	0	0
16 - 16.99	0.11	0.18	0.26	0.19	0.11	0.08	0.05	0	0.02	0	0	0	0	0	0
17 - 17.99	0.06	0.2	0.2	0.14	0.12	0.1	0.1	0	0.04	0	0	0.02	0	0	0
18 - 18.99	0	0.13	0.32	0.13	0.13	0.19	0	0.03	0	0	0	0.03	0	0.03	0
19 - 19.99	0	0.04	0.39	0.35	0.04	0.04	0.13	0	0	0	0	0	0	0	0
20 - 20.99	0	0.14	0.09	0.27	0.23	0.14	0	0.09	0	0	0	0	0.05	0	0
21 - 21.99	0	0.09	0.18	0.23	0.32	0.18	0	0	0	0	0	0	0	0	0
22 - 22.99	0	0	0.12	0.12	0.31	0.38	0.06	0	0	0	0	0	0	0	0
23 - 23.99	0	0	0.06	0.28	0.28	0.17	0.22	0	0	0	0	0	0	0	0
24 - 24.99	0	0	0.09	0.22	0.26	0.17	0.09	0.09	0.09	0	0	0	0	0	0
25 - 25.99	0	0	0.05	0.05	0.26	0.21	0.11	0.16	0.11	0.05	0	0	0	0	0
26 - 26.99	0	0	0	0.07	0.07	0.33	0	0.33	0.07	0.13	0	0	0	0	0
27 - 27.99	0	0	0	0	0.18	0.27	0.18	0	0.09	0.09	0.09	0	0.09	0	0
28 - 28.99	0	0	0	0	0.12	0.12	0.5	0.12	0.12	0	0	0	0	0	0
29 - 29.99	0	0	0	0	0	0	0	0.22	0.56	0.11	0.11	0	0	0	0
30 - 30.99	0	0	0	0	0	0	0.12	0.5	0.12	0	0.12	0.12	0	0	0
31 - 31.99	0	0	0	0	0	0	0	0	0.33	0	0	0	0.33	0	0.33
32 - 32.99	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0

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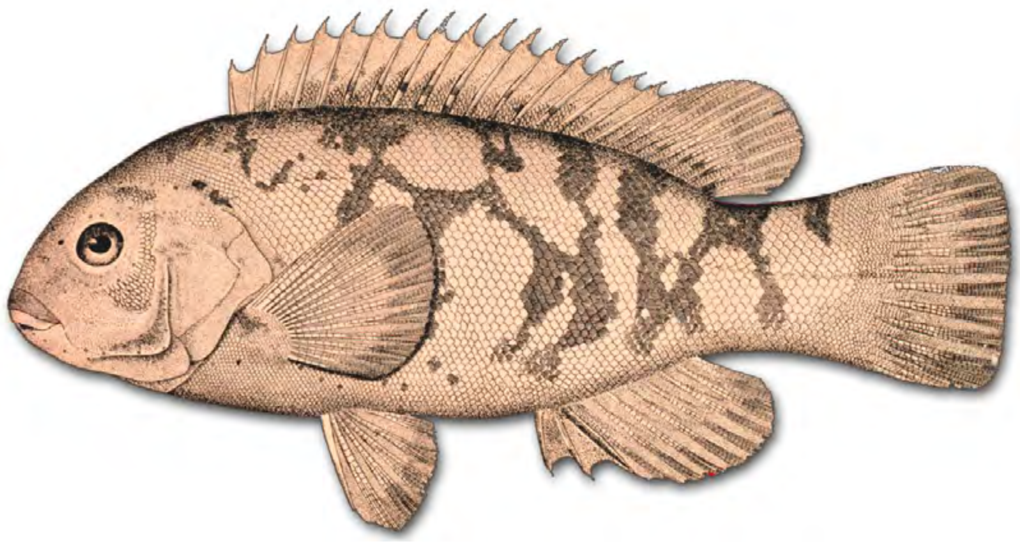
Chapter 13

TAUTOG *Tautoga onitis*



13.1 INTRODUCTION

We aged a total of 248 Tautog *Tautoga onitis*, collected by the VMRC's Biological Sampling Program in 2023. Of 248 aged fish, 242 and 6 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 2.1 and a standard error of 0.13. Thirteen age classes (2 to 13, and 15) were represented in the bay fish, comprising fish from the 2008, and 2010 to 2021 year classes. The bay fish sample in 2023 was dominated by the year classes of 2019, 2020, and 2021 with 39%, 23%, and 10%, respectively. Only 6 ocean fish were collected, 6, 8 to 9, and 15 to 16 years old, and in the year class of 2007 to 2008, 2014 to 2015, and 2017.

Of the 248 samples aged, 242 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 (242 pairs) and between otolith- and spine-ages (242 pairs), respectively.

13.2 METHODS

13.2.1 Sample Size for Ageing

We estimated sample sizes for ageing Tautog collected in both Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2023, 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 Tautog in 2023; 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 2017 to 2021. θ_a, V_a, and B_a were calculated using pooled age-length data of Tautog collected from 2017 to 2021 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 2017 to 2021 catch. A_l is number of fish to be aged for length interval l in 2023.

13.2.2 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.

13.2.3 Hardpart Preparation

13.2.3.1 *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 VMRC Ageing Lab website on how to prepare otolith thin-section for ageing Tautog using the Epoxy Resin Method.

13.2.3.2 *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 Ageing Lab website on how to prepare opercula for ageing Tautog.

13.2.3.3 *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 as 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.

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

13.2.4 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 of sectioned otoliths 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. Reader 1 aged all Tautog samples of opercula and sectioned spines. Since Reader 2 didn't have experience on ageing these two calcified structures, Reader 2 didn't age them collected in 2023. After trained in 2024, Reader 2 will be able to age the spines and opercula collected in 2024 during the early 2025.

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). 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 13.1: 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.

13.2.4.2 *Opercula*

Opercula were aged on a light table with no magnification (Figure 13.2). We didn't let Reader 2 age the opercula because Reader 2 just started the job as the Chief Technician and didn't have enough time to practice ageing them. Reader 2 will age the opercula collected in 2024 during early 2025. Therefore, Reader 1's operculum ages were also the final operculum ages of Tautog collected in 2023. Tautog opercula are also considered to have a deposition period of May through July (Hostetter and Munroe 1993), and age class assignment using

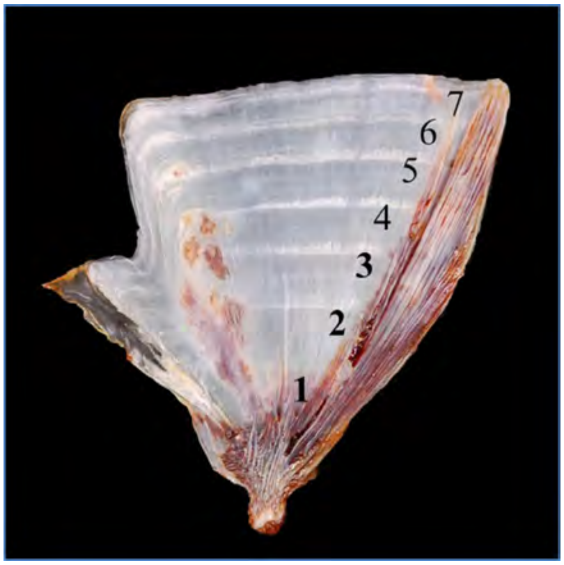


Figure 13.2: Operculum of a 7 year-old Tautog

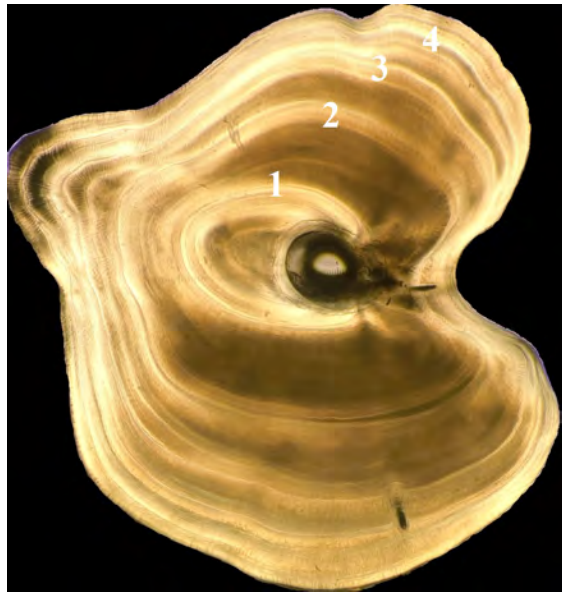


Figure 13.3: Spine of a 4 year-old Tautog

these hard-parts is conducted in the same way as otoliths.

13.2.4.3 *Spines*

All spine thin-sections were aged using an Olympus BX41 compound microscope (Figure 13.3). We didn't let Reader 2 age the spines because Reader 2 just started the job as the Chief Technician and didn't have enough time to practice ageing them. Reader 2 will age the spines collected in 2024 during early 2025. Since there were more than one sections per slide, Reader 1 used a black Sharpie dot to mark the section which was used to estimate the age of the fish. Therefore, Reader 1's spine ages were also the final spine ages of Tautog collected in 2023.

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) on the otolith ages between the two readers in the current year; 2) on the otolith ages within each reader in the current year; 3) time-series bias on the otolith ages between the current and previous years within each reader; 4) on the operculum ages within Reader 1 in the current year; 5) time-series bias on the operculum ages within Reader 1; 6) on the spine ages within Reader 1 in the current year; 7) between Reader 1's operculum ages and the final otoliths ages; and 8) between Reader 1's spine ages and the final otoliths ages. The readings on the otolith thin-sections from the entire sample for the current year were used to examine the difference between two readers. A random sub-sample of the opercula, and spine and otolith slides from 50 fish from the current year was selected for second readings to examine the difference within a reader (Reader 1 read the opercula, spine and otolith sub-samples whereas Reader 2 read only the otolith sub-samples). Fifty otoliths and opercula randomly selected from fish aged in 2000 were used to examine the time-series bias within each reader (Reader 1 read both operculum and otolith random samples whereas Reader 2 read only the otolith random samples). Since we just started to age the spines in 2022, we didn't have enough experience on ageing the spines to setup a set of the spines for time-series analysis in 2023. We expect that we may have such a set ready in a couple of years.

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 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).

13.3.1.1 *Chesapeake Bay*

We estimated a sample size of 456 bay Tautog in 2023, ranging in length interval from 8 to 26 inches (Table 13.1). 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 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 otoliths, opercula, and spines (236 fish). We aged 6 fish with opercula and spines. As a result, we aged all of 242 fishcollected by VMRC in Chesapeake Bay in 2023. We fell short in our over-all collections for this optimal length-class sampling estimate by 255 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 454 ocean Tautog in 2023, ranging in length interval from 8 to 30 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 5 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 otoliths, opercula, and spines (6 fish). As a result, we aged all of 6 fish collected by VMRC in Virginia waters of Atlantic Ocean in 2023. We fell short in our over-all collections for this optimal length-class sampling estimate by 448 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.

13.3.2 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.

13.3.2.1 *Chesapeake Bay*

Of the 242 bay Tautog aged, 13 age classes (2 to 13, and 15) were represented (Table 13.5). The average age for the sample was 4.5 years. The standard deviation and standard error were 2.1 and 0.13, respectively. Year-class data (Figure 13.4) indicates that recruitment into the fishery in Chesapeake Bay begins at age 2, which corresponds to the 2021 year-class for Tautog caught in 2023. Tautog in the sample in 2023 was dominated by the year classes of 2019, 2020, and 2021 with 39%, 23%, and 10%, respectively. The sex ratio of male to female was 1:1.17 for the bay fish.

13.3.2.2 *Atlantic Ocean*

Only 6 ocean fish was collected and aged, 6, 8 to 9, and 15 to 16 years old, and in the year class of 2007 to 2008, 2014 to 2015, and 2017 (Table 13.6).

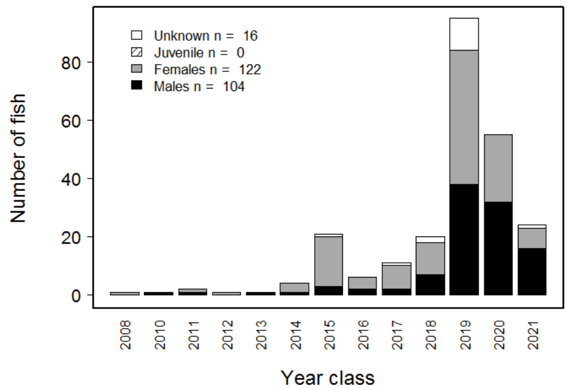


Figure 13.4: Year-class frequency distribution for Tautog collected in Chesapeake Bay, Virginia for ageing in 2023. Distribution is broken down by sex. 'Unknown' represents the fish gonads that were not available for examination or were not examined for sex during sampling.

13.3.3 Age-Length-Key (ALK)

We developed an age-length-key for bay fish (Table 13.7) using all the aged fish described in Section Sample Size. No ALK was developed for the ocean tautog because there was only 6 ocean fish collected and aged in 2023. 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.

13.3.4 Reading Precision

13.3.4.1 *Otoliths*

Reader 1 and Reader 2 aged the otoliths of 242 Tautog collected in 2023. 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 88% and a *CV* of 2.4% (test of symmetry: $\chi^2 = 6$, $df = 4$, $P = 0.1991$).There was no evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 89% (1 year or less agreement of 100%) and a *CV* of 1.8% (test of symmetry: $\chi^2 = 11.8$, $df = 9$, $P = 0.2248$) (Figure 13.5).

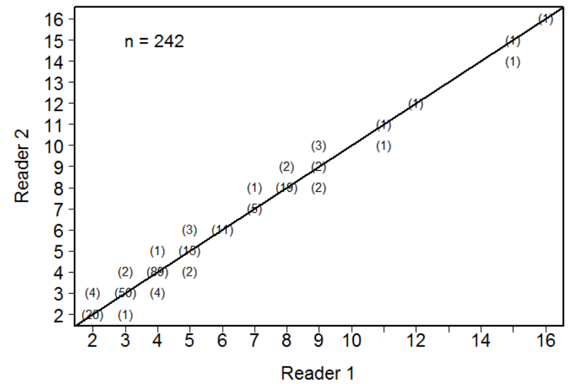


Figure 13.5: Between-reader comparison of otolith age estimates for Tautog collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2023. The number in parentheses is number of fish.

There was no time-series bias for either reader. Reader 1 had an agreement of 92% with ages of fish aged in 2000 with a *CV* of 1% (test of symmetry: $\chi^2 = 4$, $df = 2$, $P = 0.1353$). Reader 2 had an agreement of 86% with a *CV* of 1.5% (test of symmetry: $\chi^2 = 7$, $df = 3$, $P = 0.0719$).

13.3.4.2 *Opercula*

Reader 1 aged the opercula of 248 Tautog collected in 2023. There was no significant difference between the first and second readings for Reader 1 with an agreement of 80% (1 year or less agreement of 98%) and a *CV* of 3% (test of symmetry: $\chi^2 = 7.3$, $df = 6$, $P = 0.2911$).

Reader 1 had no time-series bias on ageing Tautog opercula. Reader 1 had an agreement of 58% (1 year or less agreement of 94%) with ages of fish aged in 2000 with a *CV* of 5.5% (test of symmetry: $\chi^2 = 8.3$, $df = 12$, $P = 0.7586$).

13.3.4.3 *Spines*

Reader 1 aged the spines of 248 Tautog collected in 2023. There was no significant difference between the first and second readings for Reader 1 with an agreement of 56% (1 year or less agreement of 92%) and a *CV* of 8.3% (test of symmetry: $\chi^2 = 9.2$, $df = 10$, $P = 0.5132$),

We didn't conduct time-series comparison in spine ageing. We believe that we need to practice on ageing the spines for at least more than two years before we are able to setup a set of spines for the examination of time-series precision.

13.3.5 Comparisons

13.3.5.1 *Operculum vs otolith ages*

Reader 1 aged 242 pairs of Tautog opercula and otoliths. There was no evidence of systematic disagreement between otolith and operculum ages (test of symmetry: $\chi^2 = 22.2$, $df = 20$, $P = 0.3273$) with an average *CV* of 6.4%. There was an agreement of 67% between operculum and otoliths ages whereas opercula were assigned a lower and higher age than otoliths for 16.5% and 16.1% of the fish, respectively (Figure 13.6). There was also little evidence of bias between otolith and operculum ages using an age bias plot(Figure 13.7), with no trend of either over-ageing younger or under-ageing older fish.

Figure 13.6: Comparison of operculum and otolith age estimates for Tautog collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2023. The number in parentheses is number of fish.

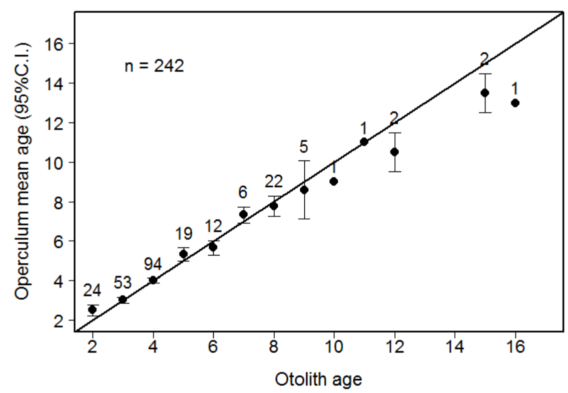


Figure 13.7: Age-bias plot for Tautog operculum and otolith age estimates in 2023. The number above the upper CI bar is number of fish.

13.3.5.2 *Spine vs otolith ages*

Reader 1 aged 242 pairs of Tautog spines and otoliths. There was an evidence of systematic disagreement between otolith and spine ages (test of symmetry: $\chi^2 = 42.1$, $df = 24$, $P = 0.0126$) with an average *CV* of 11.5%. There was an agreement of 49% between spine and otoliths ages whereas spines were assigned a lower and higher age than otoliths for 24.4% and 26.4% of the fish, respectively (Figure 13.8). There was also an evidence of bias between otolith and spine ages using an age bias plot (Figure 13.9), with spine generally assigned higher ages for younger fish and lower ages for older fish than otolith age estimates.

13.4 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.

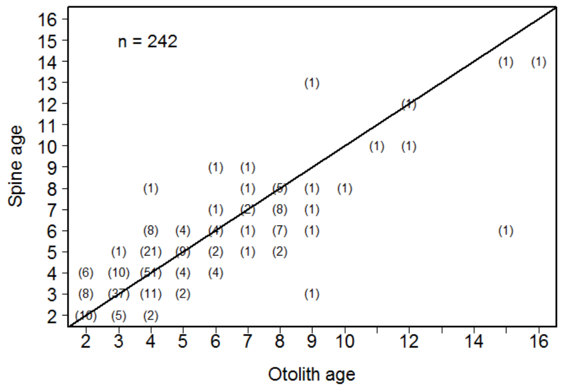


Figure 13.8: Comparison of spine and otolith age estimates for Tautog collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2023. The number in parentheses is number of fish.

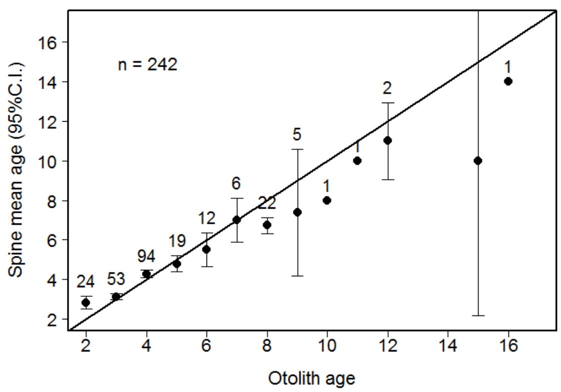


Figure 13.9: Age-bias plot for Tautog spine and otolith age estimates in 2023. The number above the upper CI bar is number of fish.

Table 13.1: Number of bay Tautog collected and aged in each 1-inch length interval in 2023. 'Target' represents the sample size for ageing estimated for 2023, 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	4	4	1
10 - 10.99	5	9	9	0
11 - 11.99	5	20	20	0
12 - 12.99	7	29	29	0
13 - 13.99	37	23	23	14
14 - 14.99	76	39	39	37
15 - 15.99	90	48	48	42
16 - 16.99	76	40	40	36
17 - 17.99	58	18	18	40
18 - 18.99	33	5	5	28
19 - 19.99	20	6	6	14
20 - 20.99	9	1	1	8
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
Totals	456	242	242	255

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Table 13.2: CV for each age estimated based on ageing the total of 456 bay Tautog in 2023. 'Percent' is the percentage of an age in the pooled age-length data of bay Tautog collected from 2017 to 2021.

Age	CV	Percent
1	>0.25	1.13
2	0.17	6.29
3	0.1	18.43
4	0.09	21.74
5	0.1	20.44
6	0.12	13.16
7	0.18	6.66
8	0.21	4.91
9	>0.25	2.84
10	>0.25	1.94
11	>0.25	0.68
12	>0.25	0.64
13	>0.25	0.6
14	>0.25	0.34
15	>0.25	0.11
16	>0.25	0.06
17	>0.25	0.02
18	>0.25	0.02

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Table 13.3: Number of ocean Tautog collected and aged in each 1-inch length interval in 2023. 'Target' represents the sample size for ageing estimated for 2023, 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	5	0	0	5
13 - 13.99	32	0	0	32
14 - 14.99	46	1	1	45
15 - 15.99	57	2	2	55
16 - 16.99	62	0	0	62
17 - 17.99	43	0	0	43
18 - 18.99	36	0	0	36
19 - 19.99	28	1	1	27
20 - 20.99	25	0	0	25
21 - 21.99	18	0	0	18
22 - 22.99	17	0	0	17
23 - 23.99	14	1	1	13
24 - 24.99	9	0	0	9
25 - 25.99	8	0	0	8
26 - 26.99	9	1	1	8
27 - 27.99	7	0	0	7
28 - 28.99	5	0	0	5
29 - 29.99	5	0	0	5
30 - 30.99	5	0	0	5
Totals	454	6	6	448

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Table 13.4: CV for each age estimated based on ageing the total of 454 ocean Tautog in 2023. 'Percent' is the percentage of an age in the pooled age-length data of ocean Tautog collected from 2017 to 2021.

Age	CV	Percent
2	>0.25	1.99
3	0.15	8.53
4	0.11	13.9
5	0.09	20.44
6	0.1	17.52
7	0.15	9.46
8	0.16	8.18
9	0.23	3.86
10	>0.25	2.69
11	>0.25	2.34
12	>0.25	1.4
13	>0.25	2.34
14	>0.25	1.29
15	>0.25	1.52
16	>0.25	1.4
17	>0.25	0.47
18	>0.25	0.7
20	>0.25	0.47
21	>0.25	0.23
22	>0.25	0.23
23	>0.25	0.47
24	>0.25	0.12
27	>0.25	0.23
30	>0.25	0.12
31	>0.25	0.12

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Table 13.5: The number of Tautog assigned to each total length-at-age category for 242 fish sampled for both otolith and operculum age determination in Chesapeake Bay, Virginia during 2023.

	Age													
Interval	2	3	4	5	6	7	8	9	10	11	12	13	15	Totals
9 - 9.99	4	0	0	0	0	0	0	0	0	0	0	0	0	4
10 - 10.99	8	1	0	0	0	0	0	0	0	0	0	0	0	9
11 - 11.99	6	7	6	1	0	0	0	0	0	0	0	0	0	20
12 - 12.99	2	16	10	1	0	0	0	0	0	0	0	0	0	29
13 - 13.99	2	12	9	0	0	0	0	0	0	0	0	0	0	23
14 - 14.99	2	11	21	1	1	2	1	0	0	0	0	0	0	39
15 - 15.99	0	4	27	9	5	1	1	1	0	0	0	0	0	48
16 - 16.99	0	3	14	7	2	2	9	2	1	0	0	0	0	40
17 - 17.99	0	0	8	1	3	1	5	0	0	0	0	0	0	18
18 - 18.99	0	1	0	0	0	0	3	0	0	0	1	0	0	5
19 - 19.99	0	0	0	0	0	0	2	1	0	1	1	1	0	6
20 - 20.99	0	0	0	0	0	0	0	0	0	0	0	0	1	1
Totals	24	55	95	20	11	6	21	4	1	1	2	1	1	242

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Table 13.6: The number of Tautog assigned to each total length-at-age category for 6 fish sampled for both otolith and operculum age determination in Virginia waters of Atlantic ocean during 2023.

	Age					
Interval	6	8	9	15	16	Totals
14 - 14.99	0	0	1	0	0	1
15 - 15.99	1	1	0	0	0	2
19 - 19.99	1	0	0	0	0	1
23 - 23.99	0	0	0	0	1	1
26 - 26.99	0	0	0	1	0	1
Totals	2	1	1	1	1	6

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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 operculum ages for Tautog sampled in Chesapeake Bay, Virginia during 2023.

	Age												
Interval	2	3	4	5	6	7	8	9	10	11	12	13	15
9 - 9.99	1	0	0	0	0	0	0	0	0	0	0	0	0
10 - 10.99	0.89	0.11	0	0	0	0	0	0	0	0	0	0	0
11 - 11.99	0.3	0.35	0.3	0.05	0	0	0	0	0	0	0	0	0
12 - 12.99	0.07	0.55	0.34	0.03	0	0	0	0	0	0	0	0	0
13 - 13.99	0.09	0.52	0.39	0	0	0	0	0	0	0	0	0	0
14 - 14.99	0.05	0.28	0.54	0.03	0.03	0.05	0.03	0	0	0	0	0	0
15 - 15.99	0	0.08	0.56	0.19	0.1	0.02	0.02	0.02	0	0	0	0	0
16 - 16.99	0	0.07	0.35	0.17	0.05	0.05	0.22	0.05	0.03	0	0	0	0
17 - 17.99	0	0	0.44	0.06	0.17	0.06	0.28	0	0	0	0	0	0
18 - 18.99	0	0.2	0	0	0	0	0.6	0	0	0	0.2	0	0
19 - 19.99	0	0	0	0	0	0	0.33	0.17	0	0.17	0.17	0.17	0
20 - 20.99	0	0	0	0	0	0	0	0	0	0	0	0	1

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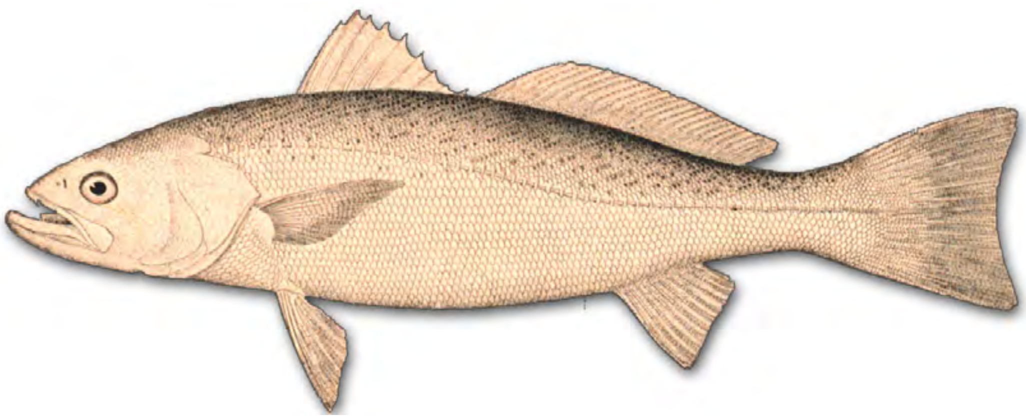
Chapter 14

WEAKFISH *Cynoscion regalis*



14.1 INTRODUCTION

We aged a total of 282 Weakfish *Cynoscion regalis*, collected by the VMRC's Biological Sampling Program for age and growth analysis in 2023. The Weakfish ages ranged from 1 to 5 years old with an average age of 2.3, a standard deviation of 0.9, and a standard error of 0.05. Five age classes (1 to 5) were represented, comprising fish of the 2018 to 2022 year-classes. The sample was dominated by fish from the year-classes of 2020, 2021, and 2022 with 43.6%, 32.3%, and 20.6%, respectively.

14.2 METHODS

14.2.1 Sample Size for Ageing

We estimated sample size for ageing Weakfish in 2023 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 Weakfish in 2023; 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 2017 to 2021. θ_a, V_a, and B_a were calculated using pooled age-length data of Weakfish collected from 2017 to 2021 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 2017 to 2021 catch. A_l is number of fish to be aged for length interval l in 2023.

14.2.2 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.

14.2.3 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 VMRC Ageing Lab website on how to prepare otolith thin-section for ageing Weakfish using the Glue Method.

14.2.4 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 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.

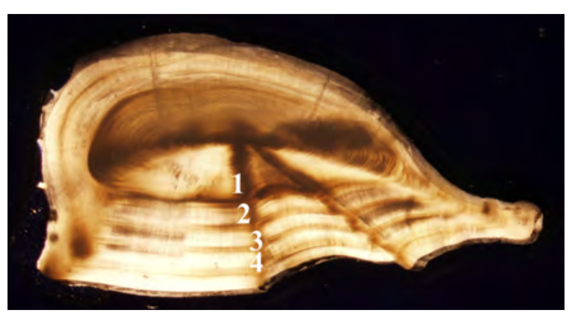


Figure 14.1: Otolith thin-section of 4 year-old Weakfish

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.

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 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).

14.3 RESULTS

14.3.1 Sample Size

We estimated a sample size of 383 for ageing Weakfish in 2023, ranging in length interval from 4 to 34 inches (Table 14.1). This sample size provided a range in *CV* for age composition approximately from the smallest *CV* of 6% for Age 2 and 3 to the *CV* of larger than 25% for the multiple minor ages (Table 14.2). In 2023, we aged 282 of 296 Weakfish (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 129 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.

14.3.2 Year Class

Of the 282 fish aged with otoliths, 5 age classes (1 to 5) were represented (Table 14.3). The average age was 2.3 years, and the standard deviation and standard error were 0.9 and 0.05, respectively. Year-class data show that the fishery was comprised of 5 year-classes: fish from the 2018 to 2022 year-classes, with fish primarily from the year-classes of 2020, 2021, and 2022 with 43.6%, 32.3%, and 20.6%, respectively. The ratio of males to females was 1:4.42 in the sample collected (Figure 14.2).

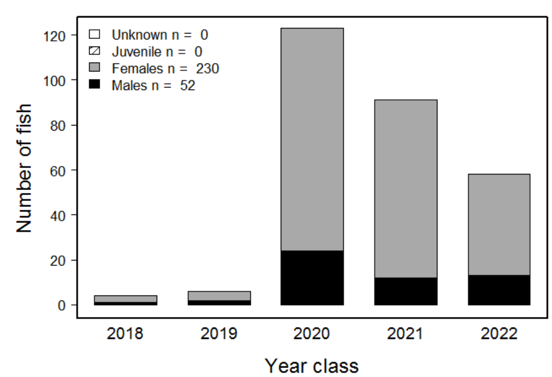


Figure 14.2: Year-class frequency distribution for Weakfish collected for ageing in 2023. Distribution is broken down by sex. 'Unknown' represents gonads that were not available for examination or were not examined for sex during sampling.

14.3.3 Age-length Key (ALK)

We developed an age-length-key (Table 14.4) 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.

14.3.4 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 3.09% (test of symmetry: $\chi^2 = 1.33$, $df = 2$, $P = 0.5134$). There was no evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 96.1% and a *CV* of 1.81% (test of symmetry: $\chi^2 = 4.8$, $df = 4$, $P = 0.3084$) (Figure 14.3).

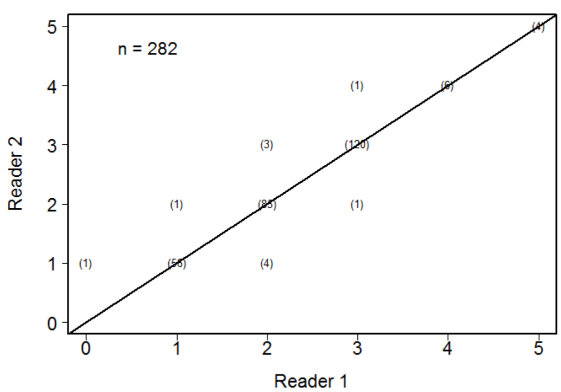


Figure 14.3: Between-reader comparison of otolith age estimates for Weakfish collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2023. The number in parentheses is number of fish.

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 0.19% (test of symmetry: $\chi^2 = 1$, $df = 1$, $P = 0.3173$). Reader 2 also had an agreement of 100%.

Table 14.1: Number of Weakfish collected and aged in each 1-inch length interval in 2023. 'Target' represents the sample size for ageing estimated for 2023, 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
6 - 6.99	5	0	0	5
7 - 7.99	5	4	4	1
8 - 8.99	12	22	22	0
9 - 9.99	34	29	29	5
10 - 10.99	54	33	33	21
11 - 11.99	47	36	36	11
12 - 12.99	39	32	32	7
13 - 13.99	26	37	33	0
14 - 14.99	19	36	26	0
15 - 15.99	24	17	17	7
16 - 16.99	16	14	14	2
17 - 17.99	11	8	8	3
18 - 18.99	9	11	11	0
19 - 19.99	7	9	9	0
20 - 20.99	5	3	3	2
21 - 21.99	5	2	2	3
22 - 22.99	5	1	1	4
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	1	1	4
27 - 27.99	5	1	1	4
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
33 - 33.99	5	0	0	5
34 - 34.99	5	0	0	5
Totals	383	296	282	129

(Go back to text)

Table 14.2: CV for each age estimated based on ageing the total of 383 Weakfish in 2023. 'Percent' is the percentage of an age in the pooled age-length data of Weakfish collected from 2017 to 2021.

Age	CV	Percent
0	>0.25	0.41
1	0.1	17.42
2	0.06	42.11
3	0.06	34.1
4	0.19	5.53
5	>0.25	0.25
6	>0.25	0.17

(Go back to text)

Table 14.3: The number of Weakfish assigned to each total length-at-age category for 282 fish sampled for otolith age determination in Virginia during 2023.

	Age					
Interval	1	2	3	4	5	Totals
7 - 7.99	4	0	0	0	0	4
8 - 8.99	15	7	0	0	0	22
9 - 9.99	7	19	3	0	0	29
10 - 10.99	9	13	11	0	0	33
11 - 11.99	4	14	17	1	0	36
12 - 12.99	6	7	19	0	0	32
13 - 13.99	5	7	21	0	0	33
14 - 14.99	5	8	13	0	0	26
15 - 15.99	3	5	7	2	0	17
16 - 16.99	0	6	8	0	0	14
17 - 17.99	0	1	6	1	0	8
18 - 18.99	0	1	9	0	1	11
19 - 19.99	0	1	6	1	1	9
20 - 20.99	0	1	2	0	0	3
21 - 21.99	0	1	0	0	1	2
22 - 22.99	0	0	1	0	0	1
26 - 26.99	0	0	0	1	0	1
27 - 27.99	0	0	0	0	1	1
Totals	58	91	123	6	4	282

(Go back to text)

Table 14.4: 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 2023.

	Age				
Interval	1	2	3	4	5
7 - 7.99	1	0	0	0	0
8 - 8.99	0.68	0.32	0	0	0
9 - 9.99	0.24	0.66	0.1	0	0
10 - 10.99	0.27	0.39	0.33	0	0
11 - 11.99	0.11	0.39	0.47	0.03	0
12 - 12.99	0.19	0.22	0.59	0	0
13 - 13.99	0.15	0.21	0.64	0	0
14 - 14.99	0.19	0.31	0.5	0	0
15 - 15.99	0.18	0.29	0.41	0.12	0
16 - 16.99	0	0.43	0.57	0	0
17 - 17.99	0	0.12	0.75	0.12	0
18 - 18.99	0	0.09	0.82	0	0.09
19 - 19.99	0	0.11	0.67	0.11	0.11
20 - 20.99	0	0.33	0.67	0	0
21 - 21.99	0	0.5	0	0	0.5
22 - 22.99	0	0	1	0	0
26 - 26.99	0	0	0	1	0
27 - 27.99	0	0	0	0	1

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Chapter 15

APPENDIX

COMPARISONS IN MENHADEN *BREVOORTIA TYRANNUS* AGES ESTIMATED USING SCALES (GLASS VS. ACETATE SLIDE) AND OTOLITHS (WHOLE OTOLITHS VS. THIN-SECTION)

15.1 INTRODUCTION

The NOAA Beaufort Laboratory, NC, has been ageing Atlantic Menhaden *Brevoortia tyrannus* using their scales since 1955. As a result, several Atlantic States, including Virginia (VMRC), have been sending their Menhaden scale samples to the Beaufor Ageing Lab for ageing Menhaden for multiple years. Currently the VMRC Ageing Lab is preparing to start ageing menhaden collected by the VMRC biological sampling program instead of sending the samples to the Beaufort Ageing Lab. During our ageing preparation and practice, we realized that the only verification of scale ageing was conducted by June and Roithmayr (1960) using known-age fish, but limited to verify the formation of the first annulus. It was reported that Age-6 and older fish were rarely observed when scales were used to age Menhaden, however, it is well-known that scale ages may underestimate ages for older fish (Liao et al. 2013), and moreover no previous studies has verified scale ageing on older Menhaden. Because known-age menhaden, especially older menhaden, are unavailable for ageing verification, and also because the previous studies found that otoliths provide more accurate and precise age estimates than scales for many fish species, we recommend to collect and age both scales and otoliths from Menhaden, and to compare their otolith and scale ages to see if the scale age underestimates the ages for older menhaden. The potential findings from this project: 1. There is no difference between scale and otolith ages, supporting that scale ages be continuously used for Menhaden stock assessment; or 2. Scale age may underestimate ages for older menhaden, providing evidence that further studies may need to identify the effects of such an ageing error on menhaden stock assessment and its fisheries management. This project will also prepare us to start ageing Menhaden in-house in the near future.

15.2 METHODS

15.2.1 Preparation

To decide which hardpart, otolith vs scale, will provide more precise estimates of Menhaden ages, we collected both scales and otoliths from each fish, and made slides from them, respectively. There are more than one ways to make scale and otolith slides. To compare which ways may provide more precise age estimates, we made two sets of scale slides and two sets of otolith slides from each fish whenever the second otoliths from a fish was available.

15.2.1.1 *Acetate slides of scale impressions*

We made an acetate slide of scales from the each fish, following the VMRC Protocol on Preparation of Scale Impressions for Age Estimation on how to make an acetate scale slide.

15.2.1.2 *Glass slides of scales*

Because the NOAA Ageing Lab at Beaufort in NC uses glass scale slides to age Menhaden for many years, for consistency between the labs, we made glass slides of Menhaden scales following the NOAA Beaufort Ageing Lab methods. More specifically, we selected and cleaned the scales (not used for the acetate slide) from the same fish used for the acetate slide following the methods listed in the protocol (Please see the protocol above). After cleaning them, instead of pressing the scales on an acetate slide using a hydraulic press, we put them between two microscope slides and taped both ends of the slides.

15.2.1.3 *Otolith thin-sections*

Because Menhaden otoliths are very small, we used the Epoxy Resin Method to section them. To increase their readability, we bake the whole otoliths before sectioning them. The details on the Epoxy Resin Method and Baking Method can be found in VMRC Protocol on Preparation of Otolith Transverse Cross-Sections for Age Estimation

15.2.1.4 *Whole otolith slide*

Using the second otolith from the same fish when it was available, we made a whole otolith slide by putting the otolith on the microscope slide and covered the otolith with a drop of Flo-texx to fix the otlith on the slide and increase its readability.

15.2.2 Readings

We aged the menhaden scales and otoliths using the methods listed in Chapter 9 (Page 9-49) in A Practical Handbook for Determining the Ages of Gulf of Mexico and Atlantic Coast Fishes (THIRD EDITION). All the slides were read by each reader independently 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 as its final age. 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 then assigned an agreed age between two readers to the fish as its final age. When the two readers were unable to agree on a final age, the fish was excluded from further analysis.

To demonstrate how a scale impression, scale between two slides, otolith thin-section, and whole otolith on a slide look like, we used the images of those slides made from the same fish with an Ageing and Growth Identification Number (AGID) of 440 in the next 4 sections.

15.2.2.1 *Scale impressions on acetate slide*

All acetate slides of scale impressions 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 (Figure 15.1). The ages estimated usig this method were hereinafter referred to as "acetate-scale age".

Figure 15.1: A scale impression on an acetate slide made from Menhaden AGID 440.

15.2.2.2 *Scales between two glass slides*

All glass slides of scales 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 (Figure 15.2). The ages estimated usig this method were hereinafter referred to as "glass-scale age".

Figure 15.2: A scale between two glass slides made from Menhaden AGID 440.

15.2.2.3 *Otolith thin-sections*

All thin-sections of otoliths were aged by two different readers using an Olympus BX41 compound microscope (Figure 15.3). The ages estimated usig this method were hereinafter referred to as "section-otolith age".

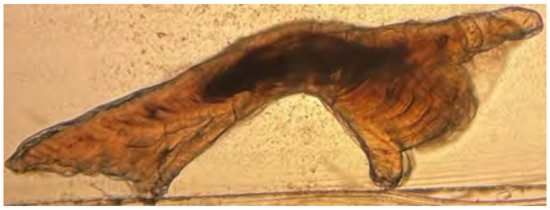

Figure 15.3: An otolith thin-section mounted on a glass slide made from Menhaden AGID 440.

15.2.2.4 *Whole otoliths*

All slides of 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 (Figure 15.4). The ages estimated usig this method were hereinafter referred to as "whole-otolith age".

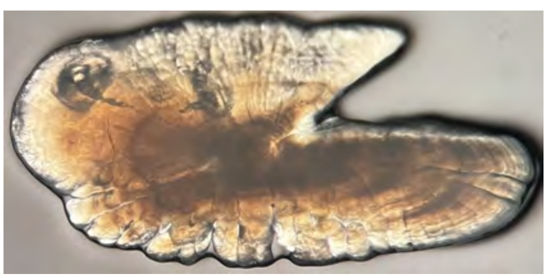

Figure 15.4: A whole otolith mounted on a glass slide made from Menhaden AGID 440.

15.2.3 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) on the acetate-scale ages within each reader; 2) on the acetate-scale ages between the two readers; 3) on the glass-scale ages within each reader; 4) on the glass-scale ages between the two readers; 5) on the section-otolith ages withn each reader; 6) on the section-otolith ages between the two readers; 7) on the whole-otolith ages within each reader; 8) on the whole-otolith ages between the two readers; 9) between the final acetate-scale and final glass-scale ages; 10) between the final section-otolith and whole-otolith ages.

Based on the comparisons above, we decided a scale age between the acetate-scale and glass-scale age as the final preferred scale age (Hereafter referred to as "prefer-scale age) and an otolith age between the section-otolith and whole-otolith age as the final preferred otolith age (Hereafter referred to as "prefer-otolith age"), respectively, for the further comparison between the prefer-scale and prefer-otolith age.

15.3 RESULTS

We collected 45 menhaden with both scales and otoliths in 2023. We made one acetate and one glass slide of scales, and one slide of thin-section otolith from each of the 45 fish. Of the 45 fish, we were able to collect the second otoliths from 35 fish, therefore, We made one whole otolith slide from each of them. Both readers aged all the slides (Table 15.1).

15.3.1 Reading Precision

15.3.1.1 *Acetate-scale age*

Reader 1 had high self-precision and Reader 2 had moderate self-precision on the acetate-scale ages. Specifically, there was no significant difference between the first and second readings for Reader 1 with an agreement of 76% (1 year or less agreement of 98%) and a mean CV of 5.11% (test of symmetry: $\chi^2 = 11$, df = 5, P = 0.0514), and there was no significant difference between the first and second readings for Reader 2 with an agreement of 64% (1 year or less agreement of 98%) and a mean CV of 14.67% (test of symmetry: $\chi^2 = 8.67$, df = 8, P = 0.3712). There was no evidence of systematic disagreement on the acetate-scale ages between Reader 1 and Reader 2 with an agreement of 60% (1 year or less agreement of 89%) and a mean CV of 10.37% (test of symmetry: χ^2 = 15.33, df = 8, P = 0.053) (Figure 15.5 and Table 15.2).

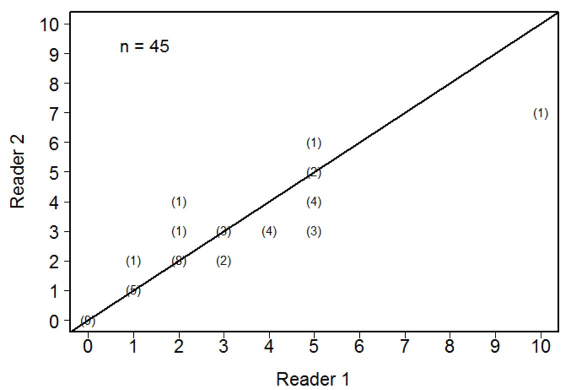

Figure 15.5: Between-reader comparison of the acetate-scale ages for Menhaden collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2023. The number in parentheses is number of fish.

15.3.1.2 *Glass-scale age*

Reader 1 had high self-precision on the glass-scale ages whereas Reader 2 had low self-precision with a systematic disagreement. Specifically, there was no significant difference between the first and second readings for Reader 1 with an agreement of 80% (1 year or less agreement of 98%) and a mean CV of 6.79% (test of symmetry: χ^2 = 6.33, df = 6, P = 0.3869), however, 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 93%) and a mean CV of 22.59% (test of symmetry: χ^2 = 16.5, df = 8, P = 0.0358). There was no evidence of systematic disagreement on the glass-scale ages between Reader 1 and Reader 2 with an agreement of 62% (1 year or less agreement of 87%) and a mean CV of 20.66% (test of symmetry: χ^2 = 14.33, df = 8, P = 0.0735) (Figure 15.6 and Table 15.2).

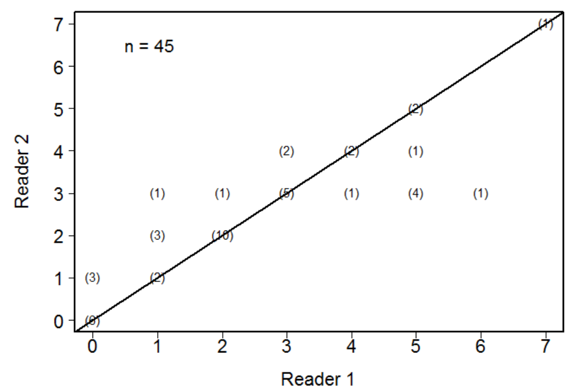

Figure 15.6: Between-reader comparison of the glass-scale ages for Menhaden collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2023. The number in parentheses is number of fish.

15.3.1.3 *Section-otolith age*

Both Reader 1 and 2 had moderate self-precision on the section-otolith ages. Specifically, there was no significant difference between the first and second readings for Reader 1 with an agreement of 44% (1 year or less agreement of 89%) and a mean CV of 21.4% (test of symmetry: χ^2 = 11.13, df = 10, P = 0.3472), and there was no significant difference between the first and second readings for Reader 2 with an agreement of 47% (1 year or less agreement of 84%) and a mean CV of 28.67% (test of symmetry: χ^2 = 16, df = 12, P = 0.1912). There was no evidence of systematic disagreement on the section-otolith ages between Reader 1 and Reader 2 with an agreement of 31% (1 year or less agreement of 76%) and a mean CV of 40.44% (test of symmetry: χ^2 = 21.67, df = 14, P = 0.0857) (Figure 15.7 and Table 15.2).

15.3.1.4 *Whole-otolith age*

Reader 1 had moderate self-precision on the whole-otolith ages whereas Reader 2 had low self-precision with a systematic disagreement. Specifically, there was no significant difference between the first and second readings for Reader 1 with an agreement of 46% (1 year or less agreement of 89%) and a mean CV of

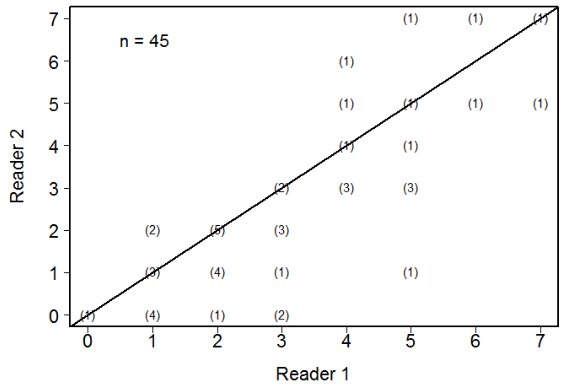

Figure 15.7: Between-reader comparison of the section-otolith ages for Menhaden collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2023. The number in parentheses is number of fish.

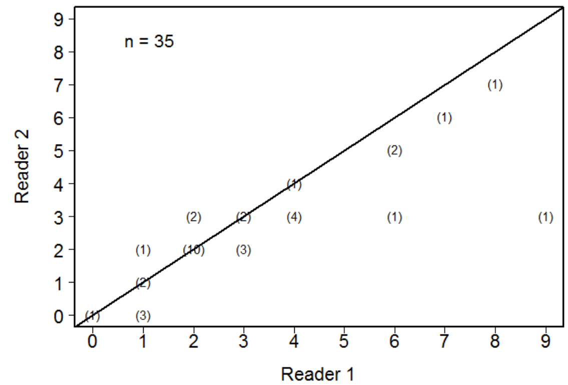

Figure 15.8: Between-reader comparison of the whole-otolith ages for Menhaden collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2023. The number in parentheses is number of fish.

26.21% (test of symmetry: χ^2 = 14.2, df = 10, P = 0.1641), however, there was a significant difference between the first and second readings for Reader 2 with an agreement of 46% (1 year or less agreement of 97%) and a mean CV of 20.24% (test of symmetry: χ^2 = 15.57, df = 7, P = 0.0293). There was no evidence of systematic disagreement on the whole-otolith ages between Reader 1 and Reader 2 with an agreement of 46% (1 year or less agreement of 94%) and a mean CV of 24.5% (test of symmetry: χ^2 = 14.2, df = 9, P = 0.1154) (Figure 15.8 and Table 15.2).

15.3.2 Comparisons

15.3.2.1 *Acetate-scale vs. glass-scale age*

We aged 45 menhaden using both acetate- and glass-slides of their scales. There was no evidence of systematic disagreement between the acetate- and glass-slide ages (test of symmetry: χ^2 = 4.33, df = 5, P = 0.5025) with an agreement of 80% (1 year or less agreement of 98%) and a mean CV of 4.56 (Figure 15.9 and Table 15.2). There was also little evidence of bias between those two kinds of scale ages using an age bias plot (Figure 15.10), with no trend of either over-ageing younger or under-ageing older fish.

15.3.2.2 *Section-otolith vs. whole-otolith age*

We aged 35 menhaden using both their otolith thin-sections and whole otoliths. There was no evidence of systematic disagreement between the otolith ages estimated using the thin-sections and whole otoliths (test of symmetry: χ^2 = 20, df = 13, P = 0.0952) with an agreement of 43% (1 year or less agreement of 63%) and a mean CV of 24.77 (Figure 15.11 and Table 15.2). There was also little evidence of bias between those two kinds of otolith ages using an age bias plot (Figure 15.12), with no trend of either over-ageing younger or under-ageing older fish.

15.3.2.3 *Acetate-scale vs. whole-otolith age*

We chose the acetate-scale ages as the prefer-scale ages because there was no systematic disagreement between the acetate- and glass-scale ages and also because it costs less to make acetate slides than glass slides and is relatively easier to store and exchange acetate slides than glass slides. We chose the whole-otolith ages as the prefer-otolith ages because there was no systematic disagreement between the section- and whole-otolith ages and also because it costs less time to make whole-otolith

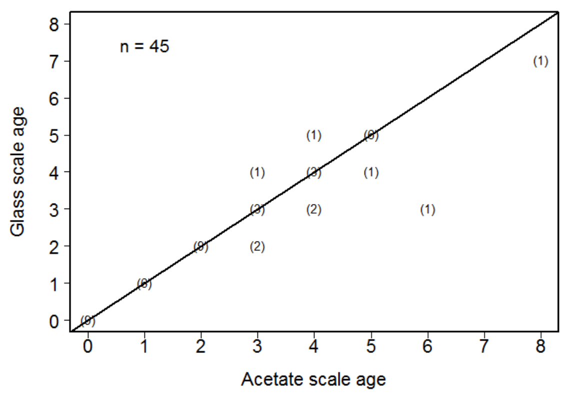

Figure 15.9: Comparison between the section- and glass-scale ages for Menhaden collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2023. The number in parentheses is number of fish.

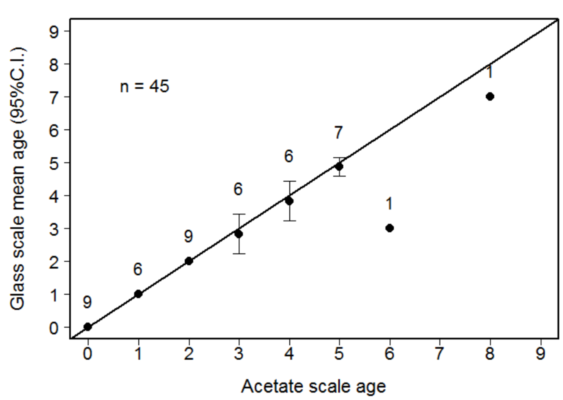

Figure 15.10: Age-bias plot for Menhaden acetate- and glass-scale ages in 2023. The number above the upper CI bar is number of fish.

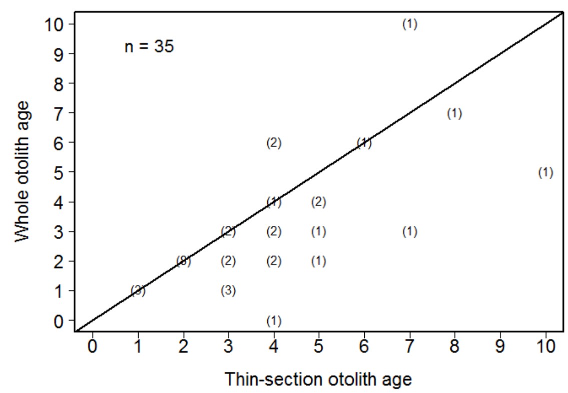

Figure 15.11: Comparison between the section- and whole-otolith ages for Menhaden collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2023. The number in parentheses is number of fish.

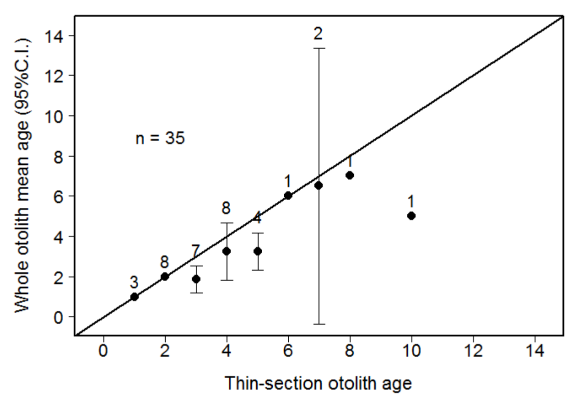

Figure 15.12: Age-bias plot for Menhaden section- and whole-otolith ages in 2023. The number above the upper CI bar is number of fish.

slides than section-otolith slides. As a result, we made comparison between the final acetate-scale and the whole-otolith ages. There was no evidence of systematic disagreement between the acetate-scale and whole-otolith ages (test of symmetry: $\chi^2 = 15.13$, df = 12, P = 0.2342) with an agreement of 31% (1 year or less agreement of 74%) and a mean CV of 29.57 (Figure 15.13 and Table 15.2). There was also little evidence of bias between the acetate-scale and whole-otolith ages using an age bias plot (Figure 15.14), with no trend of either over-ageing younger or under-ageing older fish.

15.4 DISCUSSION

Among the four different slides, it took the longest and shortest time to make the thin-section and whole otolith slide, respectively, whereas the time to make the acetate slide of scale impressions and the slide of scales between two glass slides are very similar and fall betweeen two otolith slides.

All the acetate, glass scale slides, and the whole otolith slides can be read under a Nikon SMZ1000 stereo microscope under transmitted light and dark-field polarization at between 8 and 20 times magnification, however, the thin-

Figure 15.13: Comparison between the acetate-scale and whole-otolith ages for Menhaden collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2023. The number in parentheses is number of fish.

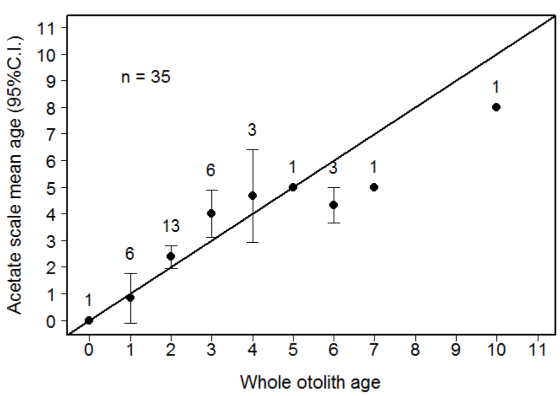

Figure 15.14: Age-bias plot for Menhaden acetate-scale and whole-otolith ages in 2023. The number above the upper CI bar is number of fish.

section otolith slides have to be read under an Olympus BX41 compound microscope, which is more difficult and time consuming.

In general, the acetate-scale ages had the highest precision within each reader as well as between the readers, followed by glass-scale ages, whole-otolith ages, and section-otoliths. This could be because the otolith thin-sections are too small to be read clearly, and the fact that both readers were inexperienced with reading them before.

There was no significant difference between the acetate- and glass-scale age, the acetate-scale age had higher within- and between-reader precsion than the glass-scale age, and it is easier to store and exchange the acetate slides between agencies, as a result, we chose to use the acetate-scale ages for comparison to the otolith ages. There was no significant difference between the section- and whole-otolith ages, however, it takes a longer time to process and age the otolith thin-sections than the whole otoliths, therefore, we chose to use the whole-otolith ages for comparison to the acetate-scale ages.

Because there is no significant difference between the acetate-scale and whole-otolith age and it costs less time and effort to collect menhaden's scales (No need to sacrifice fish) than to collect menhaden's otoliths, the acetate scale slide seems a good option to age menhaden. However, since our sample sizes are very small and we had no experience on ageing menhadem before, we recommend the acetate scale slide with caution, instead, we suggest to have more work done on this topic. For example, increase the sample sizes of paired scale and otoliths through multiple months within each year for multiple years. Such large sample sizes will allow us to identify which method will provide the highest precision for age estimates of menhaden.

Table 15.1: The final ages estimated using the acetate scale slide (Acetate), glass scale slide (Glass), otolith thin-section (Section), and whole otolith (Whole) for menhaden collected in 2023. Note that several fish don't have their second otolith for the whole otolith ageing. "Month" is when a fish was collected. "Total" is the total length in mm. "M" and "F" stands for male and female, respectively.

Fish ID	Month	Total	Sex	Acetate	Glass	Section	Whole
423	6	324	M	4	4	4	2
424	6	297	M	3	3	2	2
425	6	287	M	4	3	3	3
426	6	322	M	4	3	4	6
427	6	316	F	5	5	3	3
428	6	346	M	5	4	8	7
429	6	306	M	6	3	5	4
430	6	339	F	5	5	7	3
431	6	346	M	5	5	6	
432	6	338	F	3	3	4	4
433	6	345	F	5	5	4	6
434	6	287	M	1	1	2	2
435	6	229	M	2	2	2	2
436	6	311	F	3	3	4	2
437	6	280	M	3	2	2	2
438	6	313	M	4	4	5	3
439	6	339	F	5	5	10	5
440	6	334	M	8	7	7	10
441	6	333	M	4	5	6	6
442	6	296	M	2	2	2	2
513	7	235	F	2	2	2	2
514	7	315	F	5	5	5	4
515	7	271	F	2	2	2	2
516	7	287	F	3	4	5	2
517	7	241	M	2	2	4	3
518	7	256	M	3	2	3	1
519	7	231	F	2	2	3	2
520	7	252	F	2	2	3	2
521	7	238	F	2	2	2	2
522	7	259	F	2	2	3	
523	7	293	F	4	4	4	3
524	7	145		0	0	0	
525	7	136		0	0	3	
526	7	153		1	1	1	
527	7	145		0	0	3	
528	7	154		1	1	3	
529	7	143		1	1	1	
530	7	146		0	0	4	0
531	7	138		0	0	4	
532	7	147		0	0	1	1
533	7	141		0	0	1	1
534	7	151		0	0	1	
536	7	167		1	1	3	1
537	7	142		0	0	1	1
538	7	151		1	1	3	1

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Table 15.2: Statistics on the precisions and comparisons within each reader, between the readers, and between the ages estimated using the different methods. "One Year Agreement (%)" stands for one year or less agreement between two sets of ages.

Age	Comparison	Agreement(%)	One Year Agreement(%)	Mean CV(%)	Chi square	df	P-value
Acetate-scale	within Reader 1	76	98	5.11	11.00	5	0.0514
Acetate-scale	within Reader 2	64	98	14.67	8.67	8	0.3712
Acetate-scale	between readers	60	89	10.37	15.33	8	0.0530
Glass-scale	within Reader 1	80	98	6.79	6.33	6	0.3869
Glass-scale	within Reader 2	56	93	22.59	16.50	8	0.0358
Glass-scale	between readers	62	87	20.66	14.33	8	0.0735
Section-otolith	within Reader 1	44	89	21.40	11.13	10	0.3472
Section-otolith	within Reader 2	47	84	28.67	16.00	12	0.1912
Section-otolith	between readers	31	76	40.44	21.67	14	0.0857
Whole-otolith	within Reader 1	46	89	26.21	14.20	10	0.1641
Whole-otolith	within Reader 2	46	97	20.24	15.57	7	0.0293
Whole-otolith	between readers	46	94	24.50	14.20	9	0.1154
Glass-scale	against acetate-scale	80	98	4.56	4.33	5	0.5025
Whole-otolith	against section-otolith	43	63	24.77	20.00	13	0.0952
Acetate-scale	against whole-otolith	31	74	29.57	15.13	12	0.2342

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