2024 Final Report
Virginia ~ Chesapeake Bay Finfish Ageing and Population Analysis

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

Hongsheng Liao, Nenetl Salomon, Alicia Nelson, & Patrick Geer

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EXECUTIVE SUMMARY

This executive summary briefly summarizes what the Age and Growth Lab achieved in 2024 in terms of the objectives listed in the 2024 - 2025 proposal.

Objective 1: We propose to continue support of 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 2024. 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 2024. All fish were collected by the Virginia Marine Resources Commission's (VMRC) Biological Sampling Program in 2024 and aged in early 2025 at the Age and Growth Laboratory of VMRC. We present measures of ageing precision, graphs of year-class distributions, and age-length keys for each species.

Four calcified structures (hard-parts) are used in age determination in the Ageing and Growth Lab in 2024. Specifically, one calcified structure (Otoliths only) was used to determine fish ages of the following 11 species: Atlantic Croaker, *Micropogonias undulatus*, (n = 292); Black Drum, *Pogonias cromis*, (n = 97); Bluefish, *Pomatomus saltatrix*, (n = 369); Cobia, *Rachycentron canadum*, (n = 345); Red Drum, *Sciaenops ocellatus*, (n = 192); Sheepshead, *Archosargus probatocephalus*, (n = 275); Atlantic Spadefish, *Chaetodipterus faber*, (n = 282); Spanish Mackerel, *Scomberomorous maculates*, (n = 301); Spot, *Leiostomus xanthurus*, (n = 143); Spotted Seatrout, *Cynoscion nebulosus*, (n = 296); and Weakfish, *Cynoscion regalis*, (n = 228). Two calcified structures (Scales and otoliths) were used for determining fish ages of the following two species: Striped Bass, *Morone saxatilis*, (n = 879) and Summer Flounder, *Paralichthys dentatus*, (n = 800). Three calcified structures (Opercula, otoliths, and spines) were used for determining ages of Tautog, *Tautoga onitis*, (n = 316). Comparing alternative hard-parts allowed us to assess their usefulness in determining fish age as well as the relative precision of each structure. In total, we made 12,820 age readings from scales, otoliths, opercula, and spines collected during 2024. A summary of the age ranges for all species is presented in Table 1.

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 collected from Chesapeake Bay and Virginia waters of Atlantic in 2024. 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. By the end of 2024, we estimated the sample sizes of the above species for 2025. We didn't estimate sample sizes for ageing Cobia, Black Drum, Red Drum, and Sheepshead because we have never collected enough samples to age those species each year.

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 hard-parts collected, number of fish aged, and age readings for the 14 finfish species in 2024. The hard-parts and age readings include both otoliths and scales for Striped Bass and Summer Flounder, and otoliths, opercula, and spines for Tautog. When otolith-ages are available, they are the final ages for Striped Bass, Summer Flounder, and Tautog, otherwise, the scale-ages for Striped Bass and Summer Flounder whereas the operculum-ages for Tautog. When both otolith- and operculum-ages are unavailable, the spine-ages are the final ages for Tautog. The otolith-ages are always for other species.

Species	Number of fish collected	Number of hard-parts	Number of fish aged	Number of readings	Minimum age	Maximum age
Atlantic Croaker	321	320	292	584	0	7
Black Drum	97	97	97	194	1	50
Bluefish	682	682	369	738	0	8
Cobia	346	345	345	690	2	12
Red Drum	192	192	192	384	0	2
Sheepshead	277	275	275	550	1	33
Spadefish	345	344	282	564	1	8
Spanish Mackerel	379	378	301	602	0	8
Spot	285	252	143	286	1	3
Spotted Seatrout	381	380	296	592	0	8
Striped Bass	1,068	1,417	879	2,456	2	29
Summer Flounder	953	1,574	800	2,846	1	15
Tautog	316	939	316	1,878	2	18
Weakfish	260	257	228	456	1	6
Totals	5,902	7,452	4,815	12,820		

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 order to start to age Menhaden in-house, we not only provided Menhaden scale slides (glass slides) to the NOAA Ageing Lab at Beaufort, NC, for the Menhaden Stock Assessment but also made two scale slides (glass vs. acetate) and two otolith slides (thin-section vs. whole otolith) from 159 Menhaden collected in 2024. We have finished this project and had a preliminary result, that is, scale impression on an acetate slide could be used to age Menhaden in terms of ageing precision and cost/effectiveness (Please see APPENDIX at the end of this report).

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 2023 Ageing Lab Final Report in 2024.

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

In 2024, we developed a new web-based application (VMRC Menhade Paired Calcified Structure Images) to share images of paired hardparts from each fish of Menhaden (Thin-section of otoliths, whole otoliths, acetate scale impression, vs. whole scale). By the end of June of 2025, we uploaded the 4 images from each of 99 Menhaden collected in 2024 and aged in early 2025.

In 2024, we revised all 7 web applications posted at VMRC Ageing Lab website (VMRC Ageing Lab) in terms of their appearance, functions, and coding. We also updated age data in VMRC 5 web applications (Fish Age Estimator, Fish Growth Predictor, %MSP/%Female SPR/%SPR Estimator, VMRC Date Sharing App, and Sportfish Donation data App). These apps help fishermen to understand the importance of knowledge on fish ages and growth, and allow fish 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 2024 and aged by the lab.

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 2024. Anytime when we revised an old processing method and added a new method, we added those new information in the protocol. In early April of 2025, Dr. Hank Liao, Lab Manager, participated ASMFC QAQC Ageing Workshop held at St. Petersburg, Florida, to continue learning how to age Tautog using their spines and Menhaden using their scales. Dr. Liao also participated the conference call held by ASMFC about updating Menhaden ageing exchange program in late April of 2025.

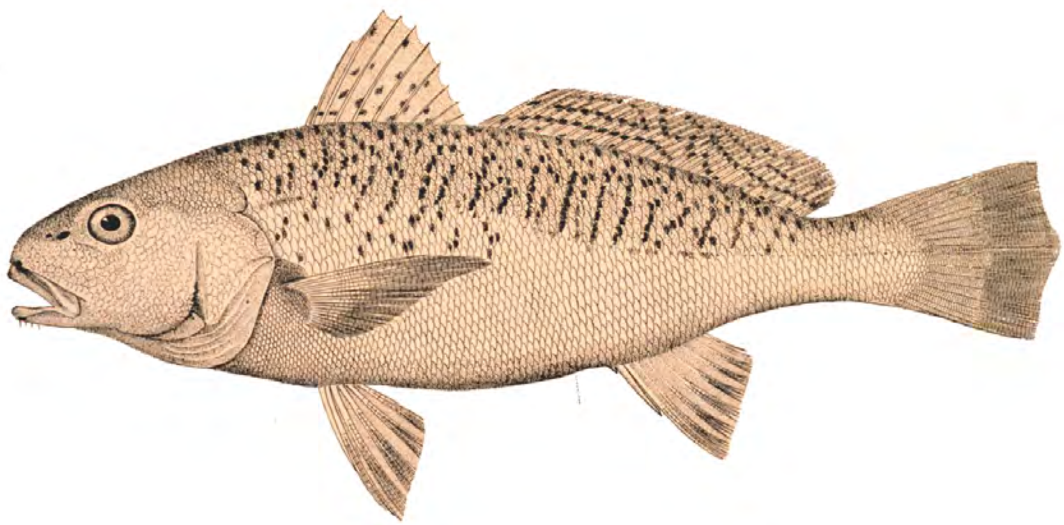
Besides above work the Age and Growth Lab did in 2024, to support environmental and wildlife agencies, and charities, we donated more than 4,808 pounds of dissected fish to the Salvation Army to feed the homeless, and Alton's Keep WildBird Rescue and Rehabilitation Center Inc., a local wildlife rescue agency which is responsible for saving injured animals found by the public. In August of 2024, the Ageing Lab participated 2024 Virginia State Fair to demonstrate what contributions the VMRC Ageing Lab make to the stock assessments and fisheries management of Virginia important fish species.

ACKNOWLEDGMENTS

We thank Marben Abutin and Natalie Baker for their technical expertise in preparing otoliths, scales, and opercula 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 Thompson, 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 292 Atlantic Croaker, *Micropogonias undulatus*, collected by the VMRC's Biological Sampling Program for age and growth analysis in 2024. Croaker ages ranged from 0 to 7 years old with an average age of 1.4, a standard deviation of 0.9, and a standard error of 0.05. Eight age classes (0 to 7) were represented, comprising fish of the 2017 to 2024 year-classes. The sample was dominated by fish from the year-classes of 2022 and 2023 with 17.1% and 73%, respectively.

1.2 METHODS

1.2.1 Sample size for ageing

We estimated sample size for ageing Croaker in 2024 using a two-stage random sampling method (Quinn and Deriso 1999) to increase precision in estimates of age composition from fish sampled efficiently and effectively. The basic equation is:

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

where A is the sample size for ageing Croaker in 2024; θ_a stands for the proportion of Age a fish in a catch; V_a, B_a, and CV_a represent the variance components within and between length intervals, and the coefficient of variation for Age a, respectively; L is the total number of Croaker used by VMRC to estimate length distribution of the catches from 2018 to 2022. θ_a, V_a, and B_a were calculated using pooled age-length data of Croaker collected from 2018 to 2022 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 2018 to 2022 catch. A_l is number of fish to be aged for length interval l in 2024.

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 glass slide with clear Crystalbond^{TM} 509 adhesive or imbedded in epoxy. 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 a pencil 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, 3-inch diameter, Norton 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 CQFE website on how to prepare otolith thin-section for ageing Atlantic Croaker.

1.2.4 Readings

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

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

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

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

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 2024 (Figure 1.1).

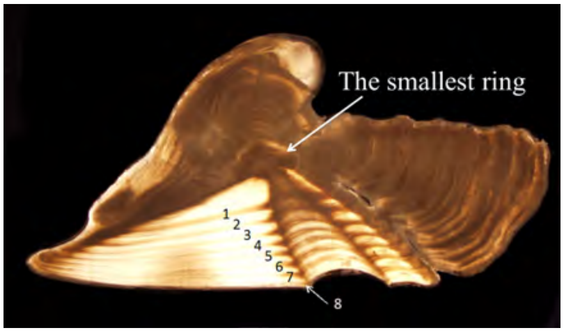


Figure 1.1: Otolith thin-sections of a 8 year-old Croaker without counting the smallest ring and with the last annulus on the edge of the thin-section

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, Reader 1 re-aged the fish with disagreement and decided a final age for the fish. This method is different from what we used before the pandemic of COVID-19 during the period of 2020 -2021 because of 6-food social distance requirement. 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 4.4.0 (R Core Team 2021).

1.3 RESULTS

1.3.1 Sample size

We estimated a sample size of 428 Atlantic Croaker in 2024, ranging in length interval from 4 to 15 inches (Table 1.1). This sample size provided a range in CV for age composition approximately from the smallest CV of 8% for the major age of Age 2 to the CV of larger than 25% for the multiple minor ages (Table 1.2). In 2024, we aged 292 of 320 Croaker (The rest of fish were either without otoliths or over-collected for certain length interval(s)) collected by VMRC. We fell short in our overall collections for this optimal length-class sampling estimate by 164 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.

1.3.2 Reading precision

Both readers had high self-precision. Specifically, there was no significant difference between the first and second readings for Reader 1 with an agreement of 98% and a CV of 0.22% (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 96% and a CV of 0.92% (test of symmetry: $\chi^2 = 2$, $df = 2$, $P = 0.3679$). There was an evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 93.15% and a CV of 2.7% (test of symmetry: $\chi^2 = 16.33$, $df = 6$, $P = 0.0121$) (Figure 1.2).

There was no time-series bias for either reader. Reader 1 had an agreement of 100% with ages of fish aged in 2003. Reader 2 also had an agreement of 100% .

1.3.3 Year class

Of the 292 fish aged with otoliths, 8 age classes (0 to 7) were represented (Table 1.3). The average age was 1.4 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 8 year-classes: fish from the 2017 to 2024 year-classes, with fish primarily from the year classes of 2022 and 2023

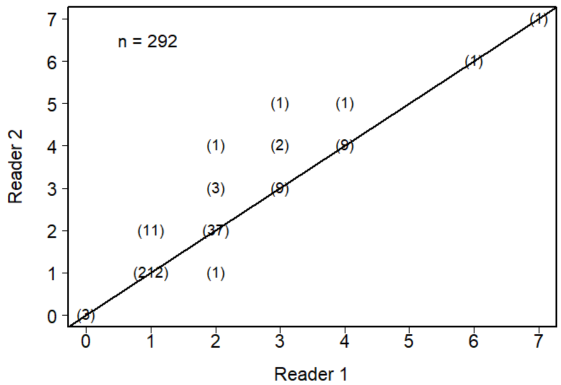


Figure 1.2: Between-reader comparison of otolith age estimates for Atlantic Croaker collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2024. The number in parentheses is number of fish.

with 17.1% and 73%, respectively. The ratio of males to females was 1:16.06 in the sample collected (Figure 1.3).

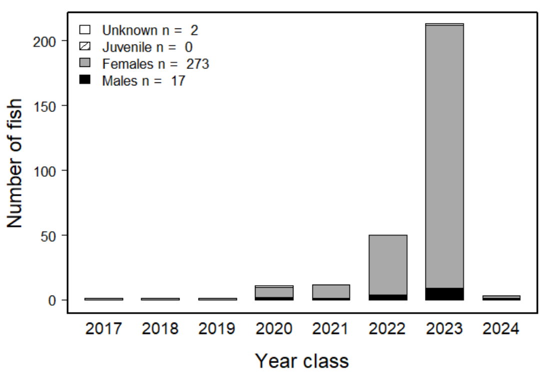


Figure 1.3: Year-class frequency distribution for Atlantic Croaker collected for ageing in 2024. Distribution is broken down by sex. 'Unknown' is for gonads that were not available for examination or were not examined for sex during sampling.

1.3.4 Age-length key (ALK)

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

Table 1.1: Number of Atlantic Croaker collected and aged in each 1-inch length interval in 2024. 'Target' represents the sample size for ageing estimated for 2024, and 'Need' represents number of fish shorted in each length interval compared to the optimum sample size for ageing and number of fish aged.

Interval	Target	Collected	Aged	Need
4 - 4.99	5	0	0	5
5 - 5.99	5	0	0	5
6 - 6.99	5	3	3	2
7 - 7.99	17	22	18	0
8 - 8.99	20	44	20	0
9 - 9.99	45	70	70	0
10 - 10.99	83	85	85	0
11 - 11.99	122	82	82	40
12 - 12.99	87	10	10	77
13 - 13.99	29	1	1	28
14 - 14.99	5	3	3	2
15 - 15.99	5	0	0	5
Totals	428	320	292	164

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Table 1.2: CV for each age estimated based on ageing the total of 428 Croaker in 2024. 'Percent' is the percentage of an age in the pooled age-length data of Croaker collected from 2018 to 2022.

Age	CV	Percent
0	0.24	2.4
1	0.09	22.32
2	0.08	27.3
3	0.12	13.82
4	0.15	9.53
5	0.16	9.01
6	0.14	11.16
7	>0.25	2.4
8	>0.25	1.63
9	>0.25	0.34
11	>0.25	0.09

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Table 1.3: The number of Atlantic Croaker assigned to each total length-at-age category for 292 fish sampled for otolith age determination in Virginia during 2024.

	Age								
Interval	0	1	2	3	4	5	6	7	Totals
6 - 6.99	1	2	0	0	0	0	0	0	3
7 - 7.99	2	12	4	0	0	0	0	0	18
8 - 8.99	0	10	5	3	2	0	0	0	20
9 - 9.99	0	39	15	6	8	1	1	0	70
10 - 10.99	0	70	11	3	1	0	0	0	85
11 - 11.99	0	74	8	0	0	0	0	0	82
12 - 12.99	0	6	4	0	0	0	0	0	10
13 - 13.99	0	0	1	0	0	0	0	0	1
14 - 14.99	0	0	2	0	0	0	0	1	3
Totals	3	213	50	12	11	1	1	1	292

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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 2024.

	Age							
Interval	0	1	2	3	4	5	6	7
6 - 6.99	0.33	0.67	0	0	0	0	0	0
7 - 7.99	0.11	0.67	0.22	0	0	0	0	0
8 - 8.99	0	0.5	0.25	0.15	0.1	0	0	0
9 - 9.99	0	0.56	0.21	0.09	0.11	0.01	0.01	0
10 - 10.99	0	0.82	0.13	0.04	0.01	0	0	0
11 - 11.99	0	0.9	0.1	0	0	0	0	0
12 - 12.99	0	0.6	0.4	0	0	0	0	0
13 - 13.99	0	0	1	0	0	0	0	0
14 - 14.99	0	0	0.67	0	0	0	0	0.33

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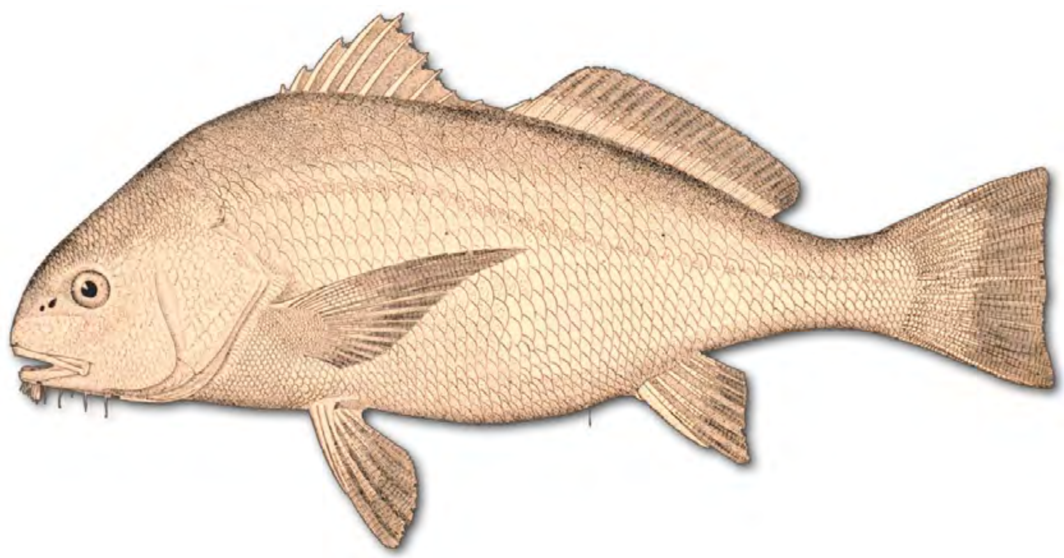
Chapter 2

BLACK DRUM *Pogonias cromis*



2.1 INTRODUCTION

We aged a total of 97 Black Drum, *Pogonias cromis*, collected by the VMRC's Biological Sampling Program for age and growth analysis in 2024. Black drum ages ranged from 1 to 50 years old with an average age of 14.8, a standard deviation of 10.9, and a standard error of 1.11. Thirty-four age classes (1 to 19, 21 to 25, 28 to 29, 31 to 34, 37 to 38, 41, and 50) were represented, comprising fish of the 1974, 1983, 1986 to 1987, 1990 to 1993, 1995 to 1996, 1999 to 2003, and 2005 to 2023 year-classes. The sample was dominated by fish from the year-classes of 1991, 1993, 1999, 2001, 2006, 2007, 2009, 2015, 2018, 2019, 2020, 2021, and 2022 with 3.1%, 3.1%, 3.1%, 4.1%, 4.1%, 8.2%, 3.1%, 10.3%, 10.3%, 7.2%, 4.1%, 6.2%, and 4.1%, 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). The left or right sagittal otolith was randomly selected and attached, distal side down, to a glass slide with Crystalbond™ 509 adhesive or embedded in epoxy. 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 a pencil 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, three inch diameter, Norton 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 CQFE website on how to prepare otolith thin-section for ageing Black Drum.

2.2.3 Readings

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

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

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

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

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, Reader 1 re-aged the fish with disagreement and decided a final age for the fish. This method is different from what we used before the pandemic of COVID-19 during the period of 2020 -2021 because of 6-food social distance requirement. 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 2.1).

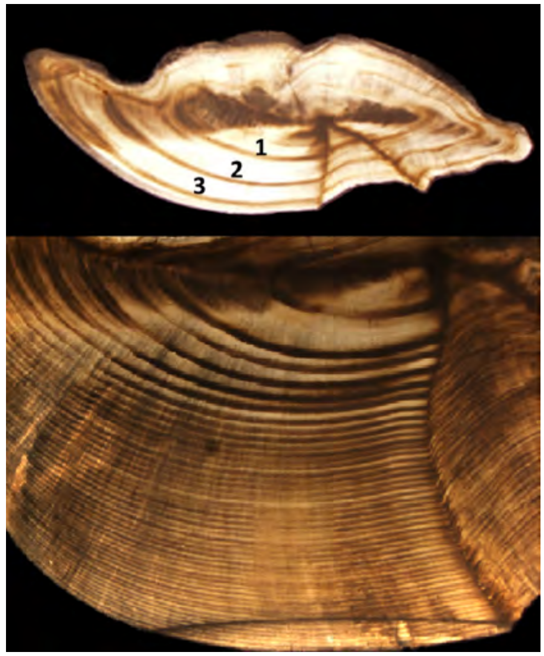


Figure 2.1: Otolith thin-sections of a 3 (Upper panel) and 47 year-old (Lower panel) Black Drum.

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 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 4.4.0 (R Core Team 2021).

2.3 RESULTS

2.3.1 Reading precision

Both readers had high self-precision. Specifically, there was no significant difference between the first and second readings for Reader 1 with an agreement of 96% and a *CV* of 0.39% (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 94% and a *CV* of 0.98% (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 95.88% and a *CV* of 0.18% (test of symmetry: $\chi^2 = 4$, *df* = 4, *P* = 0.406) (Figure 2.2).

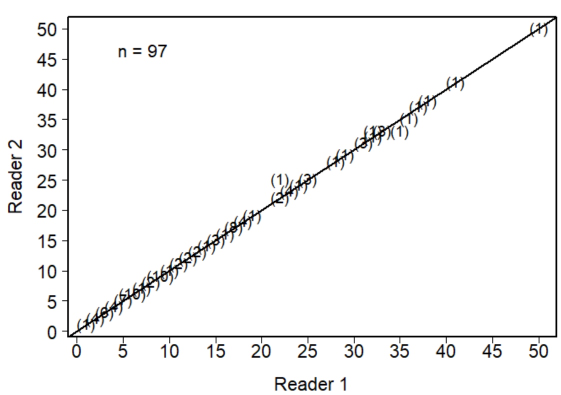


Figure 2.2: Between-reader comparison of otolith age estimates for Black Drum collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2024. The number in parentheses is number of fish.

There was no time-series bias for either reader. Reader 1 had an agreement of 88% with ages of fish aged in 2000 with a *CV* of 1.32% (test of symmetry: $\chi^2 = 6$, *df* = 6, *P* = 0.4232). Reader 2 had an agreement of 90%with a *CV* of 0.75% (test of symmetry: $\chi^2 = 5$, *df* = 5, *P* = 0.4159).

2.3.2 Year class

Of the 97 fish aged with otoliths, 34 age classes (1 to 19, 21 to 25, 28 to 29, 31 to 34, 37 to 38, 41, and 50) were represented (Table 2.1). The average age was 14.8 years, and the standard deviation and standard error were 10.9 and 1.11, respectively. Year-class data show that the fishery was comprised of 34 year-classes: fish from the 1974, 1983, 1986 to 1987, 1990 to 1993, 1995 to 1996, 1999 to 2003, and 2005 to 2023 year-classes, with fish primarily from the year classes of 1991, 1993, 1999, 2001, 2006, 2007, 2009, 2015, 2018, 2019, 2020, 2021, and 2022 with 3.1%, 3.1%, 3.1%, 4.1%, 4.1%, 8.2%, 3.1%, 10.3%, 10.3%, 7.2%, 4.1%, 6.2%, and 4.1%, respectively. The ratio of males to females was 1:1.09 in the sample collected (Figure 2.3).

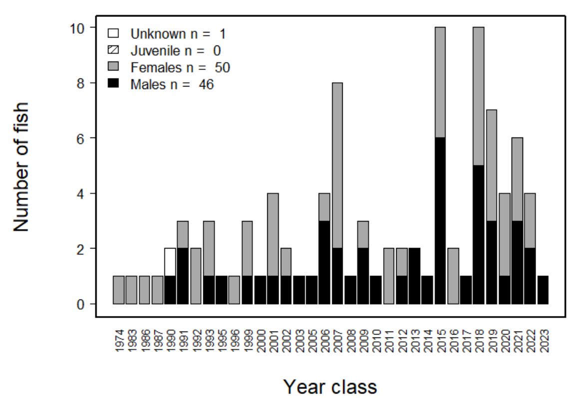


Figure 2.3: Year-class frequency distribution for Black Drum collected for ageing in 2024. Distribution is broken down by sex. 'Unknown' represents gonads that were not available for examination or were not examined for sex during sampling.

2.3.3 Age-length key (ALK)

We developed an age-length-key (Table 2.2) that can be used in the conversion of numbers-at-length in the estimated catch to numbers-at-age using otolith ages. The table is based

on VMRC's stratified sampling of landings by
total length inch intervals.

Table 2.1: The number of Black Drum assigned to each total length (inch)-at-age category for 97 fish sampled for otolith age determination in Virginia during 2024.

	Age																																	
Interval	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	21	22	23	24	25	28	29	31	32	33	34	37	38	41	50	Totals
3 - 3.99	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
4 - 4.99	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
18 - 18.99	1	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	0	0	0	0	0	0	0	0	4
21 - 21.99	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
22 - 22.99	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
23 - 23.99	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
24 - 24.99	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
25 - 25.99	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
26 - 26.99	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
27 - 27.99	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
28 - 28.99	0	0	0	0	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	0	0	0	0	0	3
29 - 29.99	0	0	0	0	1	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6
30 - 30.99	0	0	0	0	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
31 - 31.99	0	0	0	0	0	4	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5
32 - 32.99	0	0	0	0	0	0	1	1	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	0	5
33 - 33.99	0	0	0	0	0	0	0	0	0	1	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
34 - 34.99	0	0	0	0	0	0	0	0	4	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5
35 - 35.99	0	0	0	0	0	0	0	0	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
36 - 36.99	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
37 - 37.99	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	5	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	8
38 - 38.99	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5
39 - 39.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	4
40 - 40.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	0	1	2	0	0	2	0	0	0	0	0	0	0	0	0	0	8
41 - 41.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1	0	1	0	1	1	0	1	0	0	0	0	7
42 - 42.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0	3
43 - 43.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0	1	0	0	4
44 - 44.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1
45 - 45.99	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	0	0	0	2	0	0	0	0	0	3
47 - 47.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
48 - 48.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
Totals	1	4	6	4	7	10	1	2	10	1	2	2	2	1	3	1	8	4	1	1	2	4	1	3	1	1	3	2	3	2	1	1	1	1	97

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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 2024.

	Age																																	
Interval	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	21	22	23	24	25	28	29	31	32	33	34	37	38	41	50
3 - 3.99	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4 - 4.99	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18 - 18.99	0.25	0.75	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
21 - 21.99	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
22 - 22.99	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
23 - 23.99	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
24 - 24.99	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
25 - 25.99	0	0	0.5	0.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
26 - 26.99	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
27 - 27.99	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	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	0	0	0	0	0	0	0	0	0	0	0	0	0	0
29 - 29.99	0	0	0	0	0.17	0.83	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
30 - 30.99	0	0	0	0	0.33	0.33	0	0.33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
31 - 31.99	0	0	0	0	0	0.8	0	0	0.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
32 - 32.99	0	0	0	0	0	0	0.2	0.2	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	0
33 - 33.99	0	0	0	0	0	0	0	0	0	0.33	0	0.33	0	0	0.33	0	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	0	0	0.8	0	0	0	0	0	0.2	0	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	0	0	0	0.67	0	0.33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
36 - 36.99	0	0	0	0	0	0	0	0	0	0	0.5	0.5	0	0	0	0	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	0	0	0	0	0	0	0	0.12	0	0	0	0.62	0.12	0	0	0	0	0	0	0	0.12	0	0	0	0	0	0	0	0
38 - 38.99	0	0	0	0	0	0	0	0	0	0	0	0	0.2	0.2	0.2	0.2	0.2	0	0	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	0	0	0	0	0	0	0	0	0	0.25	0.25	0.25	0	0	0.25	0	0	0	0	0	0	0	0	0	0	0	0
40 - 40.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.12	0.25	0	0.12	0.25	0	0	0.25	0	0	0	0	0	0	0	0	0	0
41 - 41.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.29	0.14	0	0.14	0	0.14	0.14	0	0.14	0	0	0	0
42 - 42.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.33	0	0	0	0	0.33	0.33	0	0	0	0	0	0
43 - 43.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.25	0	0.25	0.25	0	0.25	0	0
44 - 44.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
45 - 45.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.33	0	0	0	0	0.67	0	0	0	0	0
47 - 47.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
48 - 48.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0

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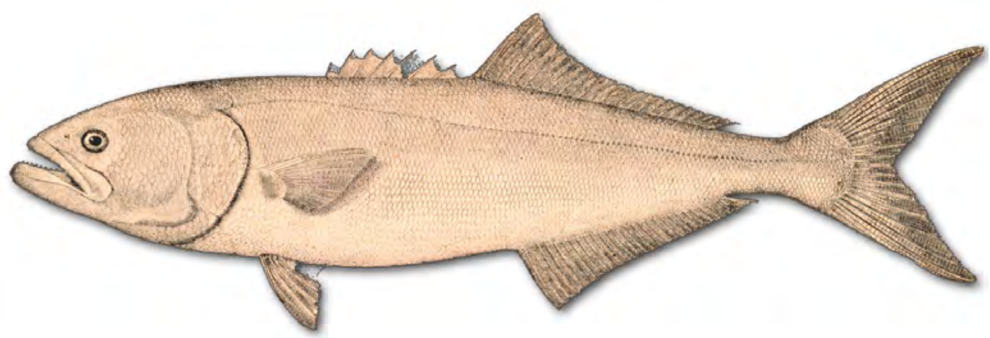
Chapter 3

BLUEFISH *Pomatomus saltatrix*



3.1 INTRODUCTION

We aged a total of 369 Bluefish, *Pomatomus saltatrix*, collected by the VMRC's Biological Sampling Program for age and growth analysis in 2024. Bluefish ages ranged from 0 to 8 years old with an average age of 2.3, a standard deviation of 1.5, and a standard error of 0.08. Nine age classes (0 to 8) were represented, comprising fish of the 2016 to 2024 year-classes. The sample was dominated by fish from the year-classes of 2021, 2022, and 2023 with 23.6%, 40.4%, and 24.1%, respectively.

3.2 METHODS

3.2.1 Sample size for ageing

We estimated sample size for ageing Bluefish in 2024 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 2024; 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 2018 to 2022. θ_a, V_a, and B_a were calculated using pooled age-length data of Bluefish collected from 2018 to 2022 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 2018 to 2022 catch. A_l is number of fish to be aged for length interval l in 2024. 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 & Growth Laboratory in labeled coin envelopes, and were sorted by date of capture. Their envelope labels were verified against VMRC's collection data, and each fish was assigned a unique Age and Growth Laboratory identification number. All otoliths were stored dry in their original labeled coin envelopes.

3.2.3 Preparation

We used our thin-section and bake 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 by eye and, when necessary, under a stereo microscope to identify the location of the core. Then, the position of the core was marked using a permanent marker 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, 3-inch diameter, Norton 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 focus. 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 CQFE website on how to prepare otolith thin-section for ageing Bluefish.

3.2.4 Readings

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

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

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

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

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 (Figure 3.1). Each reader aged all of the otolith samples.

If an otolith was properly sectioned, the sulcal groove came to a sharp point within the middle

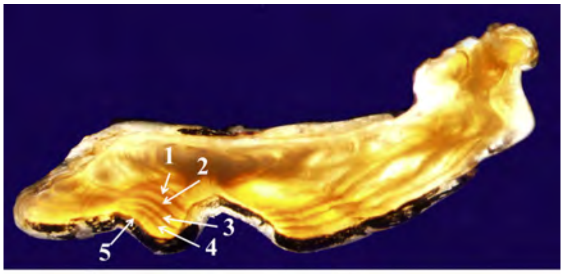


Figure 3.1: Otolith thin-section of a 5 year-old Bluefish with the last annulus on the edge of the thin-section

of the focus. Typically the first year's annulus was found by locating the focus of the otolith, which was characterized as a visually distinct dark, oblong region found in the center of the otolith. 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.

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 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 4.4.0 (R Core Team 2021).

3.3 RESULTS

3.3.1 Sample size

We estimated a sample size of 434 Bluefish in 2024, ranging in length interval from 17 to 121 centimeters (Table 3.1). This sample size provided a range in CV for age composition approximately from the smallest CV of 5% for Age 1 to the CV of larger than 25% for the multiple minor ages (Table 3.2). In 2024, we randomly selected and aged 369 fish from 682 Bluefish collected by VMRC. We fell short in our over-all collections for this optimal length-class sampling estimate by 128 fish, as a result, the precision for the estimates of major age groups would definitely be influenced significantly. Therefore, precaution should be used when developing ALK using these age data.

3.3.2 Reading precision

Reader 1 had moderate self-precision and Read 2 had low self-precision.Specifically, there was a difference between the first and second readings for Reader 1 with an agreement of 84% and a CV of 5.31% (test of symmetry: χ^2 = 8, df = 3, P = 0.046), and there was no significant difference between the first and second readings for Reader 2 with an agreement of 84% and a CV of 10.21% (test of symmetry: χ^2 = 4, df = 4, P = 0.406). There was an evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 85.09% and a CV of 6.01% (test of symmetry: χ^2 = 28.33, df = 10, P = 0.0016) (Figure 3.2).

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 3.39% (test of symmetry: χ^2 = 4, df = 2, P = 0.1353). Reader 2 had an agreement of 86% with a CV of 7.67% (test of symmetry: χ^2 = 4, df = 4, P = 0.406).

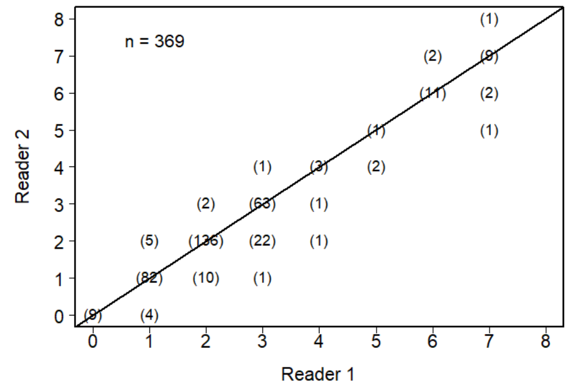


Figure 3.2: Between-reader comparison of otolith age estimates for Bluefish collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2024. The number in parentheses is number of fish.

3.3.3 Year class

Of the 369 fish aged with otoliths, 9 age classes (0 to 8) were represented (Table 3.3). The average age was 2.3 years, and the standard deviation and standard error were 1.5 and 0.08, respectively. Year-class data show that the fishery was comprised of 9 year-classes: fish from the 2016 to 2024 year-classes, with fish primarily from the year classes of 2021, 2022, and 2023 with 23.6%, 40.4%, and 24.1%, respectively. The ratio of males to females was 1:2.07 in the sample collected (Figure 3.3).

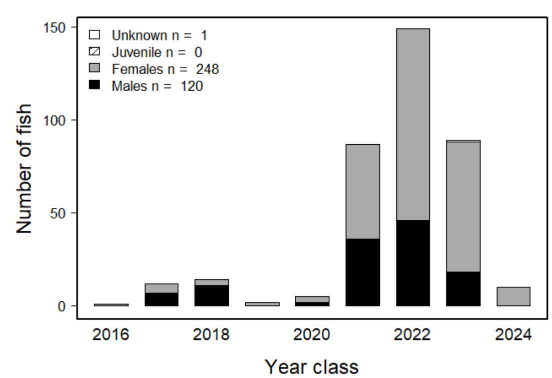


Figure 3.3: Year-class frequency distribution for Bluefish collected for ageing in 2024. Distribution is broken down by sex. 'Unknown' represents gonads that were not available for examination or were not examined for sex during sampling.

3.3.4 Age-length key (ALK)

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

Table 3.1: Number of Bluefish collected and aged in each 1-cm length interval in 2024. 'Target' represents the sample size for ageing estimated for 2024, 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
17 - 17.99	5	1	1	4
18 - 18.99	5	1	1	4
19 - 19.99	5	0	0	5
20 - 20.99	5	0	0	5
21 - 21.99	5	2	2	3
22 - 22.99	5	1	1	4
23 - 23.99	5	5	5	0
24 - 24.99	5	4	4	1
25 - 25.99	5	0	0	5
26 - 26.99	6	7	7	0
27 - 27.99	6	7	7	0
28 - 28.99	6	10	10	0
29 - 29.99	6	10	6	0
30 - 30.99	6	10	6	0
31 - 31.99	6	10	6	0
32 - 32.99	6	20	6	0
33 - 33.99	6	13	6	0
34 - 34.99	6	22	6	0
35 - 35.99	5	27	6	0
36 - 36.99	7	17	8	0
37 - 37.99	6	25	6	0
38 - 38.99	7	25	8	0
39 - 39.99	7	18	8	0
40 - 40.99	6	23	6	0
41 - 41.99	7	30	8	0
42 - 42.99	7	22	8	0
43 - 43.99	7	27	8	0
44 - 44.99	6	14	6	0
45 - 45.99	7	14	8	0
46 - 46.99	6	15	6	0
47 - 47.99	7	14	8	0
48 - 48.99	5	11	6	0
49 - 49.99	5	15	6	0
50 - 50.99	5	8	6	0
51 - 51.99	5	9	6	0
52 - 52.99	5	11	6	0
53 - 53.99	5	5	5	0
54 - 54.99	5	8	6	0
55 - 55.99	5	10	6	0
56 - 56.99	5	6	6	0
57 - 57.99	5	11	6	0
58 - 58.99	5	13	6	0

(To continue)

Table 3.1 (Continued)

Interval	Target	Collected	Aged	Need
59 - 59.99	5	8	6	0
60 - 60.99	5	15	6	0
61 - 61.99	5	8	6	0
62 - 62.99	5	16	6	0
63 - 63.99	5	8	6	0
64 - 64.99	5	18	6	0
65 - 65.99	5	14	6	0
66 - 66.99	5	19	19	0
67 - 67.99	5	14	14	0
68 - 68.99	5	6	6	0
69 - 69.99	5	8	8	0
70 - 70.99	5	6	6	0
71 - 71.99	5	6	6	0
72 - 72.99	5	1	1	4
73 - 73.99	5	0	0	5
74 - 74.99	5	4	4	1
75 - 75.99	5	0	0	5
76 - 76.99	5	1	1	4
77 - 77.99	5	2	2	3
78 - 78.99	5	0	0	5
79 - 79.99	5	2	2	3
80 - 80.99	5	3	3	2
81 - 81.99	5	7	7	0
82 - 82.99	5	4	4	1
83 - 83.99	5	2	2	3
84 - 84.99	5	5	5	0
85 - 85.99	5	3	3	2
86 - 86.99	5	0	0	5
87 - 87.99	5	0	0	5
88 - 88.99	5	0	0	5
89 - 89.99	5	0	0	5
90 - 90.99	5	0	0	5
91 - 91.99	5	1	1	4
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	434	682	369	128

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Table 3.2: CV for each age estimated based on ageing the total of 434 Bluefish in 2024. 'Percent' is the percentage of an age in the pooled age-length data of Bluefish collected from 2018 to 2022.

Age	CV	Percent
0	0.15	6.58
1	0.05	33.85
2	0.07	26.86
3	0.14	9.4
4	0.12	8.46
5	0.16	5.78
6	0.23	3.29
7	0.23	2.89
8	>0.25	2.01
9	>0.25	0.27
10	>0.25	0.54
11	>0.25	0.07

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Table 3.3: The number of Bluefish assigned to each total length (cm)-at-age category for 369 fish sampled for otolith age determination in Virginia during 2024.

	Age									
Interval	0	1	2	3	4	5	6	7	8	Totals
17 - 17.99	1	0	0	0	0	0	0	0	0	1
18 - 18.99	1	0	0	0	0	0	0	0	0	1
21 - 21.99	2	0	0	0	0	0	0	0	0	2
22 - 22.99	0	1	0	0	0	0	0	0	0	1
23 - 23.99	2	3	0	0	0	0	0	0	0	5
24 - 24.99	2	2	0	0	0	0	0	0	0	4
26 - 26.99	2	5	0	0	0	0	0	0	0	7
27 - 27.99	0	6	1	0	0	0	0	0	0	7
28 - 28.99	0	10	0	0	0	0	0	0	0	10
29 - 29.99	0	6	0	0	0	0	0	0	0	6
30 - 30.99	0	6	0	0	0	0	0	0	0	6
31 - 31.99	0	6	0	0	0	0	0	0	0	6
32 - 32.99	0	6	0	0	0	0	0	0	0	6
33 - 33.99	0	6	0	0	0	0	0	0	0	6
34 - 34.99	0	3	2	1	0	0	0	0	0	6
35 - 35.99	0	3	3	0	0	0	0	0	0	6
36 - 36.99	0	4	4	0	0	0	0	0	0	8
37 - 37.99	0	3	3	0	0	0	0	0	0	6
38 - 38.99	0	4	4	0	0	0	0	0	0	8
39 - 39.99	0	3	5	0	0	0	0	0	0	8
40 - 40.99	0	1	5	0	0	0	0	0	0	6
41 - 41.99	0	2	5	1	0	0	0	0	0	8
42 - 42.99	0	3	5	0	0	0	0	0	0	8
43 - 43.99	0	2	6	0	0	0	0	0	0	8
44 - 44.99	0	0	6	0	0	0	0	0	0	6
45 - 45.99	0	1	7	0	0	0	0	0	0	8
46 - 46.99	0	0	5	1	0	0	0	0	0	6
47 - 47.99	0	1	6	1	0	0	0	0	0	8
48 - 48.99	0	1	5	0	0	0	0	0	0	6
49 - 49.99	0	1	4	1	0	0	0	0	0	6
50 - 50.99	0	0	4	2	0	0	0	0	0	6
51 - 51.99	0	0	4	2	0	0	0	0	0	6
52 - 52.99	0	0	6	0	0	0	0	0	0	6
53 - 53.99	0	0	4	1	0	0	0	0	0	5
54 - 54.99	0	0	5	1	0	0	0	0	0	6
55 - 55.99	0	0	5	1	0	0	0	0	0	6
56 - 56.99	0	0	0	6	0	0	0	0	0	6
57 - 57.99	0	0	4	2	0	0	0	0	0	6
58 - 58.99	0	0	3	3	0	0	0	0	0	6
59 - 59.99	0	0	3	3	0	0	0	0	0	6
60 - 60.99	0	0	2	4	0	0	0	0	0	6
61 - 61.99	0	0	2	4	0	0	0	0	0	6

(To continue)

Table 3.3 (Continued)

	Age									
Interval	0	1	2	3	4	5	6	7	8	Totals
62 - 62.99	0	0	1	5	0	0	0	0	0	6
63 - 63.99	0	0	3	3	0	0	0	0	0	6
64 - 64.99	0	0	1	5	0	0	0	0	0	6
65 - 65.99	0	0	2	4	0	0	0	0	0	6
66 - 66.99	0	0	5	13	1	0	0	0	0	19
67 - 67.99	0	0	8	6	0	0	0	0	0	14
68 - 68.99	0	0	2	3	1	0	0	0	0	6
69 - 69.99	0	0	4	3	0	1	0	0	0	8
70 - 70.99	0	0	2	4	0	0	0	0	0	6
71 - 71.99	0	0	2	3	1	0	0	0	0	6
72 - 72.99	0	0	0	1	0	0	0	0	0	1
74 - 74.99	0	0	1	1	1	0	1	0	0	4
76 - 76.99	0	0	0	1	0	0	0	0	0	1
77 - 77.99	0	0	0	1	1	0	0	0	0	2
79 - 79.99	0	0	0	0	0	0	1	1	0	2
80 - 80.99	0	0	0	0	0	1	1	0	1	3
81 - 81.99	0	0	0	0	0	0	4	3	0	7
82 - 82.99	0	0	0	0	0	0	2	2	0	4
83 - 83.99	0	0	0	0	0	0	1	1	0	2
84 - 84.99	0	0	0	0	0	0	3	2	0	5
85 - 85.99	0	0	0	0	0	0	1	2	0	3
91 - 91.99	0	0	0	0	0	0	0	1	0	1
Totals	10	89	149	87	5	2	14	12	1	369

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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 2024.

	Age								
Interval	0	1	2	3	4	5	6	7	8
17 - 17.99	1	0	0	0	0	0	0	0	0
18 - 18.99	1	0	0	0	0	0	0	0	0
21 - 21.99	1	0	0	0	0	0	0	0	0
22 - 22.99	0	1	0	0	0	0	0	0	0
23 - 23.99	0.4	0.6	0	0	0	0	0	0	0
24 - 24.99	0.5	0.5	0	0	0	0	0	0	0
26 - 26.99	0.29	0.71	0	0	0	0	0	0	0
27 - 27.99	0	0.86	0.14	0	0	0	0	0	0
28 - 28.99	0	1	0	0	0	0	0	0	0
29 - 29.99	0	1	0	0	0	0	0	0	0
30 - 30.99	0	1	0	0	0	0	0	0	0
31 - 31.99	0	1	0	0	0	0	0	0	0
32 - 32.99	0	1	0	0	0	0	0	0	0
33 - 33.99	0	1	0	0	0	0	0	0	0
34 - 34.99	0	0.5	0.33	0.17	0	0	0	0	0
35 - 35.99	0	0.5	0.5	0	0	0	0	0	0
36 - 36.99	0	0.5	0.5	0	0	0	0	0	0
37 - 37.99	0	0.5	0.5	0	0	0	0	0	0
38 - 38.99	0	0.5	0.5	0	0	0	0	0	0
39 - 39.99	0	0.38	0.62	0	0	0	0	0	0
40 - 40.99	0	0.17	0.83	0	0	0	0	0	0
41 - 41.99	0	0.25	0.62	0.12	0	0	0	0	0
42 - 42.99	0	0.38	0.62	0	0	0	0	0	0
43 - 43.99	0	0.25	0.75	0	0	0	0	0	0
44 - 44.99	0	0	1	0	0	0	0	0	0
45 - 45.99	0	0.12	0.88	0	0	0	0	0	0
46 - 46.99	0	0	0.83	0.17	0	0	0	0	0
47 - 47.99	0	0.12	0.75	0.12	0	0	0	0	0
48 - 48.99	0	0.17	0.83	0	0	0	0	0	0
49 - 49.99	0	0.17	0.67	0.17	0	0	0	0	0
50 - 50.99	0	0	0.67	0.33	0	0	0	0	0
51 - 51.99	0	0	0.67	0.33	0	0	0	0	0
52 - 52.99	0	0	1	0	0	0	0	0	0
53 - 53.99	0	0	0.8	0.2	0	0	0	0	0
54 - 54.99	0	0	0.83	0.17	0	0	0	0	0
55 - 55.99	0	0	0.83	0.17	0	0	0	0	0
56 - 56.99	0	0	0	1	0	0	0	0	0
57 - 57.99	0	0	0.67	0.33	0	0	0	0	0
58 - 58.99	0	0	0.5	0.5	0	0	0	0	0
59 - 59.99	0	0	0.5	0.5	0	0	0	0	0
60 - 60.99	0	0	0.33	0.67	0	0	0	0	0
61 - 61.99	0	0	0.33	0.67	0	0	0	0	0

(To continue)

Table 3.4 (Continued)

	Age								
Interval	0	1	2	3	4	5	6	7	8
62 - 62.99	0	0	0.17	0.83	0	0	0	0	0
63 - 63.99	0	0	0.5	0.5	0	0	0	0	0
64 - 64.99	0	0	0.17	0.83	0	0	0	0	0
65 - 65.99	0	0	0.33	0.67	0	0	0	0	0
66 - 66.99	0	0	0.26	0.68	0.05	0	0	0	0
67 - 67.99	0	0	0.57	0.43	0	0	0	0	0
68 - 68.99	0	0	0.33	0.5	0.17	0	0	0	0
69 - 69.99	0	0	0.5	0.38	0	0.12	0	0	0
70 - 70.99	0	0	0.33	0.67	0	0	0	0	0
71 - 71.99	0	0	0.33	0.5	0.17	0	0	0	0
72 - 72.99	0	0	0	1	0	0	0	0	0
74 - 74.99	0	0	0.25	0.25	0.25	0	0.25	0	0
76 - 76.99	0	0	0	1	0	0	0	0	0
77 - 77.99	0	0	0	0.5	0.5	0	0	0	0
79 - 79.99	0	0	0	0	0	0	0.5	0.5	0
80 - 80.99	0	0	0	0	0	0.33	0.33	0	0.33
81 - 81.99	0	0	0	0	0	0	0.57	0.43	0
82 - 82.99	0	0	0	0	0	0	0.5	0.5	0
83 - 83.99	0	0	0	0	0	0	0.5	0.5	0
84 - 84.99	0	0	0	0	0	0	0.6	0.4	0
85 - 85.99	0	0	0	0	0	0	0.33	0.67	0
91 - 91.99	0	0	0	0	0	0	0	1	0

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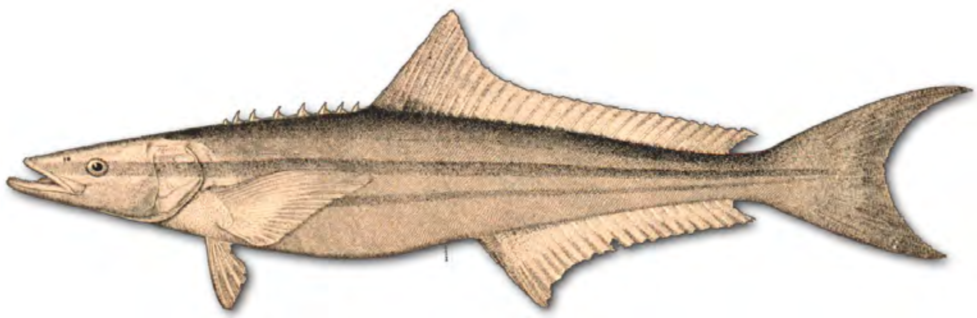
Chapter 4

COBIA *Rachycentron canadum*



4.1 INTRODUCTION

We aged a total of 345 Cobia, *Rachycentron canadum*, collected by the VMRC's Biological Sampling Program for age and growth analysis in 2024. Cobia ages ranged from 2 to 12 years old with an average age of 5.7, a standard deviation of 1.9, and a standard error of 0.1. Ten age classes (2 to 10, and 12) were represented, comprising fish of the 2012, and 2014 to 2022 year-classes. The sample was dominated by fish from the year-classes of 2016, 2018, 2019, and 2020 with 19.4%, 20%, 12.8%, and 27%, 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 a permanent marker 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, three inch diameter, Norton 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 CQFE website on how to prepare otolith thin-section for ageing Cobia.

4.2.3 Readings

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

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

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

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, Reader 1 re-aged the fish with disagreement and decided a final age for the fish. This method is different from what we used before the pandemic of COVID-19 during the period of 2020 -2021 because of 6-food social distance requirement. 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).

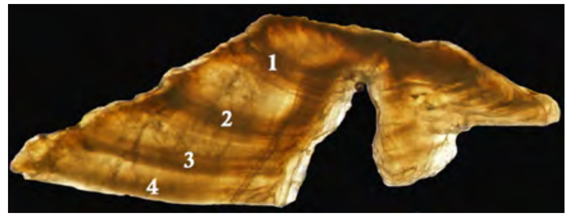


Figure 4.1: Otolith thin-section of a 4 year-old Cobia.

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 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 4.4.0 (R Core Team 2021).

4.3 RESULTS

4.3.1 Reading precision

Both readers had high self-precision. Specifically, there was no significant difference between the first and second readings for Reader 1 with an agreement of 94% and a *CV* of 1.19% (test of symmetry: $\chi^2 = 3$, $df = 3$, $P = 0.3916$), and there was no significant difference between the first and second readings for Reader 2 with an agreement of 98%

and a *CV* of 0.19% (test of symmetry: $\chi^2 = 1$, $df = 1$, $P = 0.3173$). There was no evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 94.2% and a *CV* of 0.78% (test of symmetry: $\chi^2 = 12.57$, $df = 8$, $P = 0.1275$) (Figure 4.2).

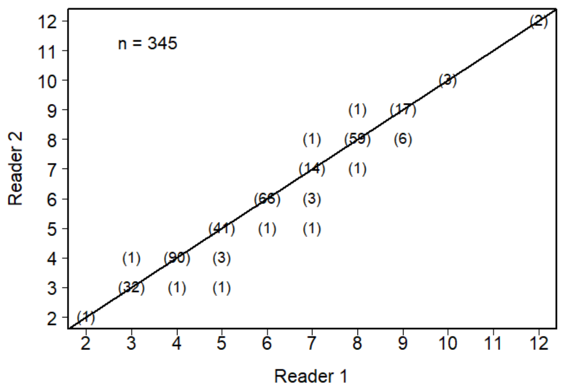


Figure 4.2: Between-reader comparison of otolith age estimates for Cobia collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2024. The number in parentheses is number of fish.

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 2.06% (test of symmetry: $\chi^2 = 9$, $df = 6$, $P = 0.1736$). Reader 2 had an agreement of 82% with a *CV* of 1.93% (test of symmetry: $\chi^2 = 7$, $df = 6$, $P = 0.3208$).

4.3.2 Year class

Of the 345 fish aged with otoliths, 10 age classes (2 to 10, and 12) were represented (Table 4.1). The average age was 5.7 years, and the standard deviation and standard error were 1.9 and 0.1, respectively. Year-class data show that the fishery was comprised of 10 year-classes: fish from the 2012, and 2014 to 2022 year-classes, with fish primarily from the year classes of 2016, 2018, 2019, and 2020 with 19.4%, 20%, 12.8%, and 27%, respectively. The ratio of males to females was 1:2.33 in the sample collected (Figure 4.3).

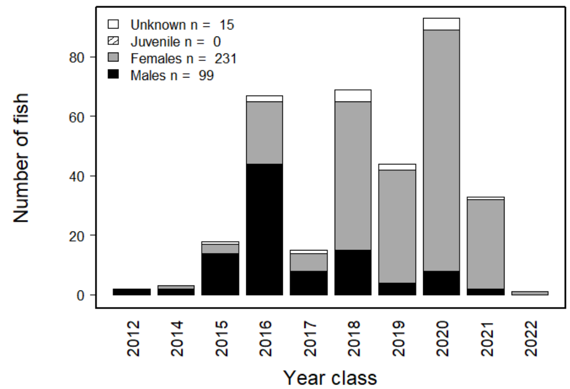


Figure 4.3: Year-class frequency distribution for Cobia collected for ageing in 2024. Distribution is broken down by sex. 'Unknown' represents gonads that were not available for examination or were not examined for sex during sampling.

4.3.3 Age-length key (ALK)

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

Table 4.1: The number of Cobia assigned to each total length (inch)-at-age category for 345 fish sampled for otolith age determination in Virginia during 2024.

	Age										
Interval	2	3	4	5	6	7	8	9	10	12	Totals
28 - 28.99	1	0	0	0	0	0	0	0	0	0	1
32 - 32.99	0	1	0	0	0	0	0	0	0	0	1
34 - 34.99	0	0	1	0	0	0	0	0	0	0	1
35 - 35.99	0	1	0	0	0	0	0	0	0	0	1
36 - 36.99	0	4	1	0	0	0	0	0	0	0	5
37 - 37.99	0	5	5	1	0	0	0	0	0	0	11
38 - 38.99	0	5	7	2	4	0	0	0	0	0	18
39 - 39.99	0	7	6	1	4	1	7	1	0	0	27
40 - 40.99	0	3	13	2	1	4	6	1	0	0	30
41 - 41.99	0	4	16	5	4	1	6	2	0	0	38
42 - 42.99	0	2	13	0	3	2	7	0	0	0	27
43 - 43.99	0	1	13	5	2	1	4	2	1	0	29
44 - 44.99	0	0	11	4	10	1	9	3	1	0	39
45 - 45.99	0	0	3	7	3	0	3	4	0	1	21
46 - 46.99	0	0	3	6	5	0	4	1	1	1	21
47 - 47.99	0	0	1	2	6	1	2	1	0	0	13
48 - 48.99	0	0	0	4	5	2	1	0	0	0	12
49 - 49.99	0	0	0	2	6	0	3	0	0	0	11
50 - 50.99	0	0	0	0	5	0	4	1	0	0	10
51 - 51.99	0	0	0	2	10	1	3	0	0	0	16
52 - 52.99	0	0	0	1	1	1	6	1	0	0	10
53 - 53.99	0	0	0	0	0	0	1	0	0	0	1
57 - 57.99	0	0	0	0	0	0	1	1	0	0	2
Totals	1	33	93	44	69	15	67	18	3	2	345

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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 2024.

	Age									
Interval	2	3	4	5	6	7	8	9	10	12
28 - 28.99	1	0	0	0	0	0	0	0	0	0
32 - 32.99	0	1	0	0	0	0	0	0	0	0
34 - 34.99	0	0	1	0	0	0	0	0	0	0
35 - 35.99	0	1	0	0	0	0	0	0	0	0
36 - 36.99	0	0.8	0.2	0	0	0	0	0	0	0
37 - 37.99	0	0.45	0.45	0.09	0	0	0	0	0	0
38 - 38.99	0	0.28	0.39	0.11	0.22	0	0	0	0	0
39 - 39.99	0	0.26	0.22	0.04	0.15	0.04	0.26	0.04	0	0
40 - 40.99	0	0.1	0.43	0.07	0.03	0.13	0.2	0.03	0	0
41 - 41.99	0	0.11	0.42	0.13	0.11	0.03	0.16	0.05	0	0
42 - 42.99	0	0.07	0.48	0	0.11	0.07	0.26	0	0	0
43 - 43.99	0	0.03	0.45	0.17	0.07	0.03	0.14	0.07	0.03	0
44 - 44.99	0	0	0.28	0.1	0.26	0.03	0.23	0.08	0.03	0
45 - 45.99	0	0	0.14	0.33	0.14	0	0.14	0.19	0	0.05
46 - 46.99	0	0	0.14	0.29	0.24	0	0.19	0.05	0.05	0.05
47 - 47.99	0	0	0.08	0.15	0.46	0.08	0.15	0.08	0	0
48 - 48.99	0	0	0	0.33	0.42	0.17	0.08	0	0	0
49 - 49.99	0	0	0	0.18	0.55	0	0.27	0	0	0
50 - 50.99	0	0	0	0	0.5	0	0.4	0.1	0	0
51 - 51.99	0	0	0	0.12	0.62	0.06	0.19	0	0	0
52 - 52.99	0	0	0	0.1	0.1	0.1	0.6	0.1	0	0
53 - 53.99	0	0	0	0	0	0	1	0	0	0
57 - 57.99	0	0	0	0	0	0	0.5	0.5	0	0

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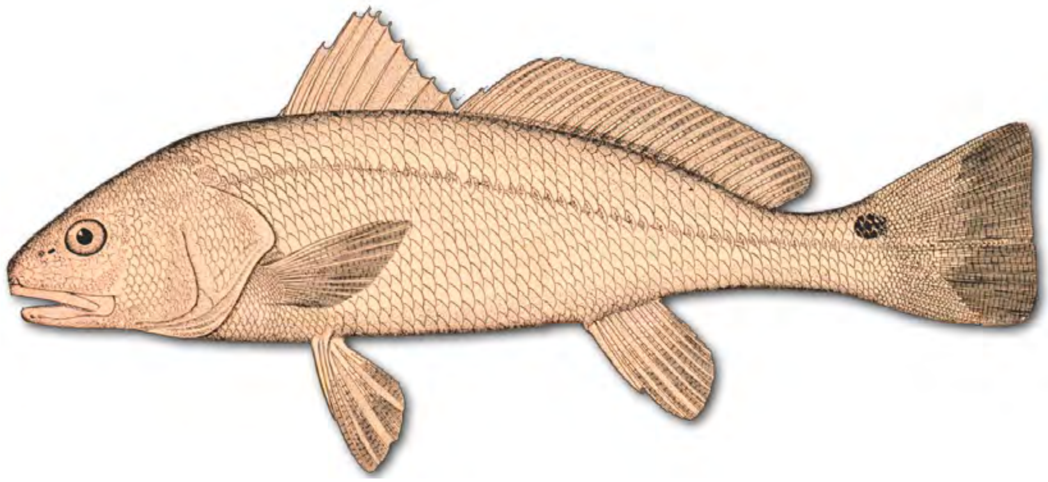
Chapter 5

RED DRUM *Sciaenops ocellatus*



5.1 INTRODUCTION

We aged a total of 192 Red Drum, *Sciaenops ocellatus*, collected by the VMRC's Biological Sampling Program for age and growth analysis in 2024. Red drum ages ranged from 0 to 2 years old with an average age of 1.1, 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 2022 to 2024 year-classes. The sample was dominated by fish from the year-class of 2023 with 80.2%.

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 dry in 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 glass slide with Crystalbond^{TM} 509 adhesive. 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 a pencil 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, three inch diameter, Norton 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 CQFE website on how to prepare otolith thin-section for ageing Red Drum.

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".

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 2024 (Figure 5.1).

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, Reader 1 re-aged the fish with disagreement and decided a final age for the fish. This method is different from what we used before the pandemic of COVID-19 during the period of 2020 -2021 because of 6-food social distance requirement. 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).

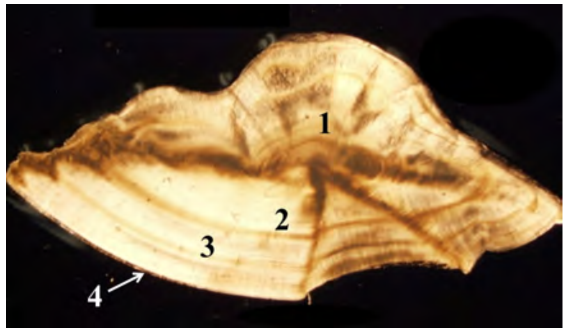


Figure 5.1: Otolith thin-section of a 3 year-old Red Drum with the last annulus on the edge of the thin-section

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 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 4.4.0 (R Core Team 2021).

5.3 RESULTS

5.3.1 Reading precision

Both readers had high self-precision. Specifically, there was no significant difference between the first and second readings for Reader 1 with an agreement of 98% and a *CV* of 0.94% (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 98.44% and a *CV* of 0.74% (test of symmetry: $\chi^2 = 3$, $df = 1$, $P = 0.0833$) (Figure 5.2).

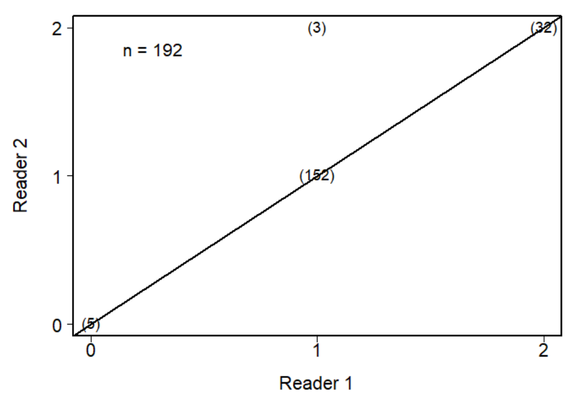


Figure 5.2: Between-reader comparison of otolith age estimates for Red Drum collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2024. The number in parentheses is number of fish.

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% .

5.3.2 Year class

Of the 192 fish aged with otoliths, 3 age classes (0 to 2) were represented (Table 5.1). The average age was 1.1 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 2022 to 2024 year-classes, with fish primarily from the year class of 2023 with 80.2%. The ratio of males to females was 1:0.57 in the sample collected (Figure 5.3).

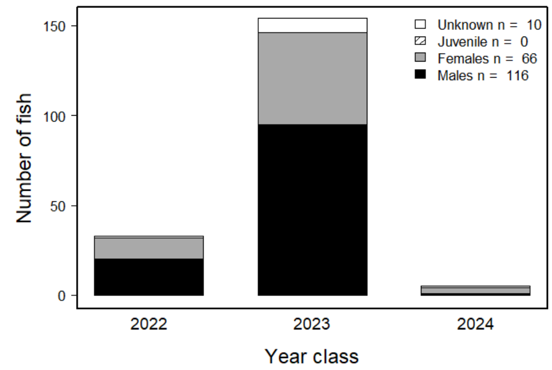


Figure 5.3: Year-class frequency distribution for Red Drum collected for ageing in 2024. Distribution is broken down by sex. 'Unknown' represents gonads that were not available for examination or were not examined for sex during sampling.

5.3.3 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.

Table 5.1: The number of Red Drum assigned to each total length (inch)-at-age category for 192 fish sampled for otolith age determination in Virginia during 2024.

	Age			
Interval	0	1	2	Totals
12 - 12.99	1	0	0	1
13 - 13.99	1	0	0	1
14 - 14.99	2	0	0	2
17 - 17.99	0	2	0	2
18 - 18.99	1	3	0	4
19 - 19.99	0	8	1	9
20 - 20.99	0	23	0	23
21 - 21.99	0	31	1	32
22 - 22.99	0	43	0	43
23 - 23.99	0	27	7	34
24 - 24.99	0	13	6	19
25 - 25.99	0	3	15	18
26 - 26.99	0	1	3	4
Totals	5	154	33	192

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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 2024.

	Age		
Interval	0	1	2
12 - 12.99	1	0	0
13 - 13.99	1	0	0
14 - 14.99	1	0	0
17 - 17.99	0	1	0
18 - 18.99	0.25	0.75	0
19 - 19.99	0	0.89	0.11
20 - 20.99	0	1	0
21 - 21.99	0	0.97	0.03
22 - 22.99	0	1	0
23 - 23.99	0	0.79	0.21
24 - 24.99	0	0.68	0.32
25 - 25.99	0	0.17	0.83
26 - 26.99	0	0.25	0.75

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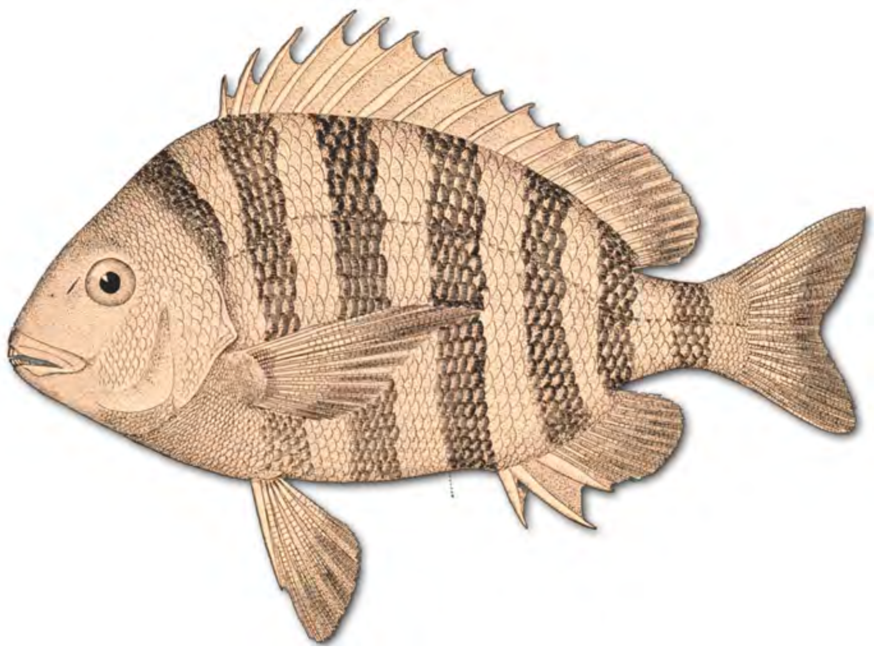
Chapter 6

SHEEPSHEAD *Archosargus probatocephalus*



6.1 INTRODUCTION

We aged a total of 275 Sheepshead, *Archosargus probatocephalus*, collected by the VMRC's Biological Sampling Program for age and growth analysis in 2024. Sheepshead ages ranged from 1 to 33 years old with an average age of 10.5, a standard deviation of 7.6, and a standard error of 0.46. twenty-eight age classes (1 to 20, 23 to 28, 30, and 33) were represented, comprising fish of the 1991, 1994, 1996 to 2001, and 2004 to 2023 year-classes. The sample was dominated by fish from the year-classes of 2007, 2011, 2012, 2015, 2016, 2019, 2020, 2021, and 2022 with 5.1%, 12.4%, 5.1%, 4.7%, 7.6%, 9.4%, 6.2%, 11.3%, and 9.1%, 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 a permanent marker across the epoxy resin surface. At least one transverse cross-section (hereafter "thin-section") was then removed from the marked core of each otolith using a Buehler IsoMet^{TM} low-speed saw equipped with two, three inch diameter, Norton 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 CQFE website on how to prepare otolith thin-section for ageing Sheepshead.

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".

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, Reader 1 re-aged the fish with disagreement and decided a final age for the fish. This method is different from what we used before the pandemic of COVID-19 during the period of 2020 -2021 because of 6-food social distance requirement. All thin-sections were aged using a Nikon SMZ1000 stereo microscope under transmitted light and dark-field polarization at between 8 and 20 times magnification (Figure 6.1).

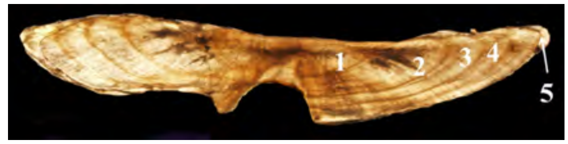


Figure 6.1: Otolith thin-section of a 5 year-old Sheepshead

6.2.4 Comparison tests

A symmetry test (Hoenig et al. 1995) and coefficient of variation (*CV*) analysis were used to detect any systematic difference and precision on age readings, respectively, for the following comparisons: 1) between the two readers in the current year, 2) within each reader in the current year, and 3) time-series bias between the current and previous years within each reader. The readings from the entire sample for the current year were used to examine the difference between two readers. A random sub-sample of 50 fish from the current year was selected for second readings to examine the difference within a reader. Fifty otoliths randomly selected from fish aged in 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 4.4.0 (R Core Team 2021).

6.3 RESULTS

6.3.1 Reading precision

Both readers had high self-precision. Specifically, there was no significant difference between the first and second readings for Reader 1 with an agreement of 84% and a *CV* of 0.66% (test of symmetry: $\chi^2 = 4$, $df = 6$, $P = 0.6767$), and there was no significant difference between the first and second readings for Reader 2 with an agreement of 74% and a *CV* of 1.78% (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 77.45% and a *CV* of 1.92% (test of symmetry: $\chi^2 = 33$, $df = 25$, $P = 0.1309$) (Figure 6.2).

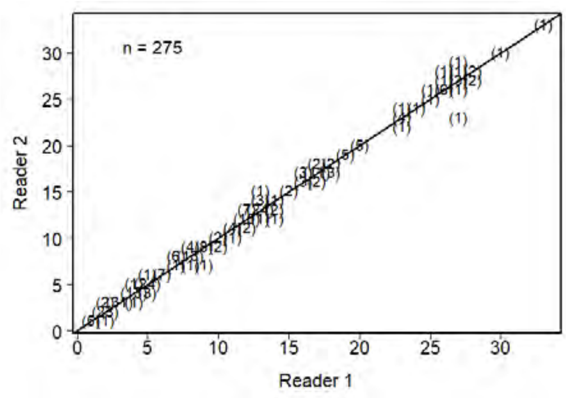


Figure 6.2: Between-reader comparison of otolith age estimates for Sheepshead collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2024. The number in parentheses is number of fish.

There was no time-series bias for either reader. Reader 1 had an agreement of 88% with ages of fish aged in 2008 with a *CV* of 1.54% (test of symmetry: $\chi^2 = 6$, $df = 6$, $P = 0.4232$). Reader 2 had an agreement of 94% with a *CV* of 1.93% (test of symmetry: $\chi^2 = 3$, $df = 3$, $P = 0.3916$).

6.3.2 Year class

Of the 275 fish aged with otoliths, 28 age classes (1 to 20, 23 to 28, 30, and 33) were represented (Table 6.1). The average age was 10.5 years, and the standard deviation and standard error were 7.6 and 0.46, respectively. Year-class data show that the fishery was comprised of 28 year-classes: fish from the 1991, 1994, 1996 to 2001, and 2004 to 2023 year-classes, with fish primarily from the year classes of 2007, 2011, 2012, 2015, 2016, 2019, 2020, 2021, and 2022 with 5.1%, 12.4%, 5.1%, 4.7%, 7.6%, 9.4%, 6.2%, 11.3%, and 9.1%, respectively. The ratio of males to females was 1:1.86 in the sample collected (Figure 6.3).

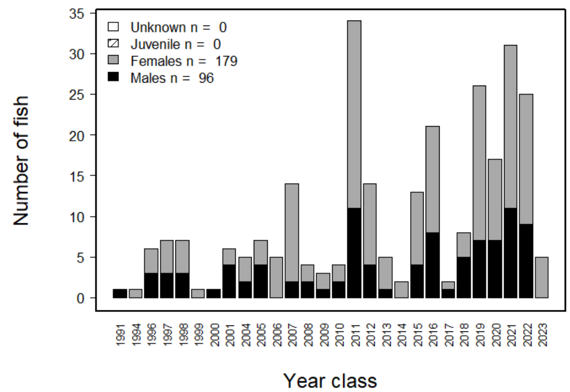


Figure 6.3: Year-class frequency distribution for Sheepshead collected for ageing in 2024. Distribution is broken down by sex. 'Unknown' represents gonads that were not available for examination or were not examined for sex during sampling.

6.3.3 Age-length key (ALK)

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

Table 6.1: The number of sheepshead assigned to each total length (inch)-at-age category for 275 fish sampled for otolith age determination in Virginia during 2024.

	Age																												
Interval	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	23	24	25	26	27	28	30	33	Totals
9 - 9.99	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
10 - 10.99	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5
11 - 11.99	3	7	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	11
12 - 12.99	2	9	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	13
13 - 13.99	0	3	7	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	11
14 - 14.99	0	0	7	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	8
15 - 15.99	0	0	11	8	3	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	23
16 - 16.99	0	0	3	3	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11
17 - 17.99	0	0	0	1	2	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4
18 - 18.99	0	0	0	2	6	5	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	14
19 - 19.99	0	0	0	0	6	0	1	3	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11
20 - 20.99	0	0	0	1	3	3	0	7	2	0	0	2	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	20
21 - 21.99	0	0	0	0	0	0	1	6	4	1	3	4	3	1	0	0	0	1	1	1	0	0	0	1	0	2	0	0	29
22 - 22.99	0	0	0	0	0	0	0	3	1	0	2	2	17	2	2	1	5	1	2	2	5	1	1	4	2	1	1	0	55
23 - 23.99	0	0	0	0	1	0	0	2	4	1	0	4	6	1	1	1	7	3	3	2	1	0	0	1	2	2	0	1	43
24 - 24.99	0	0	0	0	0	0	0	0	0	0	0	1	6	0	0	1	2	0	0	0	0	0	0	1	2	1	0	0	14
25 - 25.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	2
Totals	5	25	31	17	26	8	2	21	13	2	5	14	34	4	3	4	14	5	7	5	6	1	1	7	7	6	1	1	275

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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 2024.

	Age																											
Interval	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	23	24	25	26	27	28	30	33
9 - 9.99	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10 - 10.99	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11 - 11.99	0.27	0.64	0.09	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12 - 12.99	0.15	0.69	0.15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13 - 13.99	0	0.27	0.64	0.09	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14 - 14.99	0	0	0.88	0.12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15 - 15.99	0	0	0.48	0.35	0.13	0	0	0	0.04	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16 - 16.99	0	0	0.27	0.27	0.45	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17 - 17.99	0	0	0	0.25	0.5	0	0	0	0.25	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	0.14	0.43	0.36	0	0	0	0	0	0.07	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19 - 19.99	0	0	0	0	0.55	0	0.09	0.27	0	0	0	0	0.09	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20 - 20.99	0	0	0	0.05	0.15	0.15	0	0.35	0.1	0	0	0.1	0.05	0	0	0.05	0	0	0	0	0	0	0	0	0	0	0	0
21 - 21.99	0	0	0	0	0	0	0.03	0.21	0.14	0.03	0.1	0.14	0.1	0.03	0	0	0	0.03	0.03	0.03	0	0	0	0.03	0	0.07	0	0
22 - 22.99	0	0	0	0	0	0	0	0.05	0.02	0	0.04	0.04	0.31	0.04	0.04	0.02	0.09	0.02	0.04	0.04	0.09	0.02	0.02	0.07	0.04	0.02	0.02	0
23 - 23.99	0	0	0	0	0.02	0	0	0.05	0.09	0.02	0	0.09	0.14	0.02	0.02	0.02	0.16	0.07	0.07	0.05	0.02	0	0	0.02	0.05	0.05	0	0.02
24 - 24.99	0	0	0	0	0	0	0	0	0	0	0	0.07	0.43	0	0	0.07	0.14	0	0	0	0	0	0	0.07	0.14	0.07	0	0
25 - 25.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.5	0	0	0	0	0	0.5	0	0	0

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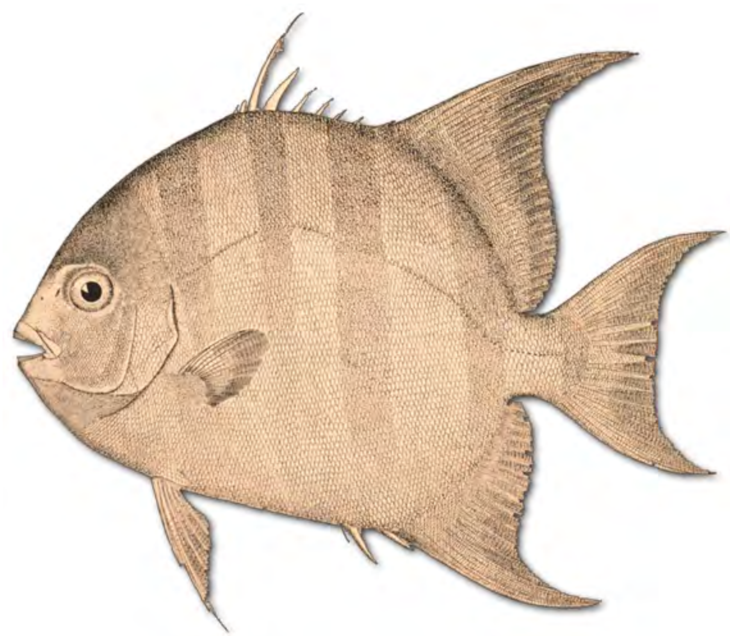
Chapter 7

ATLANTIC SPADEFISH
Chaetodipterus faber



7.1 INTRODUCTION

We aged a total of 282 Spadefish, *Chaetodipterus faber*, collected by the VMRC's Biological Sampling Program for age and growth analysis in 2024. Spadefish ages ranged from 1 to 8 years old with an average age of 2.7, a standard deviation of 1.3, and a standard error of 0.08. Eight age classes (1 to 8) were represented, comprising fish of the 2016 to 2023 year-classes. The sample was dominated by fish from the year-class of 2022 with 51.8%.

7.2 METHODS

7.2.1 Sample size for ageing

We estimated sample size for ageing Spadefish in 2024 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 2024; 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 2018 to 2022. θ_a, V_a, and B_a were calculated using pooled age-length data of Spadefish collected from 2018 to 2022 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 2018 to 2022 catch. A_l is number of fish to be aged for length interval l in 2024.

7.2.2 Handling of collections

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

7.2.3 Preparation

We used our thin-section and bake 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 a permanent marker 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, 3-inch diameter, Norton 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 focus. 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 CQFE website on how to prepare otolith thin-section for ageing Atlantic Spadefish.

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".

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, Reader 1 re-aged the fish with disagreement and decided a final age for the fish. This method is different from what we used before the pandemic of COVID-19 during the period of 2020 -2021 because of 6-food social distance requirement. 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 7.1).

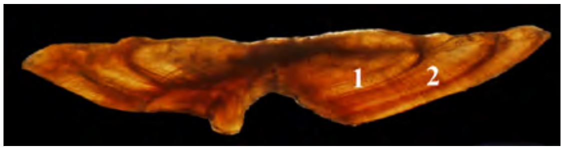


Figure 7.1: Otolith thin-section of a 2 year-old Spadefish

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) 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 4.4.0 (R Core Team 2021).

7.3 RESULTS

7.3.1 Sample size

We estimated a sample size of 348 Spadefish in 2024, ranging in length interval from 3 to 22 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 2024, we aged 282 of 344 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 89 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.

7.3.2 Reading precision

Both readers had high self-precision. Specifically, there was no significant difference between the first and second readings for Reader 1 with an agreement of 96% and a *CV* of 0.88% (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 94% and a *CV* of 1.51% (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 86.17% and a *CV* of 3.46% (test of symmetry: $\chi^2 = 8.31$, $df = 6$, $P = 0.2163$) (Figure 7.2).

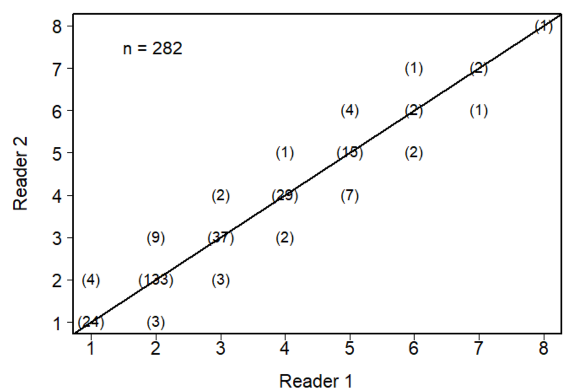


Figure 7.2: Between-reader comparison of otolith age estimates for Spadefish collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2024. The number in parentheses is number of fish.

There was no time-series bias for either reader. Reader 1 had an agreement of 80% with ages of fish aged in 2003 with a CV of 1.71% (test of symmetry: $\chi^2 = 10$, $df = 7$, $P = 0.1886$). Reader 2 had an agreement of 78% with a CV of 3.26% (test of symmetry: $\chi^2 = 11$, $df = 10$, $P = 0.3575$).

7.3.3 Year class

Of the 282 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 1.3 and 0.08, respectively. Year-class data show that the fishery was comprised of 8 year-classes: fish from the 2016 to 2023 year-classes, with fish primarily from the year class of 2022 with 51.8%. The ratio of males to females was 1:0.92 in the sample collected (Figure 7.3).

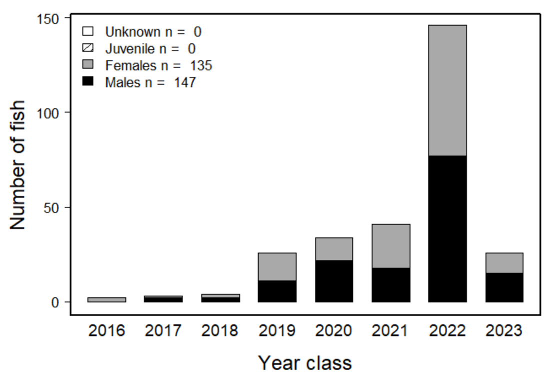


Figure 7.3: Year-class frequency distribution for Spadefish collected for ageing in 2024. Distribution is broken down by sex. 'Unknown' represents gonads that were not available for examination or were not examined for sex during sampling.

7.3.4 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.

Table 7.1: Number of Atlantic Spadefish collected and aged in each 1-inch length interval in 2024. 'Target' represents the sample size for ageing estimated for 2024, 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	21	20	20	1
6 - 6.99	41	81	51	0
7 - 7.99	41	74	42	0
8 - 8.99	29	41	41	0
9 - 9.99	20	18	18	2
10 - 10.99	17	15	15	2
11 - 11.99	16	13	13	3
12 - 12.99	21	9	9	12
13 - 13.99	23	10	10	13
14 - 14.99	18	15	15	3
15 - 15.99	17	14	14	3
16 - 16.99	13	12	12	1
17 - 17.99	20	8	8	12
18 - 18.99	15	4	4	11
19 - 19.99	9	1	1	8
20 - 20.99	5	3	3	2
21 - 21.99	5	3	3	2
22 - 22.99	5	2	2	3
Totals	348	344	282	89

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Table 7.2: CV for each age estimated based on ageing the total of 348 Spadefish in 2024. 'Percent' is the percentage of an age in the pooled age-length data of Spadefish collected from 2018 to 2022.

Age	CV	Percent
0	0.23	3.27
1	0.16	8.21
2	0.07	27.05
3	0.08	25.15
4	0.1	17.86
5	0.16	9.12
6	0.18	6.53
7	>0.25	1.75
8	>0.25	0.76
9	>0.25	0.23
10	>0.25	0.08

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Table 7.3: The number of Atlantic Spadefish assigned to each total length-at-age category for 282 fish sampled for otolith age determination in Virginia during 2024.

	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	17	3	0	0	0	0	0	0	20
6 - 6.99	8	42	1	0	0	0	0	0	51
7 - 7.99	0	42	0	0	0	0	0	0	42
8 - 8.99	0	36	5	0	0	0	0	0	41
9 - 9.99	0	16	2	0	0	0	0	0	18
10 - 10.99	0	6	8	1	0	0	0	0	15
11 - 11.99	0	0	7	5	1	0	0	0	13
12 - 12.99	0	1	4	3	1	0	0	0	9
13 - 13.99	0	0	8	2	0	0	0	0	10
14 - 14.99	0	0	3	7	5	0	0	0	15
15 - 15.99	0	0	2	8	3	1	0	0	14
16 - 16.99	0	0	1	4	6	1	0	0	12
17 - 17.99	0	0	0	3	3	1	0	1	8
18 - 18.99	0	0	0	0	3	0	1	0	4
19 - 19.99	0	0	0	0	0	0	1	0	1
20 - 20.99	0	0	0	0	1	1	1	0	3
21 - 21.99	0	0	0	1	1	0	0	1	3
22 - 22.99	0	0	0	0	2	0	0	0	2
Totals	26	146	41	34	26	4	3	2	282

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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 2024.

	Age							
Interval	1	2	3	4	5	6	7	8
4 - 4.99	1	0	0	0	0	0	0	0
5 - 5.99	0.85	0.15	0	0	0	0	0	0
6 - 6.99	0.16	0.82	0.02	0	0	0	0	0
7 - 7.99	0	1	0	0	0	0	0	0
8 - 8.99	0	0.88	0.12	0	0	0	0	0
9 - 9.99	0	0.89	0.11	0	0	0	0	0
10 - 10.99	0	0.4	0.53	0.07	0	0	0	0
11 - 11.99	0	0	0.54	0.38	0.08	0	0	0
12 - 12.99	0	0.11	0.44	0.33	0.11	0	0	0
13 - 13.99	0	0	0.8	0.2	0	0	0	0
14 - 14.99	0	0	0.2	0.47	0.33	0	0	0
15 - 15.99	0	0	0.14	0.57	0.21	0.07	0	0
16 - 16.99	0	0	0.08	0.33	0.5	0.08	0	0
17 - 17.99	0	0	0	0.38	0.38	0.12	0	0.12
18 - 18.99	0	0	0	0	0.75	0	0.25	0
19 - 19.99	0	0	0	0	0	0	1	0
20 - 20.99	0	0	0	0	0.33	0.33	0.33	0
21 - 21.99	0	0	0	0.33	0.33	0	0	0.33
22 - 22.99	0	0	0	0	1	0	0	0

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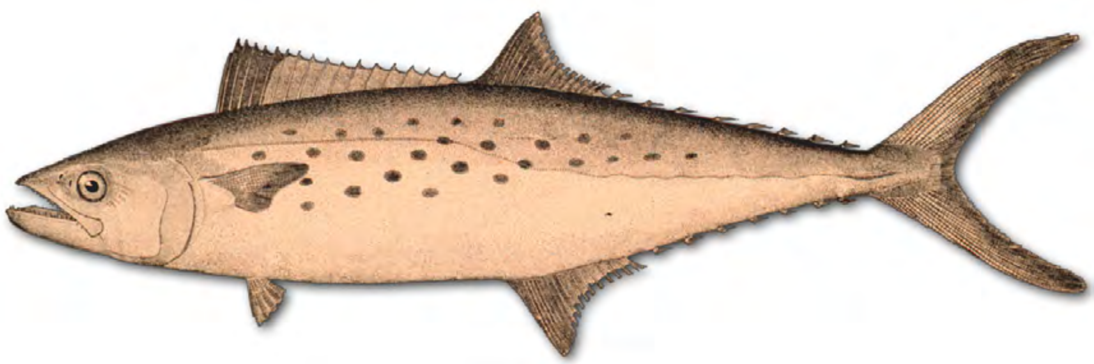
Chapter 8

SPANISH MACKEREL
Scomberomorous maculatus



8.1 INTRODUCTION

We aged a total of 301 Spanish Mackerel, *Scomberomorous maculatus*, collected by the VMRC's Biological Sampling Program for age and growth analysis in 2024. Spanish Mackerel ages ranged from 0 to 8 years old with an average age of 1.9, a standard deviation of 1.2, and a standard error of 0.07. Eight age classes (0 to 6, and 8) were represented, comprising fish of the 2016, and 2018 to 2024 year-classes. The sample was dominated by fish from the year-classes of 2022 and 2023 with 33.9% and 46.2%, respectively.

8.2 METHODS

8.2.1 Sample size for ageing

We estimated sample size for ageing Spanish Mackerel in 2024 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 2024; 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 2018 to 2022. θ_a, V_a, and B_a were calculated using pooled age-length data of Spanish Mackerel collected from 2018 to 2022 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 2018 to 2022 catch. A_l is number of fish to be aged for length interval l in 2024.

8.2.2 Handling of collections

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

8.2.3 Preparation

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 a permanent marker 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, three inch diameter, Norton 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 CQFE website on how to prepare otolith thin-section for ageing Spanish Mackerel.

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".

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, Reader 1 re-aged the fish with disagreement and decided a final age for the fish. This method is different from what we used before the pandemic of COVID-19 during the period of 2020 -2021 because of 6-food social distance requirement. 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 8.1).

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 read-

Figure 8.1: Otolith thin-section of a 3 year-old Spanish Mackerel with the last annulus on the edge of the thin-section

ers 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 4.4.0 (R Core Team 2021).

8.3 RESULTS

8.3.1 Sample size

We estimated a sample size of 329 Spanish Mackerel in 2024, ranging in length interval from 12 to 34 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 2024, we randomly selected and aged 301 fish from 378 Spanish Mackerel collected by VMRC. We fell short in our over-all collections for this optimal length-class sampling estimate by 56 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.

8.3.2 Reading precision

Reader 1 had moderate self-precision and Reader 2 had high self-precision.Specifically, there was no significant difference between the first and second readings for Reader 1 with an agreement of 84% and a *CV* of 5.08% (test of symmetry: $\chi^2 = 6$, $df = 4$, $P = 0.1991$), and there was no significant difference between the first and second readings for Reader 2 with an agreement of 88% and a *CV* of 3.99% (test of symmetry: $\chi^2 = 1.33$, $df = 3$, $P = 0.7212$). There was no evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 87.71% and a *CV* of 4.39% (test of symmetry: $\chi^2 = 9.2$, $df = 5$, $P = 0.1013$) (Figure 8.2).

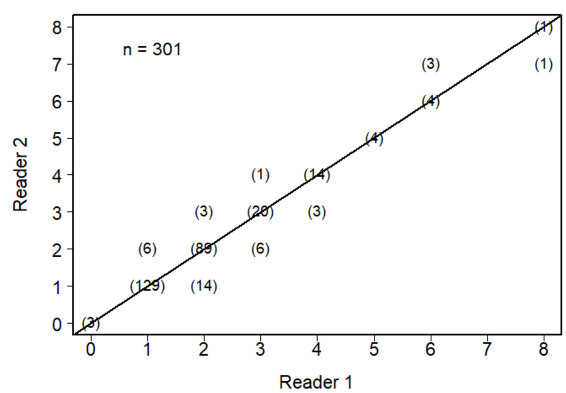


Figure 8.2: Between-reader comparison of otolith age estimates for Spanish Mackerel collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2024. The number in parentheses is number of fish.

There was no time-series bias for either reader. Reader 1 had an agreement of 96% with fish aged in 2003 with a *CV* of 1.89% (test of symmetry: $\chi^2 = 2$, $df = 1$, $P = 0.1573$). Reader 2 had an agreement of 98% with a *CV* of 0.31% (test of symmetry: $\chi^2 = 1$, $df = 1$, $P = 0.3173$).

8.3.3 Year class

Of the 301 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 1.2 and 0.07, respectively. Year-class data show that the fishery was comprised of 8 year-classes: fish from the 2016, and 2018 to 2024 year-classes, with fish primarily from the year classes of 2022 and 2023 with 33.9% and 46.2%, respectively. The ratio of males to females was 1:2.52 in the sample collected (Figure 8.3).

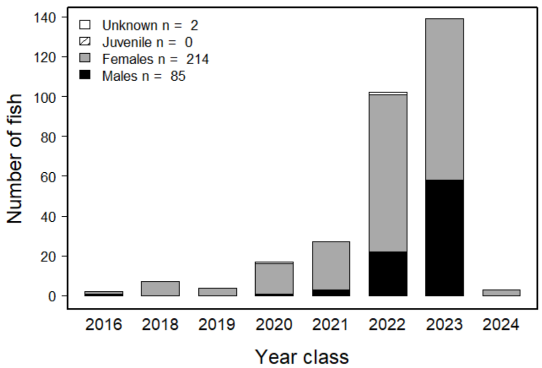


Figure 8.3: Year-class frequency distribution for Spanish Mackerel collected for ageing in 2024. Distribution is broken down by sex. 'Unknown' represents gonads that were not available for examination or were not examined for sex during sampling.

8.3.4 Age-length key (ALK)

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

Table 8.1: Number of Spanish Mackerel collected and aged in each 1-inch length interval in 2024. 'Target' represents the sample size for ageing estimated for 2024, and 'Need' represents number of fish shorted in each length interval compared to the optimum sample size for ageing and number of fish aged.

Interval	Target	Collected	Aged	Need
12 - 12.99	5	0	0	5
13 - 13.99	5	1	1	4
14 - 14.99	24	8	8	16
15 - 15.99	38	49	49	0
16 - 16.99	45	73	46	0
17 - 17.99	44	58	44	0
18 - 18.99	27	36	28	0
19 - 19.99	23	30	24	0
20 - 20.99	17	22	18	0
21 - 21.99	16	22	16	0
22 - 22.99	9	12	10	0
23 - 23.99	9	18	10	0
24 - 24.99	9	12	10	0
25 - 25.99	8	11	11	0
26 - 26.99	6	9	9	0
27 - 27.99	7	11	11	0
28 - 28.99	7	2	2	5
29 - 29.99	5	1	1	4
30 - 30.99	5	2	2	3
31 - 31.99	5	1	1	4
32 - 32.99	5	0	0	5
33 - 33.99	5	0	0	5
34 - 34.99	5	0	0	5
Totals	329	378	301	56

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Table 8.2: CV for each age estimated based on ageing the total of 329 Spanish Mackerel in 2024. 'Percent' is the percentage of an age in the pooled age-length data of Spanish Mackerel collected from 2018 to 2022.

Age	CV	Percent
0	0.23	4.61
1	0.05	42.2
2	0.09	25.42
3	0.13	13.94
4	0.15	9.03
5	>0.25	2.36
6	>0.25	1.57
7	>0.25	0.88

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Table 8.3: The number of Spanish Mackerel assigned to each total length-at-age category for 301 fish sampled for otolith age determination in Virginia during 2024.

	Age								
Interval	0	1	2	3	4	5	6	8	Totals
13 - 13.99	0	1	0	0	0	0	0	0	1
14 - 14.99	3	5	0	0	0	0	0	0	8
15 - 15.99	0	44	5	0	0	0	0	0	49
16 - 16.99	0	42	3	1	0	0	0	0	46
17 - 17.99	0	30	14	0	0	0	0	0	44
18 - 18.99	0	15	11	2	0	0	0	0	28
19 - 19.99	0	1	20	2	1	0	0	0	24
20 - 20.99	0	1	17	0	0	0	0	0	18
21 - 21.99	0	0	16	0	0	0	0	0	16
22 - 22.99	0	0	6	4	0	0	0	0	10
23 - 23.99	0	0	4	3	3	0	0	0	10
24 - 24.99	0	0	2	5	3	0	0	0	10
25 - 25.99	0	0	4	5	2	0	0	0	11
26 - 26.99	0	0	0	2	4	0	2	1	9
27 - 27.99	0	0	0	2	3	3	3	0	11
28 - 28.99	0	0	0	1	0	0	1	0	2
29 - 29.99	0	0	0	0	1	0	0	0	1
30 - 30.99	0	0	0	0	0	1	1	0	2
31 - 31.99	0	0	0	0	0	0	0	1	1
Totals	3	139	102	27	17	4	7	2	301

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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 2024.

	Age							
Interval	0	1	2	3	4	5	6	8
13 - 13.99	0	1	0	0	0	0	0	0
14 - 14.99	0.38	0.62	0	0	0	0	0	0
15 - 15.99	0	0.9	0.1	0	0	0	0	0
16 - 16.99	0	0.91	0.07	0.02	0	0	0	0
17 - 17.99	0	0.68	0.32	0	0	0	0	0
18 - 18.99	0	0.54	0.39	0.07	0	0	0	0
19 - 19.99	0	0.04	0.83	0.08	0.04	0	0	0
20 - 20.99	0	0.06	0.94	0	0	0	0	0
21 - 21.99	0	0	1	0	0	0	0	0
22 - 22.99	0	0	0.6	0.4	0	0	0	0
23 - 23.99	0	0	0.4	0.3	0.3	0	0	0
24 - 24.99	0	0	0.2	0.5	0.3	0	0	0
25 - 25.99	0	0	0.36	0.45	0.18	0	0	0
26 - 26.99	0	0	0	0.22	0.44	0	0.22	0.11
27 - 27.99	0	0	0	0.18	0.27	0.27	0.27	0
28 - 28.99	0	0	0	0.5	0	0	0.5	0
29 - 29.99	0	0	0	0	1	0	0	0
30 - 30.99	0	0	0	0	0	0.5	0.5	0
31 - 31.99	0	0	0	0	0	0	0	1

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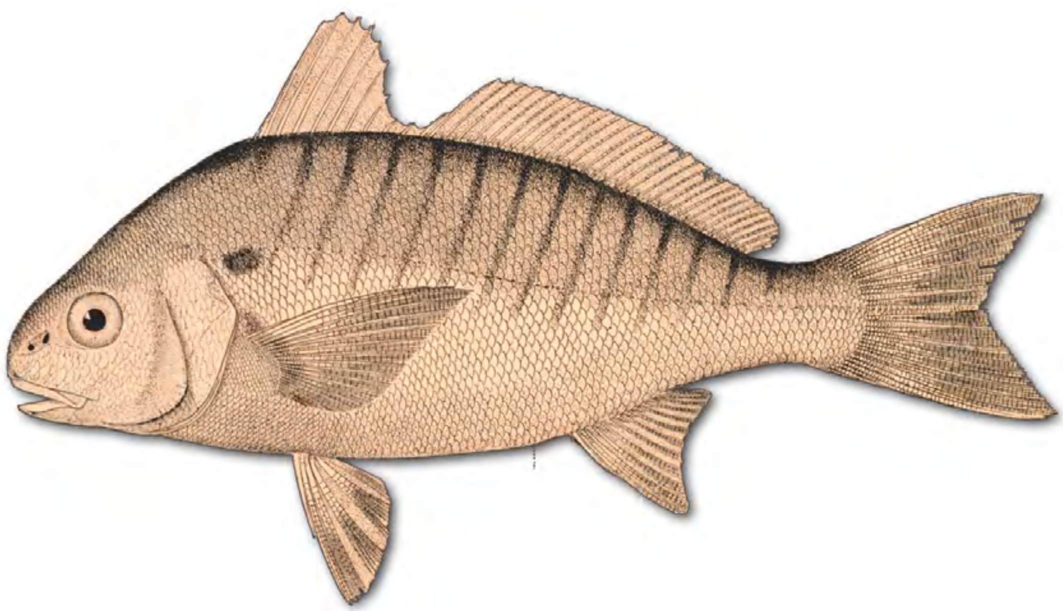
Chapter 9

SPOT *Leiostomus xanthurus*



9.1 INTRODUCTION

We aged a total of 143 Spot, *Leiostomus xanthurus*, collected by the VMRC's Biological Sampling Program for age and growth analysis in 2024. Spot ages ranged from 1 to 3 years old with an average age of 1.1, a standard deviation of 0.4, and a standard error of 0.03. Three age classes (1 to 3) were represented, comprising fish of the 2021 to 2023 year-classes. The sample was dominated by fish from the year-class of 2023 with 87.4%.

9.2 METHODS

9.2.1 Sample size for ageing

We estimated sample size for ageing Spot in 2024 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 2024; 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 2018 to 2022. θ_a, V_a, and B_a were calculated using pooled age-length data of Spot collected from 2018 to 2022 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 2018 to 2022 catch. A_l is number of fish to be aged for length interval l in 2024.

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 dry in 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 a permanent marker 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, 3-inch diameter, Norton 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 CQFE website on how to prepare otolith thin-section for ageing Spot.

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".

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, Reader 1 re-aged the fish with disagreement and decided a final age for the fish. This method is different from what we used before the pandemic of COVID-19 during the period of 2020 -2021 because of 6-food social distance requirement. 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 9.1).

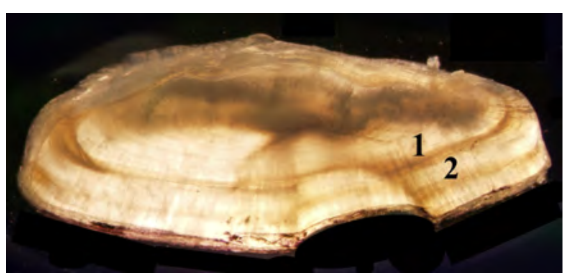


Figure 9.1: Otolith thin-section of a 2 year-old Spot

9.2.5 Comparison tests

A symmetry test (Hoenig et al. 1995) and coefficient of variation (*CV*) analysis were used to detect any systematic difference and precision on age readings, respectively, for the following comparisons: 1) between the two readers in the current year, 2) within each reader in the current year, and 3) time-series bias between the current and previous years within each reader. The readings from the entire sample for the current year were used to examine the difference between two readers. A random sub-sample of 50 fish from the current year was selected for second readings to examine the difference within a reader. Fifty otoliths randomly selected from fish aged in 2003 were used to examine the time-series bias within each reader. A figure of 1:1 equivalence was used to illustrate those differences (Campana et al. 1995). All statistics analyses were performed in R 4.4.0 (R Core Team 2021).

9.3 RESULTS

9.3.1 Sample size

We estimated a sample size of 180 Spot in 2024, ranging in length interval from 3 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 2024, we randomly selected and aged 143 fish from 252 Spot collected by VMRC. We fell short in our over-all collections for this optimal length-class sampling estimate by 59 fish. We were short of many fish from the major length intervals (The interval requires 10 or more fish), as a result, the precision for the estimates of major age groups would definitely be influenced significantly. Therefore, precaution should be used when developing ALK using these age data.

9.3.2 Reading precision

Reader 1 had high self-precision and Read 2 had moderate self-precision. Specifically, there was no significant difference between the first and second readings for Reader 1 with an agreement of 96% and a *CV* of 1.89% (test of symmetry: $\chi^2 = 2$, $df = 1$, $P = 0.1573$), and there was a difference between the first and second readings for Reader 2 with an agreement of 82% and a *CV* of 8.49% (test of symmetry: $\chi^2 = 5.44$, $df = 1$, $P = 0.0196$). There was no evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 90.21% and a *CV* of 5.14% (test of symmetry: $\chi^2 = 5$, $df = 3$, $P = 0.1718$) (Figure 9.2).

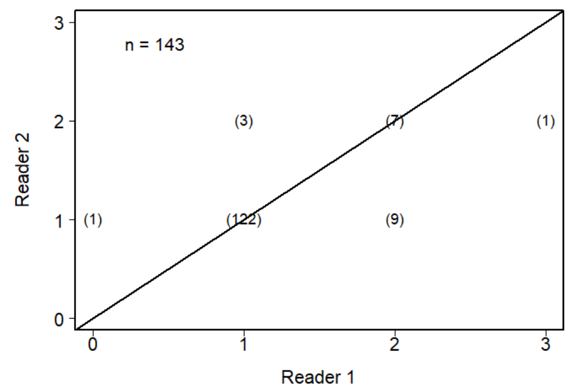


Figure 9.2: Between-reader comparison of otolith age estimates for Spot collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2024. The number in parentheses is number of fish.

There was no time-series bias for either reader. Reader 1 had an agreement of 100% with ages of fish aged in 2003. Reader 2 also had an agreement of 100% .

9.3.3 Year class

Of the 143 fish aged with otoliths, 3 age classes (1 to 3) were represented (Table 9.3). The average age was 1.1 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 2023 with 87.4%. The ratio of males to females was 1:7.19 in the sample collected (Figure 9.3).

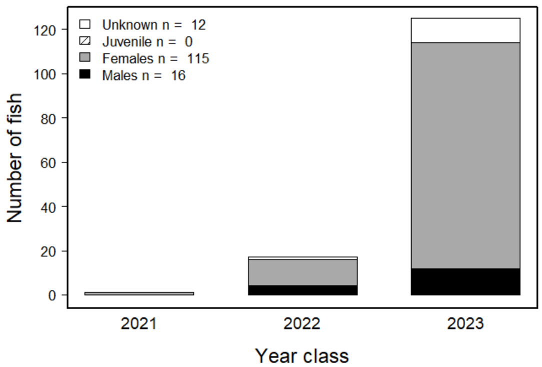


Figure 9.3: Year-class frequency distribution for Spot collected for ageing in 2024. Distribution is broken down by sex. 'Unknown' is for gonads that were not available for examination or were not examined for sex during sampling.

9.3.4 Age-length key (ALK)

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

Table 9.1: Number of Spot collected and aged in each 1-inch length interval in 2024. 'Target' represents the sample size for ageing estimated for 2024, 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	1	1	4
4 - 4.99	5	0	0	5
5 - 5.99	5	1	1	4
6 - 6.99	6	47	15	0
7 - 7.99	20	89	20	0
8 - 8.99	37	58	50	0
9 - 9.99	58	47	47	11
10 - 10.99	34	9	9	25
11 - 11.99	5	0	0	5
12 - 12.99	5	0	0	5
Totals	180	252	143	59

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Table 9.2: CV for each age estimated based on ageing the total of 180 Spot in 2024. 'Percent' is the percentage of an age in the pooled age-length data of Spot collected from 2018 to 2022.

Age	CV	Percent
0	0.21	4.7
1	0.04	76.3
2	0.16	17.53
3	>0.25	1.37
4	>0.25	0.1

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Table 9.3: The number of Spot assigned to each total length-at-age category for 143 fish sampled for otolith age determination in Virginia during 2024.

	Age			
Interval	1	2	3	Totals
3 - 3.99	1	0	0	1
5 - 5.99	1	0	0	1
6 - 6.99	14	1	0	15
7 - 7.99	19	1	0	20
8 - 8.99	39	10	1	50
9 - 9.99	43	4	0	47
10 - 10.99	8	1	0	9
Totals	125	17	1	143

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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 2024.

	Age		
Interval	1	2	3
3 - 3.99	1	0	0
5 - 5.99	1	0	0
6 - 6.99	0.93	0.07	0
7 - 7.99	0.95	0.05	0
8 - 8.99	0.78	0.2	0.02
9 - 9.99	0.91	0.09	0
10 - 10.99	0.89	0.11	0

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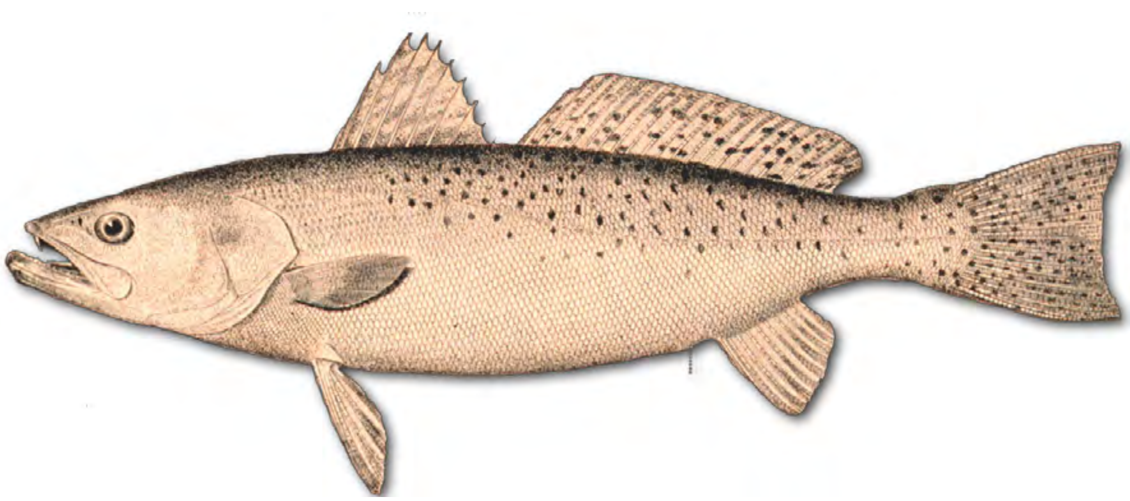
Chapter 10

SPOTTED SEATROUT *Cynoscion nebulosus*



10.1 INTRODUCTION

We aged a total of 296 Spotted Seatrout, *Cynoscion nebulosus*, collected by the VMRC's Biological Sampling Program for age and growth analysis in 2024. Spotted seatrout ages ranged from 0 to 8 years old with an average age of 2.1, 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 2016, and 2018 to 2024 year-classes. The sample was dominated by fish from the year-classes of 2022 and 2023 with 32.8% and 31.1%, respectively.

10.2 METHODS

10.2.1 Sample size for ageing

We estimated sample size for ageing Spotted Seatrout in 2024 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 2024; 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 2018 to 2022. θ_a, V_a, and B_a were calculated using pooled age-length data of Spotted Seatrout collected from 2018 to 2022 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 2018 to 2022 catch. A_l is number of fish to be aged for length interval l in 2024.

10.2.2 Handling of collections

Otoliths were received by the Age & 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 otolith was randomly selected and attached, distal side down, to a glass slide with clear Crystalbond^{TM} 509 adhesive. 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 a pencil 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, 3-inch diameter, Norton 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 CQFE website on how to prepare otolith thin-section for ageing Spotted Seatrout.

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".

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, Reader 1 re-aged the fish with disagreement and decided a final age for the fish. This method is different from what we used before the pandemic of COVID-19 during the period of 2020 -2021 because of 6-food social distance requirement. 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 10.1).

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 be-

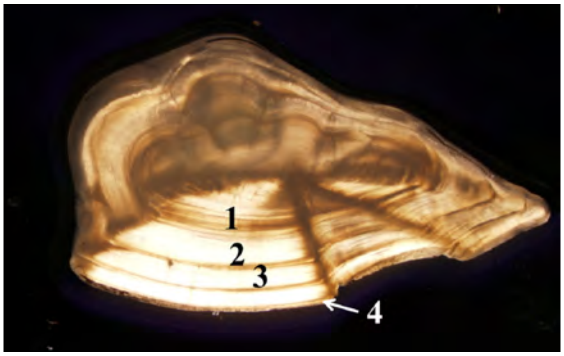


Figure 10.1: Otolith thin-section of a 4 year-old Spotted Seatrout with the last annulus on the edge of the thin-section

tween the current and previous years within each reader. The readings from the entire sample for the current year were used to examine the difference between two readers. A random sub-sample of 50 fish from the current year was selected for second readings to examine the difference within a reader. Fifty otoliths randomly selected from fish aged in 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 4.4.0 (R Core Team 2021).

10.3 RESULTS

10.3.1 Sample size

We estimated a sample size of 333 Spotted Seatrout in 2024, 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 2024, we randomly selected and aged 296 fish from 380 Spotted Seatrout collected by VMRC. We fell short in our over-all collections for this optimal length-class sampling estimate by 64 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 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 94% and a *CV* of 4.18% (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 97.64% and a *CV* of 0.94% (test of symmetry: $\chi^2 = 2.2$, $df = 3$, $P = 0.5319$) (Figure 10.2).

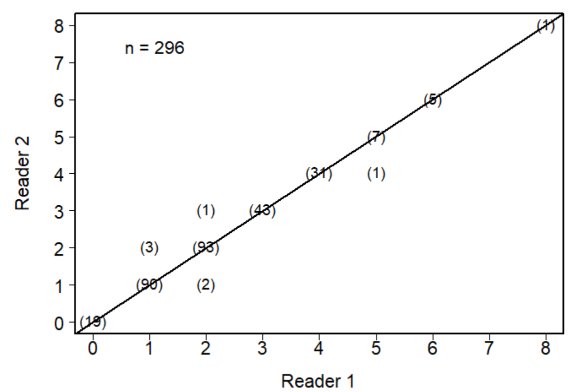


Figure 10.2: Between-reader comparison of otolith age estimates for Spotted Seatrout collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2024. The number in parentheses is number of fish.

There was no time-series bias for either reader. Reader 1 had an agreement of 100% with ages of fish aged in 2003. Reader 2 also had an agreement of 100%.

10.3.3 Year class

Of the 296 fish aged with otoliths, 8 age classes (0 to 6, and 8) were represented (Table 10.3). The average age was 2.1 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 2016, and 2018 to 2024 year-classes, with fish primarily from the year classes of 2022 and 2023 with 32.8% and 31.1%, respectively. The ratio of males to females was 1:1.43 in the sample collected (Figure 10.3).

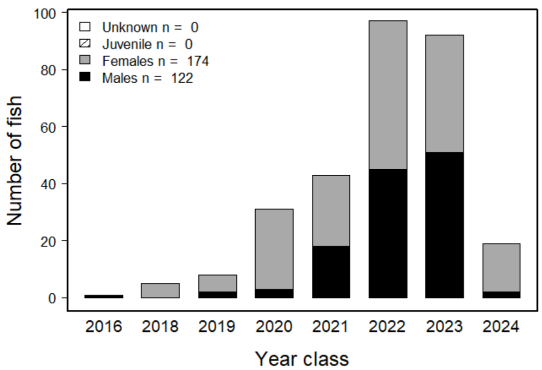


Figure 10.3: Year-class frequency distribution for Spotted Seatrout collected for ageing in 2024. Distribution is broken down by sex. 'Unknown' represents gonads that were not available for examination or were not examined for sex during sampling.

10.3.4 Age-length key (ALK)

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

Table 10.1: Number of Spotted Seatrout collected and aged in each 1-inch length interval in 2024. 'Target' represents the sample size for ageing estimated for 2024, 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	7	0	0	7
11 - 11.99	10	0	0	10
12 - 12.99	19	0	0	19
13 - 13.99	13	13	13	0
14 - 14.99	17	23	23	0
15 - 15.99	25	28	28	0
16 - 16.99	33	49	34	0
17 - 17.99	32	50	33	0
18 - 18.99	27	37	28	0
19 - 19.99	23	38	24	0
20 - 20.99	22	26	22	0
21 - 21.99	13	15	14	0
22 - 22.99	15	20	16	0
23 - 23.99	11	12	12	0
24 - 24.99	11	28	12	0
25 - 25.99	10	14	10	0
26 - 26.99	5	9	9	0
27 - 27.99	5	6	6	0
28 - 28.99	5	10	10	0
29 - 29.99	5	1	1	4
30 - 30.99	5	1	1	4
31 - 31.99	5	0	0	5
Totals	333	380	296	64

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Table 10.2: CV for each age estimated based on ageing the total of 333 Spotted Seatrout in 2024. 'Percent' is the percentage of an age in the pooled age-length data of Spotted Seatrout collected from 2018 to 2022.

Age	CV	Percent
0	0.11	10.57
1	0.05	43.95
2	0.07	30.65
3	0.13	11.2
4	>0.25	3.08
5	>0.25	0.56

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Table 10.3: The number of Spotted Seatrout assigned to each total length-at-age category for 296 fish sampled for otolith age determination in Virginia during 2024.

	Age								
Interval	0	1	2	3	4	5	6	8	Totals
13 - 13.99	7	6	0	0	0	0	0	0	13
14 - 14.99	12	10	1	0	0	0	0	0	23
15 - 15.99	0	23	4	1	0	0	0	0	28
16 - 16.99	0	25	9	0	0	0	0	0	34
17 - 17.99	0	19	14	0	0	0	0	0	33
18 - 18.99	0	6	20	1	1	0	0	0	28
19 - 19.99	0	1	17	4	2	0	0	0	24
20 - 20.99	0	1	15	6	0	0	0	0	22
21 - 21.99	0	1	9	4	0	0	0	0	14
22 - 22.99	0	0	7	7	0	2	0	0	16
23 - 23.99	0	0	1	9	2	0	0	0	12
24 - 24.99	0	0	0	7	4	0	0	1	12
25 - 25.99	0	0	0	2	8	0	0	0	10
26 - 26.99	0	0	0	1	7	1	0	0	9
27 - 27.99	0	0	0	0	4	1	1	0	6
28 - 28.99	0	0	0	1	3	4	2	0	10
29 - 29.99	0	0	0	0	0	0	1	0	1
30 - 30.99	0	0	0	0	0	0	1	0	1
Totals	19	92	97	43	31	8	5	1	296

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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 2024.

	Age							
Interval	0	1	2	3	4	5	6	8
13 - 13.99	0.54	0.46	0	0	0	0	0	0
14 - 14.99	0.52	0.43	0.04	0	0	0	0	0
15 - 15.99	0	0.82	0.14	0.04	0	0	0	0
16 - 16.99	0	0.74	0.26	0	0	0	0	0
17 - 17.99	0	0.58	0.42	0	0	0	0	0
18 - 18.99	0	0.21	0.71	0.04	0.04	0	0	0
19 - 19.99	0	0.04	0.71	0.17	0.08	0	0	0
20 - 20.99	0	0.05	0.68	0.27	0	0	0	0
21 - 21.99	0	0.07	0.64	0.29	0	0	0	0
22 - 22.99	0	0	0.44	0.44	0	0.12	0	0
23 - 23.99	0	0	0.08	0.75	0.17	0	0	0
24 - 24.99	0	0	0	0.58	0.33	0	0	0.08
25 - 25.99	0	0	0	0.2	0.8	0	0	0
26 - 26.99	0	0	0	0.11	0.78	0.11	0	0
27 - 27.99	0	0	0	0	0.67	0.17	0.17	0
28 - 28.99	0	0	0	0.1	0.3	0.4	0.2	0
29 - 29.99	0	0	0	0	0	0	1	0
30 - 30.99	0	0	0	0	0	0	1	0

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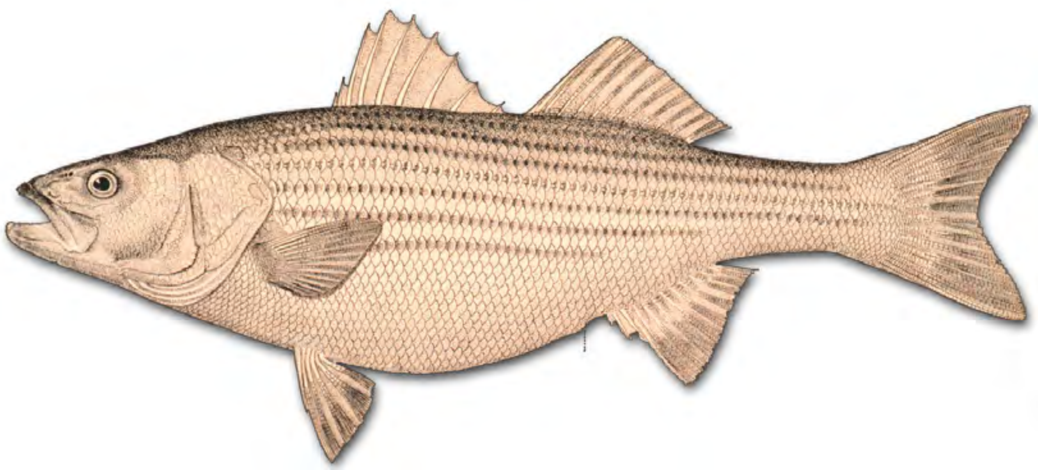
Chapter 11

STRIPED BASS *Morone saxatilis*



11.1 INTRODUCTION

We aged a total of 879 Striped Bass, *Morone saxatilis*, using the otoliths and scales of 349 fish, the otoliths of 2 fish, and the scales of 528 fish collected by the VMRC's Biological Sampling Program in 2024. Of 879 aged fish, 525 and 354 fish were collected in Chesapeake Bay (bay fish) and Virginia waters of the Atlantic Ocean (ocean fish), respectively. The average bay fish age was 7.2 years with a standard deviation of 3.6 and a standard error of 0.16. Twenty age classes (2 to 15, 17, 19 to 21, 24, and 29) were represented in the bay fish, comprising fish from the 1995, 2000, 2003 to 2005, 2007, and 2009 to 2022 year classes. The bay fish sample in 2024 was dominated by the year classes of 2015, 2018, 2019, and 2020 with 20%, 15%, 13%, and 16%, respectively. The average ocean fish age was 12.6 years with a standard deviation of 4.2 and a standard error of 0.22. Nineteen age classes (8 to 26) were represented in the ocean fish, comprising fish from the 1998 to 2016 year classes. The ocean fish sample in 2024 was dominated by the year classes of 2011, 2014, and 2015 with 10%, 18%, and 26%, respectively. The 349 otolith ages were compared to their paired 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 2024, 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:

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

where A is the sample size for ageing Striped Bass in 2024; 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 2018 to 2022. θ_a, V_a, and B_a were calculated using pooled age-length data of Striped Bass collected from 2018 to 2022 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 2018 to 2022 catch. A_l is number of fish to be aged for length interval l in 2024.

11.2.2 Handling of collection

Sagittal otoliths (hereafter, referred to as "otoliths") and scales were received by the Age & 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 thin-section and bake 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 a permanent marker 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, 3-inch diameter, Norton 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 focus. 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 CQFE website on how to prepare otolith thin-section for ageing Striped Bass.

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

11.2.4.1 *Otoliths*

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 (Figure 11.1). Each reader aged all of the otolith samples. 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. 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 Striped Bass have a tendency to split as they advance towards the distal surface. As a result, it is critical that reading path proceed in a direction down the sulcal ridge and outwards to the distal surface.

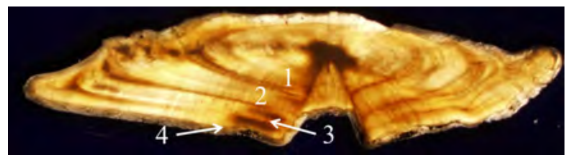


Figure 11.1: Otolith thin-section of a 4 year-old Striped Bass with the last annulus on the edge of the thin-section

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

11.2.4.2 *Scales*

We 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. Annuli on

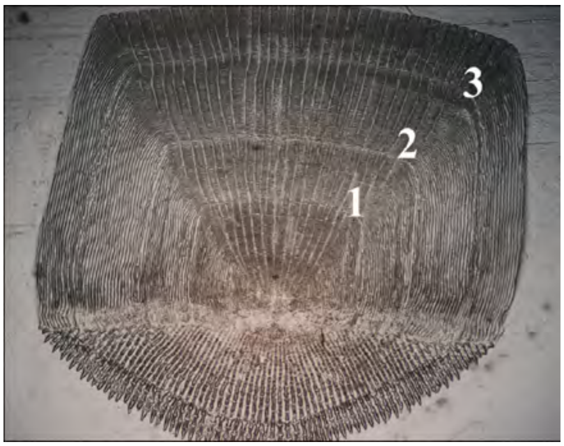


Figure 11.2: Scale impression of a 3 year-old Striped Bass.

Striped Bass scales are identified based on two scale microstructure features, "crossing over" and circuli disruption. Primarily, "crossing over" in the lateral margins near the posterior/anterior interface of the scale is used to determine the origin of the annulus. Here compressed circuli (annulus) "cross-over" the previously deposited circuli of the previous year's growth. Typically annuli of the first three years can be observed transversing this interface as dark bands. These bands remain consistent throughout the posterior field and rejoin the posterior/anterior interface on the opposite side of the focus. Annuli can also be observed in the anterior lateral field of the scale. Here the annuli typically reveal a pattern of discontinuous and suddenly breaking segmented circuli. This event can also be distinguished by the presence of concentric white lines, which are typically associated with the disruption of circuli. Annuli can also be observed bisecting the perpendicular plain of the radial striations in the anterior field of the scale. Radii emanate out from the focus of the scale towards the outer corner margins of the anterior field. These radial striations consist mainly of segmented concave circuli. The point of intersection between radii and annuli results in a "straightening out" of the concave circuli. This straightening of the circuli should be consistent throughout the entire anterior field of the scale. This event is further amplified by the presence of concave circuli neighboring both directly above and below the annulus. The first year's annulus can be difficult to locate on some scales. It is typically best identified in the lateral field of the anterior portion of the scale. The distance from the focus to the first year's annulus is typically larger with respect to the following annuli. For the annuli two through six, summer growth generally decreases proportionally. For ages greater than six, a crowding effect of the annuli near the outer margins of the scale is observed. This crowding effect creates difficulties in edge interpretation. At this point it is best to focus on the straightening of the circuli at the anterior margins of the scale.

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

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) between the two readers in the current year; 2) within each reader in the current year; 3) time-series bias between the current and previous years within each reader;

and 4) between scale and otoliths ages. The readings from the entire sample for the current year were used to examine the difference between two readers. A random sub-sample of 50 fish from the current year was selected for second readings to examine the difference within a reader. Fifty otoliths 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 4.4.0 (R Core Team 2021).

11.3 RESULTS

11.3.1 Sample size

11.3.1.1 *Chesapeake Bay*

We estimated a sample size of 500 bay Striped Bass in 2024, 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 4 and 5 to the *CV* of larger than 25% for the multiple minor ages of the bay fish (Table 11.2). We randomly selected and aged 525 fish from 714 Striped Bass collected by VMRC in Chesapeake Bay in 2024. We fell short in our over-all collections for this optimal length-class sampling estimate by 87 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 534 ocean Striped Bass in 2024, 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 and 11 to the *CV* of larger than 25% for the multiple minor ages of the ocean fish (Table 11.4). We aged all 354 Striped Bass collected by VMRC in Virginia waters of the Atlantic Ocean in 2024. We fell short in our over-all collections for this optimal length-class sampling estimate by 233 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 Reading precision

11.3.2.1 *Otoliths*

Both readers had high self-precision. Specifically, there was no significant difference between the first and second readings for Reader 1 with an agreement of 88% and a *CV* of 0.7% (test of symmetry: $\chi^2 = 2$, $df = 4$, $P = 0.7358$), and there was no significant difference between the first and second readings for Reader 2 with an agreement of 78% and a *CV* of 1.7% (test of symmetry: $\chi^2 = 9$, $df = 7$, $P = 0.2527$). There was no evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 91% (1 year or less agreement of 99%) and a *CV* of 0.6% (test of symmetry: $\chi^2 = 15.3$, $df = 17$, $P = 0.5749$) (Figure 11.3).

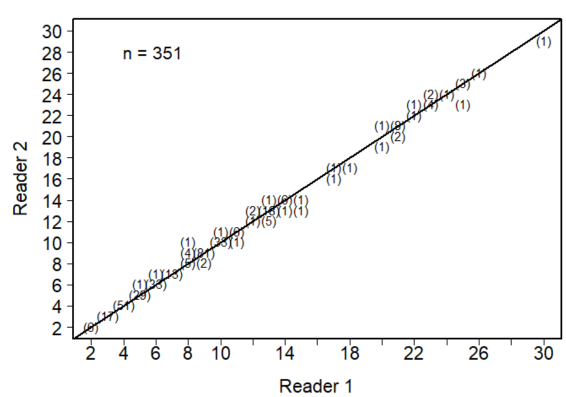


Figure 11.3: Between-reader comparison of otolith age estimates for Striped Bass collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2024. The number in parentheses is number of fish.

There was no time-series bias for either reader. Reader 1 had an agreement of 78% with ages of fish aged in 2003 with a *CV* of 2.6% (test of symmetry: $\chi^2 = 10.3$, $df = 9$, $P = 0.3242$). Reader 2 had an agreement of 77% with a *CV* of 3.1% (test of symmetry: $\chi^2 = 11.3$, $df = 11$, $P = 0.4158$).

11.3.2.2 *Scales*

Reader 1 had high self-precision and Read 2 had moderate self-precision. Specifically, there was no significant difference between the first and second readings for Reader 1 with an agreement of 70% (1 year or less agreement of 94%) and a *CV* of 2.8% (test of symmetry: $\chi^2 = 13$, $df = 13$, $P = 0.4478$), and there was no significant difference between the first and second readings for Reader 2 with an agreement of 58% (1 year or less agreement of 76%) and a *CV* of 6.2% (test of symmetry: $\chi^2 = 17$, $df = 17$, $P = 0.4544$). There was an evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 48% (1 year or less agreement of 81%) and a *CV* of 6.4% (test of symmetry: $\chi^2 = 133.1$, $df = 69$, $P < 0.0001$) (Figure 11.4).

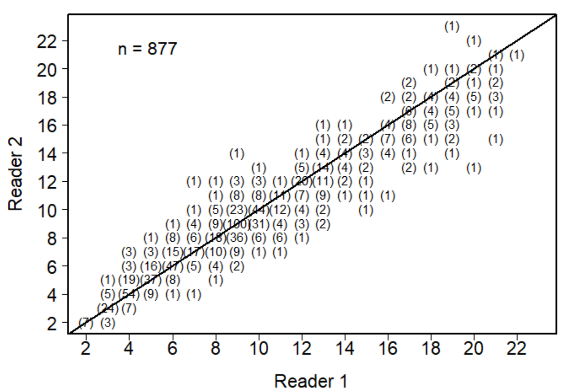


Figure 11.4: Between-reader comparison of scale age estimates for Striped Bass collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2024. The number in parentheses is number of fish.

There was no time-series bias for either reader. Reader 1 had an agreement of 58% (1 year or less agreement of 93%) with ages of fish aged in 2000 with a *CV* of 5.2% (test of symmetry: $\chi^2 = 7$, $df = 11$, $P = 0.7991$). Reader 2 had an agreement of 57% (1 year or less agreement of 90%) with a *CV* of 6% (test of symmetry: $\chi^2 = 12.5$, $df = 12$, $P = 0.409$).

11.3.3 Year class

When otolith-ages were available, they were the final ages, otherwise, scale-ages were the final ages. The final ages were used for the year-class analysis.

11.3.3.1 *Chesapeake Bay*

Of the 525 bay Striped Bass aged with 280 otoliths and 245 scales, 20 age classes (2 to 15, 17, 19 to 21, 24, and 29) were represented (Table 11.5). The average age for the sample was 7.2 years. The standard deviation and standard error were 3.6 and 0.16, respectively. Year-class data (Figure 11.5) indicates that recruitment into the fishery in Chesapeake Bay begins at age 2, which corresponds to the 2022 year-class for Striped Bass caught in 2024. Striped bass in the sample in 2024 was dominated by the year classes of 2015, 2018, 2019, and 2020 with 20%, 15%, 13%, and 16%, respectively. The sex ratio of male to female was 1:0.96 for the bay fish.

11.3.3.2 *Atlantic Ocean*

Of the 354 ocean Striped Bass aged with 71 otoliths and 283 scales, 19 age classes (8 to 26) were represented (Table 11.6). The average age for the sample was 12.6 years. The standard deviation and standard error were 4.2 and 0.22, respectively. Year-class data (Figure 11.6) indicates that recruitment into the fishery in Virginia waters of Atlantic ocean begins at age 8, which corresponds to the 2016 year-class for Striped Bass caught in 2024. Striped bass in the sample in 2024 was dominated by the year classes of 2011, 2014, and 2015 with 10%, 18%, and 26%, respectively. The sex ratio of male to female was 1:11.61 for the ocean fish.

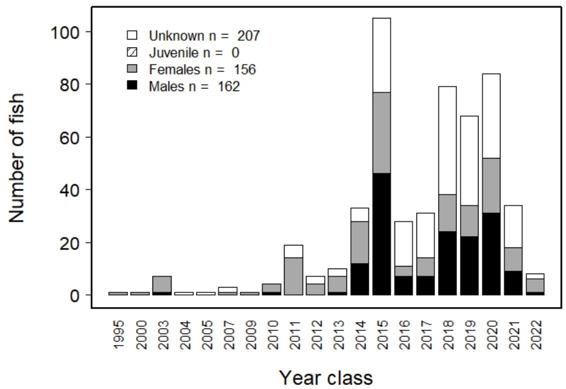


Figure 11.5: Year-class frequency distribution for Striped Bass collected in Chesapeake Bay, Virginia for ageing in 2024. Distribution is broken down by sex and estimated using scale ages. 'Unknown' represents the fish gonads that were not available for examination or were not examined for sex during sampling.

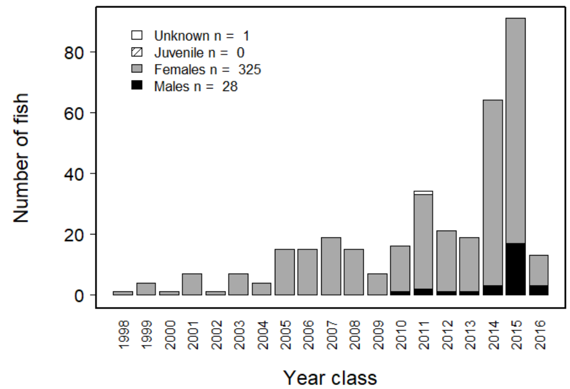


Figure 11.6: Year-class frequency distribution for Striped Bass collected in Virginia waters of the Atlantic Ocean for ageing in 2024. Distribution is broken down by sex and estimated using scale ages. 'Unknown' represents the fish gonads that were not available for examination or were not examined for sex during sampling.

11.3.4 Comparison of scale and otolith ages

We aged 349 Striped Bass using scales and otoliths (Excluding 2 fish with otolith-ages only). There was an evidence of systematic disagreement between otolith and scale ages (test of symmetry: $\chi^2 = 87.6$, $df = 41$, $P < 0.0001$) with an average CV of 4.9%. There was an agreement of 58% between scale and otoliths ages whereas scales were assigned a lower and higher age than otoliths for 27.8% and 14.3% of the fish, respectively (Figure 11.7). There was also an evidence of bias between otolith and scale ages using an age bias plot (Figure 11.8), with scale generally assigned higher ages for younger fish and lower ages for older fish than otolith age estimates.

11.3.5 Age-Length-Key (ALK)

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

11.4 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.

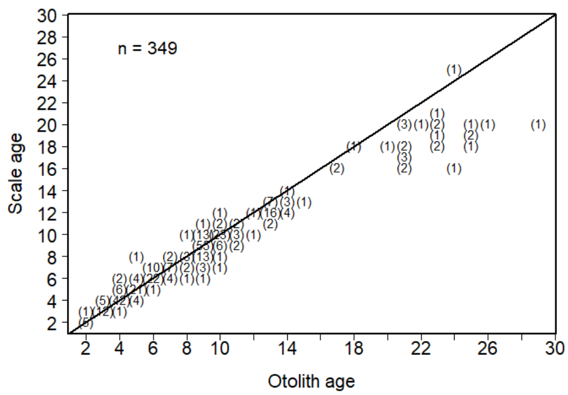


Figure 11.7: Comparison of scale and otolith age estimates for Striped Bass collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2024. The number in parentheses is number of fish.

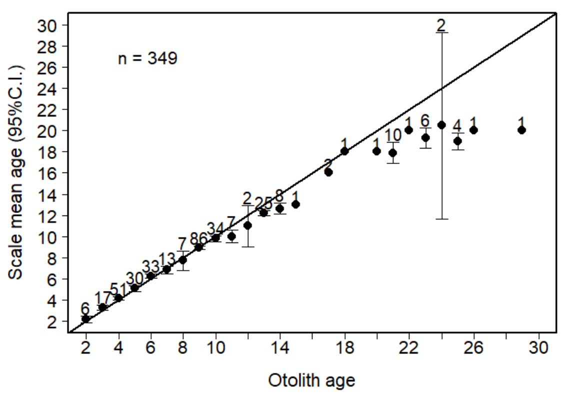


Figure 11.8: Age-bias plot for Striped Bass scale and otolith age estimates in 2024. The number above the upper CI bar is number of fish.

Table 11.1: Number of bay Striped Bass collected and aged in each 1-inch length interval in 2024. 'Target' represents the sample size for ageing estimated for 2024, 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	1	1	4
16 - 16.99	5	0	0	5
17 - 17.99	5	0	0	5
18 - 18.99	14	11	11	3
19 - 19.99	29	36	33	0
20 - 20.99	35	53	41	0
21 - 21.99	27	56	41	0
22 - 22.99	25	55	40	0
23 - 23.99	24	44	32	0
24 - 24.99	23	48	32	0
25 - 25.99	21	38	29	0
26 - 26.99	21	37	23	0
27 - 27.99	18	32	19	0
28 - 28.99	14	23	15	0
29 - 29.99	12	19	16	0
30 - 30.99	12	20	14	0
31 - 31.99	11	20	13	0
32 - 32.99	13	13	13	0
33 - 33.99	12	21	17	0
34 - 34.99	10	33	16	0
35 - 35.99	12	27	17	0
36 - 36.99	10	20	10	0
37 - 37.99	11	13	12	0
38 - 38.99	11	19	13	0
39 - 39.99	8	15	10	0
40 - 40.99	6	14	13	0
41 - 41.99	8	13	11	0
42 - 42.99	7	6	6	1
43 - 43.99	6	11	11	0
44 - 44.99	8	6	6	2
45 - 45.99	7	3	3	4
46 - 46.99	8	4	4	4
47 - 47.99	7	0	0	7
48 - 48.99	5	3	3	2
49 - 49.99	5	0	0	5
50 - 50.99	5	0	0	5
51 - 51.99	5	0	0	5
52 - 52.99	5	0	0	5
55 - 55.99	5	0	0	5
Totals	500	714	525	87

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Table 11.2: CV for each age estimated based on ageing the total of 500 bay Striped Bass in 2024. 'Percent' is the percentage of an age in the pooled age-length data of bay Striped Bass collected from 2018 to 2022.

Age	CV	Percent
1	>0.25	0.57
2	>0.25	0.64
3	0.17	5.61
4	0.1	14.21
5	0.1	15.99
6	0.11	13.28
7	0.12	11.42
8	0.15	7.85
9	0.15	7.35
10	0.21	4.18
11	0.23	3.39
12	>0.25	1.75
13	>0.25	1.46
14	>0.25	1.71
15	>0.25	1.75
16	>0.25	2.14
17	>0.25	1.57
18	>0.25	1.89
19	>0.25	1.36
20	>0.25	0.68
21	>0.25	0.64
22	>0.25	0.36
23	>0.25	0.14
24	>0.25	0.04

(Go back to text)

Table 11.3: Number of ocean Striped Bass collected and aged in each 1-inch length interval in 2024. 'Target' represents the sample size for ageing estimated for 2024, 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
28 - 28.99	5	0	0	5
29 - 29.99	5	0	0	5
30 - 30.99	5	2	2	3
31 - 31.99	5	2	2	3
32 - 32.99	8	3	3	5
33 - 33.99	13	7	7	6
34 - 34.99	21	11	11	10
35 - 35.99	32	27	27	5
36 - 36.99	52	43	43	9
37 - 37.99	64	46	46	18
38 - 38.99	67	36	36	31
39 - 39.99	56	14	14	42
40 - 40.99	47	17	17	30
41 - 41.99	39	20	20	19
42 - 42.99	26	16	16	10
43 - 43.99	17	16	16	1
44 - 44.99	13	31	31	0
45 - 45.99	6	25	25	0
46 - 46.99	9	19	19	0
47 - 47.99	9	15	15	0
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	534	354	354	233

(Go back to text)

Table 11.4: CV for each age estimated based on ageing the total of 534 ocean Striped Bass in 2024. 'Percent' is the percentage of an age in the pooled age-length data of ocean Striped Bass collected from 2018 to 2022.

Age	CV	Percent
5	>0.25	0.36
6	>0.25	0.09
7	>0.25	1.9
8	0.12	10.51
9	0.12	12.05
10	0.09	18.66
11	0.09	18.12
12	0.13	10.42
13	0.19	5.07
14	0.18	5.8
15	0.17	5.8
16	0.23	3.17
17	0.24	2.81
18	>0.25	1.45
19	>0.25	1.36
20	>0.25	1.09
21	>0.25	0.72
22	>0.25	0.18
23	>0.25	0.18
24	>0.25	0.18
25	>0.25	0.09

(Go back to text)

Table 11.5: The number of Striped Bass assigned to each total length-at-age category for 525 fish sampled for the final ages determined with 280 otolith ages and 245 scale ages in Chesapeake Bay, Virginia during 2024.

	Age																			
Interval	2	3	4	5	6	7	8	9	10	11	12	13	14	15	17	19	20	21	24	29	Totals
15 - 15.99	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
18 - 18.99	2	6	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11
19 - 19.99	4	7	16	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	33
20 - 20.99	0	4	22	4	9	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	41
21 - 21.99	0	5	18	9	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	41
22 - 22.99	1	7	10	13	7	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	40
23 - 23.99	0	4	10	9	6	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0	32
24 - 24.99	0	1	3	9	13	3	2	0	1	0	0	0	0	0	0	0	0	0	0	0	32
25 - 25.99	0	0	2	5	9	4	4	3	2	0	0	0	0	0	0	0	0	0	0	0	29
26 - 26.99	0	0	1	5	9	3	2	2	1	0	0	0	0	0	0	0	0	0	0	0	23
27 - 27.99	0	0	0	6	6	1	2	3	0	1	0	0	0	0	0	0	0	0	0	0	19
28 - 28.99	0	0	0	0	4	3	2	4	2	0	0	0	0	0	0	0	0	0	0	0	15
29 - 29.99	0	0	0	1	2	2	4	4	2	1	0	0	0	0	0	0	0	0	0	0	16
30 - 30.99	0	0	0	0	2	4	0	7	1	0	0	0	0	0	0	0	0	0	0	0	14
31 - 31.99	0	0	0	0	3	2	2	6	0	0	0	0	0	0	0	0	0	0	0	0	13
32 - 32.99	0	0	0	0	0	1	3	8	0	1	0	0	0	0	0	0	0	0	0	0	13
33 - 33.99	0	0	0	0	0	0	1	12	4	0	0	0	0	0	0	0	0	0	0	0	17
34 - 34.99	0	0	0	0	0	0	2	12	1	1	0	0	0	0	0	0	0	0	0	0	16
35 - 35.99	0	0	0	0	0	0	1	15	1	0	0	0	0	0	0	0	0	0	0	0	17
36 - 36.99	0	0	0	0	0	0	1	7	1	0	0	0	1	0	0	0	0	0	0	0	10
37 - 37.99	0	0	0	0	0	1	0	7	4	0	0	0	0	0	0	0	0	0	0	0	12
38 - 38.99	0	0	0	0	0	1	2	4	5	1	0	0	0	0	0	0	0	0	0	0	13
39 - 39.99	0	0	0	0	0	0	0	3	3	1	1	1	1	0	0	0	0	0	0	0	10
40 - 40.99	0	0	0	0	0	0	0	3	4	2	2	1	0	0	0	0	0	1	0	0	13
41 - 41.99	0	0	0	0	0	0	0	1	0	0	1	7	2	0	0	0	0	0	0	0	11
42 - 42.99	0	0	0	0	0	0	0	0	1	0	2	2	0	1	0	0	0	0	0	0	6
43 - 43.99	0	0	0	0	0	0	0	3	0	2	1	4	0	0	1	0	0	0	0	0	11
44 - 44.99	0	0	0	0	0	0	0	0	0	0	0	3	0	0	1	0	0	2	0	0	6
45 - 45.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1	0	3
46 - 46.99	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	0	0	1	4
48 - 48.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	3
Totals	8	34	84	68	79	31	28	105	33	10	7	19	4	1	3	1	1	7	1	1	525

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Table 11.6: The number of Striped Bass assigned to each total length-at-age category for 354 fish sampled for the final ages determined with 71 otolith ages and 283 scale ages) in Virginia waters of Atlantic ocean during 2024.

	Age																		
Interval	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	Totals
30 - 30.99	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
31 - 31.99	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
32 - 32.99	0	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
33 - 33.99	0	4	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7
34 - 34.99	0	8	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11
35 - 35.99	1	17	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	27
36 - 36.99	2	26	12	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	43
37 - 37.99	5	19	15	5	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	46
38 - 38.99	3	9	16	2	2	1	2	0	1	0	0	0	0	0	0	0	0	0	0	36
39 - 39.99	1	3	2	3	3	2	0	0	0	0	0	0	0	0	0	0	0	0	0	14
40 - 40.99	0	0	1	3	4	7	2	0	0	0	0	0	0	0	0	0	0	0	0	17
41 - 41.99	0	0	2	1	7	9	1	0	0	0	0	0	0	0	0	0	0	0	0	20
42 - 42.99	0	0	0	2	2	5	2	1	1	1	1	0	0	0	0	1	0	0	0	16
43 - 43.99	0	0	0	1	2	4	0	1	4	1	1	0	0	2	0	0	0	0	0	16
44 - 44.99	0	0	0	0	0	3	3	4	3	5	4	7	0	0	0	0	1	0	1	31
45 - 45.99	0	0	0	0	0	1	4	0	4	7	2	2	2	1	0	1	0	1	0	25
46 - 46.99	0	0	0	0	0	0	2	1	2	3	4	2	2	1	0	2	0	0	0	19
47 - 47.99	0	0	0	0	0	0	0	0	0	2	3	4	0	2	1	2	0	1	0	15
48 - 48.99	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	2	0	3
49 - 49.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1
Totals	13	91	64	19	21	34	16	7	15	19	15	15	4	7	1	7	1	4	1	354

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Table 11.7: Age-Length key, as proportion-at-age in each 1-inch length interval, based on the final ages for Striped Bass sampled in Chesapeake Bay, Virginia during 2024.

	Age																			
Interval	2	3	4	5	6	7	8	9	10	11	12	13	14	15	17	19	20	21	24	29
15 - 15.99	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18 - 18.99	0.18	0.55	0.18	0.09	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19 - 19.99	0.12	0.21	0.48	0.18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20 - 20.99	0	0.1	0.54	0.1	0.22	0.05	0	0	0	0	0	0	0	0	0	0	0	0	0	0
21 - 21.99	0	0.12	0.44	0.22	0.22	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
22 - 22.99	0.03	0.17	0.25	0.32	0.17	0.05	0	0	0	0	0	0	0	0	0	0	0	0	0	0
23 - 23.99	0	0.12	0.31	0.28	0.19	0.06	0	0.03	0	0	0	0	0	0	0	0	0	0	0	0
24 - 24.99	0	0.03	0.09	0.28	0.41	0.09	0.06	0	0.03	0	0	0	0	0	0	0	0	0	0	0
25 - 25.99	0	0	0.07	0.17	0.31	0.14	0.14	0.1	0.07	0	0	0	0	0	0	0	0	0	0	0
26 - 26.99	0	0	0.04	0.22	0.39	0.13	0.09	0.09	0.04	0	0	0	0	0	0	0	0	0	0	0
27 - 27.99	0	0	0	0.32	0.32	0.05	0.11	0.16	0	0.05	0	0	0	0	0	0	0	0	0	0
28 - 28.99	0	0	0	0	0.27	0.2	0.13	0.27	0.13	0	0	0	0	0	0	0	0	0	0	0
29 - 29.99	0	0	0	0.06	0.12	0.12	0.25	0.25	0.12	0.06	0	0	0	0	0	0	0	0	0	0
30 - 30.99	0	0	0	0	0.14	0.29	0	0.5	0.07	0	0	0	0	0	0	0	0	0	0	0
31 - 31.99	0	0	0	0	0.23	0.15	0.15	0.46	0	0	0	0	0	0	0	0	0	0	0	0
32 - 32.99	0	0	0	0	0	0.08	0.23	0.62	0	0.08	0	0	0	0	0	0	0	0	0	0
33 - 33.99	0	0	0	0	0	0	0.06	0.71	0.24	0	0	0	0	0	0	0	0	0	0	0
34 - 34.99	0	0	0	0	0	0	0.12	0.75	0.06	0.06	0	0	0	0	0	0	0	0	0	0
35 - 35.99	0	0	0	0	0	0	0.06	0.88	0.06	0	0	0	0	0	0	0	0	0	0	0
36 - 36.99	0	0	0	0	0	0	0.1	0.7	0.1	0	0	0	0.1	0	0	0	0	0	0	0
37 - 37.99	0	0	0	0	0	0.08	0	0.58	0.33	0	0	0	0	0	0	0	0	0	0	0
38 - 38.99	0	0	0	0	0	0.08	0.15	0.31	0.38	0.08	0	0	0	0	0	0	0	0	0	0
39 - 39.99	0	0	0	0	0	0	0	0.3	0.3	0.1	0.1	0.1	0.1	0	0	0	0	0	0	0
40 - 40.99	0	0	0	0	0	0	0	0.23	0.31	0.15	0.15	0.08	0	0	0	0	0	0.08	0	0
41 - 41.99	0	0	0	0	0	0	0	0.09	0	0	0.09	0.64	0.18	0	0	0	0	0	0	0
42 - 42.99	0	0	0	0	0	0	0	0	0.17	0	0.33	0.33	0	0.17	0	0	0	0	0	0
43 - 43.99	0	0	0	0	0	0	0	0.27	0	0.18	0.09	0.36	0	0	0.09	0	0	0	0	0
44 - 44.99	0	0	0	0	0	0	0	0	0	0	0	0.5	0	0	0.17	0	0	0.33	0	0
45 - 45.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.33	0	0	0.33	0.33	0
46 - 46.99	0	0	0	0	0	0	0	0	0	0	0	0.25	0	0	0	0.25	0.25	0	0	0.25
48 - 48.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0

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Table 11.8: Age-Length key, as proportion-at-age in each 1-inch length interval, based on the final ages for Striped Bass sampled in Virginia waters of the Atlantic Ocean during 2024.

	Age																		
Interval	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
30 - 30.99	0.5	0.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
31 - 31.99	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
32 - 32.99	0	0.67	0.33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
33 - 33.99	0	0.57	0.43	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
34 - 34.99	0	0.73	0.27	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
35 - 35.99	0.04	0.63	0.33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
36 - 36.99	0.05	0.6	0.28	0.05	0	0.02	0	0	0	0	0	0	0	0	0	0	0	0	0
37 - 37.99	0.11	0.41	0.33	0.11	0.02	0.02	0	0	0	0	0	0	0	0	0	0	0	0	0
38 - 38.99	0.08	0.25	0.44	0.06	0.06	0.03	0.06	0	0.03	0	0	0	0	0	0	0	0	0	0
39 - 39.99	0.07	0.21	0.14	0.21	0.21	0.14	0	0	0	0	0	0	0	0	0	0	0	0	0
40 - 40.99	0	0	0.06	0.18	0.24	0.41	0.12	0	0	0	0	0	0	0	0	0	0	0	0
41 - 41.99	0	0	0.1	0.05	0.35	0.45	0.05	0	0	0	0	0	0	0	0	0	0	0	0
42 - 42.99	0	0	0	0.12	0.12	0.31	0.12	0.06	0.06	0.06	0.06	0	0	0	0	0.06	0	0	0
43 - 43.99	0	0	0	0.06	0.12	0.25	0	0.06	0.25	0.06	0.06	0	0	0.12	0	0	0	0	0
44 - 44.99	0	0	0	0	0	0.1	0.1	0.13	0.1	0.16	0.13	0.23	0	0	0	0	0.03	0	0.03
45 - 45.99	0	0	0	0	0	0.04	0.16	0	0.16	0.28	0.08	0.08	0.08	0.04	0	0.04	0	0.04	0
46 - 46.99	0	0	0	0	0	0	0.11	0.05	0.11	0.16	0.21	0.11	0.11	0.05	0	0.11	0	0	0
47 - 47.99	0	0	0	0	0	0	0	0	0	0.13	0.2	0.27	0	0.13	0.07	0.13	0	0.07	0
48 - 48.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0.33	0	0	0	0.67	0
49 - 49.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0

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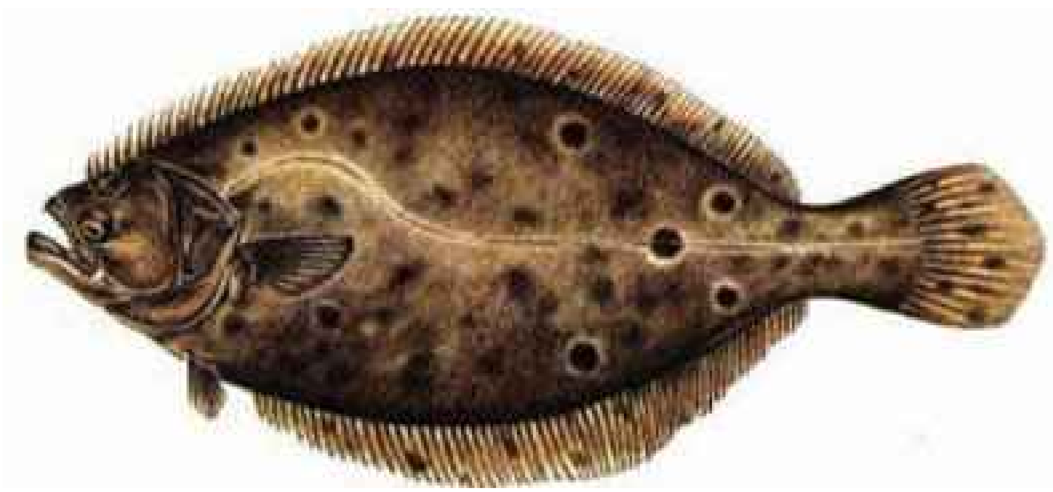
Chapter 12

SUMMER FLOUNDER *Paralichthys dentatus*



12.1 INTRODUCTION

We aged a total of 800 Summer Flounder,*Paralichthys dentatus*, using the otoliths and scales of 623 fish, the otoliths of 6 fish, and the scales of 171 fish collected by the VMRC's Biological Sampling Program in 2024. Of 800 aged fish, 309 and 491 fish were collected in Chesapeake Bay (bay fish) and Virginia waters of the Atlantic Ocean (ocean fish), respectively. The average bay fish age was 2.6 years with a standard deviation of 0.9 and a standard error of 0.05. Six age classes (1 to 6) were represented in the bay fish, comprising fish from the 2018 to 2023 year classes. The bay fish sample in 2024 was dominated by the year classes of 2021 and 2022 with 31% and 47%, respectively. The average ocean fish age was 4.8 years with a standard deviation of 2.1 and a standard error of 0.09. Fourteen age classes (1 to 12, and 14 to 15) were represented in the ocean fish, comprising fish from the 2009 to 2010, and 2012 to 2023 year classes. The ocean fish sample in 2024 was dominated by the year classes of 2017, 2018, 2019, 2020, 2021, and 2022 with 9%, 14%, 22%, 13%, 20%, and 11%, respectively. The 623 otolith ages were compared to their paired 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 2024, 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 2024; 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 2018 to 2022. θ_a, V_a, and B_a were calculated using pooled age-length data of Summer Flounder collected from 2018 to 2022 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 2018 to 2022 catch. A_l is number of fish to be aged for length interval l in 2024.

12.2.2 Handling of collection

Sagittal otoliths (hereafter, referred to as "otoliths") and scales were received by the Age & 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 thin-section and bake 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 a permanent marker 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, 3-inch diameter, Norton 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 focus. 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 CQFE website on how to prepare otolith thin-section for ageing Summer Flounder.

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

12.2.4.1 *Otoliths*

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 (Figure 12.1). Each reader aged all of the otolith samples. 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. 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

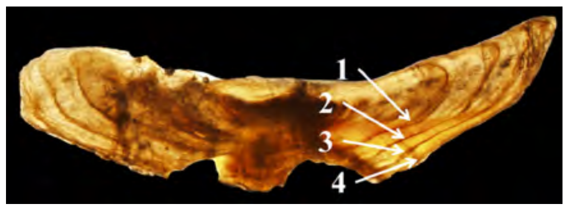


Figure 12.1: Otolith thin-section of a 4 year-old Summer Flounder with the last annulus on the edge of the thin-section

ridge and outwards to the distal surface.

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

12.2.4.2 *Scales*

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

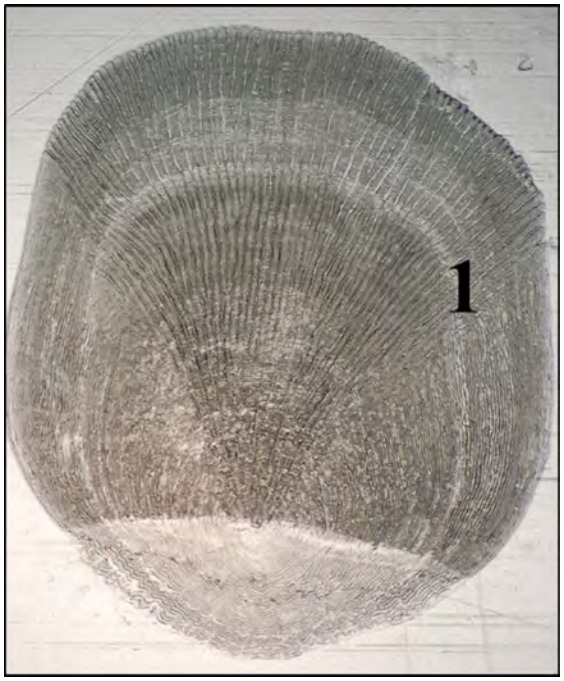


Figure 12.2: Scale impression of a 1 year-old Summer Flounder

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) between the two readers in the current year; 2) within each reader in the current year; 3) time-series bias between the current and previous years within each reader; and 4) between scale and otoliths ages. The readings from the entire sample for the current year were used to examine the difference between two readers. A random sub-sample of 50 fish from the current year was selected for second readings to examine the difference within a reader. Fifty otoliths 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 4.4.0 (R Core Team 2021).

12.3 RESULTS

12.3.1 Sample size

12.3.1.1 *Chesapeake Bay*

We estimated a sample size of 293 bay Summer Flounder in 2024, ranging in length interval from 11 to 30 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 randomly selected and aged 309 fish from 318 Summer Flounder collected by VMRC in Chesapeake Bay in 2024. We fell short in our over-all collections for this optimal length-class sampling estimate by 44 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 486 ocean Summer Flounder in 2024, 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 randomly selected and aged 491 fish from 633 Summer Flounder collected by VMRC in Virginia waters of the Atlantic Ocean in 2024. We fell short in our over-all collections for this optimal length-class sampling estimate by 45 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 Reading precision

12.3.2.1 *Otoliths*

Both readers had high self-precision. Specifically, there was no significant difference between the first and second readings for Reader 1 with an agreement of 86% and a *CV* of 2.5% (test of symmetry: $\chi^2 = 4.3$, $df = 4$, $P = 0.3628$), and there was no significant difference between the first and second readings for Reader 2 with an agreement of 88% and a *CV* of 2.1% (test of symmetry: $\chi^2 = 4$, $df = 5$, $P = 0.5494$). There was an evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 86% (1 year or less agreement of 98%) and a *CV* of 2.7% (test of symmetry: $\chi^2 = 30.6$, $df = 18$, $P = 0.0319$) (Figure 12.3).

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 0.2% (test of symmetry: $\chi^2 = 1$, $df = 1$, $P = 0.3173$). Reader 2 had an agreement of 86% with a *CV* of 2.4% (test of symmetry: $\chi^2 = 5$, $df = 4$, $P = 0.2873$).

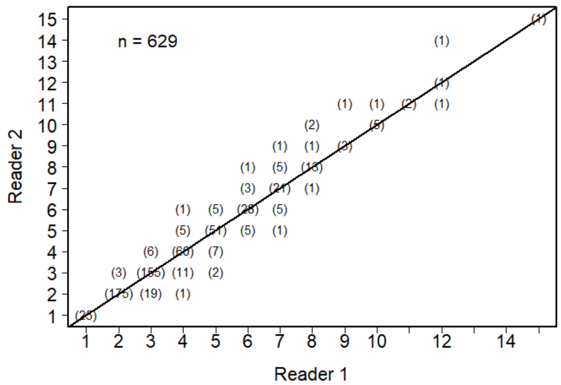


Figure 12.3: Between-reader comparison of otolith age estimates for Summer Flounder collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2024. The number in parentheses is number of fish.

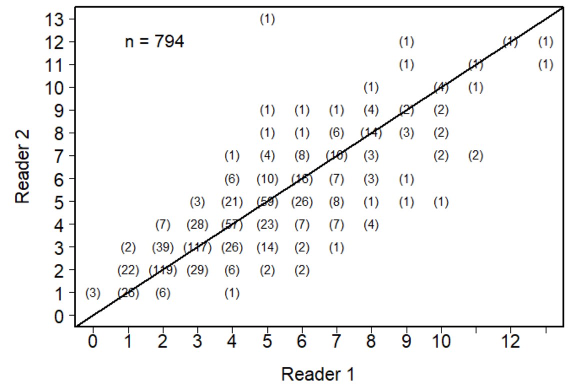


Figure 12.4: Between-reader comparison of scale age estimates for Summer Flounder collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2024. The number in parentheses is number of fish.

12.3.2.2 *Scales*

Reader 1 had moderate self-precision and Read 2 had low self-precision. Specifically, there was no significant difference between the first and second readings for Reader 1 with an agreement of 56% (1 year or less agreement of 88%) and a *CV* of 9.9% (test of symmetry: $\chi^2 = 11.5$, $df = 13$, $P = 0.5718$), and there was no significant difference between the first and second readings for Reader 2 with an agreement of 50% (1 year or less agreement of 92%) and a *CV* of 10.7% (test of symmetry: $\chi^2 = 10.1$, $df = 9$, $P = 0.339$). There was an evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 54% (1 year or less agreement of 87%) and a *CV* of 12.4% (test of symmetry: $\chi^2 = 64.5$, $df = 37$, $P = 0.0034$) (Figure 12.4).

There was no time-series bias for either reader. Reader 1 had an agreement of 70% (1 year or less agreement of 98%) with ages of fish aged in 2000 with a *CV* of 6% (test of symmetry: $\chi^2 = 3.7$, $df = 6$, $P = 0.7217$). Reader 2 had an agreement of 68% (1 year or less agreement of 94%) with a *CV* of 7.4% (test of symmetry: $\chi^2 = 9$, $df = 9$, $P = 0.4373$).

12.3.3 Year class

When otolith-ages were available, they were the final ages, otherwise, scale-ages were the final ages. The final ages were used for the year-class analysis.

12.3.3.1 *Chesapeake Bay*

Of the 309 bay Summer Flounder aged with 307 otoliths and 2 scales, 6 age classes (1 to 6) were represented (Table 12.5). The average age for the sample was 2.6 years. The standard deviation and standard error were 0.9 and 0.05, respectively. Year-class data (Figure 12.5) indicates that recruitment into the fishery in Chesapeake Bay begins at age 1, which corresponds to the 2023 year-class for Summer Flounder caught in 2024. Summer flounder in the sample in 2024 was dominated by the year classes of 2021 and 2022 with 31% and 47%, respectively. The sex ratio of male to female was 1:306 for the bay fish.

12.3.3.2 *Atlantic Ocean*

Of the 491 ocean Summer Flounder aged with 322 otoliths and 169 scales, 14 age classes (1 to 12, and 14 to 15) were represented (Table 12.6). The average age for the sample was 4.8 years. The standard deviation and standard er-

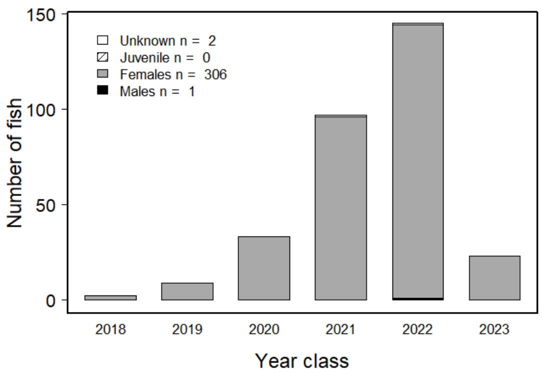


Figure 12.5: Year-class frequency distribution for Summer Flounder collected in Chesapeake Bay, Virginia for ageing in 2024. Distribution is broken down by sex. 'Unknown' represents gonads that were not available for examination or were not examined for sex during sampling.

ror were 2.1 and 0.09, respectively. Year-class data (Figure 12.6) indicates that recruitment into the fishery in Virginia waters of Atlantic ocean begins at age 1, which corresponds to the 2023 year-class for Summer Flounder caught in 2024. Summer flounder in the sample in 2024 was dominated by the year classes of 2017, 2018, 2019, 2020, 2021, and 2022 with 9%, 14%, 22%, 13%, 20%, and 11%, respectively. The sex ratio of male to female was 1:1.19 for the ocean fish.

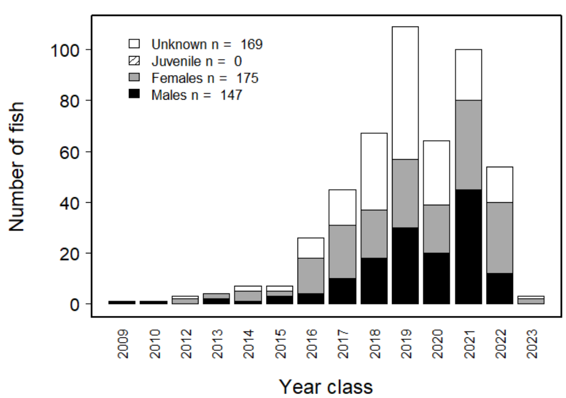


Figure 12.6: Year-class frequency distribution for Summer Flounder collected in Virginia waters of the Atlantic Ocean for ageing in 2024. Distribution is broken down by. 'Unknown' represents gonads that were not available for examination or were not examined for sex during sampling.

12.3.4 Comparison of scale and otolith ages

We aged 623 Summer Flounder using scales and otoliths (Excluding 6 fish with otolith-ages only). There was an evidence of systematic disagreement between otolith and scale ages (test of symmetry: $\chi^2 = 59.2$, $df = 31$, $P = 0.0017$) with an average CV of 13.4%. There was an agreement of 51% between scale and otoliths ages whereas scales were assigned a lower and higher age than otoliths for 27.3% and 21.5% of the fish, respectively (Figure 12.7). There was also an evidence of bias between otolith and scale ages using an age bias plot(Figure 12.8), with scale generally assigned higher ages for younger fish and lower ages for older fish than otolith age estimates.

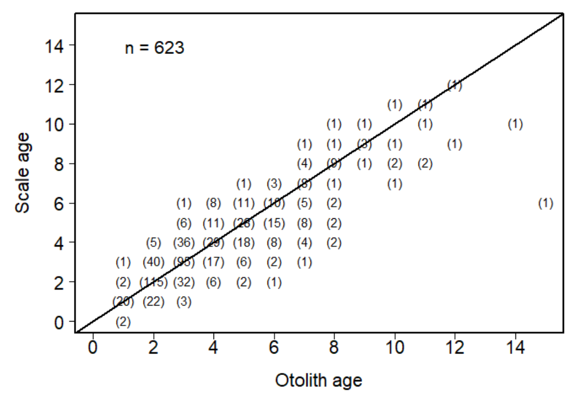


Figure 12.7: Comparison of scale and otolith age estimates for Summer Flounder collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2024. The number in parentheses is number of fish

12.3.5 Age-Length-Key (ALK)

We developed an age-length-key for both bay (Table 12.7) and ocean fish (Table 12.8) using the final 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.

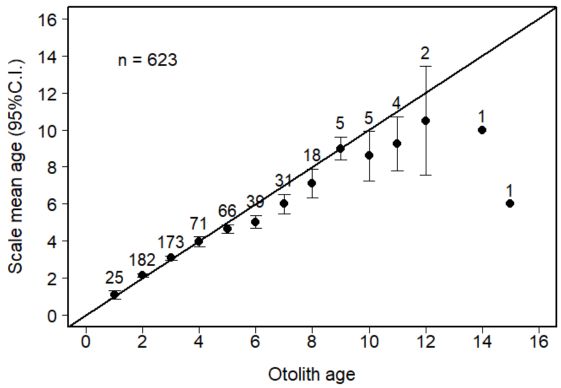


Figure 12.8: Age-bias plot for Summer Flounder scale and otolith age estimates in 2024. The number above the upper CI bar is number of fish.

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 summer 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 2024. 'Target' represents the sample size for ageing estimated for 2024, 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
11 - 11.99	5	1	1	4
12 - 12.99	5	0	0	5
13 - 13.99	5	5	5	0
14 - 14.99	57	50	50	7
15 - 15.99	48	69	68	0
16 - 16.99	35	62	56	0
17 - 17.99	35	49	47	0
18 - 18.99	29	36	36	0
19 - 19.99	22	22	22	0
20 - 20.99	18	15	15	3
21 - 21.99	9	6	6	3
22 - 22.99	5	1	1	4
23 - 23.99	5	2	2	3
24 - 24.99	5	0	0	5
25 - 25.99	5	0	0	5
30 - 30.99	5	0	0	5
Totals	293	318	309	44

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Table 12.2: CV for each age estimated based on ageing the total of 293 bay Summer Flounder in 2024. 'Percent' is the percentage of an age in the pooled age-length data of bay Summer Flounder collected from 2018 to 2022.

Age	CV	Percent
0	>0.25	0.06
1	0.19	8.19
2	0.06	44.52
3	0.11	23.73
4	0.15	12.35
5	0.21	6.93
6	>0.25	3.01
7	>0.25	0.84
8	>0.25	0.3
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 2024. 'Target' represents the sample size for ageing estimated for 2024, 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	9	6	0
14 - 14.99	38	77	50	0
15 - 15.99	57	132	73	0
16 - 16.99	60	82	60	0
17 - 17.99	56	64	57	0
18 - 18.99	43	43	43	0
19 - 19.99	31	46	35	0
20 - 20.99	24	29	24	0
21 - 21.99	22	38	30	0
22 - 22.99	24	32	32	0
23 - 23.99	22	20	20	2
24 - 24.99	22	20	20	2
25 - 25.99	19	16	16	3
26 - 26.99	17	12	12	5
27 - 27.99	14	5	5	9
28 - 28.99	11	4	4	7
29 - 29.99	6	2	2	4
30 - 30.99	5	2	2	3
31 - 31.99	5	0	0	5
32 - 32.99	5	0	0	5
Totals	486	633	491	45

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Table 12.4: CV for each age estimated based on ageing the total of 486 ocean Summer Flounder in 2024. 'Percent' is the percentage of an age in the pooled age-length data of ocean Summer Flounder collected from 2018 to 2022.

Age	CV	Percent
0	>0.25	0.04
1	>0.25	1.84
2	0.16	7.52
3	0.1	16.36
4	0.09	19.64
5	0.1	17.44
6	0.11	14.84
7	0.14	8.84
8	0.17	6.4
9	0.22	3.6
10	>0.25	1.84
11	>0.25	0.96
12	>0.25	0.52
13	>0.25	0.08
14	>0.25	0.08

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Table 12.5: The number of Summer Flounder assigned to each total length-at-age category for 309 fish sampled for the final ages determined with 307 otolith ages and 2 scale ages in Chesapeake Bay, Virginia during 2024.

	Age						
Interval	1	2	3	4	5	6	Totals
11 - 11.99	1	0	0	0	0	0	1
13 - 13.99	3	2	0	0	0	0	5
14 - 14.99	13	31	5	1	0	0	50
15 - 15.99	5	48	12	2	0	1	68
16 - 16.99	1	28	24	2	1	0	56
17 - 17.99	0	21	21	5	0	0	47
18 - 18.99	0	11	20	4	1	0	36
19 - 19.99	0	3	11	7	1	0	22
20 - 20.99	0	1	4	9	1	0	15
21 - 21.99	0	0	0	2	4	0	6
22 - 22.99	0	0	0	1	0	0	1
23 - 23.99	0	0	0	0	1	1	2
Totals	23	145	97	33	9	2	309

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Table 12.6: The number of Summer Flounder assigned to each total length-at-age category for 491 fish sampled for the final ages determined with 322 otolith ages and 169 scale ages in Virginia waters of Atlantic ocean during 2024.

	Age													
Interval	1	2	3	4	5	6	7	8	9	10	11	12	14	15	Totals
13 - 13.99	1	2	1	0	2	0	0	0	0	0	0	0	0	0	6
14 - 14.99	0	10	27	6	4	2	1	0	0	0	0	0	0	0	50
15 - 15.99	1	9	27	14	12	4	3	2	1	0	0	0	0	0	73
16 - 16.99	1	10	12	11	16	6	1	3	0	0	0	0	0	0	60
17 - 17.99	0	14	9	5	10	14	4	0	0	1	0	0	0	0	57
18 - 18.99	0	5	7	11	10	4	4	0	1	1	0	0	0	0	43
19 - 19.99	0	3	9	6	10	4	2	0	0	0	1	0	0	0	35
20 - 20.99	0	1	4	3	10	1	3	1	0	0	0	0	0	1	24
21 - 21.99	0	0	4	3	13	3	4	1	1	0	0	0	1	0	30
22 - 22.99	0	0	0	5	8	6	7	3	1	0	1	1	0	0	32
23 - 23.99	0	0	0	0	8	8	2	2	0	0	0	0	0	0	20
24 - 24.99	0	0	0	0	2	7	4	5	1	1	0	0	0	0	20
25 - 25.99	0	0	0	0	3	5	3	4	1	0	0	0	0	0	16
26 - 26.99	0	0	0	0	0	3	3	3	0	2	0	1	0	0	12
27 - 27.99	0	0	0	0	1	0	1	2	0	1	0	0	0	0	5
28 - 28.99	0	0	0	0	0	0	3	0	0	0	0	1	0	0	4
29 - 29.99	0	0	0	0	0	0	0	0	1	0	1	0	0	0	2
30 - 30.99	0	0	0	0	0	0	0	0	0	1	1	0	0	0	2
Totals	3	54	100	64	109	67	45	26	7	7	4	3	1	1	491

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Table 12.7: Age-Length key, as proportion-at-age in each 1-inch length interval, based on scale ages for Summer Flounder sampled in Chesapeake Bay, Virginia during 2024.

	Age					
Interval	1	2	3	4	5	6
11 - 11.99	1	0	0	0	0	0
13 - 13.99	0.6	0.4	0	0	0	0
14 - 14.99	0.26	0.62	0.1	0.02	0	0
15 - 15.99	0.07	0.71	0.18	0.03	0	0.01
16 - 16.99	0.02	0.5	0.43	0.04	0.02	0
17 - 17.99	0	0.45	0.45	0.11	0	0
18 - 18.99	0	0.31	0.56	0.11	0.03	0
19 - 19.99	0	0.14	0.5	0.32	0.05	0
20 - 20.99	0	0.07	0.27	0.6	0.07	0
21 - 21.99	0	0	0	0.33	0.67	0
22 - 22.99	0	0	0	1	0	0
23 - 23.99	0	0	0	0	0.5	0.5

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Table 12.8: Age-Length key, as proportion-at-age in each 1-inch length interval, based on scale ages for Summer Flounder sampled in Virginia waters of the Atlantic Ocean during 2024.

	Age													
Interval	1	2	3	4	5	6	7	8	9	10	11	12	14	15
13 - 13.99	0.17	0.33	0.17	0	0.33	0	0	0	0	0	0	0	0	0
14 - 14.99	0	0.2	0.54	0.12	0.08	0.04	0.02	0	0	0	0	0	0	0
15 - 15.99	0.01	0.12	0.37	0.19	0.16	0.05	0.04	0.03	0.01	0	0	0	0	0
16 - 16.99	0.02	0.17	0.2	0.18	0.27	0.1	0.02	0.05	0	0	0	0	0	0
17 - 17.99	0	0.25	0.16	0.09	0.18	0.25	0.07	0	0	0.02	0	0	0	0
18 - 18.99	0	0.12	0.16	0.26	0.23	0.09	0.09	0	0.02	0.02	0	0	0	0
19 - 19.99	0	0.09	0.26	0.17	0.29	0.11	0.06	0	0	0	0.03	0	0	0
20 - 20.99	0	0.04	0.17	0.12	0.42	0.04	0.12	0.04	0	0	0	0	0	0.04
21 - 21.99	0	0	0.13	0.1	0.43	0.1	0.13	0.03	0.03	0	0	0	0.03	0
22 - 22.99	0	0	0	0.16	0.25	0.19	0.22	0.09	0.03	0	0.03	0.03	0	0
23 - 23.99	0	0	0	0	0.4	0.4	0.1	0.1	0	0	0	0	0	0
24 - 24.99	0	0	0	0	0.1	0.35	0.2	0.25	0.05	0.05	0	0	0	0
25 - 25.99	0	0	0	0	0.19	0.31	0.19	0.25	0.06	0	0	0	0	0
26 - 26.99	0	0	0	0	0	0.25	0.25	0.25	0	0.17	0	0.08	0	0
27 - 27.99	0	0	0	0	0.2	0	0.2	0.4	0	0.2	0	0	0	0
28 - 28.99	0	0	0	0	0	0	0.75	0	0	0	0	0.25	0	0
29 - 29.99	0	0	0	0	0	0	0	0	0.5	0	0.5	0	0	0
30 - 30.99	0	0	0	0	0	0	0	0	0	0.5	0.5	0	0	0

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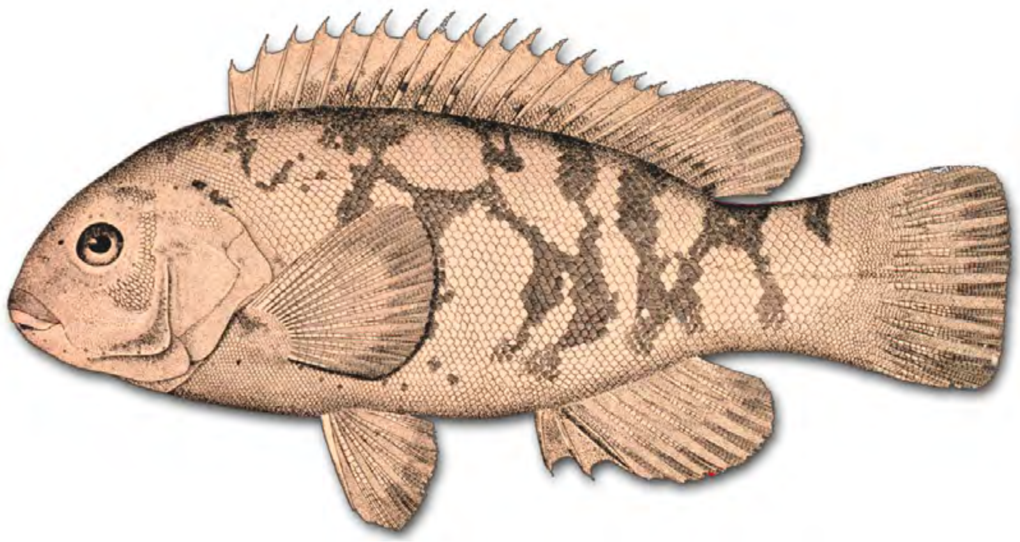
Chapter 13

TAUTOG *Tautoga onitis*



13.1 INTRODUCTION

We aged a total of 316 Tautog, *Tautoga onitis*, using the otoliths, opercula, and spines of 308 fish, the otoliths and opercula of 2 fish, the opercula and spines of 5 fish, and the opercula of 1 fish collected by the VMRC's Biological Sampling Program in 2024. Of 316 aged fish, 315 and 1 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 years with a standard deviation of 1.8 and a standard error of 0.1. Eleven age classes (2 to 10, 12, and 14) were represented in the bay fish, comprising fish from the 2010, 2012, and 2014 to 2022 year classes. The bay fish sample in 2024 was dominated by the year classes of 2019, 2020, 2021, and 2022 with 16%, 27%, 27%, and 18%, respectively. Only one ocean fish was collected, 18 years old, and in the year class of 2006.

Of 316 fish aged, the otolith ages were compared to the operculum (310 paired) and spine ages (308 paired), respectively, to examine how close both operculum and spine ages were to otolith ages (see details in Results).

13.2 METHODS

13.2.1 Sample size for ageing

We estimated sample sizes for ageing Tautog collected in Chesapeake Bay in 2024(but not for those collected in Virginia waters of the Atlantic Ocean because only several specimen collected in Atlantic Ocean 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:

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

where A is the sample size for ageing Tautog in 2024; 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 2018 to 2022. θ_a, V_a, and B_a were calculated using pooled age-length data of Tautog collected from 2018 to 2022 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 2018 to 2022 catch. A_l is number of fish to be aged for length interval l in 2024.

13.2.2 Handling of collection

Sagittal otoliths (hereafter, referred to as "otoliths") and opercula were received by the Age & 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 opercula were stored dry within their original labeled coin envelopes; otoliths were contained inside protective Axygen 2.0 ml microtubes.

13.2.3 Preparation

13.2.3.1 *Otoliths*

We used our thin-section and bake 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 a permanent marker 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, 3-inch diameter, Norton 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 focus. 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 CQFE website on how to prepare otolith thin-section for ageing Tautog.

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 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 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, Tautog otolith 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.

Tautog opercula are also considered to have a deposition period of May through July (Hostetter and Munroe 1993), and age class assignment using these hard-parts is conducted in the same way as otoliths.

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

13.2.4.1 *Otoliths*

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 (Figure 13.1). Each reader aged all of the otolith samples.

Figure 13.1: Otolith thin-section of 6 year-old Tautog

13.2.4.2 *Opercula*

All prepared opercula were aged by two different readers in chronological order based on collection date, without knowledge of previously estimated ages or the specimen lengths. Opercula were aged on a light table with no magnification (Figure 13.2).

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

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

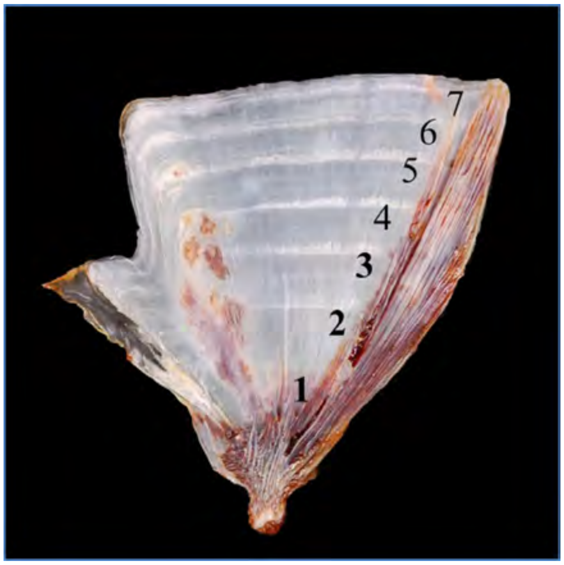


Figure 13.2: Operculum of a 7 year-old Tautog

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.

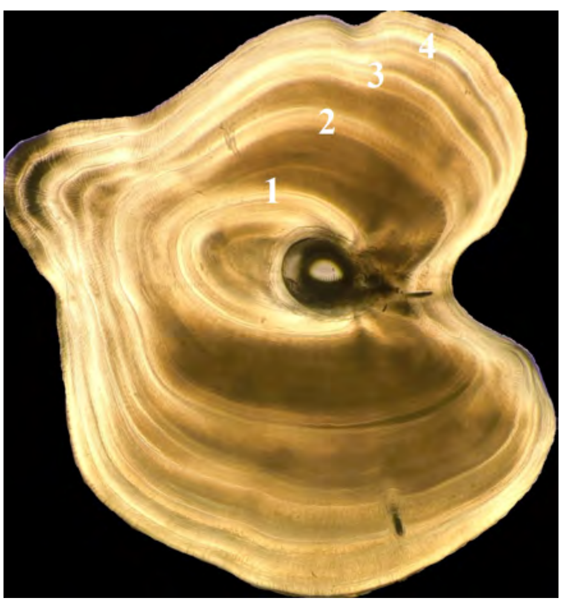


Figure 13.3: Spine of a 4 year-old Tautog

13.2.5 Comparison Tests

A symmetry test (Hoenig et al. 1995) and coefficient of variation (CV) analysis were used to detect any systematic difference and precision on age readings, respectively, for following comparisons: 1) between the two readers in the current year; 2) within each reader in the current year; 3) time-series bias between the current and previous years within each reader; 4) between operculum and otoliths ages; and 5) between spine and otoliths ages. The readings from the entire sample for the current year were used to examine the difference between two readers. A random sub-sample of 50 fish from the current year was selected for second readings to examine the difference within a reader. Fifty otoliths 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 4.4.0 (R Core Team 2021).

13.3 RESULTS

13.3.1 Sample size

13.3.1.1 *Chesapeake Bay*

We estimated a sample size of 456 bay Tautog in 2024, ranging in length interval from 8 to 24 inches (Table 13.1). This sample size provided a range in CV for age composition approximately from the smallest CV of 8% for the major age of Age 5 to the CV of larger than 25% for the multiple minor ages of the bay fish (Table 13.2). We aged all 315 Tautog collected by VMRC in Chesapeake Bay in 2024. We fell short in our over-all collections for this optimal length-class sampling estimate by 209 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*

There were only several specimen collected in Atlantic Ocean in 2023, therefore, it was not necessary to estimate the sample size for ageing Tautog collected in 2024.

13.3.2 Reading precision

13.3.2.1 *Otoliths*

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 0.9% (test of symmetry: χ^2 = 3, *df* = 3, P = 0.3916), and there was no significant difference between the first and second readings for Reader 2 with an agreement of 88% and a *CV* of 2.8% (test of symmetry: χ^2 = 4, *df* = 5, P = 0.5494). There was no evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 93% (1 year or less agreement of 100%) and a *CV* of 1.5% (test of symmetry: χ^2 = 11, *df* = 8, P = 0.2017) (Figure 13.4). There was no time-series bias for either reader. Reader 1 had an agreement of 92% with ages of fish aged in 2003 with a *CV* of 1% (test of symmetry: χ^2 = 4, *df* = 2, P = 0.1353). Reader 2 had an agreement of 86% with a *CV* of 1.6% (test of symmetry: χ^2 = 7, *df* = 3, P = 0.0719).

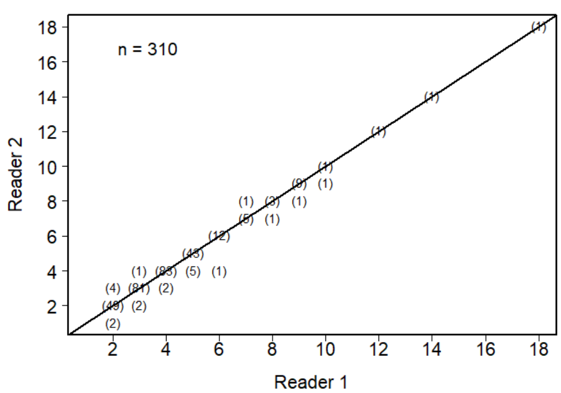


Figure 13.4: Between-reader comparison of otolith age estimates for Tautog collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2024. The number in parentheses is number of fish.

13.3.2.2 *Opercula*

Reader 1 had moderate self-precision and Read 2 had low self-precision. Specifically, there was no significant difference between the first and second readings for Reader 1 with an agreement of 74% (1 year or less agreement of 100%) and a *CV* of 5.4% (test of symmetry: χ^2 = 4.2, *df* = 5, P = 0.521), and there was no significant difference between the first and second readings for Reader 2 with an agreement of 56% (1 year or less agreement of 90%) and a *CV* of 10.6% (test of symmetry: χ^2 = 13, *df* = 11, P = 0.2933). There was an evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 45% (1 year or less agreement of 88%) and a *CV* of 13.5% (test of symmetry: χ^2 = 54.5, *df* = 22, P = 0.0001) (Figure 13.5).

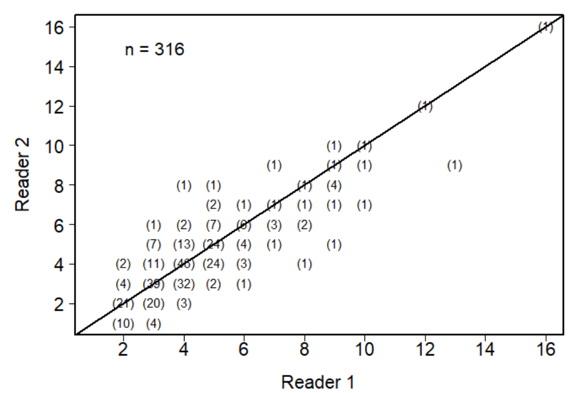


Figure 13.5: Between-reader comparison of operculum age estimates for Tautog collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2024. The number in parentheses is number of fish.

There was no time-series bias for either reader. Reader 1 had an agreement of 62% (1 year or less agreement of 96%) with ages of fish aged in 2000 with a *CV* of 5.2% (test of symmetry: χ^2 = 9.7, *df* = 10, P = 0.4702). Reader 2 had an agreement of 54% (1 year or less agreement of 82%) with a *CV* of 9.4% (test of symmetry: χ^2 = 15.3, *df* = 14, P = 0.3558).

13.3.2.3 *Spines*

Both readers had low self-precision. 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 80%) and a *CV* of 15.2% (test of symmetry: $\chi^2 = 15.6$, $df = 12$, $P = 0.2093$), and there was no significant difference between the first and second readings for Reader 2 with an agreement of 50% (1 year or less agreement of 92%) and a *CV* of 10.8% (test of symmetry: $\chi^2 = 13.8$, $df = 9$, $P = 0.1296$). There was an evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 39% (1 year or less agreement of 86%) and a *CV* of 14.2% (test of symmetry: $\chi^2 = 95.5$, $df = 25$, $P < 0.0001$) (Figure 13.6).

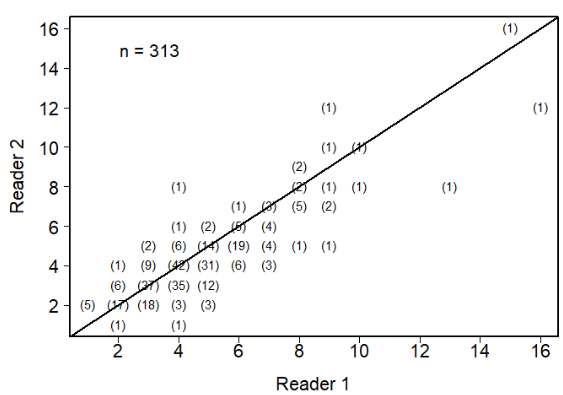


Figure 13.6: Between-reader comparison of spine age estimates for Tautog collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2024. The number in parentheses is number of fish.

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

13.3.3 Year class

When otolith-ages were available, they were the final ages, otherwise, operculum-ages were the final ages. When neither otolith- nor operculum-ages were available, the spine-ages were the final ages. The final ages were used for the year-class analysis.

13.3.3.1 *Chesapeake Bay*

Of the 315 bay Tautog aged with 309 otoliths and 6 opercula, 11 age classes (2 to 10, 12, and 14) were represented (Table 13.3). The average age for the sample was 4 years. The standard deviation and standard error were 1.8 and 0.1, respectively. Year-class data (Figure 13.7) indicates that recruitment into the fishery in Chesapeake Bay begins at age 2, which corresponds to the 2022 year-class for Tautog caught in 2024. Tautog in the sample in 2024 was dominated by the year classes of 2019, 2020, 2021, and 2022 with 16%, 27%, 27%, and 18%, respectively. The sex ratio of male to female was 1:2.25 for the bay fish.

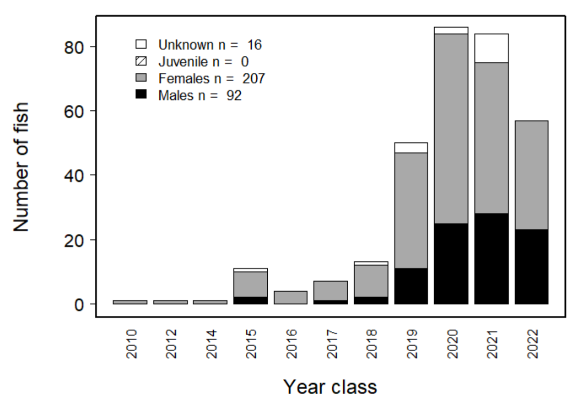


Figure 13.7: Year-class frequency distribution for Tautog collected in Chesapeake Bay, Virginia for ageing in 2024. Distribution is broken down by sex. 'Unknown' represents the fish gonads that were not available for examination or were not examined for sex during sampling.

13.3.3.2 *Atlantic Ocean*

Only one ocean Tautog was collected in 2024, 18 years old, and in the year class of 2006.

13.3.4 Comparison

13.3.4.1 *Operculum vs. otolith ages*

We aged 310 Tautog using their opercula and otoliths. There was an evidence of systematic disagreement between otolith and operculum ages (test of symmetry: $\chi^2 = 39.2$, $df = 19$, $P = 0.0042$) with an average CV of 7.3%. There was an agreement of 65% between operculum and otoliths ages whereas opercula were assigned a lower and higher age than otoliths for 10.6% and 23.9% of the fish, respectively (Figure 13.8). There was also an evidence of bias between otolith and operculum ages using an age bias plot (Figure 13.9), with operculum generally assigned higher ages for younger fish and lower ages for older fish than otolith age estimates.

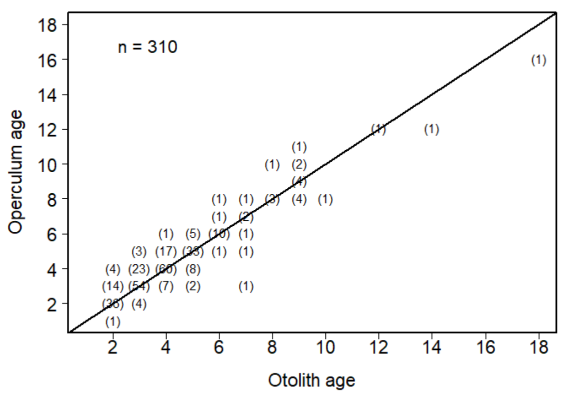


Figure 13.8: Comparison of operculum and otolith age estimates for Tautog collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2024. The number in parentheses is number of fish.

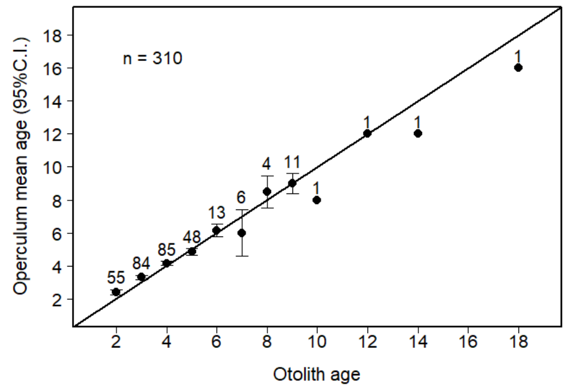


Figure 13.9: Age-bias plot for Tautog operculum and otolith age estimates in 2024. The number above the upper CI bar is number of fish.

13.3.4.2 *Spine vs. otolith ages*

We aged 308 Tautog using their spines and otoliths. There was an evidence of systematic disagreement between otolith and spine ages (test of symmetry: $\chi^2 = 80.9$, $df = 27$, $P < 0.0001$) with an average CV of 13.7%. There was an agreement of 42% between spine and otoliths ages whereas spines were assigned a lower and higher age than otoliths for 12.3% and 46.1% of the fish, respectively (Figure 13.10). There was also an evidence of bias between otolith and spine ages using an age bias plot (Figure 13.11), with spine generally assigned higher ages for younger fish and lower ages for older fish than otolith age estimates.

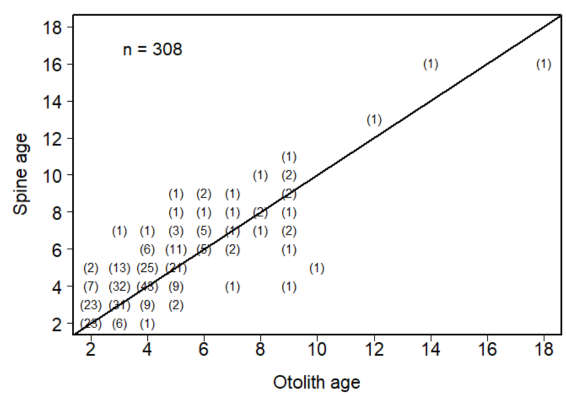


Figure 13.10: Comparison of spine and otolith age estimates for Tautog collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2024. The number in parentheses is number of fish.

13.3.5 Age-Length-Key (ALK)

We developed an age-length-key for bay fish (Table 13.4) using the final ages. No ALK was developed for the ocean tautog because there was only one ocean fish collected and aged in 2024. The ALK can be used in the conversion of numbers-at-length in the estimated catch to

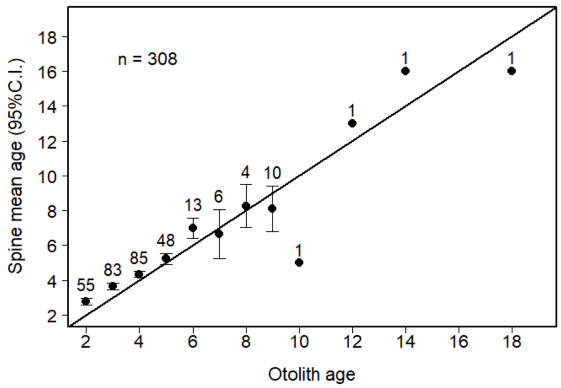


Figure 13.11: Age-bias plot for Tautog spine and otolith age estimates in 2024. The number above the upper CI bar is number of fish.

numbers-at-age using operculum ages. The table is based on VMRC's stratified sampling of landings by total length inch intervals.

Table 13.1: Number of bay Tautog collected and aged in each 1-inch length interval in 2024. 'Target' represents the sample size for ageing estimated for 2024, 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	8	8	8	0
11 - 11.99	11	35	35	0
12 - 12.99	24	43	43	0
13 - 13.99	24	49	49	0
14 - 14.99	64	59	59	5
15 - 15.99	116	56	56	60
16 - 16.99	93	35	35	58
17 - 17.99	60	18	18	42
18 - 18.99	18	8	8	10
19 - 19.99	8	1	1	7
20 - 20.99	5	1	1	4
21 - 21.99	5	1	1	4
22 - 22.99	5	1	1	4
24 - 24.99	5	0	0	5
Totals	456	315	315	209

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Table 13.2: CV for each age estimated based on ageing the total of 456 bay Tautog in 2024. 'Percent' is the percentage of an age in the pooled age-length data of bay Tautog collected from 2018 to 2022.

Age	CV	Percent
1	>0.25	0.88
2	0.24	2.93
3	0.14	9.08
4	0.1	19.91
5	0.08	25.04
6	0.1	17.72
7	0.15	9.52
8	0.17	7.03
9	0.22	4.39
10	>0.25	1.76
11	>0.25	0.73
12	>0.25	0.15
13	>0.25	0.59
14	>0.25	0.15
15	>0.25	0.15

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Table 13.3: The number of Tautog assigned to each total length-at-age category for 315 fish sampled for the final ages determined with 309 otolith ages and 6 operculum ages in Chesapeake Bay, Virginia during 2024.

	Age											
Interval	2	3	4	5	6	7	8	9	10	12	14	Totals
10 - 10.99	7	1	0	0	0	0	0	0	0	0	0	8
11 - 11.99	27	5	3	0	0	0	0	0	0	0	0	35
12 - 12.99	12	12	15	3	0	1	0	0	0	0	0	43
13 - 13.99	5	27	15	1	1	0	0	0	0	0	0	49
14 - 14.99	4	18	23	7	2	2	1	1	0	1	0	59
15 - 15.99	1	17	18	16	1	1	2	0	0	0	0	56
16 - 16.99	1	3	8	11	4	3	1	4	0	0	0	35
17 - 17.99	0	1	3	9	3	0	0	2	0	0	0	18
18 - 18.99	0	0	1	3	1	0	0	3	0	0	0	8
19 - 19.99	0	0	0	0	1	0	0	0	0	0	0	1
20 - 20.99	0	0	0	0	0	0	0	0	1	0	0	1
21 - 21.99	0	0	0	0	0	0	0	1	0	0	0	1
22 - 22.99	0	0	0	0	0	0	0	0	0	0	1	1
Totals	57	84	86	50	13	7	4	11	1	1	1	315

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Table 13.4: Age-Length key, as proportion-at-age in each 1-inch length interval, based on the final ages for Tautog sampled in Chesapeake Bay, Virginia during 2024.

	Age										
Interval	2	3	4	5	6	7	8	9	10	12	14
10 - 10.99	0.88	0.12	0	0	0	0	0	0	0	0	0
11 - 11.99	0.77	0.14	0.09	0	0	0	0	0	0	0	0
12 - 12.99	0.28	0.28	0.35	0.07	0	0.02	0	0	0	0	0
13 - 13.99	0.1	0.55	0.31	0.02	0.02	0	0	0	0	0	0
14 - 14.99	0.07	0.31	0.39	0.12	0.03	0.03	0.02	0.02	0	0.02	0
15 - 15.99	0.02	0.3	0.32	0.29	0.02	0.02	0.04	0	0	0	0
16 - 16.99	0.03	0.09	0.23	0.31	0.11	0.09	0.03	0.11	0	0	0
17 - 17.99	0	0.06	0.17	0.5	0.17	0	0	0.11	0	0	0
18 - 18.99	0	0	0.12	0.38	0.12	0	0	0.38	0	0	0
19 - 19.99	0	0	0	0	1	0	0	0	0	0	0
20 - 20.99	0	0	0	0	0	0	0	0	1	0	0
21 - 21.99	0	0	0	0	0	0	0	1	0	0	0
22 - 22.99	0	0	0	0	0	0	0	0	0	0	1

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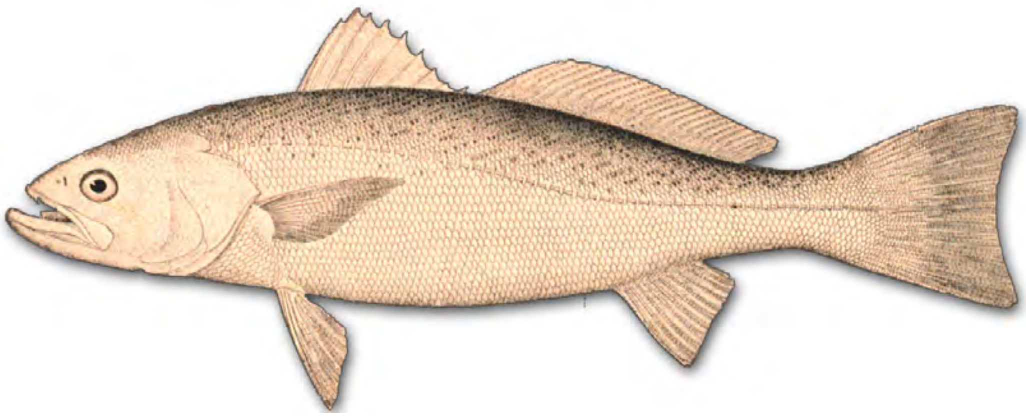
Chapter 14

WEAKFISH *Cynoscion regalis*



14.1 INTRODUCTION

We aged a total of 228 Weakfish, *Cynoscion regalis*, collected by the VMRC's Biological Sampling Program for age and growth analysis in 2024. The Weakfish ages ranged from 1 to 6 years old with an average age of 2.1, a standard deviation of 0.9, and a standard error of 0.06. Five age classes (1 to 4, and 6) were represented, comprising fish of the 2018, and 2020 to 2023 year-classes. The sample was dominated by fish from the year-class of 2022 with 49.1%.

14.2 METHODS

14.2.1 Sample size for ageing

We estimated sample size for ageing Weakfish in 2024 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:

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

where A is the sample size for ageing Weakfish in 2024; 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 2018 to 2022. θ_a, V_a, and B_a were calculated using pooled age-length data of Weakfish collected from 2018 to 2022 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 2018 to 2022 catch. A_l is number of fish to be aged for length interval l in 2024.

14.2.2 Handling of collections

Otoliths were received by the Age & 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 otolith was randomly selected and attached, distal side down, to a glass slide with clear Crystalbond™ 509 adhesive. 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 a pencil 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, 3-inch diameter, Norton 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 CQFE website on how to prepare otolith thin-section for ageing Weakfish.

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".

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, Reader 1 re-aged the fish with disagreement and decided a final age for the fish. This method is different from what we used before the pandemic of COVID-19 during the period of 2020 -2021 because of 6-food social distance requirement. 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 14.1).

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 fol-

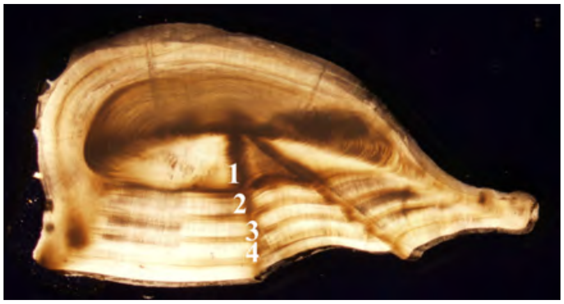


Figure 14.1: Otolith thin-section of 4 year-old Weakfish

lowing 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 4.4.0 (R Core Team 2021).

14.3 RESULTS

14.3.1 Sample size

We estimated a sample size of 392 for ageing Weakfish in 2024, 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 2024, we aged 228 of 257 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 181 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 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 3.39% (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 98.25% and a *CV* of 0.74% (test of symmetry: $\chi^2 = 1.33$, $df = 2$, $P = 0.5134$) (Figure 14.2).

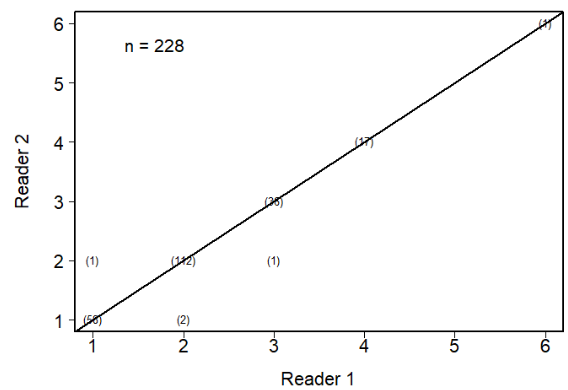


Figure 14.2: Between-reader comparison of otolith age estimates for Weakfish collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2024. The number in parentheses is number of fish.

There was no time-series bias for either reader. Reader 1 had an agreement of 100% with ages of fish aged in 2003. Reader 2 had an agreement of 94% with a *CV* of 1.01% (test of symmetry: $\chi^2 = 3$, $df = 3$, $P = 0.3916$).

14.3.3 Year class

Of the 228 fish aged with otoliths, 5 age classes (1 to 4, and 6) were represented (Table 14.3).

The average age was 2.1 years, and the standard deviation and standard error were 0.9 and 0.06, respectively. Year-class data show that the fishery was comprised of 5 year-classes: fish from the 2018, and 2020 to 2023 year-classes, with fish primarily from the year-class of 2022 with 49.1%. The ratio of males to females was 1:3.3 in the sample collected (Figure 14.3).

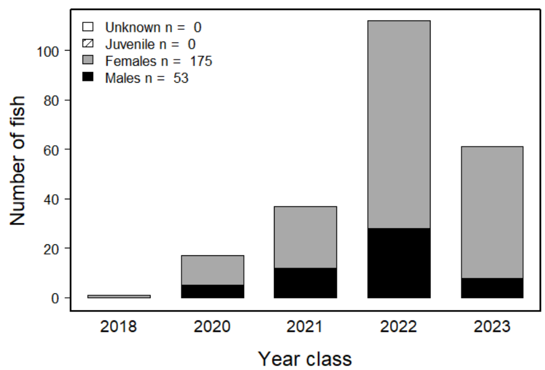


Figure 14.3: Year-class frequency distribution for Weakfish collected for ageing in 2024. Distribution is broken down by sex. 'Unknown' represents gonads that were not available for examination or were not examined for sex during sampling.

14.3.4 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.

Table 14.1: Number of Weakfish collected and aged in each 1-inch length interval in 2024. 'Target' represents the sample size for ageing estimated for 2024, 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	15	51	22	0
9 - 9.99	36	46	46	0
10 - 10.99	53	19	19	34
11 - 11.99	48	29	29	19
12 - 12.99	43	30	30	13
13 - 13.99	28	20	20	8
14 - 14.99	21	13	13	8
15 - 15.99	25	18	18	7
16 - 16.99	15	12	12	3
17 - 17.99	13	5	5	8
18 - 18.99	9	2	2	7
19 - 19.99	6	1	1	5
20 - 20.99	5	1	1	4
21 - 21.99	5	0	0	5
22 - 22.99	5	1	1	4
23 - 23.99	5	0	0	5
24 - 24.99	5	2	2	3
25 - 25.99	5	0	0	5
26 - 26.99	5	1	1	4
27 - 27.99	5	2	2	3
28 - 28.99	5	0	0	5
29 - 29.99	5	0	0	5
30 - 30.99	5	0	0	5
33 - 33.99	5	0	0	5
34 - 34.99	5	0	0	5
Totals	392	257	228	181

(Go back to text)

Table 14.2: CV for each age estimated based on ageing the total of 392 Weakfish in 2024. 'Percent' is the percentage of an age in the pooled age-length data of Weakfish collected from 2018 to 2022.

Age	CV	Percent
0	>0.25	0.42
1	0.1	16.85
2	0.06	41.83
3	0.06	34.2
4	0.17	6.45
5	>0.25	0.25

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Table 14.3: The number of Weakfish assigned to each total length-at-age category for 228 fish sampled for otolith age determination in Virginia during 2024.

	Age					
Interval	1	2	3	4	6	Totals
7 - 7.99	3	1	0	0	0	4
8 - 8.99	17	5	0	0	0	22
9 - 9.99	23	23	0	0	0	46
10 - 10.99	7	10	2	0	0	19
11 - 11.99	6	18	5	0	0	29
12 - 12.99	2	17	11	0	0	30
13 - 13.99	3	13	3	1	0	20
14 - 14.99	0	8	3	2	0	13
15 - 15.99	0	9	3	6	0	18
16 - 16.99	0	5	4	3	0	12
17 - 17.99	0	2	3	0	0	5
18 - 18.99	0	1	1	0	0	2
19 - 19.99	0	0	1	0	0	1
20 - 20.99	0	0	0	1	0	1
22 - 22.99	0	0	1	0	0	1
24 - 24.99	0	0	0	2	0	2
26 - 26.99	0	0	0	1	0	1
27 - 27.99	0	0	0	1	1	2
Totals	61	112	37	17	1	228

(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 2024.

	Age				
Interval	1	2	3	4	6
7 - 7.99	0.75	0.25	0	0	0
8 - 8.99	0.77	0.23	0	0	0
9 - 9.99	0.5	0.5	0	0	0
10 - 10.99	0.37	0.53	0.11	0	0
11 - 11.99	0.21	0.62	0.17	0	0
12 - 12.99	0.07	0.57	0.37	0	0
13 - 13.99	0.15	0.65	0.15	0.05	0
14 - 14.99	0	0.62	0.23	0.15	0
15 - 15.99	0	0.5	0.17	0.33	0
16 - 16.99	0	0.42	0.33	0.25	0
17 - 17.99	0	0.4	0.6	0	0
18 - 18.99	0	0.5	0.5	0	0
19 - 19.99	0	0	1	0	0
20 - 20.99	0	0	0	1	0
22 - 22.99	0	0	1	0	0
24 - 24.99	0	0	0	1	0
26 - 26.99	0	0	0	1	0
27 - 27.99	0	0	0	0.5	0.5

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Chapter 15

APPENDIX

Comparisons in Menhaden *Brevoortia tyrannus* Ages Estimated Using Scales, Scale Acetate Impressions, Whole Otoliths, and Otolith Thin-Sections)

Jemelyn Grace Baldisimo, Gabriel Salomon,

Ethan Simpson, and Hongsheng Liao

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. Since 2023, 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. Which hardpart and what ageing method are more practical for us to age Menhaden in-house in the near future; 2. There is no difference between scale and otolith ages, supporting that scale ages be continuously used for Menhaden stock assessment; and 3. 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.

15.2 METHODS

15.2.1 Preparation

To decide which hardpart, otolith vs scale, will provide more precise estimates of Menhaden ages, we did a pilot study on comparing 4 different ageing methods, pressing scales on an acetate slide (hereafter "acetate-scale slide"), fixing scales between two microscopic slides (hereafter "glass-scale slide), thin-sectioning otoliths (hereafter "section-otolith slide"), and mounting the whole otolith on a microscopic slide (hereafter "whole-otolith slide"), using the menhaden collected in 2023. However, our 2023 study was able to collect only 45 menhaden and 2023 was also the first year in which our lab started learning how to age menhaden, as a result, the results from the 2023 study was inconclusive (Please see the Appendix in Virginia Marine Resources Commission Ageing Lab 2023 Annual Report). In 2024, we continued to collect both scales and otoliths from Menhaden, and made slides from them as we did in the 2023 study.

15.2.1.1 *Acetate-scale slide*

We made an acetate-scale slide from 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-scale slide*

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-scale slides of Menhaden 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 heated hydraulic press, we put them between two microscope slides and taped both ends of the slides.

15.2.1.3 *Section-otolith slide*

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 Flotexx to fix the otolith 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 an acetate-scale slide, a glass-scale slide, a section-otolith slide, and a whole-otolith slide look like, we used the images of those slides made from the same fish (AGID 100) collected in 2024 as the examples in the next 4 sections. An AGID is an Ageing and Growth Identification Number (AGID) assigned to a fish by year in our lab.

15.2.2.1 *Acetate-scale slide*

All the acetate-scale slides 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 using this method were hereinafter referred to as "acetate-scale age".

15.2.2.2 *Glass-scale slide*

All the glass-scale slides 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 using this method were hereinafter referred to as "glass-scale age".

15.2.2.3 *Section-otolith slide*

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

15.2.2.4 *Whole-otolith slide*

All the whole-otolith slides 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 using this method were hereinafter referred to as "whole-otolith age".

15.2.3 Comparison Test

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 each of 4 different ages within each reader (self-precision);
2. On each of 4 different ages between the two readers (between-reader precision);

3. Between any 2 of 4 different ages (between-age comparison).

As defined in the previous section, 4 different ages stand for acetate-scale, glass-scale, section-otolith, and whole-otolith age, respectively.

15.3 RESULTS

We collected 159 menhaden for ageing in 2024. We intended to make four different hardpart slides from each of 159 samples, however, due to the quality or loss of hardparts we were unable to do so from some of the samples. Table 15.1 summarizes the sample sizes with 1, 2, 3, and 4 slides. All the ages estimated from the slides above were used to analyze the precision between two readers and the comparison among the ages estimated. A random selection of 50 samples from each of 4 slide types was used for the precision within each reader.

15.3.1 Reading Precision

15.3.1.1 *Reader 1*

Among 4 different ages, Reader 1 had the highest self-precision on the acetate-scale ages with an agreement of 58% (1 year or less agreement of 86%) and a mean CV of 8.3% (Figure 15.5), and had no systematic difference between the first and second readings (test of symmetry: $\chi^2 = 10.33$, df = 8, P = 0.2424) (Figure 15.6). Reader 1's self-precision were very similar on the section-otolith age (An agreement of 38%; 1 year or less agreement of 88%; and a mean CV of 11.8%) and glass-scale age (an agreement of 36%; 1 year or less agreement of 90%; and a mean CV of 10.9%) (Figure 15.5). For Reader 1, there was no systematic difference on the section-otolith age (test of symmetry: $\chi^2 = 14.6$, df = 9, P = 0.1026), however, there was a systematic difference on the glass-scale age (test of symmetry: $\chi^2 = 22.4$, df = 8, P = 0.0042) (Figure 15.6). Reader 1 had the lowest self-precision on the whole-otolith age with an agreement of 18% (1 year or less agreement of 48%) and a mean CV of 24.6% (Figure 15.5), and had a systematic difference between the first and second readings (test of symmetry: $\chi^2 = 37.67$, df = 16, P = 0.0017) (Figure 15.6)

15.3.1.2 *Reader 2*

Among 4 different ages, Reader 2 had the highest self-precision on the section-otolith ages with an agreement of 56% (1 year or less agreement of 94%) and a mean CV of 9.5% (Figure 15.7), and had no systematic difference between the first and second readings (test of symmetry: $\chi^2 = 8.33$, df = 6, P = 0.2147) (Figure 15.8). Reader 2's self-precision became less in an order of the whole-otolith age (an agreement of 34% (1 year or less agreement of 76%) and a mean CV of 16.5%), acetate-scale age (an agreement of 18% (1 year or less agreement of 68%) and a mean CV of 20.8%), and glass-scale age (an agreement of 14% (1 year or less agreement of 44%) and a mean CV of 23.8% (Figure 15.7). For Reader 2, there was no systematic difference on the whole-otolith ages between the first and second readings (test of symmetry: $\chi^2 = 18.9$, df = 12, P = 0.0909), however, there was a systematic difference on the acetate- (test of symmetry: $\chi^2 = 41$, df = 10, P < 0.0001) and glass-scale ages (test of symmetry: $\chi^2 = 43$, df = 11, P < 0.0001), respectively (Figure 15.8).

15.3.1.3 *Between readers*

The between-reader precision was the highest for the glass-scale ages (an agreement of 35% (1 year or less agreement of 82%) and a mean CV of 14.8%), followed by the whole-otolith ages (an agreement of 35% (1 year or less agreement of 80%) and a mean CV of 16.5%), whereas, the acetate-scale (an agreement of 28% (1 year or less agreement of 70%) and a mean CV of 19.1%) and section-otolith ages (an agreement of 31% (1 year or less agreement of 66%) and a mean CV of 19.3%) fell behind but were very similar (Figure 15.9). However, both the section- (test of symmetry: $\chi^2 = 31.55$, df = 21, P = 0.0649) and whole-otolith ages (test of symmetry: $\chi^2 = 12.12$, df = 15, P = 0.6696) had no systematic difference between two readers whereas both the acetate- (test of symmetry: $\chi^2 = 76.05$, df = 12, P < 0.0001) and glass-scale ages (test of symmetry: $\chi^2 = 64.75$, df = 14, P < 0.0001) had systematic differences (Figure 15.10).

15.3.2 Comparison test

When comparing the ages estimated using two different methods, we found that the precision was the highest between the acetate- and glass-scale ages (an agreement of 51% (1 year or less agreement of 86%) and a mean CV of 8.9%) (Figure 15.11) and there was no systematic difference between the two (test of symmetry: $\chi^2 = 15.35$, df = 10, P = 0.1198) (Figure 15.12).

The precision were less between the section-otolith and acetate-scale ages (an agreement of 31% (1 year or less agreement of 74%) and a mean CV of 15.3%) and between the section-otolith and glass-scale ages (an agreement of 32% (1 year or less agreement of 71%) and a mean CV of 15.8%) (Figure 15.11) with no systematic differences for both pairs (section-otolith vs. acetate-scale age: $\chi^2 = 24.68$, df = 19, P 0.1713; section-otolith vs. glass-scale age: $\chi^2 = 25.7$, df = 18, P = 0.1067) (Figure 15.12).

The pairs with the lowest precision were the whole-otolith vs. acetate-scale ages (an agreement of 21% (1 year or less agreement of 50% and a mean CV of 21.1%), the whole-otolith vs. glass-scale ages (an agreement of 16% (1 year or less agreement of 50% and a mean CV of 22%), and the whole- vs. section-otolith age (an agreement of 17% (1 year or less agreement of 51%) and a mean CV of 23%) (Figure 15.11) with systematic differences for all three pairs (whole-otolith vs. acetate-scale age: $\chi^2 = 43.1$, df = 20, P = 0.002; whole-otolith vs. glass-scale age: $\chi^2 = 60.56$, df = 22, P < 0.0001; whole- vs. section-otolith age: $\chi^2 = 44.21$, df = 24, P = 0.0072) (Figure 15.12).

Table 15.2 groups all the precision and comparisons with a p-value larger than 0.05 in the upper section and those with a p-value smaller than or equal to 0.05 in the lower section. Each of 4 different ages has the precision and comparison analysis for 6 times (1 time for between-reader precision, 2 times for self-precision, and 3 times for between-age comparison).

The section-otolith age has 5 of 6 times with a p-value larger than 0.05 (no systematic differences), the acetate-scale age has 3 of 6 times with a p-value larger than 0.05, and both the whole-otolith and glass-scale age has only 2 of 6 times with a p-value larger than 0.05. Such a trend indicates that the section-otolith age may provide the most consistent age estimate, in contrast, the whole-otolith and glass-scale age may provide the least consistent age estimates while the acetate-scale age falls between the two (Table 15.2).

15.4 DISCUSSION

Of 8 self-precision tests, two had moderate precision (< 10%) with non-systematic difference, Acetate-scale within Reader 1 (8.3%) and Section-otolith within Reader 2 (9.5%), implying that the precision could be due to either the different methods (Acetate-scale vs. Section-otolith), the readers (Reader 1 vs. Reader 2), or both. As a result, we are unable to decide which method may provide more precise age estimates for Menhaden.

Within non-systematic difference comparisons between two different ages, the acetate- against glass-scale age has the highest precision (mean CV = 8.9%), indicating that the acetate- and glass-scale age may provide very similar age estimates for Menhaden. Between these two methods, we would recommend the acetate-scale method due to the consideration of financial costs on and practice of both methods. In general, one acetate slide costs less than two glass slides (which are used to make one glass-scale slide), \$0.29 vs. \$0.41 currently in our lab. Even though making and storing acetate-scale slides require a heated-hydraulic press and slide boxes, respectively, whereas making glass-scale slides requires just tapes and no slide box needed, we believe that an acetate-scale slide won't cost more than a glass-scale slide since our lab has two heated-hydraulic presses already for processing Striped Bass and Summer Flounder scales. However, in terms of practice, we prefer the acetate- to glass-scale slide for following reasons:

1. An acetate-scale slide is more permanent than a glass-scale slide because the scales can slide out between two glass slides after a while, resulting in a confusion on which scales belong to which slides;

2. It is much easier to retrieve a specific acetate-scale slide from a slide box than to retrieve a glass slide from a stack of glass slides, making ageing process more efficient;

3. It is much safer to transport (such as for an ageing exchange program between ageing labs) and store acetate-scale slides because they are unbreakable.

There is a clear winner between the section- and whole-otolith ages. More specifically, the average CV of three precision (2 self-precision and 1 between-reader precision) is 13.5% for the section-otolith ages whereas 19.4% for the whole-otolith ages. Moreover, there is no systematic difference between the first and second readings within each reader and between two readers for the section-otolith ages, in contrast, there is a systematic difference between the first and second readings within Reader 1's whole-otolith ages with the highest mean CV (25.1%) estimated in this study. Therefore, the section-otolith method may provide more consistent age estimates for Menhaden than the whole-otolith method.

The discussions above have left us two options: the acetate-scale or section-otolith method. Within non-systematic difference comparisons between two different ages, the section-otolith against the acetate-scale ages has the second lowest mean CV (15.3%), indicating that the section-otolith and acetate-scale ages may provide very similar age estimates for Menhaden. However, it is well-known that it costs much less to make an acetate-scale slide than a section-otolith slide, therefore, the acetate-scale method may have an advantage over the section-otolith method here.

However, because our two readers had only two-year experience on ageing Menhaden using the 4 methods, the differences on the ages observed in this study are most likely attributed to not only the different ageing methods but also the lack of ageing experience from each reader. Therefore, this study is unable to identify if the scale age may overestimate and underestimate ages for younger and older Menhaden, respectively, observed in other species (Liao et al. 2013), recommending to use the results from this study with caution. We will continue to collect both scales and otoliths from Menhaden in 2025, process them using the 4 methods and conduct the same tests on the 4 different ages. We expect that our third-year study will provide more conclusive results and help us to decide which method we should use to age Menhaden at the VMRC Ageing Lab.

15.5 ACKNOWLEDGMENTS

We thank Amanda Rezek at the NOAA Beaufort Laboratory, NC, for training our staff on using Menhaden scales for age determination. The training established the foundation upon which the VMRC Ageing Lab was able to initiate this project. We would like also to thank the VMRC field technicians, Richard Hancock, Myra Thompson, and Chris Williams, for their many efforts in collecting samples for this cooperative project. A special note of appreciation is extended to the Ageing Lab Technicians, Marben Abutin and Natalie Baker, for their efforts to learn new techniques and to process Menhaden scales and otoliths beyond their routine lab assignment. Jemelyn Grace Baldisimo worked on this project through the Commonwealth of Virginia Engineering and Science Fellowship funded by the Virginia Academy of Science, Engineering & Medicine.

Table 15.1: The summary of the sample sizes from which we were able to make different hardpart slides in 2024. "Y" and "N" stand for with and without slides, respectively.

	Slide Type			
Sample Size	Acetate-scale	Glass-scale	Section-otolith	Whole-otolith
103	Y	Y	Y	Y
33	Y	Y	Y	N
17	Y	Y	N	Y
3	N	Y	Y	Y
1	Y	N	Y	Y
1	Y	Y	N	N
1	N	N	N	Y

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Table 15.2: Statistics on the precision within each reader and between the readers, and the comparisons between the ages estimated using the different methods. "One Year Agreement (%)" stands for one year or less agreement between two sets of ages. There are two sections in the table, the p-values larger than 0.05 are in the upper section whereas smaller than and equal to 0.05 in the lower section. Within each section the mean CVs (%)) are listed from the smallest (highest precision) to largest (lowest precision).

Precision/Comparison	Agreement(%)	One Year Agreement(%)	Mean CV(%)	Chi square	df	P-value
		P-value > 0.05				
Acetate-scale within Reader 1	58	86	8.3	10.33	8	0.2424
Acetate-scale against glass-scale	51	86	8.9	15.35	10	0.1198
Section-otolith within Reader 2	56	94	9.5	8.33	6	0.2147
Section-otolith within Reader 1	38	88	11.8	14.6	9	0.1026
Section-otolith against acetate-scale	31	74	15.3	24.68	19	0.1713
Section-otolith against glass-scale	32	71	15.8	25.7	18	0.1067
Whole-otolith within Reader 2	34	76	16.5	18.9	12	0.0909
Whole-otolith between readers	35	80	16.5	12.12	15	0.6696
Section-otolith between readers	31	66	19.3	31.55	21	0.0649
		P-value <= 0.05				
Glass-scale within Reader 1	36	90	10.9	22.4	8	0.0042
Glass-scale between readers	35	82	14.8	64.75	14	< 0.0001
Acetate-scale between readers	28	70	19.1	76.05	12	< 0.0001
Acetate-scale within Reader 2	18	68	20.8	41	10	< 0.0001
Whole-otolith against acetate-scale	21	50	21.1	43.1	20	0.002
Whole-otolith against glass-scale	16	50	22	60.56	22	< 0.0001
Whole-otolith against section-otolith	17	51	23	44.21	24	0.0072
Glass-scale within Reader 2	14	44	23.8	43	11	< 0.0001
Whole-otolith within Reader 1	18	48	24.6	37.67	16	0.0017

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Figure 15.1: A scale impression on an acetate slide made from Menhaden AGID 100 collected in 2024.
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Figure 15.2: A scale between two glass slides made from Menhaden AGID 100 collected in 2024.
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Figure 15.3: An otolith thin-section mounted on a glass slide made from Menhaden AGID 100 collected in 2024.
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Figure 15.4: A whole otolith mounted on a glass slide made from Menhaden AGID 100 collected in 2024.
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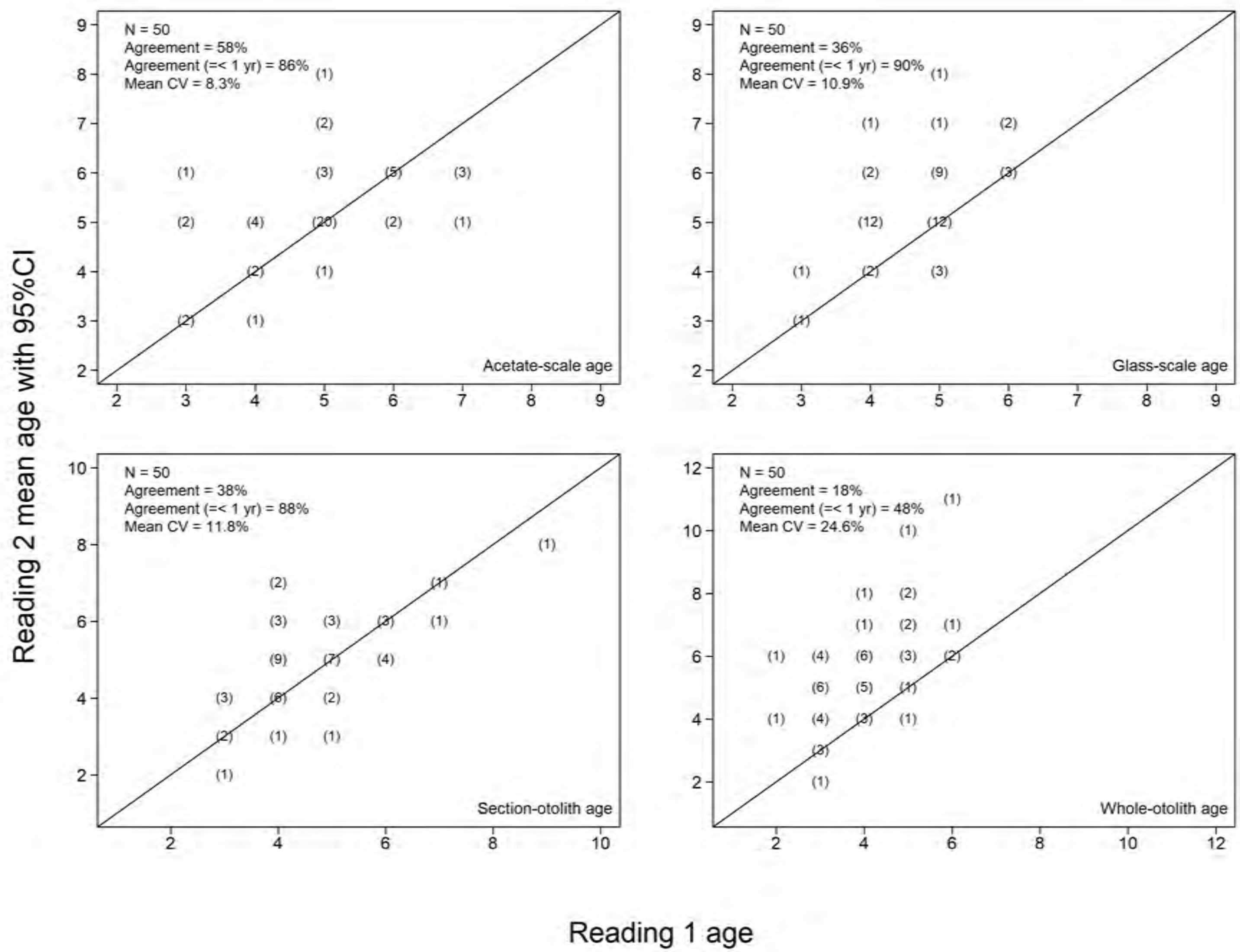

Figure 15.5: Reading 2 mean age with 95% confidence interval against Reading 1 of Reader 1 for the acetate scale age, glass scale age, sectioned otolith age, and whole otolith age, respectively. N stands for the total sample size and the number in parenthesis is the number of fish at a given reading age.

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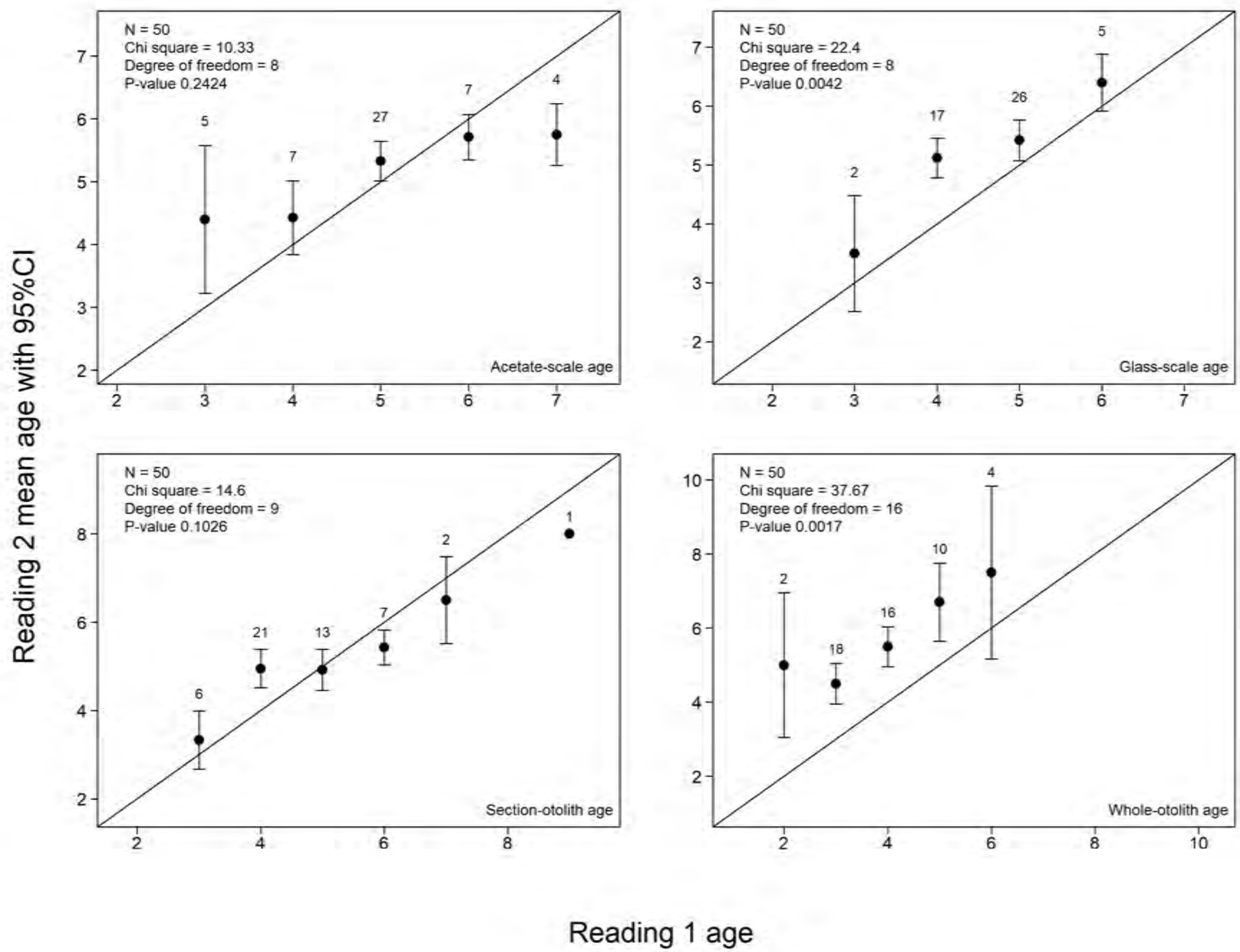

Figure 15.6: Symmetry test results between Reading 1 and 2 of Reader 1 on the acetate scale age, glass scale age, sectioned otolith age, and whole otolith age, respectively. N stands for the total sample size and the number above 95% confidence interval is the number of fish at a given reading age.

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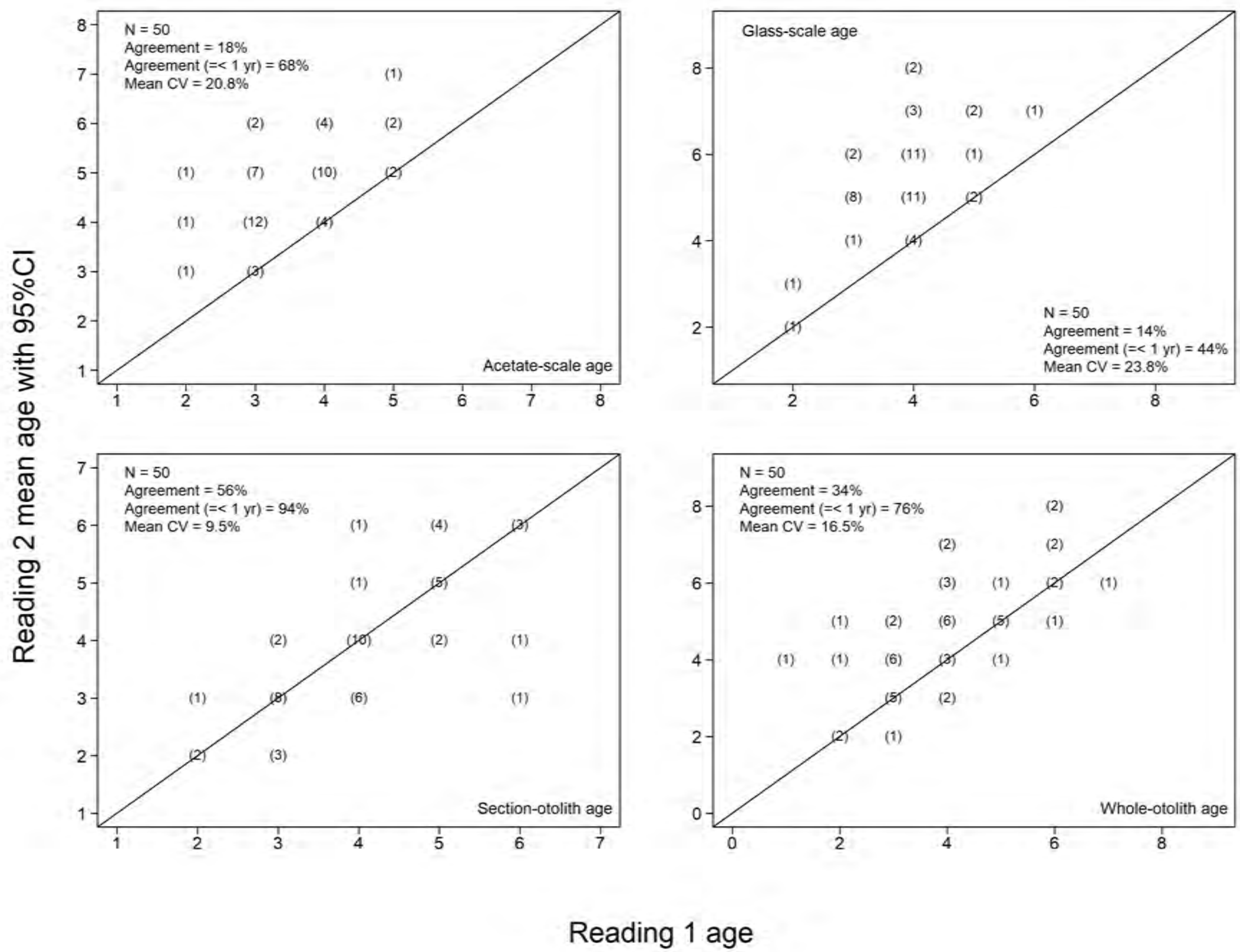

Figure 15.7: Reading 2 mean age with 95% confidence interval against Reading 1 of Reader 2 for the acetate scale age, glass scale age, sectioned otolith age, and whole otolith age, respectively. N stands for the total sample size and the number in parenthesis is the number of fish at a given reading age.

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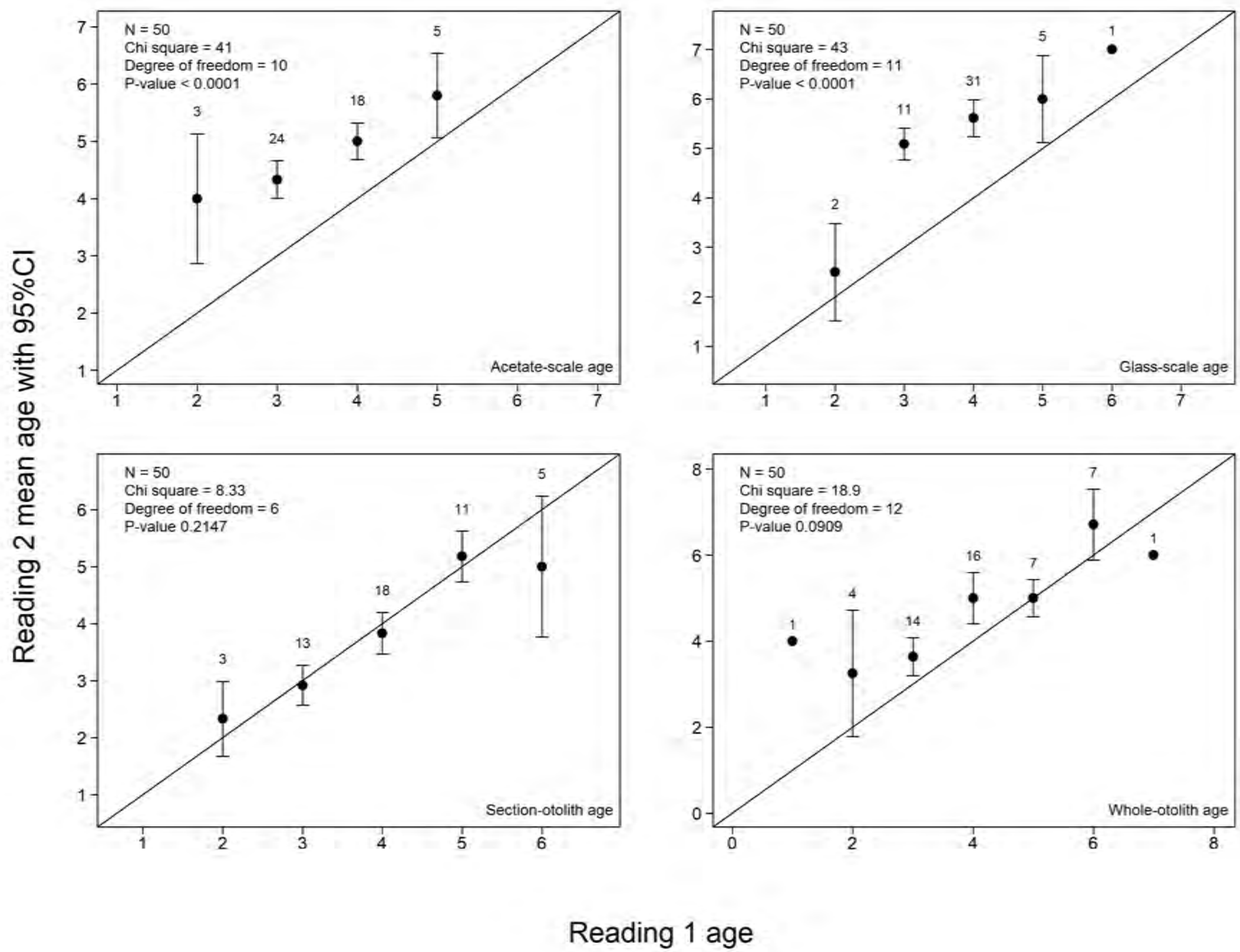

Figure 15.8: Symmetry test results between Reading 1 and 2 of Reader 2 on the acetate scale age, glass scale age, sectioned otolith age, and whole otolith age, respectively. N stands for the total sample size and the number above 95% confidence interval is the number of fish at a given reading age.

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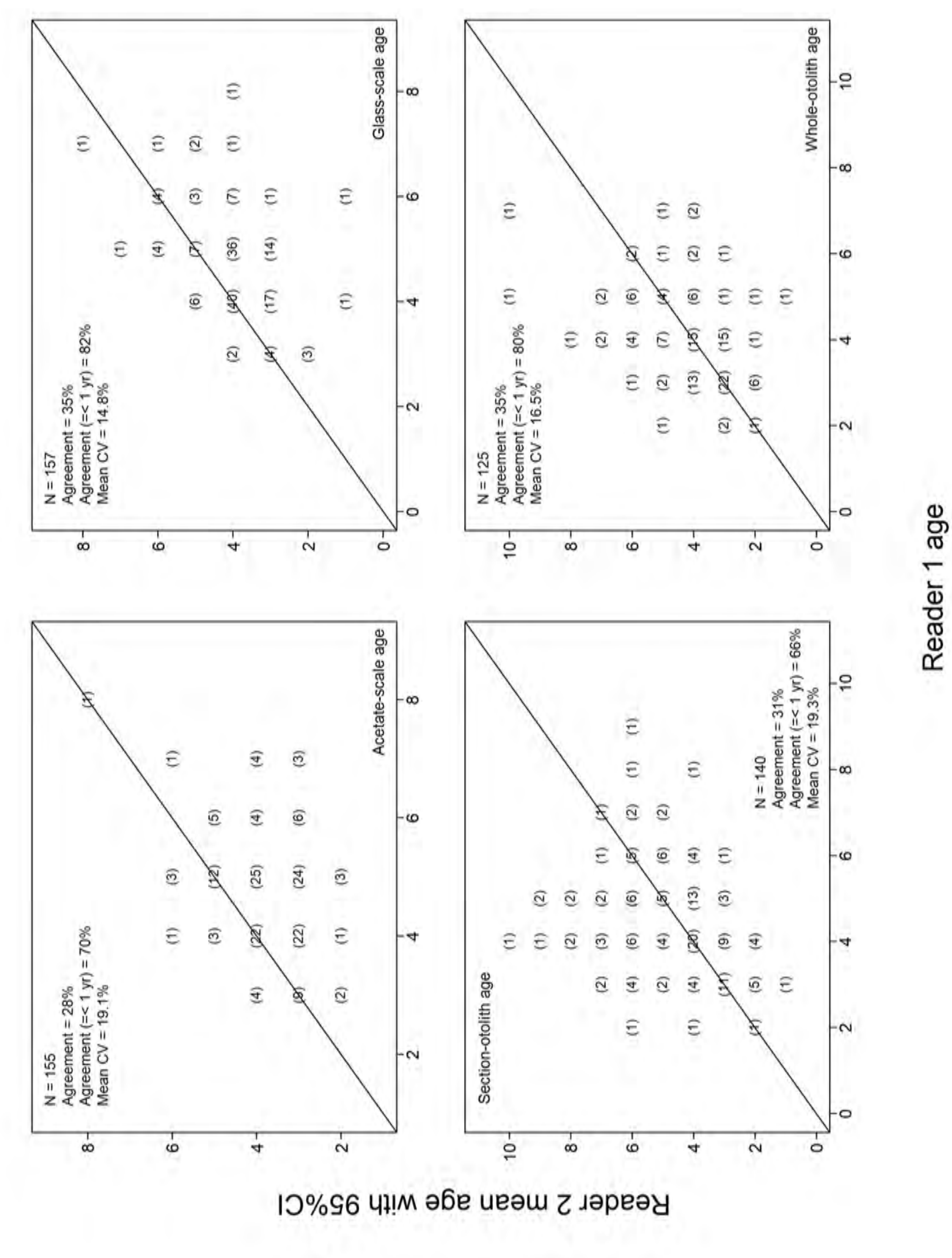

Figure 15.9: Between-reader precision for the acetate scale age, glass scale age, sectioned otolith age, and whole otolith age, respectively. N stands for the total sample size and the number in parenthesis is the number fish at a given reading age.
(Go back to text)

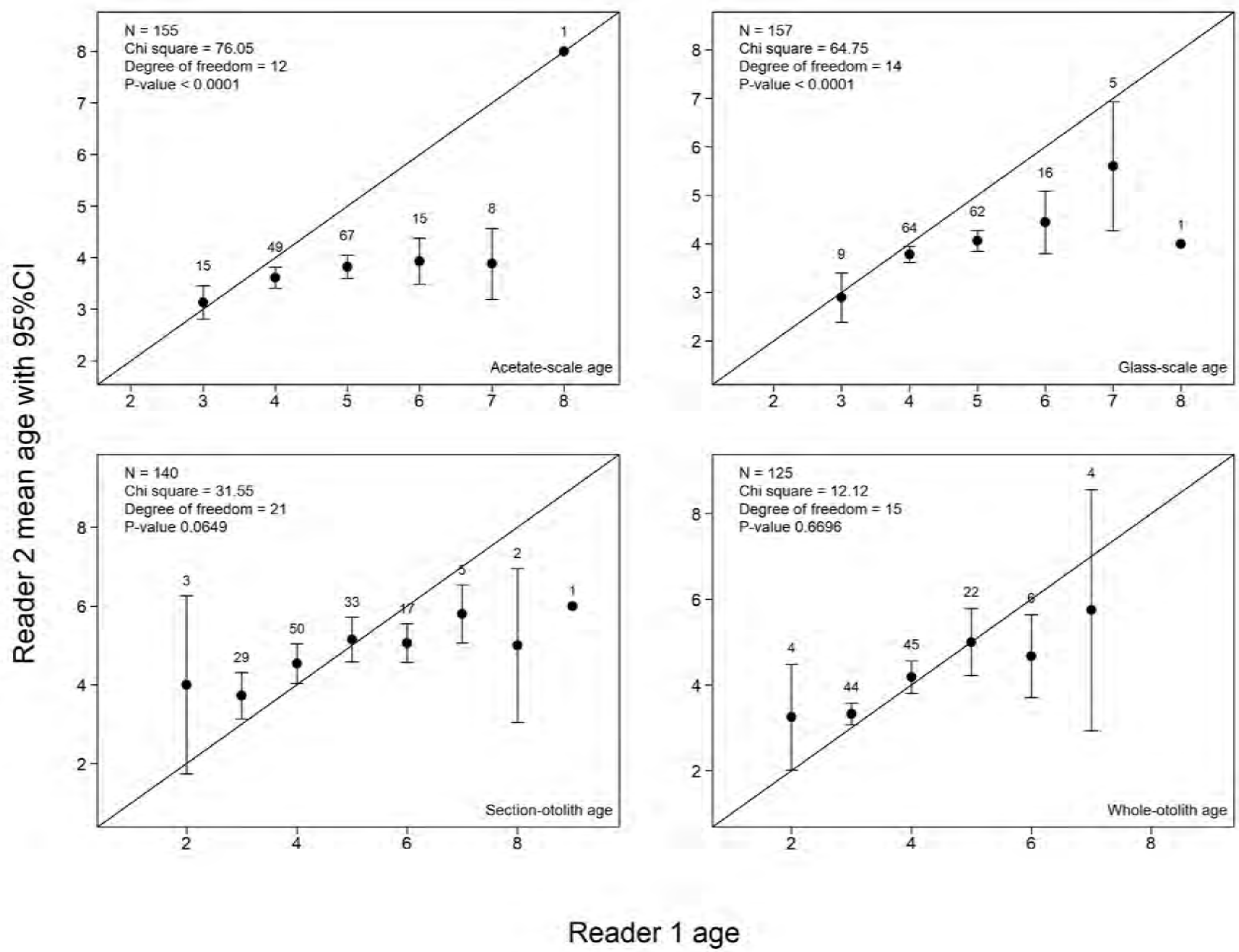

Figure 15.10: Symmetry test results between two readers on the acetate scale age, glass scale age, sectioned otolith age, and whole otolith age, respectively. N stands for the total sample size and the number above 95% confidence interval is the number of fish at a given reading age.
(Go back to text)

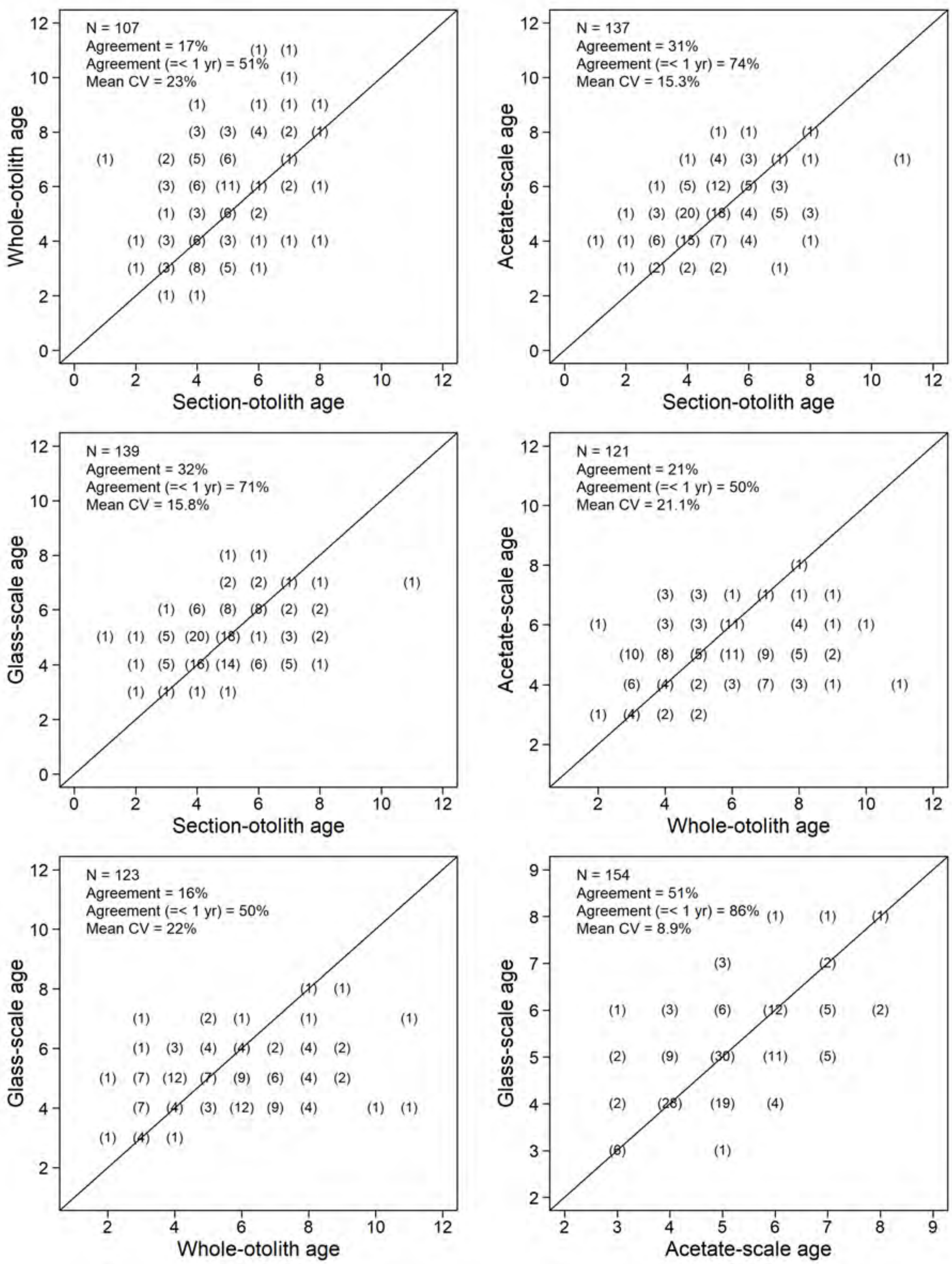

Figure 15.11: The precision between any two different ages of the acetate scale age, glass scale age, sectioned otolith age, and whole otolith age. N stands for the total sample size and the number in parenthesis is the number of fish at a given reading age.

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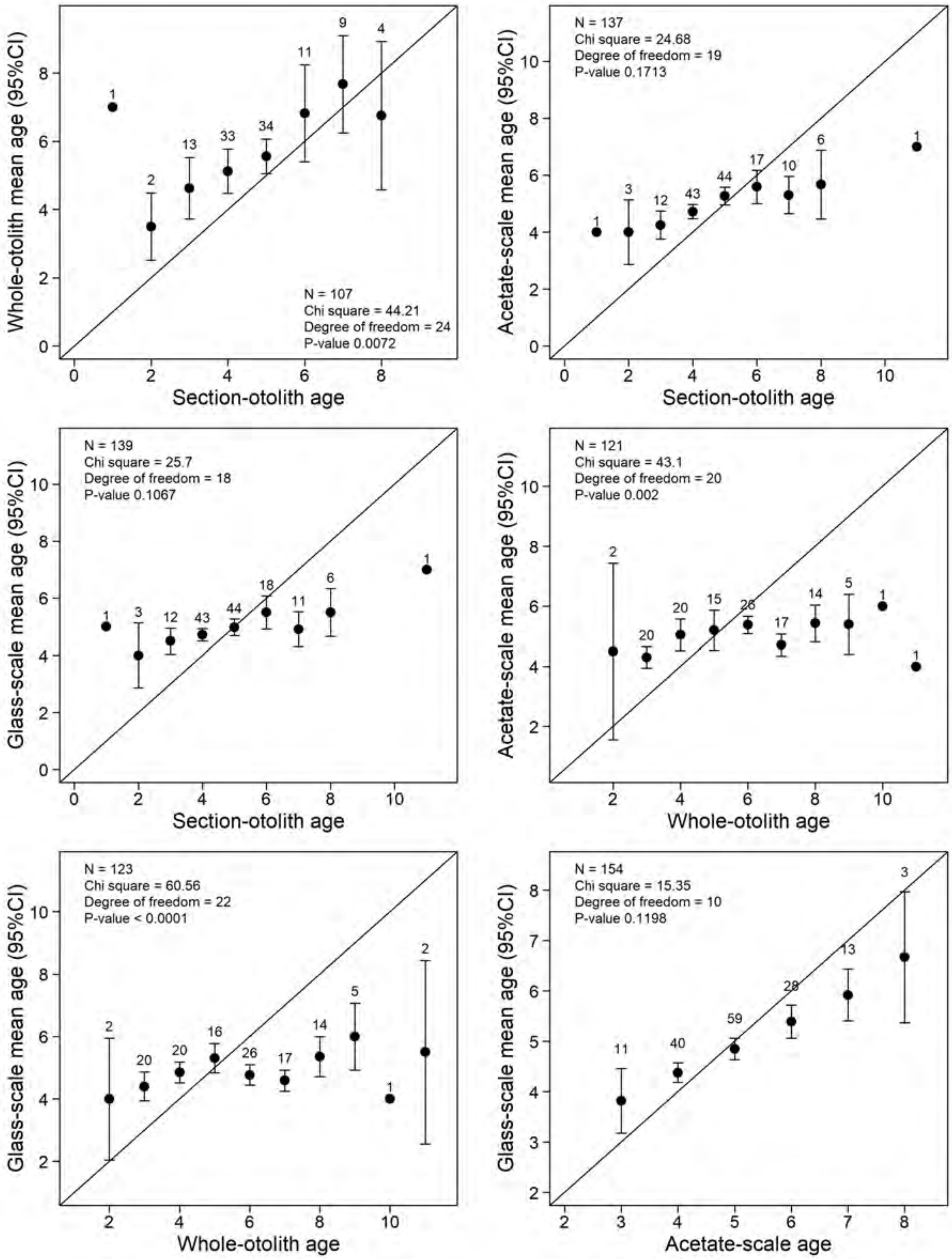

Figure 15.12: The symmetry test results between any two different ages of the acetate scale age, glass scale age, sectioned otolith age, and whole otolith age. N stands for the total sample size and the number above 95% confidence interval is the number of fish at a given reading age.

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