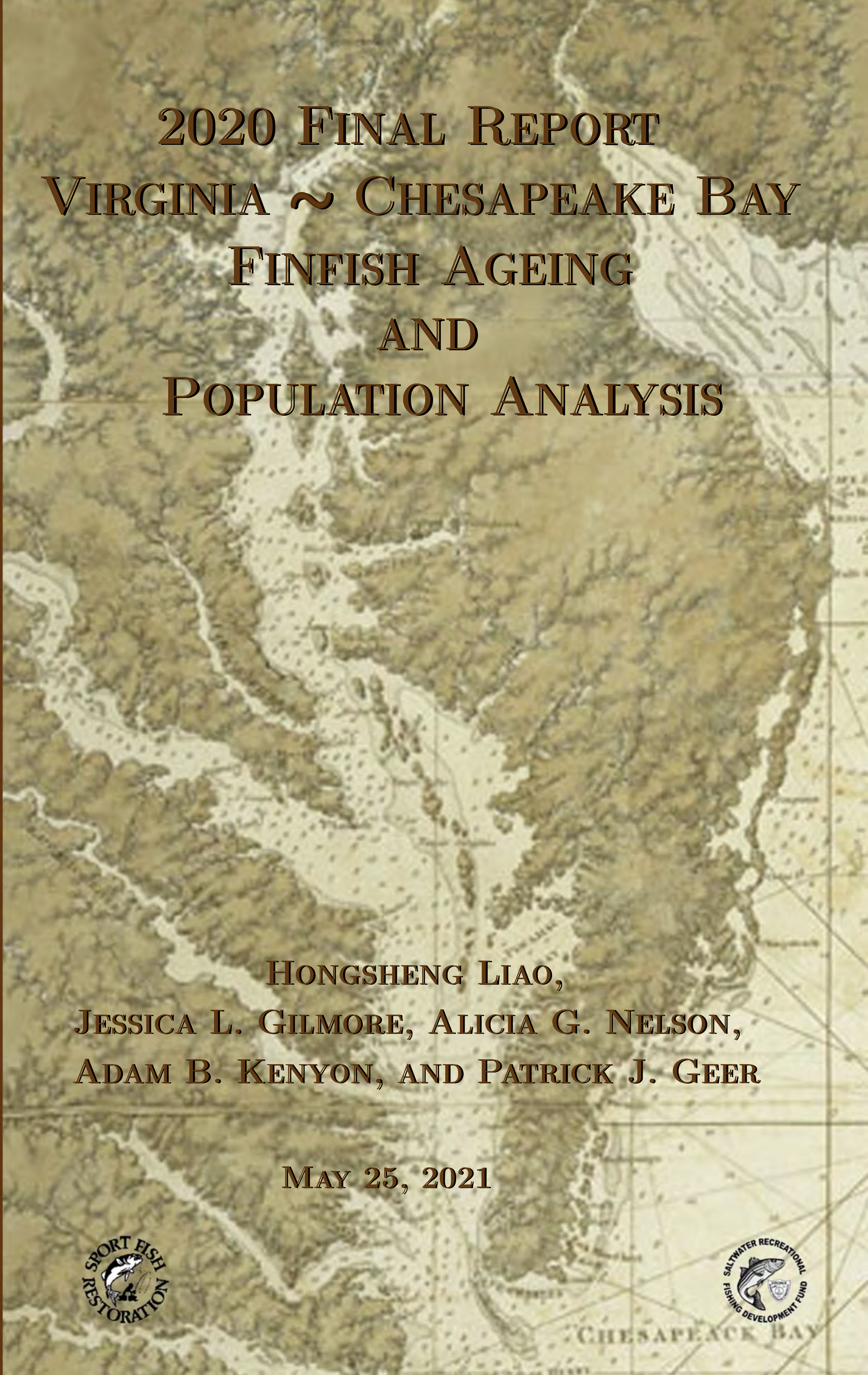

2020 FINAL REPORT
VIRGINIA ~ CHESAPEAKE BAY
FINFISH AGEING
AND
POPULATION ANALYSIS

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MAY 25, 2021

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

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EXECUTIVE SUMMARY

This executive summary briefly summarizes what the Age and Growth Lab achieved in 2020 in terms of the objectives listed in the 2019-2020 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 2020. 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 2020. All fish were collected by the Virginia Marine Resources Commission's (VMRC) Stock Assessment Program in 2020 and aged in 2021 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.

Three calcified structures (hard-parts) are used in age determination. Specifically, two calcified structures were used for determining fish ages of the following three species: Striped Bass, *Morone saxatilis*, (n = 614); Summer Flounder, *Paralichthys dentatus*, (n = 707); and Tautog, *Tautoga onitis*, (n = 109). Scales and otoliths were used to age Striped Bass and Summer Flounder, opercula and otoliths were used to age Tautog. Comparing alternative hard-parts allowed us to assess their usefulness in determining fish age as well as the relative precision of each structure. Ages were determined from otoliths only for the following species: Atlantic Croaker, *Micropogonias undulatus*, (n = 193); Black Drum, *Pogonias cromis*, (n = 13); Bluefish, *Pomatomus saltatrix*, (n = 218); Cobia, *Rachycentron canadum*, (n = 390); Red Drum, *Sciaenops ocellatus*, (n = 62); Sheepshead, *Archosargus probatocephalus*, (n = 43); Atlantic Spadefish, *Chaetodipterus faber*, (n = 190); Spanish Mackerel, *Scomberomorous maculates*, (n = 200); Spot, *Leiostomus xanthurus*, (n = 203); Spotted Seatrout, *Cynoscion nebulosus*, (n = 270); and Weakfish, *Cynoscion regalis*, (n = 246). In total, we made 7,798 age readings from scales, otoliths and opercula collected during 2020. A summary of the age ranges for all species aged is presented in Table 1.

Two readers aged all the samples separately. However, due to the 6-foot social distancing during the pandemic of COVID-19, only the primary reader (Reader 1) re-aged the fish with the disagreement between the two readers and assigned the final ages to those fish. Please see each chapter for the details.

Objective 2: VMRC will continue to develop sampling methods that are cost effective and representative of landings in the fisheries. This will produce accurate estimates of catch and effort. We have been using two-stage sampling to decide sample sizes for ageing 10 of our 14 species, which have helped to minimize costs on ageing while maximizing precision on estimates of catch-at-age.

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

Objective 3: VMRC will develop routine stock assessments based on age-structured models (such 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 the ASMFC. Currently the Lab Manager, Dr. Hongsheng Liao, is a member of Atlantic Striped Bass Stock Assessment Subcommittee (SAS). However, the SAS just finished the Striped Bass Benchmark Stock Assessment in 2018, therefore, there was no activity from the SAS in 2020. In 2020, Dr. Liao continued to update %MSP%fSPR%SPR Estimator by adding more functions in the estimator. This model is used to estimate the maximum spawning potential of a fish population given a certain fisheries management policy. for example, once Black Drum and Spotted Seatrout stock assessments are completely, we are planning to use the estimator with the stock assessment results to explore potential fisheries management policy for Virginia Black Drum and Spotted Seatrout commercial and recreational fisheries.

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.

Since the lab was transferred from ODU to VMRC in September of 2019, we have transferred some of the lab information from ODU Ageing Laboratory website to VMRC Ageing Laboratory website. At the VMRC Age and Growth Lab website, we published six annual reports by the Age and Growth Lab (including this report) and six web-based applications. Among the six applications, two were developed and published in 2020 (Please see Objective 5 below).

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

In 2020, we continued to update two web-based applications and have them posted at VMRC website, VMRC Ageing Lab Data Sharing App and %MSP%fSPR%SPR Estimator. The first application is designed to share VMRC age data with other state, federal, and academic agencies. For example, in 2020 we shared our Striped Bass otolith thin-sections and their age data with University of Massachusetts. The second application can be used by fisheries management to identify the relationships between management options and stock maximum spawning potential. All six applications developed at the Ageing Lab since 2017 have been playing an important role in maximizing utilization of the VMRC age data among fisheries scientists and the public.

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 2020. Anytime when we revised an old processing method and added a new method, we added those new information in the protocol.

Besides above work the Age and Growth Lab did in 2020, to support environmental and wildlife agencies, and charities, we donated more than 240 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.

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 2020. The hard-parts and age readings include both scales and otoliths for Striped Bass and Summer Flounder. For Tautog, the hard-parts include opercula, otoliths, and pelvic spines, but the age readings include opercula and otoliths only.

Species	Number of fish collected	Number of hard-parts	Numnber of fish aged	Number of readings	Minimun age	Maximum age
Atlantic Croaker	300	300	193	386	0	7
Black Drum	13	13	13	26	1	19
Bluefish	304	304	218	436	0	3
Cobia	392	390	390	780	2	10
Red Drum	62	62	62	124	1	22
Sheepshead	43	43	43	86	1	23
Spadefish	207	207	190	380	0	8
Spanish Mackerel	262	262	200	400	0	6
Spot	238	238	203	406	0	3
Spotted Seatrout	412	412	270	540	0	5
Striped Bass	736	921	614	1,596	2	22
Summer Flounder	710	853	707	1,710	0	13
Tautog	109	0	109	436	1	14
Weakfish	265	264	246	492	0	5
Totals	4,053	4,269	3,458	7,798		

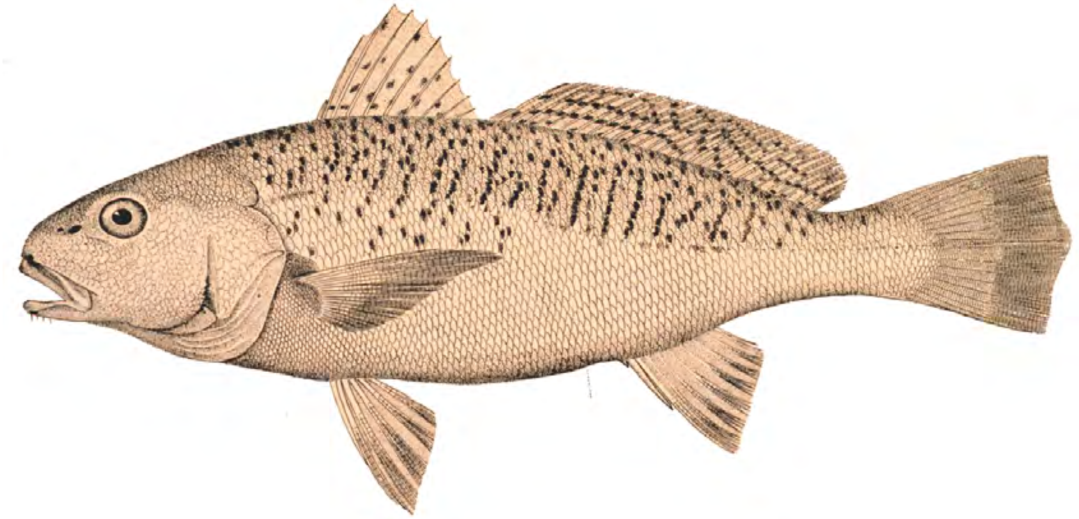
(Go back to text)

ACKNOWLEDGMENTS

We thank our Lab Technician Marben Abutin, Savanah Davidson, and Emily Davis for their technical expertise in preparing otoliths, scales, and opercula for age determination, and our Field Technician Richard Hancock, Myra Thompson, and Chris Williams for their many efforts in biological sampling. They all put in long hours processing "tons" of fish in our lab. We would like to thank VMRC supervisor Ethan Simpson for his help in processing fish and hard-parts whenever we fell short of hands.

CHAPTER 1

ATLANTIC CROAKER *Micropogonias undulatus*



1.1 INTRODUCTION

We aged a total of 193 Atlantic Croaker, *Micropogonias undulatus*, collected by the VMRC's Biological Sampling Program for age and growth analysis in 2020. Croaker ages ranged from 0 to 7 years old with an average age of 2.6, a standard deviation of 1.5, and a standard error of 0.11. Eight age classes (0 to 7) were represented, comprising fish of the 2013 to 2020 year-classes. The sample was dominated by fish from the year-classes of 2016, 2017, 2018, and 2019 with 14%, 31.1%, 13.5%, and 25.9%, respectively.

1.2 METHODS

1.2.1 Sample size for ageing

We estimated sample size for ageing Croaker in 2020 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:

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

where A is the sample size for ageing Croaker in 2020; θ_a stands for the proportion of age a fish in a catch. V_a and B_a represent variance components within and between length intervals for age a, respectively; CV is the coefficient of variation; L was the total number of Croaker used by VMRC to estimate length distribution of the catches from 2014 to 2018. θ_a, V_a, B_a, and CV were calculated using pooled age-length data of Croaker collected from 2014 to 2018 and using equations in Quinn and Deriso (1999). For simplicity, the equations are not listed here. The equation (1.1) indicates that the more fish that are aged, the smaller the CV (or higher precision) that will be obtained. Therefore, the criterion to age A (number) of fish is that A should be a number above which there is only a 1% CV reduction for the most major 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 fish aged in the lab between 2014 and 2018. A_l is number of fish to be aged for length interval l in 2020.

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$^{\text{TM}}$ 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$^{\text{TM}}$ 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 2020 (Figure 1.1).

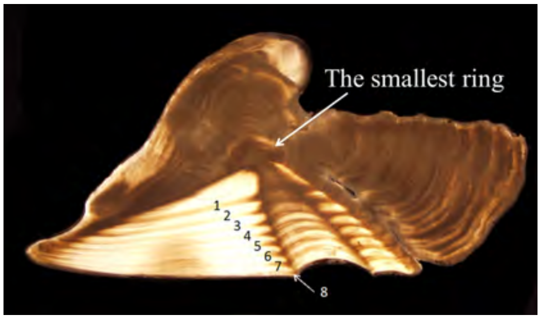


Figure 1.1: Otolith thin-sections of a 8 year-old Croaker without counting the smallest ring and with the last annulus on the edge of the thin-section

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, within the reader, for the following comparisons: 1) in the current year and 2) time-series bias between the current and previous years. The readings from the entire sample for the current year were used to examine the difference and precision in the current. A random sub-sample of 50 fish from the current year was selected for second readings of the reader. Fifty otoliths randomly selected from the fish aged in 2003 were used to examine the time-series bias within the reader. All statistics analyses were performed in R 3.6.1 (R Core Team 2019).

1.3 RESULTS

1.3.1 Sample size

We estimated a sample size of 395 Atlantic Croaker in 2020, ranging in length intervals from 4 to 17 inches (Table 1.1). This sample size provided a range in (*CV*) for age composition approximately from the smallest (*CV*) of 0.08% for Age 4 to the largest (*CV*) of 0.25% for Age 1. In 2020, we aged 193 of 300 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 over-all collections for this optimal length-class sampling estimate by 211 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.

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 100%, and there was no significant difference between the first and second readings for Reader 2 with an agreement of 100%. There was no evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 97.93% and a *CV* of 0.67% (test of symmetry: $\chi^2 = 2$, $df = 3$, $P = 0.5724$) (Figure 1.2).

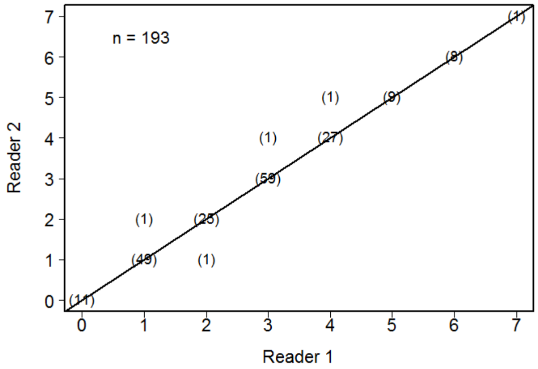


Figure 1.2: Between-reader comparison of otolith age estimates for Atlantic Croaker collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2020.

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

1.3.3 Year class

Of the 193 fish aged with otoliths, 8 age classes (0 to 7) were represented (Table 1.2). The average age was 2.6 years, and the standard deviation and standard error were 1.5 and 0.11, respectively. Year-class data show that the fishery was comprised of 8 year-classes: fish

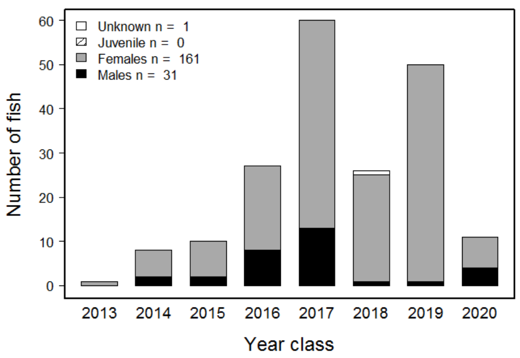


Figure 1.3: Year-class frequency distribution for Atlantic Croaker collected for ageing in 2020. Distribution is broken down by sex. 'Unknown' represents gonads that were not available for examination or were not examined for sex during sampling.

from the 2013 to 2020 year-classes, with fish primarily from the year classes of 2016, 2017, 2018, and 2019 with 14%, 31.1%, 13.5%, and 25.9%, respectively. The ratio of males to females was 1:5.19 in the sample collected (Figure 1.3).

1.3.4 Age-length-key (ALK)

We developed an age-length-key (Table 1.3) 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 2020. 'Target' represents the sample size for ageing estimated for 2020, 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	1	1	4
7 - 7.99	10	12	10	0
8 - 8.99	8	17	8	0
9 - 9.99	23	39	24	0
10 - 10.99	41	123	42	0
11 - 11.99	72	79	79	0
12 - 12.99	106	23	23	83
13 - 13.99	65	6	6	59
14 - 14.99	32	0	0	32
15 - 15.99	13	0	0	13
16 - 16.99	5	0	0	5
17 - 17.99	5	0	0	5
Totals	395	300	193	211

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Table 1.2: The number of Atlantic Croaker assigned to each total length-at-age category for 193 fish sampled for otolith age determination in Virginia during 2020.

	Age								
Interval	0	1	2	3	4	5	6	7	Totals
6 - 6.99	1	0	0	0	0	0	0	0	1
7 - 7.99	9	1	0	0	0	0	0	0	10
8 - 8.99	0	1	1	3	1	1	1	0	8
9 - 9.99	1	4	0	13	5	0	1	0	24
10 - 10.99	0	6	4	16	10	5	1	0	42
11 - 11.99	0	24	15	22	10	2	5	1	79
12 - 12.99	0	11	6	5	1	0	0	0	23
13 - 13.99	0	3	0	1	0	2	0	0	6
Totals	11	50	26	60	27	10	8	1	193

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Table 1.3: 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 2020.

	Age							
Interval	0	1	2	3	4	5	6	7
6 - 6.99	1	0	0	0	0	0	0	0
7 - 7.99	0.9	0.1	0	0	0	0	0	0
8 - 8.99	0	0.12	0.12	0.38	0.12	0.12	0.12	0
9 - 9.99	0.04	0.17	0	0.54	0.21	0	0.04	0
10 - 10.99	0	0.14	0.1	0.38	0.24	0.12	0.02	0
11 - 11.99	0	0.3	0.19	0.28	0.13	0.03	0.06	0.01
12 - 12.99	0	0.48	0.26	0.22	0.04	0	0	0
13 - 13.99	0	0.5	0	0.17	0	0.33	0	0

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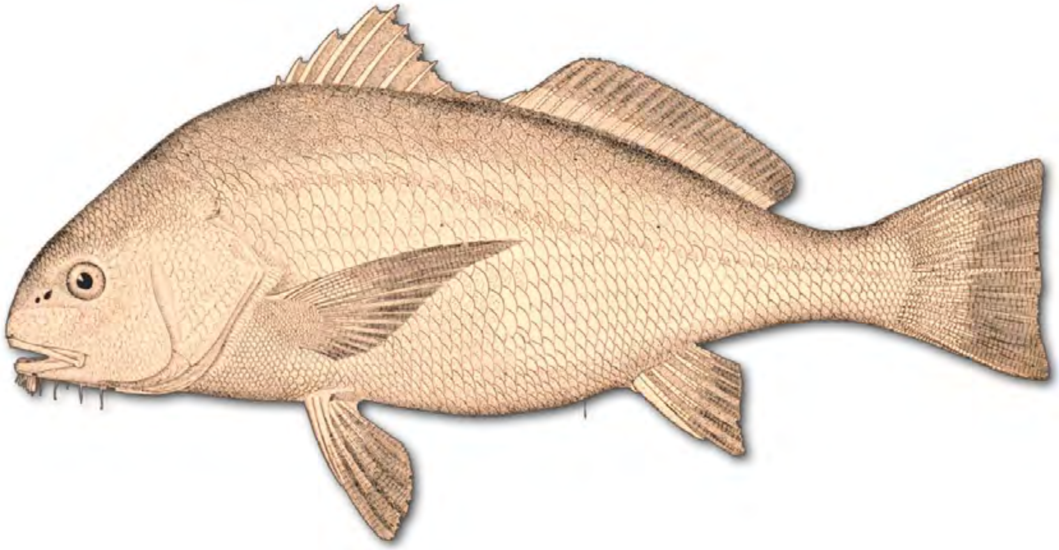
CHAPTER 2

BLACK DRUM *Pogonias cromis*



2.1 INTRODUCTION

We aged a total of 13 Black Drum, *Pogonias cromis*, collected by the VMRC's Biological Sampling Program for age and growth analysis in 2020. Black Drum ages ranged from 1 to 19 years old with an average age of 7.8, a standard deviation of 6.7, and a standard error of 1.86. Nine age classes (1 to 2, 4, 6 to 7, 13 to 14, and 18 to 19) were represented, comprising fish of the 2001 to 2002, 2006 to 2007, 2013 to 2014, 2016, and 2018 to 2019 year-classes. The sample was dominated by fish from the year-classes of 2007, 2007, and 2018 with 15.4%, 15.4%, and 23.1%, 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$^{\mathrm{TM}}$ 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$^{\mathrm{TM}}$ 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 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).

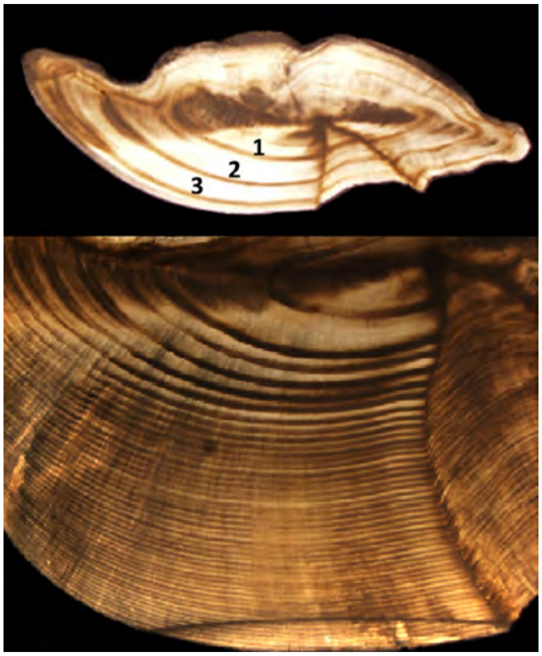


Figure 2.1: Otolith thin-sections of a 3 (Upper panel) and 47 year-old (Lower panel) Black Drum.

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, within the reader, for the following comparisons: 1) in the current year and 2) time-series bias between the current and previous years. The readings from the entire sample for the current year were used to examine the difference and precision in the current. When the sample size for the current year was smaller than 50, the entire sample was read by each reader for the second time to examine the difference within a reader. Fifty otoliths randomly selected from the fish aged in 2003 were used to examine the time-series bias within the reader. All statistics analyses were performed in R 3.6.1 (R Core Team 2019).

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 100%, and there was no significant difference between the first and second readings for Reader 2 with an agreement of 100%. There was no evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 100% (Figure 2.2).

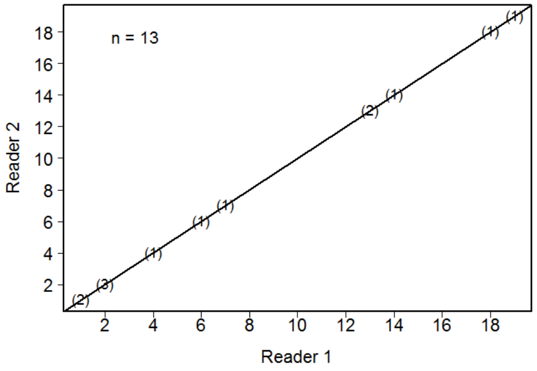


Figure 2.2: Between-reader comparison of otolith age estimates for Black Drum collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2020.

There was no time-series bias for either reader. Reader 1 had an agreement of 72% with ages of fish aged in 2000 with a *CV* of 1.26% (test of symmetry: $\chi^2 = 14$, $df = 13$, $P = 0.3738$) and, Reader 2 had an agreement of 86% with a *CV* of 5.13% (test of symmetry: $\chi^2 = 7$, $df = 7$, $P = 0.4289$).

2.3.2 Year class

Of the 13 fish aged with otoliths, 9 age classes (1 to 2, 4, 6 to 7, 13 to 14, and 18 to 19) were represented (Table 2.1). The average age was 7.8 years, and the standard deviation and standard error were 6.7 and 1.86, respectively. Year-class data show that the fishery was comprised of 9 year-classes: fish from the 2001 to 2002, 2006 to 2007, 2013 to 2014, 2016, and 2018 to 2019 year-classes, with fish primarily from the year classes of 2007, 2007, and 2018 with 15.4%, 15.4%, and 23.1%, respectively. The ratio of males to females was 1:0.62 in the sample collected (Figure 2.3).

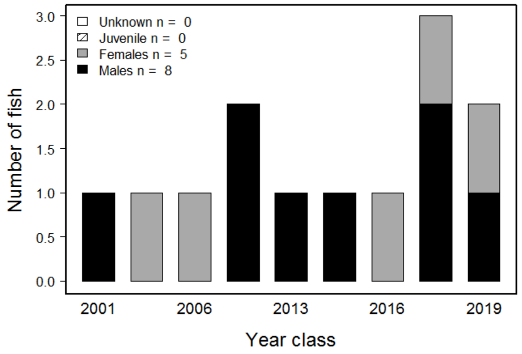


Figure 2.3: Year-class frequency distribution for Black Drum collected for ageing in 2020. Distribution is broken down by sex. 'Unknown' represents gonads that were not available for examination or were not examined for sex during sampling.

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 13 fish sampled for otolith age determination in Virginia during 2020.

	Age									
Interval	1	2	4	6	7	13	14	18	19	Totals
10 - 10.99	0	0	0	1	0	0	0	0	0	1
12 - 12.99	0	0	0	0	1	0	0	0	0	1
14 - 14.99	1	0	0	0	0	0	0	0	0	1
17 - 17.99	0	0	0	0	0	1	0	0	0	1
18 - 18.99	0	1	0	0	0	1	0	1	0	3
19 - 19.99	0	1	0	0	0	0	1	0	0	2
20 - 20.99	1	1	0	0	0	0	0	0	0	2
24 - 24.99	0	0	1	0	0	0	0	0	0	1
39 - 39.99	0	0	0	0	0	0	0	0	1	1
Totals	2	3	1	1	1	2	1	1	1	13

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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 2020.

	Age								
Interval	1	2	4	6	7	13	14	18	19
10 - 10.99	0	0	0	1	0	0	0	0	0
12 - 12.99	0	0	0	0	1	0	0	0	0
14 - 14.99	1	0	0	0	0	0	0	0	0
17 - 17.99	0	0	0	0	0	1	0	0	0
18 - 18.99	0	0.33	0	0	0	0.33	0	0.33	0
19 - 19.99	0	0.5	0	0	0	0	0.5	0	0
20 - 20.99	0.5	0.5	0	0	0	0	0	0	0
24 - 24.99	0	0	1	0	0	0	0	0	0
39 - 39.99	0	0	0	0	0	0	0	0	1

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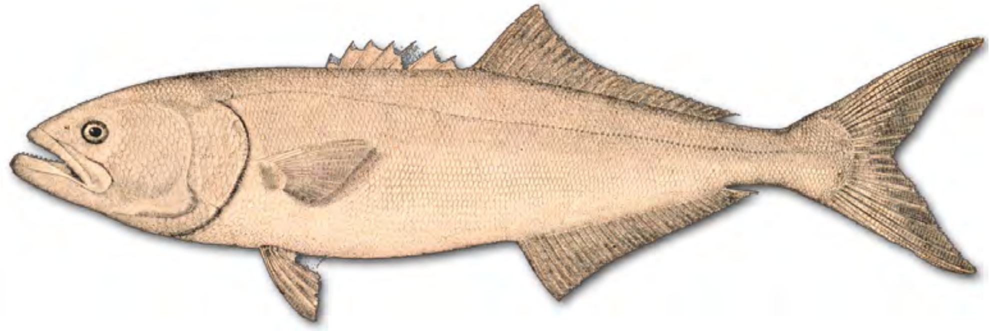
CHAPTER 3

BLUEFISH *Pomatomus saltatrix*



3.1 INTRODUCTION

We aged a total of 218 Bluefish, *Pomatomus saltatrix*, collected by the VMRC's Biological Sampling Program for age and growth analysis in 2020. Bluefish ages ranged from 0 to 3 years old with an average age of 1.4, a standard deviation of 0.8, and a standard error of 0.05. Four age classes (0 to 3) were represented, comprising fish of the 2017 to 2020 year-classes. The sample was dominated by fish from the year-classes of 2018 and 2019 with 50% and 30.3%, respectively.

3.2 METHODS

3.2.1 Sample size for ageing

We estimated sample size for ageing Bluefish in 2020 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:

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

where A is the sample size for ageing Bluefish in 2020; θ_a stands for the proportion of age a fish in a catch. V_a and B_a represent variance components within and between length intervals for age a, respectively; CV is the coefficient of variation; L was the total number of Bluefish used by VMRC to estimate length distribution of the catches from 2014 to 2018. θ_a, V_a, B_a, and CV were calculated using pooled age-length data of Bluefish collected from 2014 to 2018 and using equations in Quinn and Deriso (1999). For simplicity, the equations are not listed here. The equation (3.1) indicates that the more fish that are aged, the smaller the CV (or higher precision) that will be obtained. Therefore, the criterion to age A (number) of fish is that A should be a number above which there is only a 1% CV reduction for the most major age in catch by aging 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 fish aged in the lab between 2014 and 2018. A_l is number of fish to be aged for length interval l in 2020. Based on VMRC's request in 2010, we used 1-cm length interval for Bluefish, which differed from other species (1-inch).

3.2.2 Handling of collections

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

3.2.3 Preparation

We used our 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$^{\text{TM}}$ 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 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 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

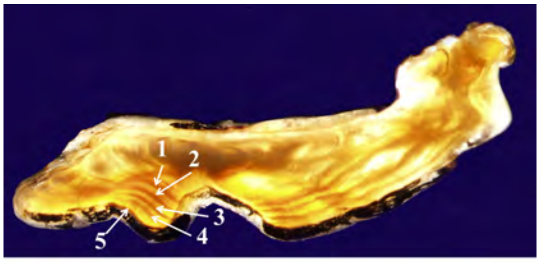


Figure 3.1: Otolith thin-section of a 5 year-old Bluefish with the last annulus on the edge of the thin-section

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 the 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 3.6.1 (R Core Team 2019).

3.3 RESULTS

3.3.1 Sample size

We estimated a sample size of 444 Bluefish in 2020, ranging in length intervals from 14 to 121 centimeters (Table 3.1). This sample size provided a range in (CV) for age composition approximately from the smallest (CV) of 0.06% for Age 1 to the largest (CV) of 0.22% for Age 8. In 2020, we aged 218 of 304 Bluefish (The rest of fish were either without otoliths or over-collected for certain length interval(s)) collected by VMRC. We fell short in our overall collections for this optimal length-class sampling estimate by 245 fish. We were short some fish from the major length intervals (the interval requires more than 5 fish), as a result, the precision for the estimates of major age groups would possibly be influenced significantly.

3.3.2 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 3.77% (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 4.34% (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 93.12% and a CV of 4.11% (test of symmetry: $\chi^2 = 6.62$, $df = 3$, $P = 0.0851$) (Figure 3.2).

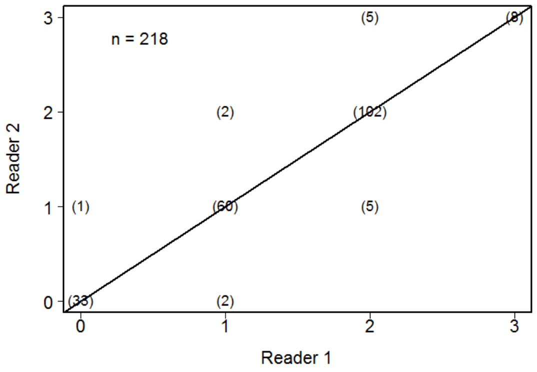


Figure 3.2: Between-reader comparison of otolith age estimates for Bluefish collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2020.

There was no time-series bias for either reader. Reader 1 had an agreement of 96% with ages of fish aged in 2000 with a CV of 1.08% (test of symmetry: $\chi^2 = 2$, $df = 2$, $P = 0.3679$), and Reader 2 had an agreement of 98% with a CV of 0.94% (test of symmetry: $\chi^2 = 1$, $df = 1$, $P = 0.3173$).

3.3.3 Year class

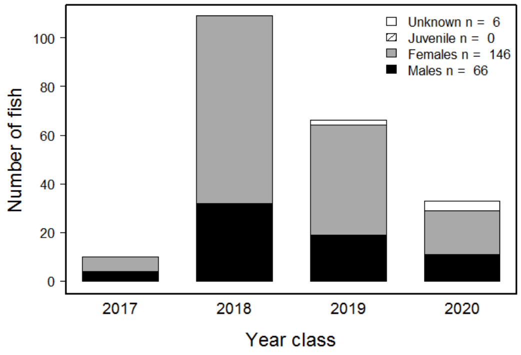


Figure 3.3: Year-class frequency distribution for Bluefish collected for ageing in 2020. Distribution is broken down by sex. 'Unknown' represents gonads that were not available for examination or were not examined for sex during sampling.

Of the 218 fish aged with otoliths, 4 age classes (0 to 3) were represented (Table 3.2). The average age was 1.4 years, and the standard deviation and standard error were 0.8 and 0.05, respectively. Year-class data show that the fishery was comprised of 4 year-classes: fish from the 2017 to 2020 year-classes, with fish primarily from the year classes of 2018 and 2019 with 50% and 30.3%, respectively. The ratio of males to females was 1:2.21 in the sample collected (Figure 3.3).

3.3.4 Age-length-key (ALK)

We developed an age-length-key (Table 3.3) 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 2020. 'Target' represents the sample size for ageing estimated for 2020, and 'Need' represents number of fish shorted in each length interval compared to the optimum sample size for ageing and number of fish aged.

Interval	Target	Collected	Aged	Need
14 - 14.99	5	0	0	5
16 - 16.99	5	0	0	5
17 - 17.99	5	0	0	5
18 - 18.99	5	0	0	5
19 - 19.99	5	0	0	5
20 - 20.99	5	1	1	4
21 - 21.99	5	0	0	5
22 - 22.99	5	3	3	2
23 - 23.99	5	2	2	3
24 - 24.99	5	5	5	0
25 - 25.99	5	4	4	1
26 - 26.99	5	7	6	0
27 - 27.99	5	10	5	0
28 - 28.99	5	11	6	0
29 - 29.99	5	10	6	0
30 - 30.99	5	8	6	0
31 - 31.99	5	8	6	0
32 - 32.99	5	5	5	0
33 - 33.99	5	6	6	0
34 - 34.99	5	8	6	0
35 - 35.99	5	2	2	3
36 - 36.99	7	9	8	0
37 - 37.99	7	4	4	3
38 - 38.99	6	6	6	0
39 - 39.99	7	7	7	0
40 - 40.99	7	5	5	2
41 - 41.99	6	15	6	0
42 - 42.99	7	13	8	0
43 - 43.99	7	15	8	0
44 - 44.99	5	18	6	0
45 - 45.99	7	7	7	0
46 - 46.99	6	10	6	0
47 - 47.99	6	8	6	0
48 - 48.99	5	10	6	0
49 - 49.99	5	10	6	0
50 - 50.99	5	8	6	0
51 - 51.99	5	12	6	0
52 - 52.99	5	9	6	0
53 - 53.99	5	6	6	0
54 - 54.99	5	11	6	0
55 - 55.99	5	5	5	0
56 - 56.99	5	4	4	1
57 - 57.99	5	5	5	0
58 - 58.99	5	7	6	0
59 - 59.99	5	3	3	2
60 - 60.99	5	2	2	3
61 - 61.99	5	3	3	2
62 - 62.99	5	1	1	4

(To continue)

Table 3.1 (Continued)

Interval	Target	Collected	Aged	Need
63 - 63.99	5	0	0	5
64 - 64.99	5	0	0	5
65 - 65.99	5	0	0	5
66 - 66.99	5	0	0	5
67 - 67.99	5	0	0	5
68 - 68.99	5	0	0	5
69 - 69.99	5	0	0	5
70 - 70.99	5	0	0	5
71 - 71.99	5	1	1	4
72 - 72.99	5	0	0	5
73 - 73.99	5	0	0	5
74 - 74.99	5	0	0	5
75 - 75.99	5	0	0	5
76 - 76.99	5	0	0	5
77 - 77.99	5	0	0	5
78 - 78.99	5	0	0	5
79 - 79.99	5	0	0	5
80 - 80.99	5	0	0	5
81 - 81.99	6	0	0	6
82 - 82.99	5	0	0	5
83 - 83.99	5	0	0	5
84 - 84.99	5	0	0	5
85 - 85.99	5	0	0	5
86 - 86.99	5	0	0	5
87 - 87.99	5	0	0	5
88 - 88.99	5	0	0	5
89 - 89.99	5	0	0	5
90 - 90.99	5	0	0	5
91 - 91.99	5	0	0	5
92 - 92.99	5	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
97 - 97.99	5	0	0	5
98 - 98.99	5	0	0	5
121 - 121.99	5	0	0	5
Totals	444	304	218	245

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Table 3.2: The number of Bluefish assigned to each total length (cm)-at-age category for 218 fish sampled for otolith age determination in Virginia during 2020.

	Age				
Interval	0	1	2	3	Totals
20 - 20.99	1	0	0	0	1
22 - 22.99	2	1	0	0	3
23 - 23.99	0	2	0	0	2
24 - 24.99	2	3	0	0	5
25 - 25.99	4	0	0	0	4
26 - 26.99	4	2	0	0	6
27 - 27.99	3	2	0	0	5
28 - 28.99	3	3	0	0	6
29 - 29.99	1	4	1	0	6
30 - 30.99	3	3	0	0	6
31 - 31.99	4	2	0	0	6
32 - 32.99	2	3	0	0	5
33 - 33.99	2	4	0	0	6
34 - 34.99	1	3	2	0	6
35 - 35.99	0	2	0	0	2
36 - 36.99	1	6	1	0	8
37 - 37.99	0	4	0	0	4
38 - 38.99	0	4	2	0	6
39 - 39.99	0	5	2	0	7
40 - 40.99	0	3	2	0	5
41 - 41.99	0	2	4	0	6
42 - 42.99	0	2	6	0	8
43 - 43.99	0	0	8	0	8
44 - 44.99	0	0	5	1	6
45 - 45.99	0	2	5	0	7
46 - 46.99	0	1	5	0	6
47 - 47.99	0	1	4	1	6
48 - 48.99	0	1	3	2	6
49 - 49.99	0	0	5	1	6
50 - 50.99	0	1	4	1	6
51 - 51.99	0	0	5	1	6
52 - 52.99	0	0	6	0	6
53 - 53.99	0	0	6	0	6
54 - 54.99	0	0	5	1	6
55 - 55.99	0	0	5	0	5
56 - 56.99	0	0	4	0	4
57 - 57.99	0	0	5	0	5
58 - 58.99	0	0	5	1	6
59 - 59.99	0	0	3	0	3
60 - 60.99	0	0	2	0	2
61 - 61.99	0	0	3	0	3
62 - 62.99	0	0	1	0	1
71 - 71.99	0	0	0	1	1
Totals	33	66	109	10	218

(Go back to text)

Table 3.3: 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 2020.

	Age			
Interval	0	1	2	3
20 - 20.99	1	0	0	0
22 - 22.99	0.67	0.33	0	0
23 - 23.99	0	1	0	0
24 - 24.99	0.4	0.6	0	0
25 - 25.99	1	0	0	0
26 - 26.99	0.67	0.33	0	0
27 - 27.99	0.6	0.4	0	0
28 - 28.99	0.5	0.5	0	0
29 - 29.99	0.17	0.67	0.17	0
30 - 30.99	0.5	0.5	0	0
31 - 31.99	0.67	0.33	0	0
32 - 32.99	0.4	0.6	0	0
33 - 33.99	0.33	0.67	0	0
34 - 34.99	0.17	0.5	0.33	0
35 - 35.99	0	1	0	0
36 - 36.99	0.12	0.75	0.12	0
37 - 37.99	0	1	0	0
38 - 38.99	0	0.67	0.33	0
39 - 39.99	0	0.71	0.29	0
40 - 40.99	0	0.6	0.4	0
41 - 41.99	0	0.33	0.67	0
42 - 42.99	0	0.25	0.75	0
43 - 43.99	0	0	1	0
44 - 44.99	0	0	0.83	0.17
45 - 45.99	0	0.29	0.71	0
46 - 46.99	0	0.17	0.83	0
47 - 47.99	0	0.17	0.67	0.17
48 - 48.99	0	0.17	0.5	0.33
49 - 49.99	0	0	0.83	0.17
50 - 50.99	0	0.17	0.67	0.17
51 - 51.99	0	0	0.83	0.17
52 - 52.99	0	0	1	0
53 - 53.99	0	0	1	0
54 - 54.99	0	0	0.83	0.17
55 - 55.99	0	0	1	0
56 - 56.99	0	0	1	0
57 - 57.99	0	0	1	0
58 - 58.99	0	0	0.83	0.17
59 - 59.99	0	0	1	0
60 - 60.99	0	0	1	0
61 - 61.99	0	0	1	0
62 - 62.99	0	0	1	0
71 - 71.99	0	0	0	1

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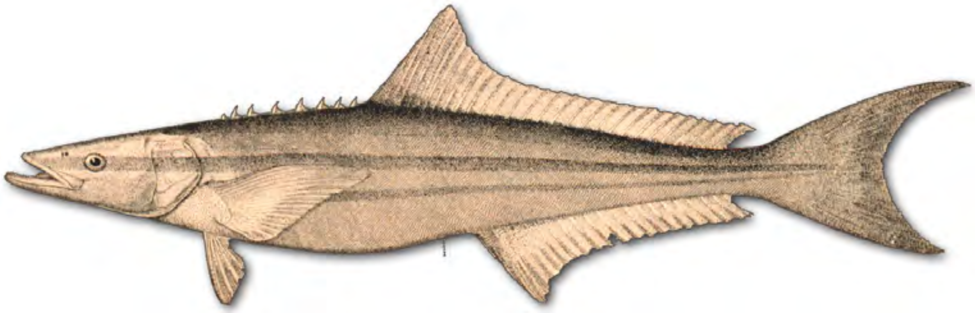
CHAPTER 4

COBIA *Rachycentron canadum*



4.1 INTRODUCTION

We aged a total of 390 Cobia, *Rachycentron canadum*, collected by the VMRC's Biological Sampling Program for age and growth analysis in 2020. Cobia ages ranged from 2 to 10 years old with an average age of 4.9, a standard deviation of 1.5, and a standard error of 0.08. Nine age classes (2 to 10) were represented, comprising fish of the 2010 to 2018 year-classes. The sample was dominated by fish from the year-classes of 2015 and 2016 with 30.8% and 38.5%, 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$^{\text{TM}}$ 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 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).

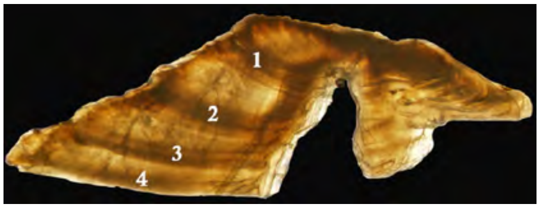


Figure 4.1: Otolith thin-section of a 4 year-old Cobia.

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, within the reader, for the following comparisons: 1) in the current year and 2) time-series bias between the current and previous years. The readings from the entire sample for the current year were used to examine the difference and precision in the current. A random sub-sample of 50 fish from the current year was selected for second readings of the reader. Fifty otoliths randomly selected from the fish aged in 2003 were used to examine the time-series bias within the reader. All statistics analyses were performed in R 3.6.1 (R Core Team 2019).

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 0.98% (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 100%. There was no evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 98.21% and a *CV* of 0.26% (test of symmetry: $\chi^2 = 7,\ df = 4,\ P = 0.1359$) (Figure 4.2).

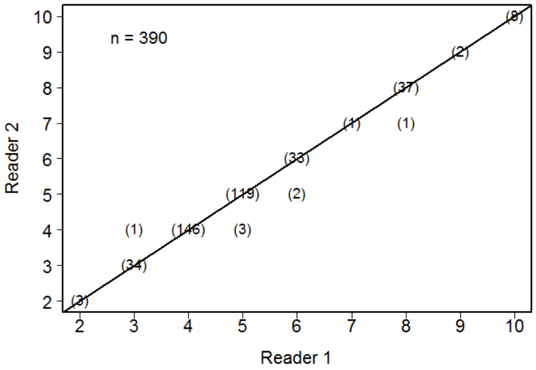


Figure 4.2: Between-reader comparison of otolith age estimates for Cobia collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2020.

There was no time-series bias for either reader. Reader 1 had an agreement of 90% with ages of fish aged in 2000 with a *CV* of 0.93% (test of symmetry: $\chi^2 = 5,\ df = 5,\ P = 0.4159$), and Reader 2 had an agreement of 88% with a *CV* of 0.88% (test of symmetry: $\chi^2 = 6,\ df = 4,\ P = 0.1991$).

4.3.2 Year class

Of the 390 fish aged with otoliths, 9 age classes (2 to 10) were represented (Table 4.1). The average age was 4.9 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 2010 to 2018 year-classes, with fish primarily from the year classes of 2015 and 2016 with 30.8% and 38.5%, respectively. The ratio of males to females was 1:1.19 in the sample collected (Figure 4.3).

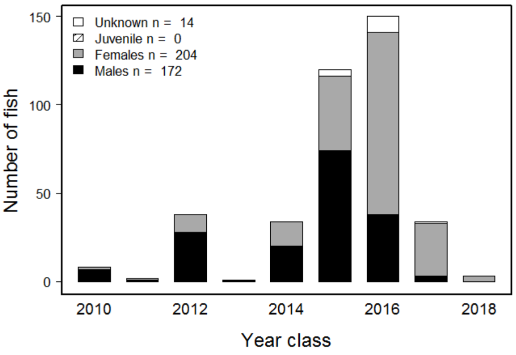


Figure 4.3: Year-class frequency distribution for Cobia collected for ageing in 2020. Distribution is broken down by sex. 'Unknown' represents gonads that were not available for examination or were not examined for sex during sampling.

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 390 fish sampled for otolith age determination in Virginia during 2020.

				Age						
Interval	2	3	4	5	6	7	8	9	10	Totals
36 - 36.99	1	0	1	0	0	0	0	0	0	2
37 - 37.99	1	4	10	0	0	0	0	0	0	15
38 - 38.99	1	7	11	4	2	0	0	0	0	25
39 - 39.99	0	7	18	13	3	0	1	0	0	42
40 - 40.99	0	7	19	17	2	0	0	0	0	45
41 - 41.99	0	5	21	19	6	0	2	0	0	53
42 - 42.99	0	1	25	14	0	0	6	0	0	46
43 - 43.99	0	0	12	8	1	0	6	0	1	28
44 - 44.99	0	1	17	4	6	0	5	0	0	33
45 - 45.99	0	2	9	4	2	1	6	0	1	25
46 - 46.99	0	0	3	5	0	0	3	0	2	13
47 - 47.99	0	0	4	7	1	0	0	0	1	13
48 - 48.99	0	0	0	8	3	0	1	0	2	14
49 - 49.99	0	0	0	6	1	0	0	1	0	8
50 - 50.99	0	0	0	5	0	0	2	0	0	7
51 - 51.99	0	0	0	3	3	0	1	1	0	8
52 - 52.99	0	0	0	1	2	0	1	0	0	4
53 - 53.99	0	0	0	2	0	0	1	0	0	3
55 - 55.99	0	0	0	0	1	0	1	0	0	2
56 - 56.99	0	0	0	0	1	0	0	0	0	1
57 - 57.99	0	0	0	0	0	0	1	0	0	1
58 - 58.99	0	0	0	0	0	0	1	0	0	1
60 - 60.99	0	0	0	0	0	0	0	0	1	1
Totals	3	34	150	120	34	1	38	2	8	390

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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 2020.

	Age								
Interval	2	3	4	5	6	7	8	9	10
36 - 36.99	0.5	0	0.5	0	0	0	0	0	0
37 - 37.99	0.07	0.27	0.67	0	0	0	0	0	0
38 - 38.99	0.04	0.28	0.44	0.16	0.08	0	0	0	0
39 - 39.99	0	0.17	0.43	0.31	0.07	0	0.02	0	0
40 - 40.99	0	0.16	0.42	0.38	0.04	0	0	0	0
41 - 41.99	0	0.09	0.4	0.36	0.11	0	0.04	0	0
42 - 42.99	0	0.02	0.54	0.3	0	0	0.13	0	0
43 - 43.99	0	0	0.43	0.29	0.04	0	0.21	0	0.04
44 - 44.99	0	0.03	0.52	0.12	0.18	0	0.15	0	0
45 - 45.99	0	0.08	0.36	0.16	0.08	0.04	0.24	0	0.04
46 - 46.99	0	0	0.23	0.38	0	0	0.23	0	0.15
47 - 47.99	0	0	0.31	0.54	0.08	0	0	0	0.08
48 - 48.99	0	0	0	0.57	0.21	0	0.07	0	0.14
49 - 49.99	0	0	0	0.75	0.12	0	0	0.12	0
50 - 50.99	0	0	0	0.71	0	0	0.29	0	0
51 - 51.99	0	0	0	0.38	0.38	0	0.12	0.12	0
52 - 52.99	0	0	0	0.25	0.5	0	0.25	0	0
53 - 53.99	0	0	0	0.67	0	0	0.33	0	0
55 - 55.99	0	0	0	0	0.5	0	0.5	0	0
56 - 56.99	0	0	0	0	1	0	0	0	0
57 - 57.99	0	0	0	0	0	0	1	0	0
58 - 58.99	0	0	0	0	0	0	1	0	0
60 - 60.99	0	0	0	0	0	0	0	0	1

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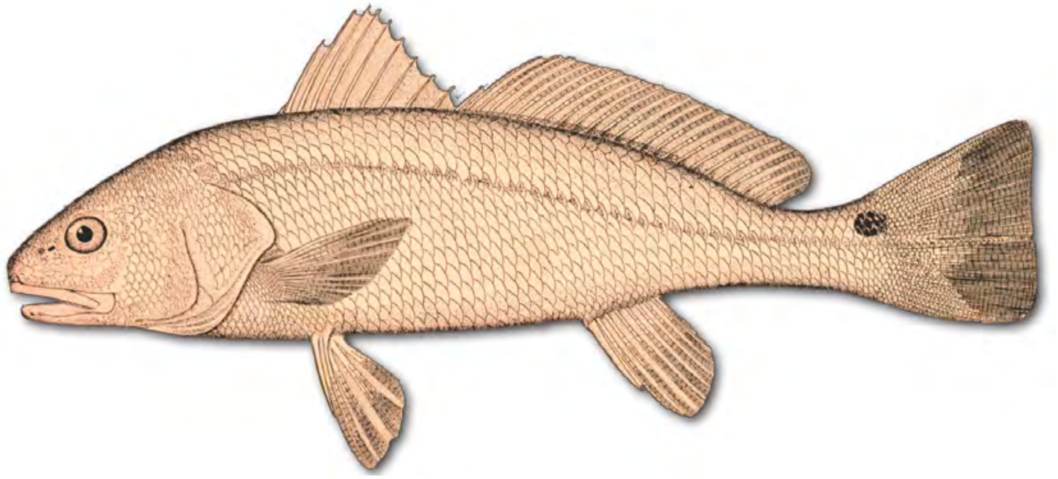
CHAPTER 5

RED DRUM *Sciaenops ocellatus*



5.1 INTRODUCTION

We aged a total of 62 Red Drum, *Sciaenops ocellatus*, collected by the VMRC's Biological Sampling Program for age and growth analysis in 2020. Red Drum ages ranged from 1 to 22 years old with an average age of 1.4, a standard deviation of 2.7, and a standard error of 0.34. Three age classes (1 to 2, and 22) were represented, comprising fish of the 1998, and 2018 to 2019 year-classes. The sample was dominated by fish from the year-class of 2019 with 93.6%.

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 modified by 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$^{\text{TM}}$ 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$^{\text{TM}}$ 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 2020 (Figure 5.1).

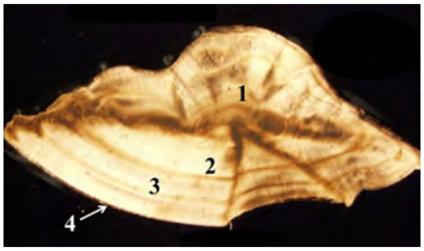


Figure 5.1: Otolith thin-section of a 3 year-old Red Drum with the last annulus on the edge of the thin-section

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).

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, within the reader, for the following comparisons: 1) in the current year and 2) time-series bias between the current and previous years. The readings from the entire sample for the current year were used to examine the difference and precision in the current. A random sub-sample of 50 fish from the current year was selected for second readings of the reader. Fifty otoliths randomly selected from the fish aged in 2003 were used to examine the time-series bias within the reader. All statistics analyses were performed in R 3.6.1 (R Core Team 2019).

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 96% and a *CV* of 1.51% (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 2.83% (test of symmetry: $\chi^2 = 0.33$, $df = 1$, $P = 0.5637$). There was evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 91.94% and a *CV* of 3.8% (test of symmetry: $\chi^2 = 5$, $df = 1$, $P = 0.0253$) (Figure 5.2).

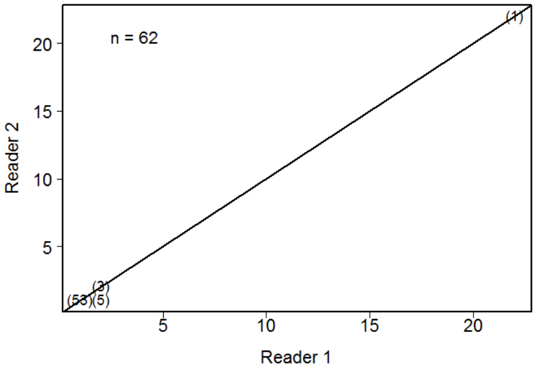


Figure 5.2: Between-reader comparison of otolith age estimates for Red Drum collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2020.

There was no time-series bias for either reader. Reader 1 had an agreement of 96% with ages of fish aged in 2000 with a *CV* of 1.51% (test of symmetry: $\chi^2 = 2$, $df = 2$, $P = 0.3679$), and Reader 2 had an agreement of 100%.

5.3.2 Year class

Of the 62 fish aged with otoliths, 3 age classes (1 to 2, and 22) were represented (Table 5.1). The average age was 1.4 years, and the standard deviation and standard error were 2.7 and 0.34, respectively. Year-class data show that the fishery was comprised of 3 year-classes: fish from the 1998, and 2018 to 2019 year-classes, with fish primarily from the year class of 2019 with 93.6%. The ratio of males to females was 1:0.32 in the sample collected (Figure 5.3).

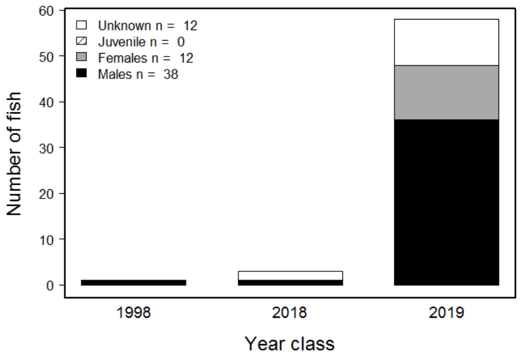


Figure 5.3: Year-class frequency distribution for Red Drum collected for ageing in 2020. Distribution is broken down by sex. 'Unknown' represents gonads that were not available for examination or were not examined for sex during sampling.

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 62 fish sampled for otolith age determination in Virginia during 2020.

	Age			
Interval	1	2	22	Totals
17 - 17.99	2	0	0	2
18 - 18.99	3	1	0	4
19 - 19.99	5	0	0	5
20 - 20.99	4	1	0	5
21 - 21.99	2	0	0	2
22 - 22.99	9	1	0	10
23 - 23.99	10	0	0	10
24 - 24.99	13	0	0	13
25 - 25.99	4	0	0	4
26 - 26.99	4	0	0	4
27 - 27.99	2	0	0	2
48 - 48.99	0	0	1	1
Totals	58	3	1	62

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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 2020.

	Age		
Interval	1	2	22
17 - 17.99	1	0	0
18 - 18.99	0.75	0.25	0
19 - 19.99	1	0	0
20 - 20.99	0.8	0.2	0
21 - 21.99	1	0	0
22 - 22.99	0.9	0.1	0
23 - 23.99	1	0	0
24 - 24.99	1	0	0
25 - 25.99	1	0	0
26 - 26.99	1	0	0
27 - 27.99	1	0	0
48 - 48.99	0	0	1

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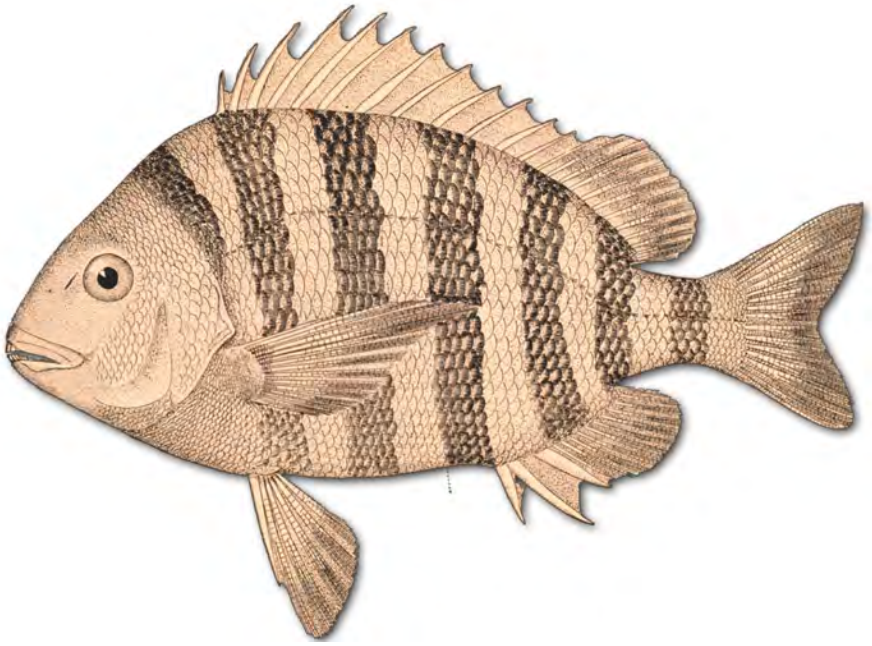
CHAPTER 6

SHEEPSHEAD *Archosargus probatocephalus*



6.1 INTRODUCTION

We aged a total of 43 Sheepshead, *Archosargus probatocephalus*, collected by the VMRC's Biological Sampling Program for age and growth analysis in 2020. Sheepshead ages ranged from 1 to 23 years old with an average age of 3.4, a standard deviation of 5.2, and a standard error of 0.79. Seven age classes (1 to 2, 4 to 5, 9, 13, and 23) were represented, comprising fish of the 1997, 2007, 2011, 2015 to 2016, and 2018 to 2019 year-classes. The sample was dominated by fish from the year-class of 2019 with 62.8%.

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, within the reader, for the following comparisons: 1) in the current year and 2) time-series bias between the current and previous years. The readings from the entire sample for the current year were used to examine the difference and precision in the current. When the sample size for the current year was smaller than 50, the entire sample was read by the reader for the second time to examine the difference within the reader. Fifty otoliths randomly selected from the fish aged in 2003 were used to examine the time-series bias within the reader. All statistics analyses were performed in R 3.6.1 (R Core Team 2019).

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 86% and a *CV* of 2.94% (test of symmetry: $\chi^2 = 4$, $df = 5$, $P = 0.5494$), 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 86.05% and a *CV* of 2.94% (test of symmetry: $\chi^2 = 6$, $df = 5$, $P = 0.3062$) (Figure 6.2).

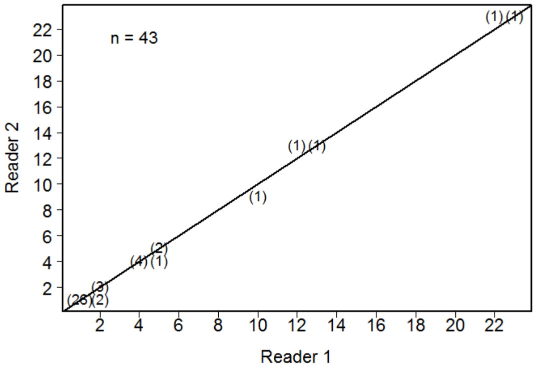


Figure 6.2: Between-reader comparison of otolith age estimates for Sheepshead collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2020.

There was no time-series bias for either reader. Both Reader 1 and Reader 2 had an agreement of 100% with ages of fish aged in 2008.

6.3.2 Year class

Of the 43 fish aged with otoliths, 7 age classes (1 to 2, 4 to 5, 9, 13, and 23) were represented (Table 6.1). The average age was 3.4 years, and the standard deviation and standard error were 5.2 and 0.79, respectively. Year-class data show that the fishery was comprised of 7 year-classes: fish from the 1997, 2007, 2011, 2015 to 2016, and 2018 to 2019 year-classes, with fish primarily from the year class of 2019 with 62.8%. The ratio of males to females was 1:1.22 in the sample collected (Figure 6.3).

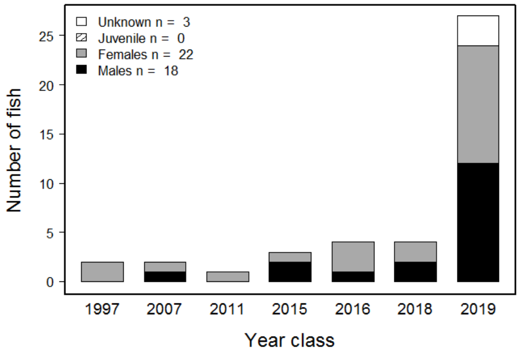


Figure 6.3: Year-class frequency distribution for Sheepshead collected for ageing in 2020. Distribution is broken down by sex. 'Unknown' represents gonads that were not available for examination or were not examined for sex during sampling.

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 43 fish sampled for otolith age determination in Virginia during 2020.

	Age							
Interval	1	2	4	5	9	13	23	Totals
8 - 8.99	1	0	0	0	0	0	0	1
9 - 9.99	6	0	0	0	0	0	0	6
10 - 10.99	10	1	0	0	0	0	0	11
11 - 11.99	8	0	0	0	0	0	0	8
12 - 12.99	1	0	0	0	0	0	0	1
13 - 13.99	1	1	0	0	0	0	0	2
14 - 14.99	0	1	0	0	0	0	0	1
15 - 15.99	0	1	0	0	0	0	0	1
16 - 16.99	0	0	1	0	0	0	0	1
17 - 17.99	0	0	2	2	0	0	0	4
18 - 18.99	0	0	0	1	0	0	0	1
19 - 19.99	0	0	1	0	0	1	0	2
21 - 21.99	0	0	0	0	1	0	0	1
24 - 24.99	0	0	0	0	0	1	0	1
25 - 25.99	0	0	0	0	0	0	2	2
Totals	27	4	4	3	1	2	2	43

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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 2020.

	Age						
Interval	1	2	4	5	9	13	23
8 - 8.99	1	0	0	0	0	0	0
9 - 9.99	1	0	0	0	0	0	0
10 - 10.99	0.91	0.09	0	0	0	0	0
11 - 11.99	1	0	0	0	0	0	0
12 - 12.99	1	0	0	0	0	0	0
13 - 13.99	0.5	0.5	0	0	0	0	0
14 - 14.99	0	1	0	0	0	0	0
15 - 15.99	0	1	0	0	0	0	0
16 - 16.99	0	0	1	0	0	0	0
17 - 17.99	0	0	0.5	0.5	0	0	0
18 - 18.99	0	0	0	1	0	0	0
19 - 19.99	0	0	0.5	0	0	0.5	0
21 - 21.99	0	0	0	0	1	0	0
24 - 24.99	0	0	0	0	0	1	0
25 - 25.99	0	0	0	0	0	0	1

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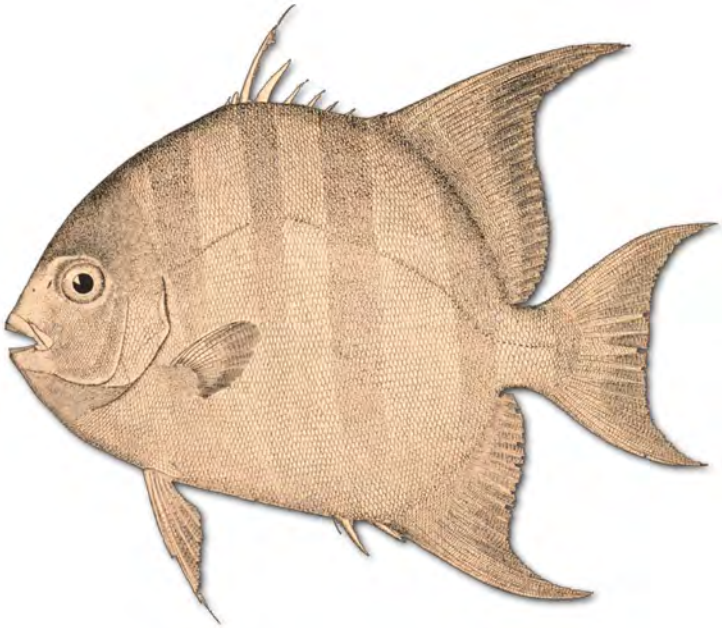
CHAPTER 7

ATLANTIC SPADEFISH
Chaetodipterus faber



7.1 INTRODUCTION

We aged a total of 190 Spadefish, *Chaetodipterus faber*, collected by the VMRC's Biological Sampling Program for age and growth analysis in 2020. Spadefish ages ranged from 0 to 8 years old with an average age of 3.8, a standard deviation of 1.4, and a standard error of 0.1. Eight age classes (0 to 6, and 8) were represented, comprising fish of the 2012, and 2014 to 2020 year-classes. The sample was dominated by fish from the year-classes of 2015 and 2016 with 31.6% and 36.3%, respectively.

7.2 METHODS

7.2.1 Sample size for ageing

We estimated sample size for ageing Spadefish in 2020 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:

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

where A is the sample size for ageing Spadefish in 2020; θ_a stands for the proportion of age a fish in a catch. V_a and B_a represent variance components within and between length intervals for age a, respectively; CV is the coefficient of variation; L was the total number of Spadefish used by VMRC to estimate length distribution of the catches from 2014 to 2018. θ_a, V_a, B_a, and CV were calculated using pooled age-length data of Spadefish collected from 2014 to 2018 and using equations in Quinn and Deriso (1999). For simplicity, the equations are not listed here. The equation (7.1) indicates that the more fish that are aged, the smaller the CV (or higher precision) that will be obtained. Therefore, the criterion to age A (number) of fish is that A should be a number above which there is only a 1% CV reduction for the most major age in catch by aging 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 fish aged in the lab between 2014 and 2018. A_l is number of fish to be aged for length interval l in 2020.

7.2.2 Handling of collections

Otoliths were received by the Age and Growth Laboratory in labeled coin envelopes, and were sorted by date of capture. Their envelope labels were verified against VMRC's collection data, and each fish was assigned a unique Age and Growth Laboratory identification number. All otoliths were stored 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$^{\mathrm{TM}}$ 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).

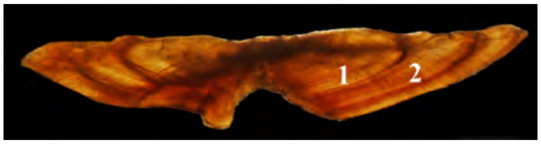


Figure 7.1: Otolith thin-section of a 2 year-old Spadefish

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, within the reader, for the following comparisons: 1) in the current year and 2) time-series bias between the current and previous years. The readings from the entire sample for the current year were used to examine the difference and precision in the current. A random sub-sample of 50 fish from the current year was selected for second readings of the reader. Fifty otoliths randomly selected from the fish aged in 2003 were used to examine the time-series bias within the reader. All statistics analyses were performed in R 3.6.1 (R Core Team 2019).

7.3 RESULTS

7.3.1 Sample size

We estimated a sample size of 346 Spadefish in 2020, ranging in length intervals 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 0.06% for Age 2 to the largest (*CV*) of 0.19% for Age 6. In 2020, we aged 190 of 207 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 173 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.

7.3.2 Reading precision

Reader 1 had moderate self-precision and Read 2 had high self-precision. Specifically, there was no significant difference between the first and second readings for Reader 1 with an agreement of 80% and a *CV* of 6.29% (test of symmetry: $\chi^2 = 7.33$, $df = 5$, $P = 0.197$), and there was no significant difference between the first and second readings for Reader 2 with an agreement of 92% and a *CV* of 1.69% (test of symmetry: $\chi^2 = 2$, $df = 3$, $P = 0.5724$). There was no evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 90.53% and a *CV* of 2.1% (test of symmetry: $\chi^2 = 3.33$, $df = 6$, $P = 0.766$) (Figure 7.2).

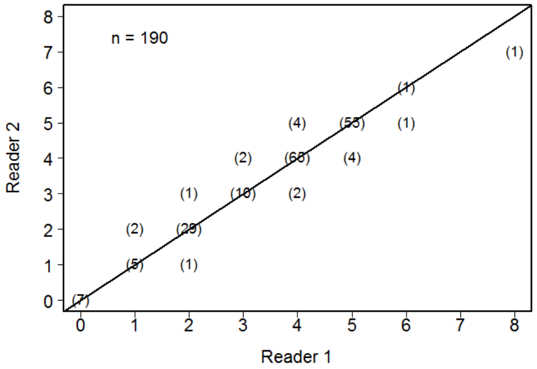


Figure 7.2: Between-reader comparison of otolith age estimates for Spadefish collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2020.

There was no time-series bias for either reader. Reader 1 had an agreement of 86% with ages of fish aged in 2003 with a *CV* of 1.89% (test of symmetry: $\chi^2 = 7$, $df = 7$, $P = 0.4289$), and Reader 2 had an agreement of 92% with a *CV* of 1.3% (test of symmetry: $\chi^2 = 4$, $df = 4$, $P = 0.406$).

7.3.3 Year class

Of the 190 fish aged with otoliths, 8 age classes (0 to 6, and 8) were represented (Table 7.2). The average age was 3.8 years, and the standard deviation and standard error were 1.4 and 0.1, respectively. Year-class data show that the fishery was comprised of 8 year-classes: fish from the 2012, and 2014 to 2020 year-classes, with fish primarily from the year classes of 2015 and 2016 with 31.6% and 36.3%, respectively. The ratio of males to females was 1:1.23 in the sample collected (Figure 7.3).

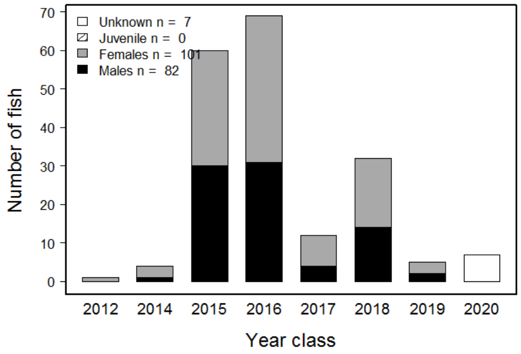


Figure 7.3: Year-class frequency distribution for Spadefish collected for ageing in 2020. Distribution is broken down by sex. 'Unknown' represents gonads that were not available for examination or were not examined for sex during sampling.

7.3.4 Age-length-key (ALK)

We developed an age-length-key (Table 7.3) 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 2020. 'Target' represents the sample size for ageing estimated for 2020, 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	2	2	3
4 - 4.99	6	5	5	1
5 - 5.99	12	7	7	5
6 - 6.99	43	8	8	35
7 - 7.99	48	12	12	36
8 - 8.99	34	10	10	24
9 - 9.99	24	3	3	21
10 - 10.99	17	4	4	13
11 - 11.99	18	7	7	11
12 - 12.99	23	19	19	4
13 - 13.99	17	27	27	0
14 - 14.99	17	28	18	0
15 - 15.99	15	23	16	0
16 - 16.99	14	16	16	0
17 - 17.99	19	22	22	0
18 - 18.99	12	8	8	4
19 - 19.99	7	5	5	2
20 - 20.99	5	0	0	5
21 - 21.99	5	1	1	4
22 - 22.99	5	0	0	5
Totals	346	207	190	173

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Table 7.2: The number of Atlantic Spadefish assigned to each total length-at-age category for 190 fish sampled for otolith age determination in Virginia during 2020.

	Age								
Interval	0	1	2	3	4	5	6	8	Totals
3 - 3.99	2	0	0	0	0	0	0	0	2
4 - 4.99	5	0	0	0	0	0	0	0	5
5 - 5.99	0	4	3	0	0	0	0	0	7
6 - 6.99	0	1	7	0	0	0	0	0	8
7 - 7.99	0	0	12	0	0	0	0	0	12
8 - 8.99	0	0	8	1	1	0	0	0	10
9 - 9.99	0	0	2	1	0	0	0	0	3
10 - 10.99	0	0	0	1	2	1	0	0	4
11 - 11.99	0	0	0	3	3	0	1	0	7
12 - 12.99	0	0	0	2	14	3	0	0	19
13 - 13.99	0	0	0	2	18	7	0	0	27
14 - 14.99	0	0	0	0	12	5	1	0	18
15 - 15.99	0	0	0	2	7	6	1	0	16
16 - 16.99	0	0	0	0	3	12	1	0	16
17 - 17.99	0	0	0	0	5	17	0	0	22
18 - 18.99	0	0	0	0	2	6	0	0	8
19 - 19.99	0	0	0	0	2	3	0	0	5
21 - 21.99	0	0	0	0	0	0	0	1	1
Totals	7	5	32	12	69	60	4	1	190

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Table 7.3: 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 2020.

	Age							
Interval	0	1	2	3	4	5	6	8
3 - 3.99	1	0	0	0	0	0	0	0
4 - 4.99	1	0	0	0	0	0	0	0
5 - 5.99	0	0.57	0.43	0	0	0	0	0
6 - 6.99	0	0.12	0.88	0	0	0	0	0
7 - 7.99	0	0	1	0	0	0	0	0
8 - 8.99	0	0	0.8	0.1	0.1	0	0	0
9 - 9.99	0	0	0.67	0.33	0	0	0	0
10 - 10.99	0	0	0	0.25	0.5	0.25	0	0
11 - 11.99	0	0	0	0.43	0.43	0	0.14	0
12 - 12.99	0	0	0	0.11	0.74	0.16	0	0
13 - 13.99	0	0	0	0.07	0.67	0.26	0	0
14 - 14.99	0	0	0	0	0.67	0.28	0.06	0
15 - 15.99	0	0	0	0.12	0.44	0.38	0.06	0
16 - 16.99	0	0	0	0	0.19	0.75	0.06	0
17 - 17.99	0	0	0	0	0.23	0.77	0	0
18 - 18.99	0	0	0	0	0.25	0.75	0	0
19 - 19.99	0	0	0	0	0.4	0.6	0	0
21 - 21.99	0	0	0	0	0	0	0	1

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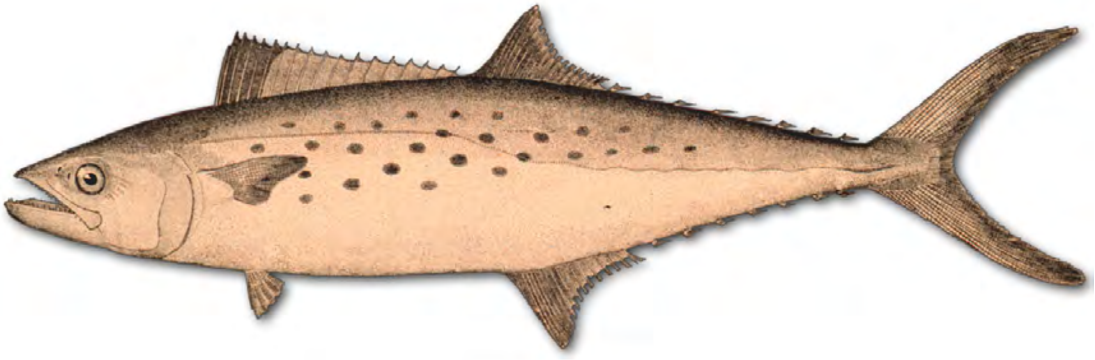
CHAPTER 8

SPANISH MACKEREL
Scomberomorous maculatus



8.1 INTRODUCTION

We aged a total of 200 Spanish Mackerel, *Scomberomorous maculatus*, collected by the VMRC's Biological Sampling Program for age and growth analysis in 2020. Spanish Mackerel ages ranged from 0 to 6 years old with an average age of 1.8, a standard deviation of 1, and a standard error of 0.07. Seven age classes (0 to 6) were represented, comprising fish of the 2014 to 2020 year-classes. The sample was dominated by fish from the year-classes of 2018 and 2019 with 49.5% and 32.5%, respectively.

8.2 METHODS

8.2.1 Sample size for ageing

We estimated sample size for ageing Spanish Mackerel in 2020 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:

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

where A is the sample size for ageing Spanish Mackerel in 2020; θ_a stands for the proportion of age a fish in a catch. V_a and B_a represent variance components within and between length intervals for age a, respectively; CV is the coefficient of variation; L was the total number of Spanish Mackerel used by VMRC to estimate length distribution of the catches from 2014 to 2018. θ_a, V_a, B_a, and CV were calculated using pooled age-length data of Spanish Mackerel collected from 2014 to 2018 and using equations in Quinn and Deriso (1999). For simplicity, the equations are not listed here. The equation (8.1) indicates that the more fish that are aged, the smaller the CV (or higher precision) that will be obtained. Therefore, the criterion to age A (number) of fish is that A should be a number above which there is only a 1% CV reduction for the most major age in catch by aging 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 fish aged in the lab between 2014 and 2018. A_l is number of fish to be aged for length interval l in 2020.

8.2.2 Handling of collections

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

8.2.3 Preparation

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$^{\mathrm{TM}}$ 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 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).

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

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, within the reader, for the following comparisons: 1) in the current year and 2) time-series bias between the current and previous years. The readings from the entire sample for the current year were used to examine the difference and precision in the current. A random sub-sample of 50 fish from the current year was selected for second readings of the reader. Fifty otoliths randomly selected from the fish aged in 2003 were used to examine the time-series bias within the reader. All statistics analyses were performed in R 3.6.1 (R Core Team 2019).

8.3 RESULTS

8.3.1 Sample size

We estimated a sample size of 226 Spanish Mackerel in 2020, ranging in length intervals from 12 to 32 inches (Table 8.1). This sample size provided a range in (CV) for age composition approximately from the smallest (CV) of 0.04% for Age 1 to the largest (CV) of 0.17% for Age 3. In 2020, we randomly selected and aged 200 fish from 262 Spanish Mackerel (The rest of fish were either without otoliths or over-collected for certain length interval(s)) collected by VMRC. We fell short in our over-all collections for this optimal length-class sampling estimate by 42 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.

8.3.2 Reading precision

Reader 1 had moderate self-precision and Read 2 had high self-precision. Specifically, there was no significant difference between the first and second readings for Reader 1 with an agreement of 82% and a CV of 7.83% (test of symmetry: $\chi^2 = 5.8$, $df = 3$, $P = 0.1218$), and there was no significant difference between the first and second readings for Reader 2 with an agreement of 94% and a CV of 1.82% (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 89% and a CV of 4.88% (test of symmetry: $\chi^2 = 7.27$, $df = 7$, $P = 0.401$) (Figure 8.2).

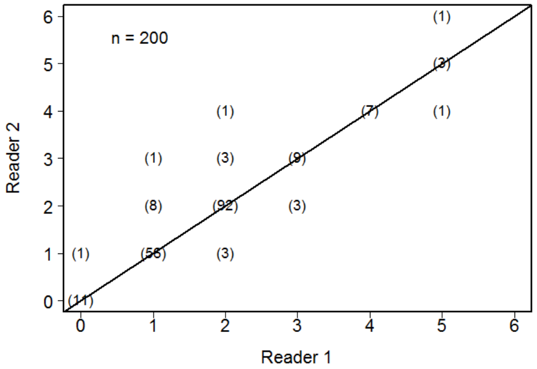


Figure 8.2: Between-reader comparison of otolith age estimates for Spanish Mackerel collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2020.

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 92% with a CV of 7.54% (test of symmetry: $\chi^2 = 1.33$, $df = 2$, $P = 0.5134$).

8.3.3 Year class

Of the 200 fish aged with otoliths, 7 age classes (0 to 6) were represented (Table 8.2). The average age was 1.8 years, and the standard deviation and standard error were 1 and 0.07, respectively. Year-class data show that the fishery was comprised of 7 year-classes: fish from the 2014 to 2020 year-classes, with fish primarily from the year classes of 2018 and 2019 with 49.5% and 32.5%, respectively. The ratio of males to females was 1:2.33 in the sample collected (Figure 8.3).

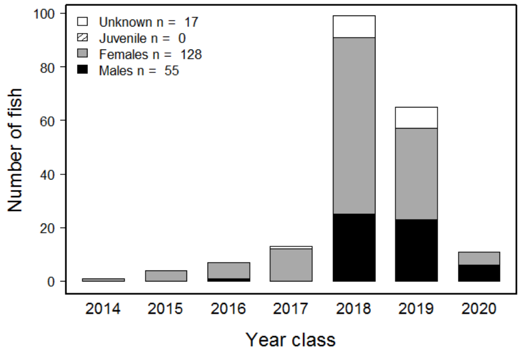


Figure 8.3: Year-class frequency distribution for Spanish Mackerel collected for ageing in 2020. Distribution is broken down by sex. 'Unknown' represents gonads that were not available for examination or were not examined for sex during sampling.

8.3.4 Age-length-key (ALK)

We developed an age-length-key (Table 8.3) 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 2020. 'Target' represents the sample size for ageing estimated for 2020, 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	16	14	14	2
15 - 15.99	29	35	35	0
16 - 16.99	33	57	34	0
17 - 17.99	29	45	30	0
18 - 18.99	17	25	18	0
19 - 19.99	14	17	14	0
20 - 20.99	11	22	12	0
21 - 21.99	11	14	12	0
22 - 22.99	6	8	6	0
23 - 23.99	5	8	8	0
24 - 24.99	5	7	7	0
25 - 25.99	5	3	3	2
26 - 26.99	5	2	2	3
27 - 27.99	5	1	1	4
28 - 28.99	5	1	1	4
29 - 29.99	5	1	1	4
30 - 30.99	5	1	1	4
31 - 31.99	5	0	0	5
32 - 32.99	5	0	0	5
Totals	226	262	200	42

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Table 8.2: The number of Spanish Mackerel assigned to each total length-at-age category for 200 fish sampled for otolith age determination in Virginia during 2020.

	Age							
Interval	0	1	2	3	4	5	6	Totals
13 - 13.99	1	0	0	0	0	0	0	1
14 - 14.99	6	8	0	0	0	0	0	14
15 - 15.99	4	22	9	0	0	0	0	35
16 - 16.99	0	22	12	0	0	0	0	34
17 - 17.99	0	12	18	0	0	0	0	30
18 - 18.99	0	1	17	0	0	0	0	18
19 - 19.99	0	0	14	0	0	0	0	14
20 - 20.99	0	0	11	1	0	0	0	12
21 - 21.99	0	0	7	4	1	0	0	12
22 - 22.99	0	0	3	3	0	0	0	6
23 - 23.99	0	0	4	3	1	0	0	8
24 - 24.99	0	0	3	2	2	0	0	7
25 - 25.99	0	0	0	0	2	1	0	3
26 - 26.99	0	0	1	0	1	0	0	2
27 - 27.99	0	0	0	0	0	1	0	1
28 - 28.99	0	0	0	0	0	0	1	1
29 - 29.99	0	0	0	0	0	1	0	1
30 - 30.99	0	0	0	0	0	1	0	1
Totals	11	65	99	13	7	4	1	200

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Table 8.3: 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 2020.

	Age						
Interval	0	1	2	3	4	5	6
13 - 13.99	1	0	0	0	0	0	0
14 - 14.99	0.43	0.57	0	0	0	0	0
15 - 15.99	0.11	0.63	0.26	0	0	0	0
16 - 16.99	0	0.65	0.35	0	0	0	0
17 - 17.99	0	0.4	0.6	0	0	0	0
18 - 18.99	0	0.06	0.94	0	0	0	0
19 - 19.99	0	0	1	0	0	0	0
20 - 20.99	0	0	0.92	0.08	0	0	0
21 - 21.99	0	0	0.58	0.33	0.08	0	0
22 - 22.99	0	0	0.5	0.5	0	0	0
23 - 23.99	0	0	0.5	0.38	0.12	0	0
24 - 24.99	0	0	0.43	0.29	0.29	0	0
25 - 25.99	0	0	0	0	0.67	0.33	0
26 - 26.99	0	0	0.5	0	0.5	0	0
27 - 27.99	0	0	0	0	0	1	0
28 - 28.99	0	0	0	0	0	0	1
29 - 29.99	0	0	0	0	0	1	0
30 - 30.99	0	0	0	0	0	1	0

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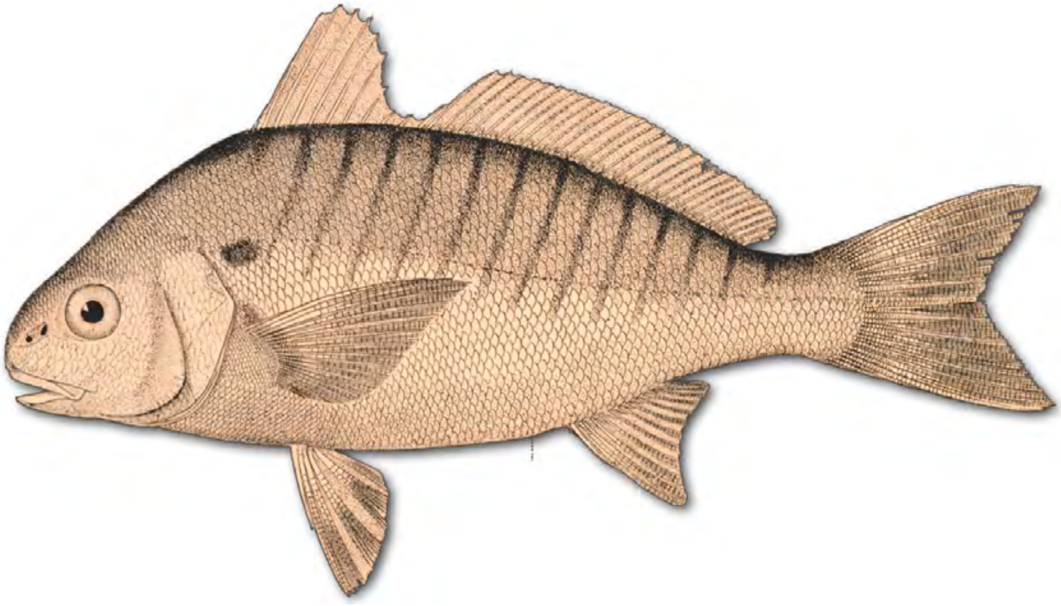
CHAPTER 9

SPOT *Leiostomus xanthurus*



9.1 INTRODUCTION

We aged a total of 203 Spot, *Leiostomus xanthurus*, collected by the VMRC's Biological Sampling Program for age and growth analysis in 2020. Spot ages ranged from 0 to 3 years old with an average age of 1, a standard deviation of 0.3, and a standard error of 0.02. Four age classes (0 to 3) were represented, comprising fish of the 2017 to 2020 year-classes. The sample was dominated by fish from the year-class of 2019 with 89.7%.

9.2 METHODS

9.2.1 Sample size for ageing

We estimated sample size for ageing Spot in 2020 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:

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

where A is the sample size for ageing Spot in 2020; θ_a stands for the proportion of age a fish in a catch. V_a and B_a represent variance components within and between length intervals for age a, respectively; CV is the coefficient of variation; L was the total number of Spot used by VMRC to estimate length distribution of the catches from 2014 to 2018. θ_a, V_a, B_a, and CV were calculated using pooled age-length data of Spot collected from 2014 to 2018 and using equations in Quinn and Deriso (1999). For simplicity, the equations are not listed here. The equation (1.1) indicates that the more fish that are aged, the smaller the CV (or higher precision) that will be obtained. Therefore, the criterion to age A (number) of fish is that A should be a number above which there is only a 1% CV reduction for the most major 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 fish aged in the lab between 2014 and 2018. A_l is number of fish to be aged for length interval l in 2020.

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$^{\text{TM}}$ 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).

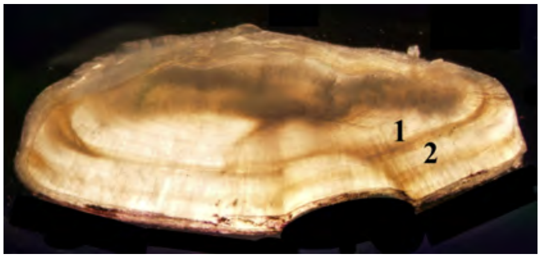


Figure 9.1: Otolith thin-section of a 2 year-old Spot

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, within the reader, for the following comparisons: 1) in the current year and 2) time-series bias between the current and previous years. The readings from the entire sample for the current year were used to examine the difference and precision in the current. A random sub-sample of 50 fish from the current year was selected for second readings of the reader. Fifty otoliths randomly selected from the fish aged in 2003 were used to examine the time-series bias within the reader. All statistics analyses were performed in R 3.6.1 (R Core Team 2019).

9.3 RESULTS

9.3.1 Sample size

We estimated a sample size of 220 Spot in 2020, ranging in length intervals from 4 to 12 inches (Table 9.1). This sample size provided a range in (*CV*) for age composition approximately from the smallest (*CV*) of 0.05% for Age 1 to the largest (*CV*) of 0.2% for Age 0. In 2020, we randomly selected and aged 203 fish from 238 Spot (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 36 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.

9.3.2 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 0.94% (test of symmetry: χ^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 98.52% and a *CV* of 0.7% (test of symmetry: χ^2 = 0.33, *df* = 1, *P* = 0.5637) (Figure 9.2).

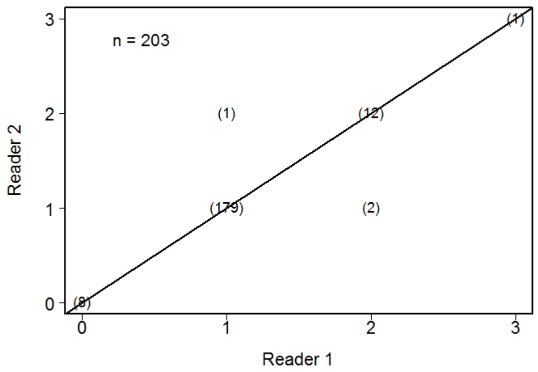


Figure 9.2: Between-reader comparison of otolith age estimates for Spot collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2020.

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 0.94% (test of symmetry: χ^2 = 1, *df* = 1, *P* = 0.3173), and Reader 2 had an agreement of 100%.

9.3.3 Year class

Of the 203 fish aged with otoliths, 4 age classes (0 to 3) were represented (Table 9.2). The average age was 1 years, and the standard deviation and standard error were 0.3 and 0.02, respectively. Year-class data show that the fishery was comprised of 4 year-classes: fish from the 2017 to 2020 year-classes, with fish primarily from the year class of 2019 with 89.7%. The ratio of males to females was 1:12.77 in the sample collected (Figure 9.3).

9.3.4 Age-length-key (ALK)

We developed an age-length-key (Table 9.3) that can be used in the conversion of numbers-at-length in the estimated catch to numbers-at-age using otolith ages. The table is based on VMRC's stratified sampling of landings by total length inch intervals.

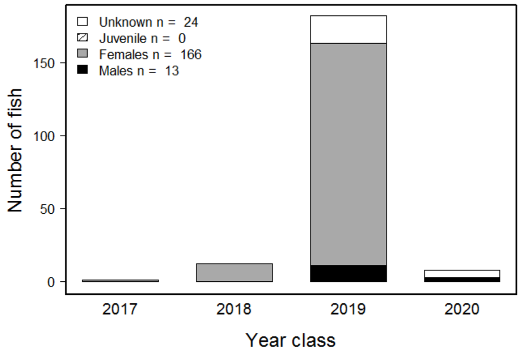


Figure 9.3: Year-class frequency distribution for Spot collected for ageing in 2020. Distribution is broken down by sex. 'Unknown' is for gonads that were not available for examination or were not examined for sex during sampling.

Table 9.1: Number of Spot collected and aged in each 1-inch length interval in 2020. 'Target' represents the sample size for ageing estimated for 2020, 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	2	2	3
5 - 5.99	6	6	6	0
6 - 6.99	6	22	9	0
7 - 7.99	25	37	26	0
8 - 8.99	47	59	48	0
9 - 9.99	66	80	80	0
10 - 10.99	51	32	32	19
11 - 11.99	9	0	0	9
12 - 12.99	5	0	0	5
Totals	220	238	203	36

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Table 9.2: The number of Spot assigned to each total length-at-age category for 203 fish sampled for otolith age determination in Virginia during 2020.

	Age				
Interval	0	1	2	3	Totals
4 - 4.99	2	0	0	0	2
5 - 5.99	6	0	0	0	6
6 - 6.99	0	9	0	0	9
7 - 7.99	0	24	2	0	26
8 - 8.99	0	45	3	0	48
9 - 9.99	0	74	6	0	80
10 - 10.99	0	30	1	1	32
Totals	8	182	12	1	203

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Table 9.3: 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 2020.

	Age			
Interval	0	1	2	3
4 - 4.99	1	0	0	0
5 - 5.99	1	0	0	0
6 - 6.99	0	1	0	0
7 - 7.99	0	0.92	0.08	0
8 - 8.99	0	0.94	0.06	0
9 - 9.99	0	0.92	0.07	0
10 - 10.99	0	0.94	0.03	0.03

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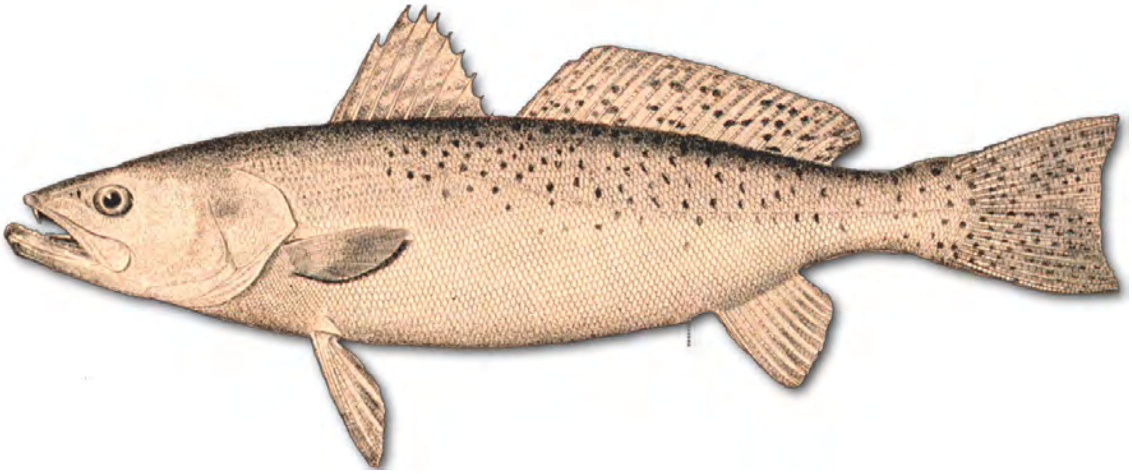
CHAPTER 10

SPOTTED SEATROUT *Cynoscion nebulosus*



10.1 INTRODUCTION

We aged a total of 270 Spotted Seatrout, *Cynoscion nebulosus*, collected by the VMRC's Biological Sampling Program for age and growth analysis in 2020. Spotted Seatrout ages ranged from 0 to 5 years old with an average age of 1.4, a standard deviation of 0.9, and a standard error of 0.05. Six age classes (0 to 5) were represented, comprising fish of the 2015 to 2020 year-classes. The sample was dominated by fish from the year-classes of 2018 and 2019 with 39.3% and 39.6%, respectively.

10.2 METHODS

10.2.1 Sample size for ageing

We estimated sample size for ageing Spotted Seatrout in 2020 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:

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

where A is the sample size for ageing Spotted Seatrout in 2020; θ_a stands for the proportion of age a fish in a catch. V_a and B_a represent variance components within and between length intervals for age a, respectively; CV is the coefficient of variation; L was the total number of Spotted Seatrout used by VMRC to estimate length distribution of the catches from 2014 to 2018. θ_a, V_a, B_a, and CV were calculated using pooled age-length data of Spotted Seatrout collected from 2014 to 2018 and using equations in Quinn and Deriso (1999). For simplicity, the equations are not listed here. The equation (10.1) indicates that the more fish that are aged, the smaller the CV (or higher precision) that will be obtained. Therefore, the criterion to age A (number) of fish is that A should be a number above which there is only a 1% CV reduction for the most major age in catch by aging 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 fish aged in the lab between 2014 and 2018. A_l is number of fish to be aged for length interval l in 2020.

10.2.2 Handling of collections

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

10.2.3 Preparation

Sagittal otoliths, hereafter, referred to as "otoliths", were processed for age determination. The left or right otolith was randomly selected and attached, distal side down, to a glass slide with clear Crystalbond$^{\mathrm{TM}}$ 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$^{\mathrm{TM}}$ 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).

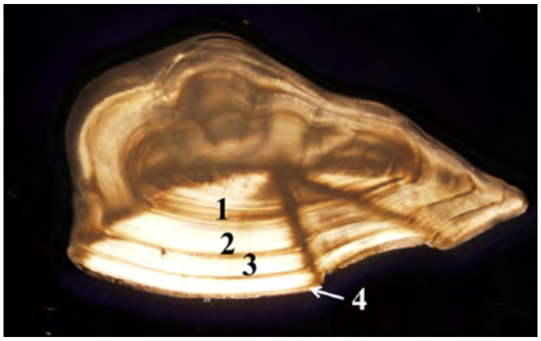


Figure 10.1: Otolith thin-section of a 4 year-old Spotted Seatrout with the last annulus on the edge of the thin-section

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, within the reader, for the following comparisons: 1) in the current year and 2) time-series bias between the current and previous years. The readings from the entire sample for the current year were used to examine the difference and precision in the current. A random sub-sample of 50 fish from the current year was selected for second readings of the reader. Fifty otoliths randomly selected from the fish aged in 2003 were used to examine the time-series bias within the reader. All statistics analyses were performed in R 3.6.1 (R Core Team 2019).

10.3 RESULTS

10.3.1 Sample size

We estimated a sample size of 320 Spotted Seatrout in 2020, ranging in length intervals from 7 to 34 inches (Table 10.1). This sample size provided a range in (*CV*) for age composition approximately from the smallest (*CV*) of 0.06% for Age 1 to the largest (*CV*) of 0.17% for Age 4. In 2020, we randomly selected and aged 270 fish from 412 Spotted Seatrout (The rest of fish were either without otoliths or over-collected for certain length interval(s)) collected by VMRC. We fell short in our over-all collections for this optimal length-class sampling estimate by 83 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.

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 100%. There was no evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 99.63% and a *CV* of 0.07% (test of symmetry: $\chi^2 = 1$, $df = 1$, $P = 0.3173$) (Figure 10.2).

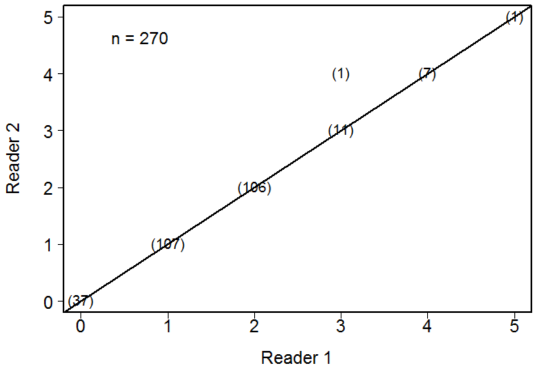


Figure 10.2: Between-reader comparison of otolith age estimates for Spotted Seatrout collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2020.

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

10.3.3 Year class

Of the 270 fish aged with otoliths, 6 age classes (0 to 5) were represented (Table 10.2). 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 6 year-classes: fish from the 2015 to 2020 year-classes, with fish primarily from the year classes of 2018 and 2019 with 39.3% and 39.6%, respectively. The ratio of males to females was 1:1.08 in the sample collected (Figure 10.3).

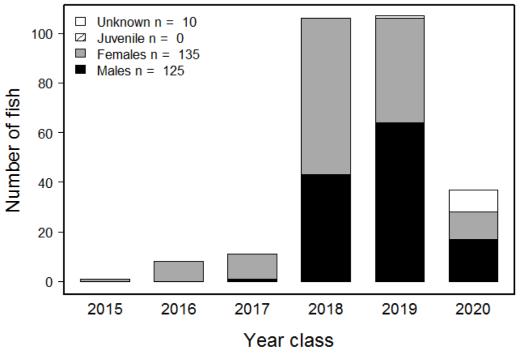


Figure 10.3: Year-class frequency distribution for Spotted Seatrout collected for ageing in 2020. Distribution is broken down by sex. 'Unknown' represents gonads that were not available for examination or were not examined for sex during sampling.

10.3.4 Age-length-key (ALK)

We developed an age-length-key (Table 10.3) 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 2020. 'Target' represents the sample size for ageing estimated for 2020, 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	3	3	2
9 - 9.99	5	9	6	0
10 - 10.99	5	15	6	0
11 - 11.99	5	21	21	0
12 - 12.99	22	2	2	20
13 - 13.99	14	13	13	1
14 - 14.99	15	55	17	0
15 - 15.99	22	64	22	0
16 - 16.99	30	44	30	0
17 - 17.99	30	51	30	0
18 - 18.99	24	39	24	0
19 - 19.99	21	16	16	5
20 - 20.99	22	16	16	6
21 - 21.99	11	17	17	0
22 - 22.99	11	18	18	0
23 - 23.99	10	8	8	2
24 - 24.99	10	7	7	3
25 - 25.99	8	5	5	3
26 - 26.99	5	3	3	2
27 - 27.99	5	3	3	2
28 - 28.99	5	1	1	4
29 - 29.99	5	0	0	5
30 - 30.99	5	1	1	4
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	320	412	270	83

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Table 10.2: The number of Spotted Seatrout assigned to each total length-at-age category for 270 fish sampled for otolith age determination in Virginia during 2020.

	Age						
Interval	0	1	2	3	4	5	Totals
8 - 8.99	3	0	0	0	0	0	3
9 - 9.99	6	0	0	0	0	0	6
10 - 10.99	6	0	0	0	0	0	6
11 - 11.99	20	1	0	0	0	0	21
12 - 12.99	2	0	0	0	0	0	2
13 - 13.99	0	13	0	0	0	0	13
14 - 14.99	0	17	0	0	0	0	17
15 - 15.99	0	22	0	0	0	0	22
16 - 16.99	0	22	8	0	0	0	30
17 - 17.99	0	17	13	0	0	0	30
18 - 18.99	0	9	15	0	0	0	24
19 - 19.99	0	3	13	0	0	0	16
20 - 20.99	0	0	16	0	0	0	16
21 - 21.99	0	2	14	1	0	0	17
22 - 22.99	0	1	17	0	0	0	18
23 - 23.99	0	0	7	1	0	0	8
24 - 24.99	0	0	3	3	1	0	7
25 - 25.99	0	0	0	4	1	0	5
26 - 26.99	0	0	0	1	2	0	3
27 - 27.99	0	0	0	0	3	0	3
28 - 28.99	0	0	0	1	0	0	1
30 - 30.99	0	0	0	0	0	1	1
31 - 31.99	0	0	0	0	1	0	1
Totals	37	107	106	11	8	1	270

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Table 10.3: 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 2020.

	Age					
Interval	0	1	2	3	4	5
8 - 8.99	1	0	0	0	0	0
9 - 9.99	1	0	0	0	0	0
10 - 10.99	1	0	0	0	0	0
11 - 11.99	0.95	0.05	0	0	0	0
12 - 12.99	1	0	0	0	0	0
13 - 13.99	0	1	0	0	0	0
14 - 14.99	0	1	0	0	0	0
15 - 15.99	0	1	0	0	0	0
16 - 16.99	0	0.73	0.27	0	0	0
17 - 17.99	0	0.57	0.43	0	0	0
18 - 18.99	0	0.38	0.62	0	0	0
19 - 19.99	0	0.19	0.81	0	0	0
20 - 20.99	0	0	1	0	0	0
21 - 21.99	0	0.12	0.82	0.06	0	0
22 - 22.99	0	0.06	0.94	0	0	0
23 - 23.99	0	0	0.88	0.12	0	0
24 - 24.99	0	0	0.43	0.43	0.14	0
25 - 25.99	0	0	0	0.8	0.2	0
26 - 26.99	0	0	0	0.33	0.67	0
27 - 27.99	0	0	0	0	1	0
28 - 28.99	0	0	0	1	0	0
30 - 30.99	0	0	0	0	0	1
31 - 31.99	0	0	0	0	1	0

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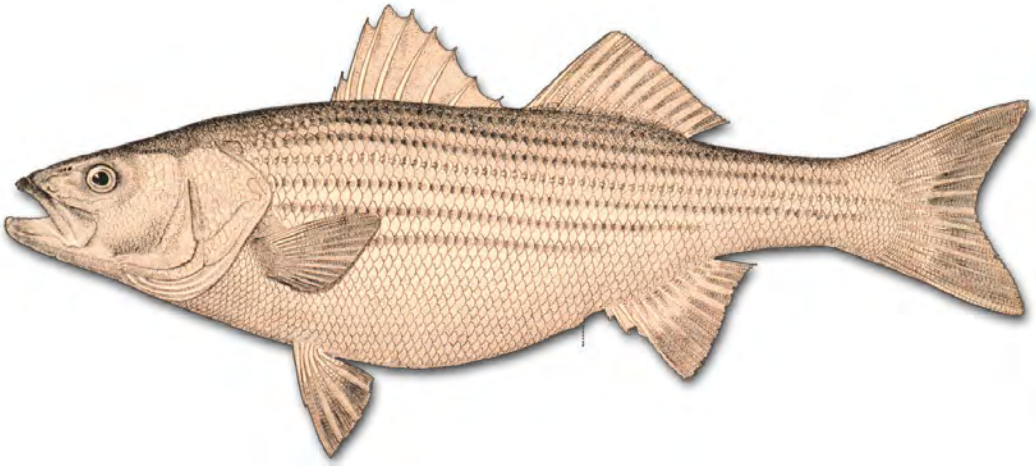
CHAPTER 11

STRIPED BASS *Morone saxatilis*



11.1 INTRODUCTION

We aged a total of 614 Striped Bass, using their scales collected by the VMRC's Biological Sampling Program in 2020. Of 614 aged fish, 614 and 0 fish were collected in Chesapeake Bay (bay fish) and Virginia waters of the Atlantic Ocean (ocean fish), respectively. The average bay fish age was 7.3 years with a standard deviation of 3.8 and a standard error of 0.15. Twenty-one age classes (2 to 22) were represented in the bay fish, comprising fish from the 1998 to 2018 year classes. The bay fish sample in 2020 was dominated by the year classes of 2011, 2011, 2015, and 2016 with 15%, 15%, 25%, and 14%, respectively. We also aged a total of 184 fish using their otoliths in addition to ageing their scales. The otolith ages were compared to the scale ages to examine how close both ages were to one another (see details in Results).

11.2 METHODS

11.2.1 Sample size for ageing

We estimated sample sizes for ageing Striped Bass collected in both Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2020, 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:

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

where A is the sample size for ageing Striped Bass in 2020; θ_a stands for the proportion of age a fish in a catch. V_a and B_a represent variance components within and between length intervals for age a, respectively; CV is the coefficient of variation; L was the total number of Striped Bass used by VMRC to estimate length distribution of the catches from 2014 to 2018. θ_a, V_a, B_a, and CV were calculated using pooled age-length data of Striped Bass collected from 2014 to 2018 and using equations in Quinn and Deriso (1999). For simplicity, the equations are not listed here. The equation (11.1) indicates that the more fish that are aged, the smaller the CV (or higher precision) that will be obtained. Therefore, the criterion to age A (number) of fish is that A should be a number above which there is only a 1% CV reduction for the most major age in catch by aging 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 fish aged in the lab between 2014 and 2018. A_l is number of fish to be aged for length interval l in 2020.

11.2.2 Handling of collection

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

11.2.3 Preparation

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 CQFE website on how to prepare scale impression for ageing Striped Bass.

Otoliths

We used our thin-section and bake technique to process Striped Bass 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.

11.2.4 Readings

The VMRC 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.

In 2020 the new notation method recommended by ASMFC was not used to assign ages on Striped Bass for two reasons:

1. Growth widths among annuli on Striped Bass scales don't grow uniformly unlike on Striped Bass otoliths, therefore, it is not practical to use the margin codes on the scales;

2. Although the margin codes can be applied to Striped Bass otoliths, it is more reasonable that the same ageing notation is used on both scales and otoliths versus using two different ageing notations on scales and otoliths, separately, keeping a consistent ageing notation between two hard-parts of the same species.

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, 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.

Scales

We determined fish age by viewing acetate impressions of scales (Figure 11.1) with a standard Bell and Howell R-735 microfiche reader equipped with 20 and 29 mm lenses. Annuli on Striped Bass scales are identified based on two scale microstructure features, "crossing over" and circuli disruption. Primarily, "crossing over" in the lateral margins near the posterior/anterior interface of the scale is used to determine the origin of the annulus. Here compressed circuli (annulus) "cross-over" the previously deposited circuli of the previous year's growth. Typically annuli of the first three years can be observed transversing this interface as dark bands. These bands remain consistent throughout the posterior field and rejoin the posterior/anterior interface on the opposite side of the focus. Annuli can also be observed in the anterior lateral field of the scale. Here the annuli typically reveal a pattern of discontinuous and suddenly breaking segmented circuli. This event can also be distinguished by the presence of concentric white lines, which are typically associated with the disruption of circuli.

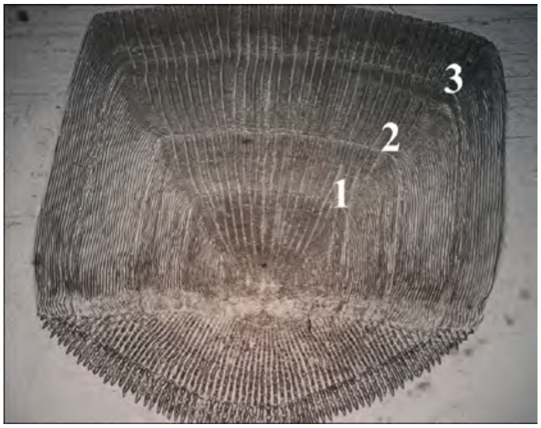


Figure 11.1: Scale impression of a 3 year-old Striped Bass.

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.

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 11.2). Each reader aged all of the otolith samples.

By convention an annulus is identified as the narrow opaque zone, or winter growth. Typically the

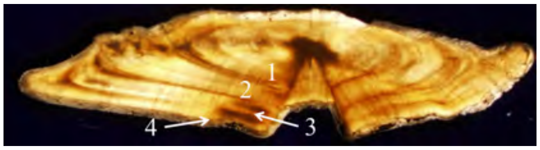


Figure 11.2: Otolith thin-section of a 4 year-old Striped Bass with the last annulus on the edge of the thin-section

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.

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

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 the 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 3.6.1 (R Core Team 2019).

11.3 RESULTS

11.3.1 Sample size

We estimated a sample size of 552 bay Striped Bass in 2020, ranging in length intervals from 17 to 52 inches (Table 11.1). This sample size provided a range in *CV* for age composition approximately from the smallest *CV* of 10% for Age 4 and 5 to the largest *CV* of 25% for Age 14 of the bay fish. We randomly selected and aged 614 fish from 736 Striped Bass (The rest of fish were either without otoliths or over-collected for certain length interval(s)) collected by VMRC in Chesapeake Bay in 2020. We fell short in our over-all collections for this optimal length-class sampling estimate by 91 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.

We estimated a sample size of 476 ocean Striped Bass in 2020, ranging in length intervals from 28 to 51 inches. However, we were not able to collect any samples from Virginia waters of Atlantic ocean in 2020.

11.3.2 Scales

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 76% (1 year or less agreement of 98%) and a *CV* of 2.78% (test of symmetry: $\chi^2 = 6.67$, $df = 5$, $P = 0.2466$), and there was no significant difference between the first and second readings for Reader 2 with an agreement of 68% (1 year or less agreement of 98%) and a *CV* of 3.98% (test of symmetry: $\chi^2 = 6.67$, $df = 7$, $P = 0.4644$). There was evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 60% (1 year or less agreement of 89%) and a *CV* of 5.26% (test of symmetry: $\chi^2 = 174.72$, $df = 40$, $P < 0.0001$) (Figure 11.3).

There was no time-series bias for either reader. Reader 1 had an agreement of 67% (1 year or less agreement of 97%) with ages of fish aged in 2000

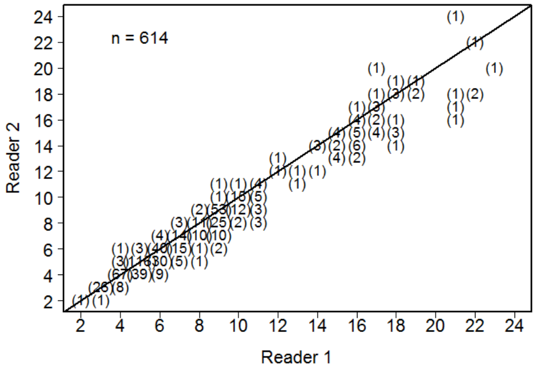


Figure 11.3: Between-reader comparison of scale age estimates for Striped Bass collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2020.

with a *CV* of 3.58% (test of symmetry: $\chi^2 = 6.67$, $df = 10$, $P = 0.7565$), and Reader 2 had an agreement of 68% (1 year or less agreement of 97%) with a *CV* of 3.44% (test of symmetry: $\chi^2 = 10.67$, $df = 11$, $P = 0.4716$).

Of the 614 bay Striped Bass aged with scales, 21 age classes (2 to 22) were represented (Table 11.2). The average age for the sample was 7.3 years. The standard deviation and standard error were 3.8 and 0.15, respectively. Year-class data (Figure 11.4) indicates that recruitment into the fishery in Chesapeake Bay begins at age 2, which corresponds to the 2018 year-class for Striped Bass caught in 2020. Striped Bass in the sample in 2020 was dominated by the year classes of 2011, 2011, 2015, and 2016 with 15%, 15%, 25%, and 14%, respectively. The sex ratio of male to female was 1:1.39 for the bay fish.

11.3.3 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 78% and a *CV* of 1.07% (test of symmetry: $\chi^2 = 9$, $df = 9$, $P = 0.4373$), and there was no significant difference between the first and second readings for Reader 2 with an agreement of 82% and a *CV* of 0.76% (test of symmetry: $\chi^2 = 7$, $df = 8$, $P = 0.5366$). There was no evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 90% (1 year or less agreement of 97%) and a *CV* of 0.54% (test of symmetry: $\chi^2 = 18$, $df = 16$, $P = 0.3239$) (Figure 11.5).

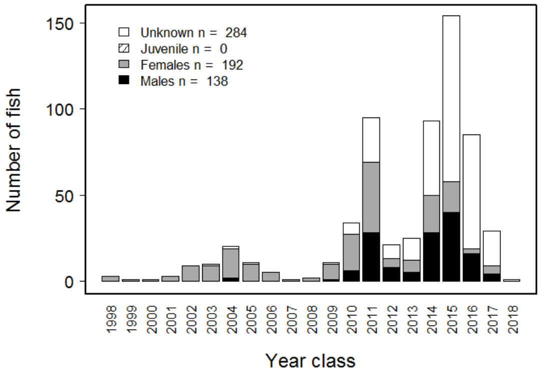


Figure 11.4: Year-class frequency distribution for Striped Bass collected in Chesapeake Bay, Virginia for ageing in 2020. Distribution is broken down by sex and estimated using scale ages. 'Unknown' represents the fish gonads that were not available for examination or were not examined for sex during sampling.

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 1.91% (test of symmetry: $\chi^2 = 12$, $df = 7$, $P = 0.1006$), and Reader 2 had an agreement of 80% with a *CV* of 1.88% (test of symmetry: $\chi^2 = 12$, $df = 7$, $P = 0.1006$).

Of the 184 Striped Bass aged with otoliths, 23 age classes (3 to 11, and 13 to 26) were represented (Table 11.3). The average age for the sample was 9.9 years. The standard deviation and standard error were 5.9 and 0.43, respectively.

11.3.4 Comparison of scale and otolith ages

We aged 184 Striped Bass using paired scales and otoliths. There was no evidence of systematic disagreement between otolith and scale ages (test of symmetry: $\chi^2 = 53.4$, $df = 40$, $P = 0.0763$) with an average *CV* of 4.7%. There was an agreement of 60% between scale and otoliths ages whereas scales were assigned a lower and higher age than otoliths for 26% and 14% of the fish, respectively (Figure 11.6). There was also little evidence of bias between otolith and scale ages using an age bias plot (Figure 11.7), with no trend of either over-ageing younger or under-ageing older fish.

11.3.5 Age-Length-Key (ALK)

We developed an age-length-key for bay fish (Table 11.4) using scale ages. The ALK can be used in the

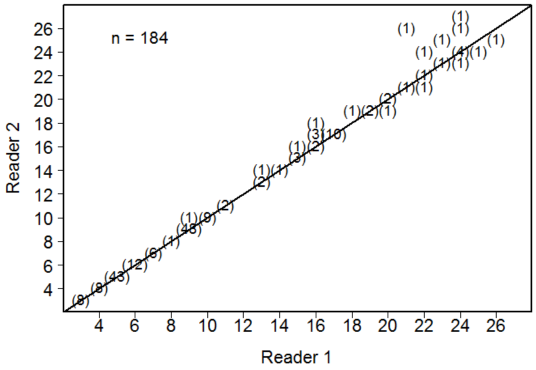


Figure 11.5: Between-reader comparison of otolith age estimates for Striped Bass collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2020.

Figure 11.6: Comparison of paired scale and otolith age estimates for Striped Bass collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2020.

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.

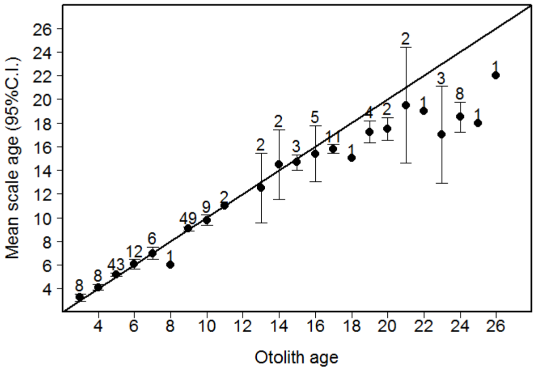


Figure 11.7: Age-bias plot for Striped Bass scale and otolith age estimates in 2020.

Table 11.1: Number of bay Striped Bass collected and aged in each 1-inch length interval in 2020. 'Target' represents the sample size for ageing estimated for 2020, 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	11	30	25	0
19 - 19.99	21	79	67	0
20 - 20.99	27	96	78	0
21 - 21.99	28	46	44	0
22 - 22.99	28	50	37	0
23 - 23.99	30	51	33	0
24 - 24.99	27	41	28	0
25 - 25.99	25	35	26	0
26 - 26.99	25	36	26	0
27 - 27.99	22	25	22	0
28 - 28.99	19	15	15	4
29 - 29.99	18	9	9	9
30 - 30.99	14	17	17	0
31 - 31.99	16	12	12	4
32 - 32.99	21	10	10	11
33 - 33.99	19	10	10	9
34 - 34.99	18	11	11	7
35 - 35.99	17	20	10	7
36 - 36.99	21	22	13	8
37 - 37.99	22	17	17	5
38 - 38.99	16	14	14	2
39 - 39.99	9	12	12	0
40 - 40.99	9	9	9	0
41 - 41.99	8	9	9	0
42 - 42.99	8	10	10	0
43 - 43.99	8	8	8	0
44 - 44.99	11	8	8	3
45 - 45.99	9	8	8	1
46 - 46.99	10	9	9	1
47 - 47.99	5	7	7	0
48 - 48.99	5	4	4	1
49 - 49.99	5	3	3	2
50 - 50.99	5	2	2	3
51 - 51.99	5	0	0	5
52 - 52.99	5	0	0	5
Totals	552	736	614	91

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Table 11.2: The number of Striped Bass assigned to each total length-at-age category for 614 fish sampled for scale age determination in Chesapeake Bay, Virginia during 2020.

	Age																				
Interval	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	Totals
17 - 17.99	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
18 - 18.99	1	5	13	2	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	25
19 - 19.99	0	8	24	28	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	67
20 - 20.99	0	6	29	32	11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	78
21 - 21.99	0	5	10	22	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	44
22 - 22.99	0	2	2	16	14	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	37
23 - 23.99	0	2	4	14	12	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	33
24 - 24.99	0	0	1	12	9	4	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	28
25 - 25.99	0	1	1	11	8	2	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	26
26 - 26.99	0	0	0	9	6	5	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0	26
27 - 27.99	0	0	1	3	5	5	1	5	2	0	0	0	0	0	0	0	0	0	0	0	0	22
28 - 28.99	0	0	0	2	2	1	3	5	1	1	0	0	0	0	0	0	0	0	0	0	0	15
29 - 29.99	0	0	0	1	2	2	1	0	2	0	1	0	0	0	0	0	0	0	0	0	0	9
30 - 30.99	0	0	0	1	1	0	3	8	4	0	0	0	0	0	0	0	0	0	0	0	0	17
31 - 31.99	0	0	0	1	1	0	4	4	2	0	0	0	0	0	0	0	0	0	0	0	0	12
32 - 32.99	0	0	0	0	1	0	3	5	0	1	0	0	0	0	0	0	0	0	0	0	0	10
33 - 33.99	0	0	0	0	0	0	3	7	0	0	0	0	0	0	0	0	0	0	0	0	0	10
34 - 34.99	0	0	0	0	0	0	1	8	2	0	0	0	0	0	0	0	0	0	0	0	0	11
35 - 35.99	0	0	0	0	0	1	1	7	0	1	0	0	0	0	0	0	0	0	0	0	0	10
36 - 36.99	0	0	0	0	2	1	0	7	2	1	0	0	0	0	0	0	0	0	0	0	0	13
37 - 37.99	0	0	0	0	0	0	1	10	6	0	0	0	0	0	0	0	0	0	0	0	0	17
38 - 38.99	0	0	0	0	0	0	0	9	3	1	0	0	0	1	0	0	0	0	0	0	0	14
39 - 39.99	0	0	0	0	0	0	0	4	5	1	1	0	0	0	1	0	0	0	0	0	0	12
40 - 40.99	0	0	0	0	0	0	0	3	4	2	0	0	0	0	0	0	0	0	0	0	0	9
41 - 41.99	0	0	0	0	0	0	0	2	1	1	0	0	1	1	3	0	0	0	0	0	0	9
42 - 42.99	0	0	0	0	0	0	0	0	0	2	0	1	0	1	4	2	0	0	0	0	0	10
43 - 43.99	0	0	0	0	0	0	0	0	0	0	0	0	2	3	1	1	1	0	0	0	0	8
44 - 44.99	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	0	3	0	0	1	1	8
45 - 45.99	0	0	0	0	0	0	0	0	0	0	0	0	1	1	4	2	0	0	0	0	0	8
46 - 46.99	0	0	0	0	0	0	0	0	0	0	0	0	1	2	3	1	2	0	0	0	0	9
47 - 47.99	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	2	1	1	0	0	1	7
48 - 48.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	0	0	4
49 - 49.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	3
50 - 50.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	2
Totals	1	29	85	154	93	25	21	95	34	11	2	1	5	11	20	10	9	3	1	1	3	614

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Table 11.3: The number of Striped Bass assigned to each total length-at-age category for 184 fish sampled for otolith age determination in Chesapeake Bay and Virginia waters of Atlantic Ocean during 2020.

	Age																						
Interval	3	4	5	6	7	8	9	10	11	13	14	15	16	17	18	19	20	21	22	23	24	25	26	Totals
18 - 18.99	3	2	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9
19 - 19.99	0	2	6	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9
20 - 20.99	3	2	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8
21 - 21.99	1	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5
22 - 22.99	1	0	4	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6
23 - 23.99	0	0	4	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5
24 - 24.99	0	0	4	1	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7
25 - 25.99	0	1	5	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8
26 - 26.99	0	0	6	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9
27 - 27.99	0	0	2	2	1	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8
28 - 28.99	0	0	2	0	1	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5
29 - 29.99	0	0	0	1	2	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5
30 - 30.99	0	0	0	1	0	0	4	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6
31 - 31.99	0	0	0	1	1	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4
32 - 32.99	0	0	0	0	0	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4
33 - 33.99	0	0	0	0	0	0	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4
34 - 34.99	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
35 - 35.99	0	0	0	0	0	0	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4
36 - 36.99	0	0	0	2	0	0	3	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	6
37 - 37.99	0	0	0	0	0	0	4	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6
38 - 38.99	0	0	0	0	0	0	3	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	5
39 - 39.99	0	0	0	0	0	0	5	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	7
40 - 40.99	0	0	0	0	0	0	3	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	5
41 - 41.99	0	0	0	0	0	0	1	2	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	5
42 - 42.99	0	0	0	0	0	0	0	0	1	0	1	0	1	1	0	1	0	0	0	0	1	0	0	6
43 - 43.99	0	0	0	0	0	0	0	0	0	0	0	2	0	3	0	0	0	0	0	0	0	0	0	5
44 - 44.99	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1	0	1	2	0	0	6
45 - 45.99	0	0	0	0	0	0	0	0	0	1	0	0	1	3	0	0	0	0	0	0	0	0	0	5
46 - 46.99	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	0	0	2	0	0	0	5
47 - 47.99	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0	1	0	0	2	0	0	6
48 - 48.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	0	0	0	3
49 - 49.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1	0	3
50 - 50.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	2
Totals	8	8	43	12	6	1	49	9	2	2	2	3	5	11	1	4	2	2	1	3	8	1	1	184

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Table 11.4: Age-Length key, as proportion-at-age in each 1-inch length interval, based on scale ages for Striped Bass sampled in Chesapeake Bay, Virginia during 2020.

	Age																			
Interval	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
17 - 17.99	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18 - 18.99	0.04	0.2	0.52	0.08	0.16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19 - 19.99	0	0.12	0.36	0.42	0.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20 - 20.99	0	0.08	0.37	0.41	0.14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
21 - 21.99	0	0.11	0.23	0.5	0.16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
22 - 22.99	0	0.05	0.05	0.43	0.38	0.08	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
23 - 23.99	0	0.06	0.12	0.42	0.36	0.03	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
24 - 24.99	0	0	0.04	0.43	0.32	0.14	0	0.07	0	0	0	0	0	0	0	0	0	0	0	0	0
25 - 25.99	0	0.04	0.04	0.42	0.31	0.08	0	0.12	0	0	0	0	0	0	0	0	0	0	0	0	0
26 - 26.99	0	0	0	0.35	0.23	0.19	0	0.23	0	0	0	0	0	0	0	0	0	0	0	0	0
27 - 27.99	0	0	0.05	0.14	0.23	0.23	0.05	0.23	0.09	0	0	0	0	0	0	0	0	0	0	0	0
28 - 28.99	0	0	0	0.13	0.13	0.07	0.2	0.33	0.07	0.07	0	0	0	0	0	0	0	0	0	0	0
29 - 29.99	0	0	0	0.11	0.22	0.22	0.11	0	0.22	0	0.11	0	0	0	0	0	0	0	0	0	0
30 - 30.99	0	0	0	0.06	0.06	0	0.18	0.47	0.24	0	0	0	0	0	0	0	0	0	0	0	0
31 - 31.99	0	0	0	0.08	0.08	0	0.33	0.33	0.17	0	0	0	0	0	0	0	0	0	0	0	0
32 - 32.99	0	0	0	0	0.1	0	0.3	0.5	0	0.1	0	0	0	0	0	0	0	0	0	0	0
33 - 33.99	0	0	0	0	0	0	0.3	0.7	0	0	0	0	0	0	0	0	0	0	0	0	0
34 - 34.99	0	0	0	0	0	0	0.09	0.73	0.18	0	0	0	0	0	0	0	0	0	0	0	0
35 - 35.99	0	0	0	0	0	0.1	0.1	0.7	0	0.1	0	0	0	0	0	0	0	0	0	0	0
36 - 36.99	0	0	0	0	0.15	0.08	0	0.54	0.15	0.08	0	0	0	0	0	0	0	0	0	0	0
37 - 37.99	0	0	0	0	0	0	0.06	0.59	0.35	0	0	0	0	0	0	0	0	0	0	0	0
38 - 38.99	0	0	0	0	0	0	0	0.64	0.21	0.07	0	0	0	0.07	0	0	0	0	0	0	0
39 - 39.99	0	0	0	0	0	0	0	0.33	0.42	0.08	0.08	0	0	0	0.08	0	0	0	0	0	0
40 - 40.99	0	0	0	0	0	0	0	0.33	0.44	0.22	0	0	0	0	0	0	0	0	0	0	0
41 - 41.99	0	0	0	0	0	0	0	0.22	0.11	0.11	0	0	0.11	0.11	0.33	0	0	0	0	0	0
42 - 42.99	0	0	0	0	0	0	0	0	0	0.2	0	0.1	0	0.1	0.4	0.2	0	0	0	0	0
43 - 43.99	0	0	0	0	0	0	0	0	0	0	0	0	0.25	0.38	0.12	0.12	0.12	0	0	0	0
44 - 44.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0.12	0.25	0	0.38	0	0	0.12	0.12
45 - 45.99	0	0	0	0	0	0	0	0	0	0	0	0	0.12	0.12	0.5	0.25	0	0	0	0	0
46 - 46.99	0	0	0	0	0	0	0	0	0	0	0	0	0.11	0.22	0.33	0.11	0.22	0	0	0	0
47 - 47.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0.14	0.14	0.29	0.14	0.14	0	0	0.14
48 - 48.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.25	0.25	0.25	0.25	0	0	0
49 - 49.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.33	0.33	0.33	0	0	0
50 - 50.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.5	0	0.5

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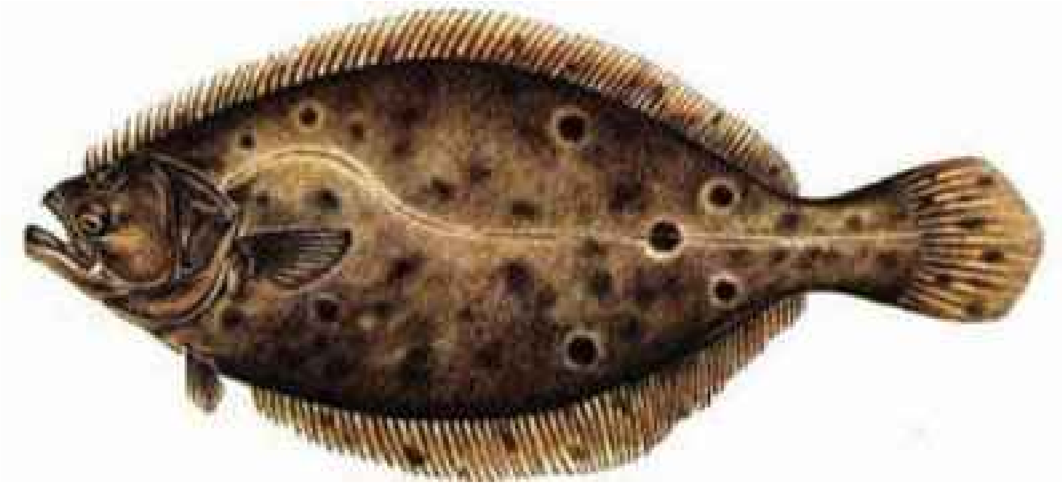
CHAPTER 12

SUMMER FLOUNDER *Paralichthys dentatus*



12.1 INTRODUCTION

We aged a total of 704 Summer Flounder (excluding 3 fish with otolith-ages only), using their scales collected by the VMRC's Biological Sampling Program in 2020. Of 704 aged fish, 292 and 415 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 1 and a standard error of 0.06. Eight age classes (0 to 7) were represented in the bay fish, comprising fish from the 2013 to 2020 year classes. The bay fish sample in 2020 was dominated by the year class of 2018 with 52%. The average ocean fish age was 5.3 years with a standard deviation of 2 and a standard error of 0.1. Twelve age classes (2 to 13) were represented in the ocean fish, comprising fish from the 2007 to 2018 year classes. The ocean fish sample in 2020 was dominated by the year classes of 2013, 2014, 2014, 2015, and 2016 with 13%, 18%, 18%, 14%, and 22%, respectively. We also aged a total of 148 fish using their otoliths in addition to ageing their scales. The otolith ages were compared to the scale ages to examine how close both ages were to one another (see details in Results).

12.2 METHODS

12.2.1 Sample size for ageing

We estimated sample sizes for ageing Summer Flounder collected in both Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2020, 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:

$$A = \frac{V_a}{\theta_a^2 CV^2 + B_a/L} \tag{12.1}$$

where A is the sample size for ageing Summer Flounder in 2020; θ_a stands for the proportion of age a fish in a catch. V_a and B_a represent variance components within and between length intervals for age a, respectively; CV is the coefficient of variation; L was the total number of Summer Flounder used by VMRC to estimate length distribution of the catches from 2014 to 2018. θ_a, V_a, B_a, and CV were calculated using pooled age-length data of Summer Flounder collected from 2014 to 2018 and using equations in Quinn and Deriso (1999). For simplicity, the equations are not listed here. The equation (12.1) indicates that the more fish that are aged, the smaller the CV (or higher precision) that will be obtained. Therefore, the criterion to age A (number) of fish is that A should be a number above which there is only a 1% CV reduction for the most major age in catch by aging 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 fish aged in the lab between 2014 and 2018. A_l is number of fish to be aged for length interval l in 2020.

12.2.2 Handling of collection

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

12.2.3 Preparation

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 CQFE website on how to prepare scale impression for ageing Summer Flounder.

Otoliths

We used our thin-section and bake technique to process Summer Flounder 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$^{\text{TM}}$ 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 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/ODU), and age class assignment using these hard-parts is conducted in the same way as otoliths.

In 2020 the new notation method recommended by

ASMFC was not used to assign ages on Summer Flounder for two reasons:

1. Growth widths among annuli on Summer Flounder scales don't grow uniformly unlike on Summer Flounder otoliths, therefore, it is not practical to use the margin codes on the scales;
2. Although the margin codes can be applied to Summer Flounder otoliths, it is more reasonable that the same ageing notation is used on both scales and otoliths versus using two different ageing notations on scales and otoliths, separately, keeping a consistent ageing notation between two hard-parts of the same species.

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, 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.

Scales

We determined fish age by viewing acetate impressions of scales (Figure 12.1) 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.

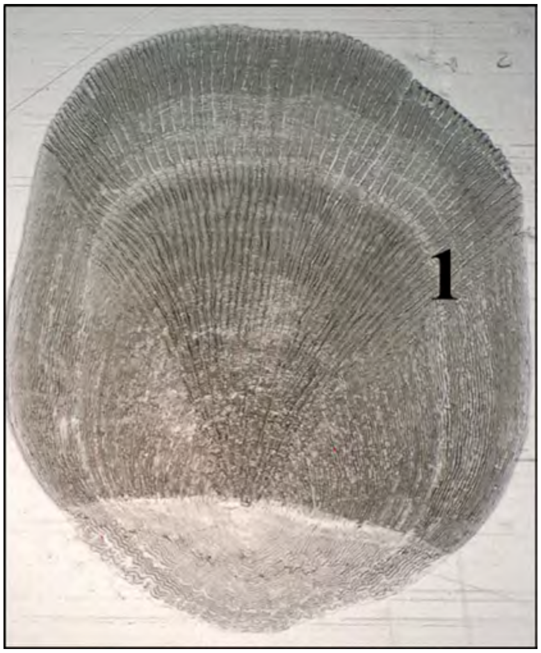


Figure 12.1: Scale impression of a 1 year-old Summer Flounder

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.

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 12.2). 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 ridge and outwards to the distal surface.

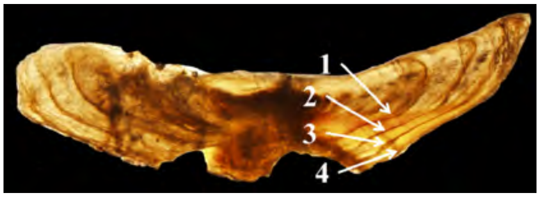


Figure 12.2: Otolith thin-section of a 4 year-old Summer Flounder with the last annulus on the edge of the thin-section

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

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 the 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 3.6.1 (R Core Team 2019).

12.3 RESULTS

12.3.1 Sample size

We estimated a sample size of 433 bay Summer Flounder in 2020, ranging in length intervals from 8 to 28 inches (Table 12.1). This sample size provided a range in CV for age composition approximately from the smallest CV of 7% for Age 2 to the largest CV of 18% for Age 6 of the bay fish. We aged all 295 Summer Flounder (including 3 fish with otoliths only) collected by VMRC in Chesapeake Bay in 2020. We fell short in our over-all collections for this optimal length-class sampling estimate by 149 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.

We estimated a sample size of 486 ocean Summer Flounder in 2020, ranging in length intervals from 13 to 32 inches (Table 12.2). This sample size provided a range in CV for age composition approximately from the smallest CV of 9% for Age 4 and 5 to the largest CV of 25% for Age 9 of the ocean fish. We aged all 415 Summer Flounder collected by VMRC in Virginia waters of the Atlantic Ocean in 2020. We fell short in our over-all collections for this optimal length-class sampling estimate by

82 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 Scales

Reader 1 had moderate self-precision and Read 2 had high self-precision. Specifically, there was no significant difference between the first and second readings for Reader 1 with an agreement of 60% (1 year or less agreement of 96%) and a *CV* of 8.58% (test of symmetry: $\chi^2 = 9.33$, $df = 9$, $P = 0.4071$), and there was no significant difference between the first and second readings for Reader 2 with an agreement of 82% (1 year or less agreement of 100%) and a *CV* of 3.52% (test of symmetry: $\chi^2 = 9$, $df = 5$, $P = 0.1091$). There was evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 76% (1 year or less agreement of 97%) and a *CV* of 4.27% (test of symmetry: $\chi^2 = 123.19$, $df = 18$, $P < 0.0001$) (Figure 12.3).

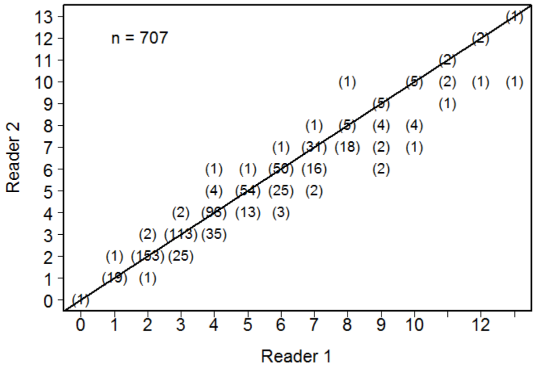


Figure 12.3: Between-reader comparison of scale age estimates for Summer Flounder collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2020.

Reader 1 had no time series bias while Read 2 does. Reader 1 had an agreement of 74% (1 year or less agreement of 100%) with ages of fish aged in 2000 with a *CV* of 4.71% (test of symmetry: $\chi^2 = 7.5$, $df = 5$, $P = 0.186$), and Reader 2 had an agreement of 82% (1 year or less agreement of 100%) with a *CV* of 3.71% (test of symmetry: $\chi^2 = 9$, $df = 3$, $P = 0.0293$).

Of the 292 bay Summer Flounder aged with scales, 8 age classes (0 to 7) were represented (Table 12.3). The average age for the sample was 2.6 years. The standard deviation and standard error were 1 and 0.06, respectively. Year-class data (Figure 12.4) indicates that recruitment into the fishery in Chesapeake Bay begins at age 0, which corresponds to the 2020 year-class for Summer Flounder caught in 2020. Summer Flounder in the sample in 2020 was dominated by the year class of 2018 with 52%. There was no male bay fish collected in 2019.

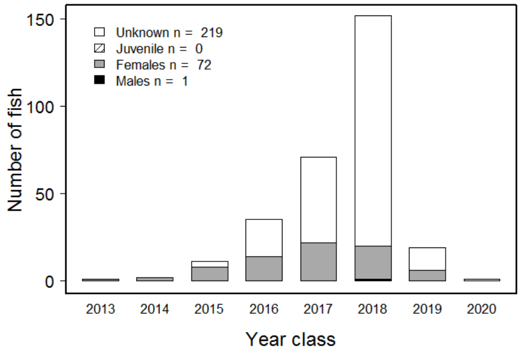


Figure 12.4: Year-class frequency distribution for Summer Flounder collected in Chesapeake Bay, Virginia for ageing in 2020. Distribution is broken down by sex and estimated using scale ages. 'Unknown' represents gonads that were not available for examination or were not examined for sex during sampling.

Of the 415 ocean Summer Flounder aged with scales, 12 age classes (2 to 13) were represented (Table 12.4). The average age for the sample was 5.3 years. The standard deviation and standard error were 2 and 0.1, respectively. Year-class data (Figure 12.5) indicates that recruitment into the fishery in Virginia waters of Atlantic ocean begins at age 2, which corresponds to the 2018 year-class for Summer Flounder caught in 2020. Summer Flounder in the sample in 2020 was dominated by the year classes of 2013, 2014, 2014, 2015, and 2016 with 13%, 18%, 18%, 14%, and 22%, respectively. The sex ratio of male to female was 1:2.29 for the ocean fish.

12.3.3 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 1.12% (test of symmetry: $\chi^2 = 3$, $df = 2$, $P = 0.2231$), and there was no significant difference

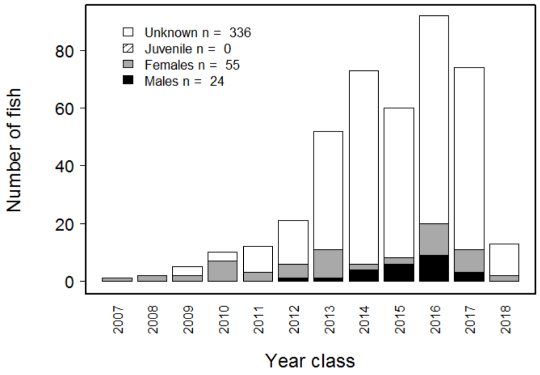


Figure 12.5: Year-class frequency distribution for Summer Flounder collected in Virginia waters of the Atlantic Ocean for ageing in 2020. Distribution is broken down by sex and estimated using scale ages. 'Unknown' represents gonads that were not available for examination or were not examined for sex during sampling.

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 97% (1 year or less agreement of 100%) and a *CV* of 0.72% (test of symmetry: $\chi^2 = 4$, $df = 4$, $P = 0.406$) (Figure 12.6).

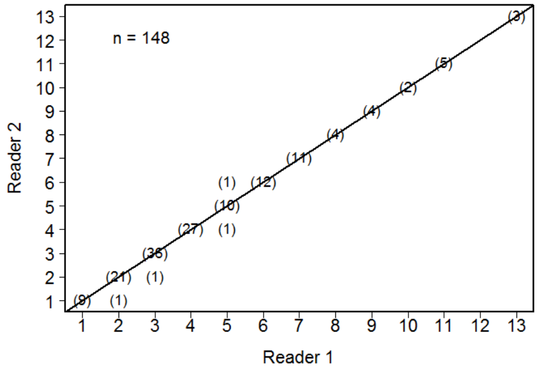


Figure 12.6: Between-reader comparison of otolith age estimates for Summer Flounder collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2020.

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 2.39% (test of symmetry: $\chi^2 = 4$, $df = 3$, $P = 0.2615$), and Reader 2 had an agreement of 94% with a *CV* of 1.37% (test of symmetry: $\chi^2 = 3$, $df = 2$, $P = 0.2231$).

Of the 148 Summer Flounder aged with otoliths, 12 age classes (1 to 11, and 13) were represented (Table 12.5). The average age for the sample was 4.5 years. The standard deviation and standard error were 2.7 and 0.22, respectively.

12.3.4 Comparison of scale and otolith ages

We aged 145 Summer Flounder using scales and otoliths (excluding 3 fish with otolith-ages only). There was no evidence of systematic disagreement between otolith and scale ages (test of symmetry: $\chi^2 = 22.33$, $df = 21$, $P = 0.3808$) with an average *CV* of 10.04%. There was an agreement of 54% between scale and otoliths ages whereas scales were assigned a lower and higher age than otoliths for 21% and 24% of the fish, respectively (Figure 12.7). There was also little evidence of bias between otolith and scale ages using an age bias plot (Figure 12.8), with no trend of either over-ageing younger or under-ageing older fish.

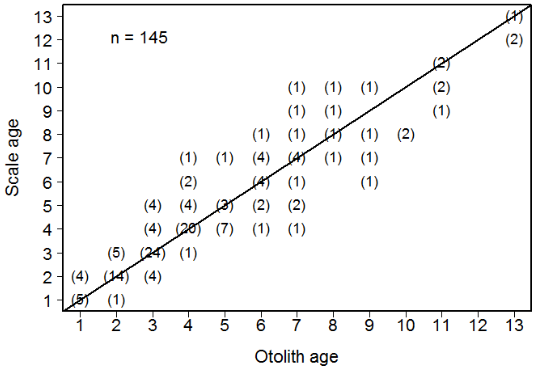


Figure 12.7: Comparison of paired scale and otolith age estimates for Summer Flounder collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2020.

12.3.5 Age-Length-Key (ALK)

We developed an age-length-key for both bay (Table 12.6) and ocean fish (Table 12.7) using scale ages, separately. The ALK can be used in the conversion of numbers-at-length in the estimated catch to numbers-at-age using scale ages. The table is based on VMRC's stratified sampling of landings by total length inch intervals.

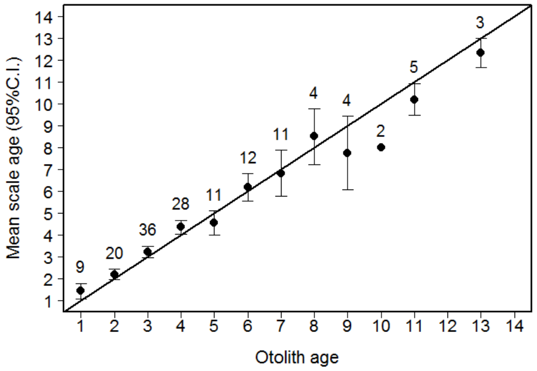


Figure 12.8: Age-bias plot for Summer Flounder scale and otolith age estimates in 2020.

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 2020. 'Target' represents the sample size for ageing estimated for 2020, 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
12 - 12.99	5	0	0	5
13 - 13.99	5	0	0	5
14 - 14.99	86	69	69	17
15 - 15.99	66	74	74	0
16 - 16.99	52	45	45	7
17 - 17.99	46	46	46	0
18 - 18.99	39	31	31	8
19 - 19.99	35	13	13	22
20 - 20.99	32	5	5	27
21 - 21.99	19	4	4	15
22 - 22.99	10	4	4	6
23 - 23.99	8	1	1	7
24 - 24.99	5	0	0	5
25 - 25.99	5	0	0	5
26 - 26.99	5	0	0	5
27 - 27.99	5	0	0	5
28 - 28.99	5	0	0	5
Totals	433	292	292	149

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Table 12.2: Number of ocean Summer Flounder collected and aged in each 1-inch length interval in 2020. 'Target' represents the sample size for ageing estimated for 2020, 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	4	4	1
14 - 14.99	42	37	37	5
15 - 15.99	66	46	46	20
16 - 16.99	62	45	45	17
17 - 17.99	54	45	45	9
18 - 18.99	38	38	38	0
19 - 19.99	26	27	27	0
20 - 20.99	26	21	21	5
21 - 21.99	19	27	27	0
22 - 22.99	24	20	20	4
23 - 23.99	25	21	21	4
24 - 24.99	21	20	20	1
25 - 25.99	18	20	20	0
26 - 26.99	16	12	12	4
27 - 27.99	13	13	13	0
28 - 28.99	9	6	6	3
29 - 29.99	7	7	7	0
30 - 30.99	5	5	5	0
31 - 31.99	5	1	1	4
32 - 32.99	5	0	0	5
Totals	486	415	415	82

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Table 12.3: The number of Summer Flounder assigned to each total length-at-age category for 292 fish sampled for scale age determination in Chesapeake Bay, Virginia during 2020.

	Age								
Interval	0	1	2	3	4	5	6	7	Totals
14 - 14.99	1	9	51	7	1	0	0	0	69
15 - 15.99	0	5	57	9	3	0	0	0	74
16 - 16.99	0	4	24	15	1	1	0	0	45
17 - 17.99	0	1	14	19	8	4	0	0	46
18 - 18.99	0	0	6	13	9	2	1	0	31
19 - 19.99	0	0	0	5	7	1	0	0	13
20 - 20.99	0	0	0	1	2	1	0	1	5
21 - 21.99	0	0	0	1	1	1	1	0	4
22 - 22.99	0	0	0	1	3	0	0	0	4
23 - 23.99	0	0	0	0	0	1	0	0	1
Totals	1	19	152	71	35	11	2	1	292

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Table 12.4: The number of Summer Flounder assigned to each total length-at-age category for 415 fish sampled for scale age determination in Virginia waters of Atlantic ocean during 2020.

	Age												
Interval	2	3	4	5	6	7	8	9	10	11	12	13	Totals
13 - 13.99	1	1	2	0	0	0	0	0	0	0	0	0	4
14 - 14.99	4	23	9	1	0	0	0	0	0	0	0	0	37
15 - 15.99	5	18	20	3	0	0	0	0	0	0	0	0	46
16 - 16.99	2	9	23	7	3	1	0	0	0	0	0	0	45
17 - 17.99	1	13	10	13	6	2	0	0	0	0	0	0	45
18 - 18.99	0	7	7	10	10	4	0	0	0	0	0	0	38
19 - 19.99	0	2	5	7	8	3	2	0	0	0	0	0	27
20 - 20.99	0	1	8	5	4	1	2	0	0	0	0	0	21
21 - 21.99	0	0	6	6	8	5	0	0	1	1	0	0	27
22 - 22.99	0	0	1	3	9	6	0	1	0	0	0	0	20
23 - 23.99	0	0	1	2	6	8	2	1	1	0	0	0	21
24 - 24.99	0	0	0	2	9	5	2	1	1	0	0	0	20
25 - 25.99	0	0	0	1	6	6	3	3	1	0	0	0	20
26 - 26.99	0	0	0	0	1	7	3	0	0	1	0	0	12
27 - 27.99	0	0	0	0	3	4	4	1	1	0	0	0	13
28 - 28.99	0	0	0	0	0	0	1	3	1	1	0	0	6
29 - 29.99	0	0	0	0	0	0	2	2	1	1	1	0	7
30 - 30.99	0	0	0	0	0	0	0	0	3	1	1	0	5
31 - 31.99	0	0	0	0	0	0	0	0	0	0	0	1	1
Totals	13	74	92	60	73	52	21	12	10	5	2	1	415

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Table 12.5: The number of Summer Flounder assigned to each total length-at-age category for 148 fish sampled for otolith age determination in Chesapeake Bay and Virginia waters of Atlantic Ocean during 2020.

	Age												
Interval	1	2	3	4	5	6	7	8	9	10	11	13	Totals
14 - 14.99	7	5	2	2	0	0	0	0	0	0	0	0	16
15 - 15.99	2	9	4	4	1	0	0	0	0	0	0	0	20
16 - 16.99	0	6	6	1	2	4	1	0	0	0	0	0	20
17 - 17.99	0	1	14	2	1	0	1	0	0	0	0	0	19
18 - 18.99	0	0	5	6	1	0	1	0	0	0	0	0	13
19 - 19.99	0	0	4	4	0	0	1	0	1	0	0	0	10
20 - 20.99	0	0	1	5	0	0	0	0	1	0	0	0	7
21 - 21.99	0	0	1	2	1	2	0	1	1	0	0	0	8
22 - 22.99	0	1	0	2	3	2	0	0	0	0	0	0	8
23 - 23.99	0	0	0	0	1	1	0	2	0	0	0	0	4
24 - 24.99	0	0	0	0	1	1	1	0	0	0	0	0	3
25 - 25.99	0	0	0	0	0	1	2	0	0	0	0	0	3
26 - 26.99	0	0	0	0	0	1	1	0	1	0	0	0	3
27 - 27.99	0	0	0	0	0	0	2	0	0	1	0	0	3
28 - 28.99	0	0	0	0	0	0	1	1	0	1	0	0	3
29 - 29.99	0	0	0	0	0	0	0	0	0	0	2	1	3
30 - 30.99	0	0	0	0	0	0	0	0	0	0	3	1	4
31 - 31.99	0	0	0	0	0	0	0	0	0	0	0	1	1
Totals	9	22	37	28	11	12	11	4	4	2	5	3	148

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Table 12.6: 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 2020.

	Age							
Interval	0	1	2	3	4	5	6	7
14 - 14.99	0.01	0.13	0.74	0.1	0.01	0	0	0
15 - 15.99	0	0.07	0.77	0.12	0.04	0	0	0
16 - 16.99	0	0.09	0.53	0.33	0.02	0.02	0	0
17 - 17.99	0	0.02	0.3	0.41	0.17	0.09	0	0
18 - 18.99	0	0	0.19	0.42	0.29	0.06	0.03	0
19 - 19.99	0	0	0	0.38	0.54	0.08	0	0
20 - 20.99	0	0	0	0.2	0.4	0.2	0	0.2
21 - 21.99	0	0	0	0.25	0.25	0.25	0.25	0
22 - 22.99	0	0	0	0.25	0.75	0	0	0
23 - 23.99	0	0	0	0	0	1	0	0

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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 Virginia waters of the Atlantic Ocean during 2020.

	Age											
Interval	2	3	4	5	6	7	8	9	10	11	12	13
13 - 13.99	0.25	0.25	0.5	0	0	0	0	0	0	0	0	0
14 - 14.99	0.11	0.62	0.24	0.03	0	0	0	0	0	0	0	0
15 - 15.99	0.11	0.39	0.43	0.07	0	0	0	0	0	0	0	0
16 - 16.99	0.04	0.2	0.51	0.16	0.07	0.02	0	0	0	0	0	0
17 - 17.99	0.02	0.29	0.22	0.29	0.13	0.04	0	0	0	0	0	0
18 - 18.99	0	0.18	0.18	0.26	0.26	0.11	0	0	0	0	0	0
19 - 19.99	0	0.07	0.19	0.26	0.3	0.11	0.07	0	0	0	0	0
20 - 20.99	0	0.05	0.38	0.24	0.19	0.05	0.1	0	0	0	0	0
21 - 21.99	0	0	0.22	0.22	0.3	0.19	0	0	0.04	0.04	0	0
22 - 22.99	0	0	0.05	0.15	0.45	0.3	0	0.05	0	0	0	0
23 - 23.99	0	0	0.05	0.1	0.29	0.38	0.1	0.05	0.05	0	0	0
24 - 24.99	0	0	0	0.1	0.45	0.25	0.1	0.05	0.05	0	0	0
25 - 25.99	0	0	0	0.05	0.3	0.3	0.15	0.15	0.05	0	0	0
26 - 26.99	0	0	0	0	0.08	0.58	0.25	0	0	0.08	0	0
27 - 27.99	0	0	0	0	0.23	0.31	0.31	0.08	0.08	0	0	0
28 - 28.99	0	0	0	0	0	0	0.17	0.5	0.17	0.17	0	0
29 - 29.99	0	0	0	0	0	0	0.29	0.29	0.14	0.14	0.14	0
30 - 30.99	0	0	0	0	0	0	0	0	0.6	0.2	0.2	0
31 - 31.99	0	0	0	0	0	0	0	0	0	0	0	1

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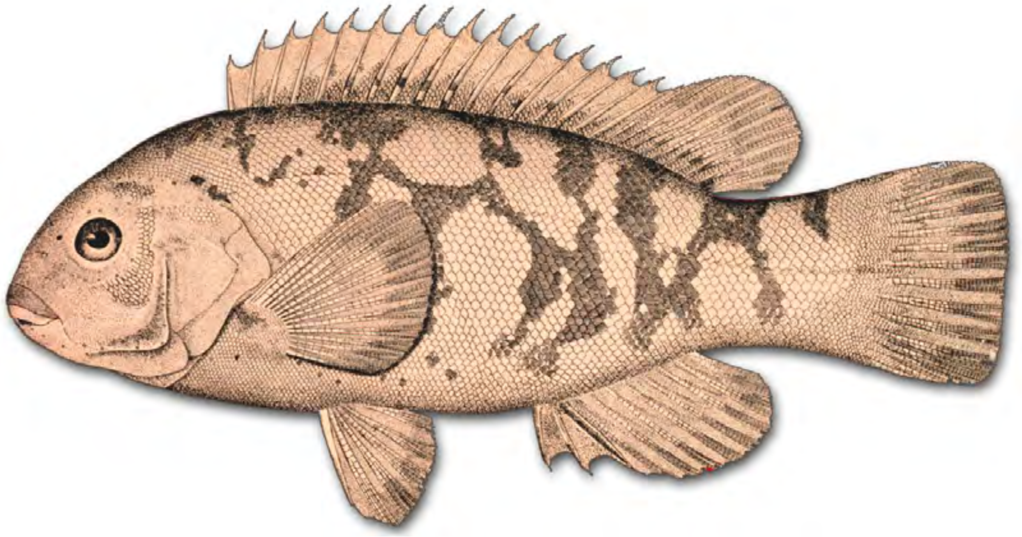
CHAPTER 13

TAUTOG *Tautoga onitis*



13.1 INTRODUCTION

We aged a total of 109 Tautog, using their opercula collected by the VMRC's Biological Sampling Program in 2020. Of 109 aged fish, 105 and 4 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 5.9 years with a standard deviation of 2.2 and a standard error of 0.21. Thirteen age classes (1, and 3 to 14) were represented in the bay fish, comprising fish from the 2006 to 2017, and 2019 year classes. The bay fish sample in 2020 was dominated by the year class of 2015 with 46%. There was one ocean fish in 2019 sample, it was Age 10 and from 2009 year class. We also aged a total of 109 fish using their otoliths in addition to ageing their opercula. The otolith ages were compared to the operculum ages to examine how close both ages were to one another (see details in Results).

13.2 METHODS

13.2.1 Sample size for ageing

We estimated sample sizes for ageing Tautog collected in both Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2020, 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:

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

where A is the sample size for ageing Tautog in 2020; θ_a stands for the proportion of age a fish in a catch. V_a and B_a represent variance components within and between length intervals for age a, respectively; CV is the coefficient of variation; L was the total number of Tautog used by VMRC to estimate length distribution of the catches from 2014 to 2018. θ_a, V_a, B_a, and CV were calculated using pooled age-length data of Tautog collected from 2014 to 2018 and using equations in Quinn and Deriso (1999). For simplicity, the equations are not listed here. The equation (13.1) indicates that the more fish that are aged, the smaller the CV (or higher precision) that will be obtained. Therefore, the criterion to age A (number) of fish is that A should be a number above which there is only a 1% CV reduction for the most major age in catch by aging 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 fish aged in the lab between 2014 and 2018. A_l is number of fish to be aged for length interval l in 2020.

13.2.2 Handling of collection

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

13.2.3 Preparation

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 CQFE website on how to prepare operculum for ageing Tautog.

Otoliths

We used our thin-section and bake technique to process Tautog 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$^{\text{TM}}$ 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 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.

In 2020 the new notation method recommended by ASMFC was not used to assign ages on Tautog for two reasons:

1. Growth widths among annuli on Tautog opercula don't grow uniformly unlike on Tautog otoliths, therefore, it is not practical to use the margin codes on the opercula;
2. Although the margin codes can be applied to Summer Tautog otoliths, it is more reasonable that the same ageing notation is used on both opercula and otoliths versus using two different ageing notations on opercula and otoliths, separately, keeping a consistent ageing notation between two hard-parts of the same species.

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, 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.

Opercula

All opercula were aged in chronological order based on collection date, without knowledge of the specimen lengths, using a light table with no magnification (Figure 13.1).

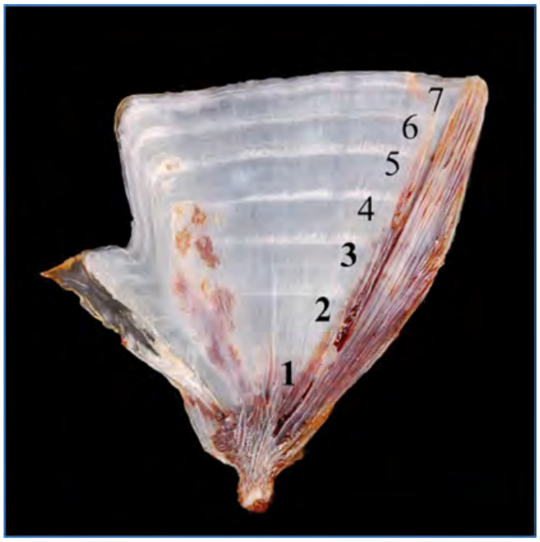


Figure 13.1: Operculum of a 7 year-old Tautog

Otoliths

All thin-sections were aged in chronological order based on collection date, without knowledge of the specimen lengths, using a Nikon SMZ1000 stereo microscope under transmitted light and dark-field polarization at between 8 and 20 times magnification (Figure 13.2).

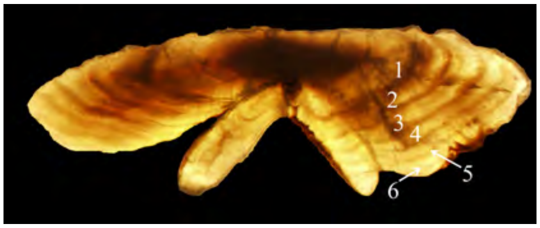


Figure 13.2: Otolith thin-section of 6 year-old Tautog

After Virginia state office closures on March 15, 2020 due to the COVID-19 virus, one of two readers had to switch from ageing opercula and otoliths to sectioning otoliths because other technicians had no equipment to section otoliths at home. As a result, all Tautog samples (opercula and sectioned otoliths) were aged by one reader only. Because there was one reader, the age estimated by the reader became the final age and was assigned to the fish.

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, within the reader, for the following comparisons: 1) in the current year and 2) time-series bias between the current and previous years; 3) between opercula and otoliths ages.. The readings from the entire sample for the current year were used to examine the difference and precision in the current. A random sub-sample of 50 fish from the current year was selected for second readings of the reader. Fifty otoliths randomly selected from the fish aged in 2000 were used to examine the time-series bias within the reader. A figure of 1:1 equivalence was used to illustrate the difference between opercula and otoliths ages (Campana et al. 1995). All statistics analyses were performed in R 3.6.1 (R Core Team 2019).

13.3 RESULTS

13.3.1 Sample size

We estimated a sample size of 430 bay Tautog in 2020, ranging in length intervals from 8 to 26 inches (Table 13.1). This sample size provided a range in *CV* for age composition approximately from the smallest *CV* of 8% for Age 5 to the largest *CV* of 20% for Age 3 of the bay fish. We aged all 105 Tautog collected by VMRC in Chesapeake Bay in 2020. We fell short in our over-all collections for this optimal length-class sampling estimate by 325 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.

We estimated a sample size of 401 ocean Tautog in 2020, ranging in length intervals from 14 to 30 inches (Table 13.2). This sample size provided a range in *CV* for age composition approximately from the smallest *CV* of 10% for Age 6 to the largest *CV* of 25% for Age 10 of the ocean fish. Only four Tautog was collected and aged in Virginia waters of Atlantic ocean, therefore, no ALK was developed for the ocean fish collected in 2020. We aged all 4 Tautog collected by VMRC in Virginia waters of the Atlantic Ocean in 2020. We fell short in our over-all collections for this optimal length-class sampling estimate by 397 fish. We were short many fish from the major length intervals (the interval requires 10 or more fish), as a result, the precision for the estimates of major age groups would definitely be influenced significantly. Therefore, precaution should be used when developing ALK using these age data.

13.3.2 Opercula

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 76% (1 year or less agreement of 98%) and a *CV* of 2.8% (test of symmetry: χ^2 = 7.33, df = 6, P = 0.2911), and there was no significant difference between the first and second readings for Reader 2 with an agreement of 94% (1 year or less agreement of 96%) and a *CV* of 0.94% (test of symmetry: χ^2 = 3, df = 3, P = 0.3916). There was no evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 76% (1 year or less agreement of 94%) and a *CV* of 3.43% (test of symmetry: χ^2 = 20.67, df = 12, P = 0.0555) (Figure 13.3).

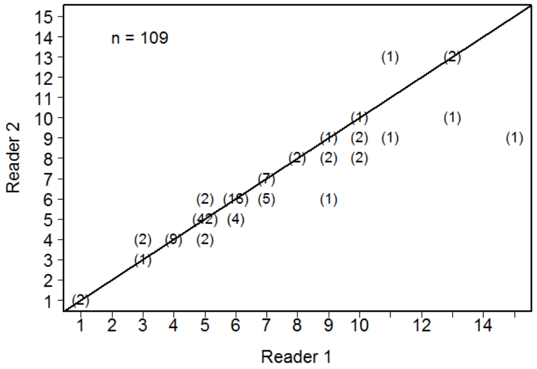


Figure 13.3: Between-reader comparison of operculum age estimates for Tautog collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2020.

There was no time-series bias for either reader. Reader 1 had an agreement of 64% (1 year or less agreement of 98%) with ages of fish aged in 2000 with a *CV* of 4.92% (test of symmetry: χ^2 = 7, df = 8, P = 0.5366), and Reader 2 had an agreement of 72% (1 year or less agreement of 96%) with a *CV* of 4.1% (test of symmetry: χ^2 = 8, df = 9, P = 0.5341).

Of the 105 bay Tautog aged with opercula, 13 age classes (1, and 3 to 14) were represented (Table 13.3). The average age for the sample was 5.9 years. The standard deviation and standard error were 2.2 and 0.21, respectively. Year-class data (Figure 13.4) indicates that recruitment into the fishery in Chesapeake Bay begins at age 1, which corresponds to the 2019 year-class for Tautog caught in 2020. Tautog in the sample in 2020 was dominated by the year class of 2015 with 46%. The sex ratio of male to female was 1:1.54 for the bay fish.

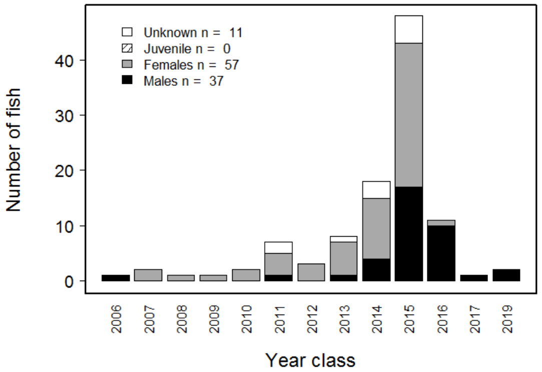


Figure 13.4: Year-class frequency distribution for Tautog collected in Chesapeake Bay, Virginia for ageing in 2020. Distribution is broken down by sex and estimated using operculum ages. 'Unknown' represents the fish gonads that were not available for examination or were not examined for sex during sampling.

Of the 4 ocean Tautog aged with opercula, 4 age classes (5 to 6, 8, and 12) were represented (Table 13.4). The average age for the sample was 7.8 years. The standard deviation and standard error were 3.1 and 1.55, respectively. Year-class data (Figure 13.5) indicates that recruitment into the fishery in Virginia waters of Atlantic ocean begins at age 5, which corresponds to the 2015 year-class for Tautog caught in 2020. Tautog in the sample in 2020 was dominated by the year class of 0 with 0%. The sex ratio of male to female was 1:1 for the ocean fish.

13.3.3 Otoliths

Both readers had high self-precision. Specifically, there was no significant difference between the first and second readings for

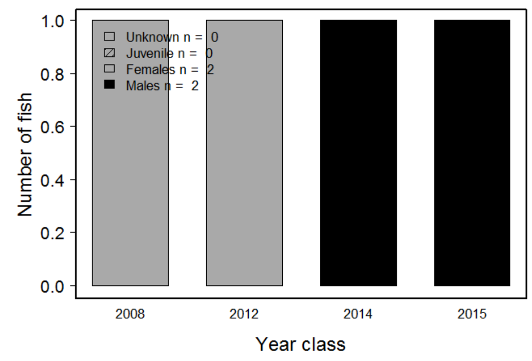


Figure 13.5: Year-class frequency distribution for Tautog collected in Virginia waters of the Atlantic Ocean for ageing in 2020. Distribution is broken down by sex and estimated using operculum ages. 'Unknown' represents the fish gonads that were not available for examination or were not examined for sex during sampling.

Reader 1 with an agreement of 96% and a *CV* of 0.25% (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 0.53% (test of symmetry: $\chi^2 = 3$, $df = 3$, $P = 0.3916$). There was no evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 94% (1 year or less agreement of 99%) and a *CV* of 0.87% (test of symmetry: $\chi^2 = 7$, $df = 5$, $P = 0.2206$) (Figure 13.6).

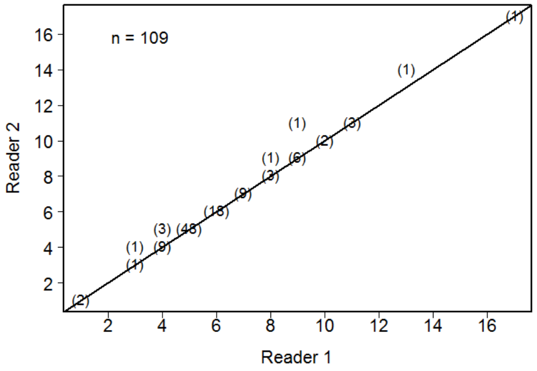


Figure 13.6: Between-reader comparison of otolith age estimates for Tautog collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2020.

There was no time-series bias for either reader. Reader 1 had an agreement of 86% with ages of fish aged in 2003 with a *CV* of 1.57% (test of symmetry: $\chi^2 = 5$, $df = 3$, $P = 0.1718$), and Reader 2 had an agreement of 90% with a *CV* of 1.15% (test of symmetry: $\chi^2 = 5$, $df = 2$, $P = 0.0821$).

Of the 109 Tautog aged with otoliths, 12 age classes (1, 3 to 11, 13, and 17) were represented (Table 13.5). The average age for the sample was 5.9 years. The standard deviation and standard error were 2.2 and 0.21, respectively.

13.3.4 Comparison of operculum and otolith ages

We aged 109 Tautog using both opercula and otoliths. There was no evidence of systematic disagreement between otolith and operculum ages (test of symmetry: $\chi^2 = 14.67$, $df = 13$, $P = 0.3286$) with an average *CV* of 3.51%. There was an agreement of 76% between operculum and otoliths ages whereas opercula were assigned a lower and higher age than otoliths for 8% and 16% of the fish, respectively (Figure 13.7). There was also little evidence of bias between otolith and operculum ages using an age bias plot(Figure 13.8), with no trend of either over-ageing younger or under-ageing older fish.

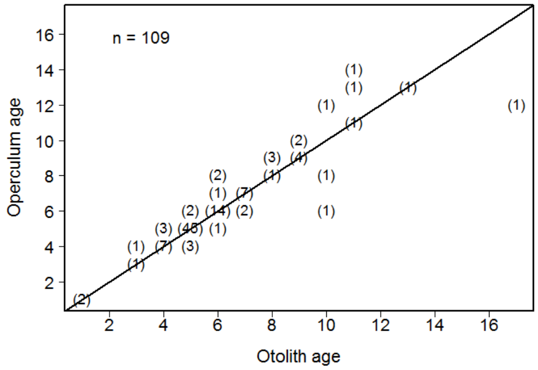


Figure 13.7: Comparison of paired operculum and otolith age estimates for Tautog collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2020.

13.3.5 Age-Length-Key (ALK)

We developed an age-length-key for the bay fish (Table 13.6). The ALK can be used in the conversion of numbers-at-length in the estimated catch to numbers-at-age using operculum ages. The table is based on VMRC's stratified sampling of landings by total length inch intervals.

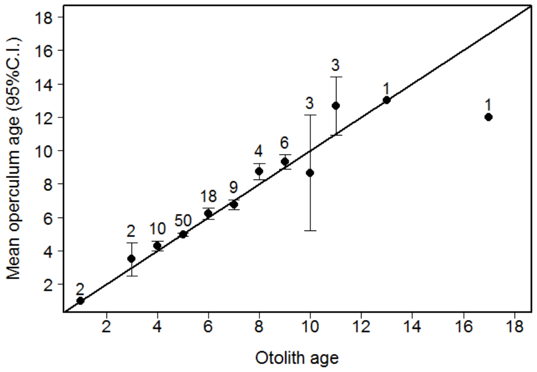


Figure 13.8: Age-bias plot for Tautog operculum and otolith age estimates in 2020.

Table 13.1: Number of bay Tautog collected and aged in each 1-inch length interval in 2020. 'Target' represents the sample size for ageing estimated for 2020, 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	1	4
9 - 9.99	5	0	1	4
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	1	4
14 - 14.99	31	0	21	10
15 - 15.99	100	0	30	70
16 - 16.99	102	0	26	76
17 - 17.99	68	0	15	53
18 - 18.99	39	0	5	34
19 - 19.99	22	0	2	20
20 - 20.99	8	0	2	6
21 - 21.99	5	0	0	5
22 - 22.99	5	0	0	5
23 - 23.99	5	0	0	5
24 - 24.99	5	0	1	4
25 - 25.99	5	0	0	5
26 - 26.99	5	0	0	5
Totals	430	0	105	325

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Table 13.2: Number of ocean Tautog collected and aged in each 1-inch length interval in 2020. 'Target' represents the sample size for ageing estimated for 2020, and 'Need' represents number of fish shorted in each length interval compared to the optimum sample size for ageing and number of fish aged.

Interval	Target	Collected	Aged	Need
14 - 14.99	5	0	0	5
15 - 15.99	30	0	0	30
16 - 16.99	43	0	1	42
17 - 17.99	43	0	1	42
18 - 18.99	25	0	0	25
19 - 19.99	43	0	0	43
20 - 20.99	30	0	1	29
21 - 21.99	33	0	0	33
22 - 22.99	23	0	0	23
23 - 23.99	23	0	0	23
24 - 24.99	23	0	1	22
25 - 25.99	20	0	0	20
26 - 26.99	15	0	0	15
27 - 27.99	25	0	0	25
28 - 28.99	10	0	0	10
29 - 29.99	5	0	0	5
30 - 30.99	5	0	0	5
Totals	401	0	4	397

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Table 13.3: The number of Tautog assigned to each total length-at-age category for 105 fish sampled for operculum age determination in Chesapeake Bay, Virginia during 2020.

	Age													
Interval	1	3	4	5	6	7	8	9	10	11	12	13	14	Totals
8 - 8.99	1	0	0	0	0	0	0	0	0	0	0	0	0	1
9 - 9.99	1	0	0	0	0	0	0	0	0	0	0	0	0	1
13 - 13.99	0	0	0	1	0	0	0	0	0	0	0	0	0	1
14 - 14.99	0	0	3	15	2	1	0	0	0	0	0	0	0	21
15 - 15.99	0	1	3	20	3	1	1	0	0	1	0	0	0	30
16 - 16.99	0	0	3	6	8	4	2	1	2	0	0	0	0	26
17 - 17.99	0	0	2	5	4	1	0	3	0	0	0	0	0	15
18 - 18.99	0	0	0	1	0	1	0	1	0	0	1	1	0	5
19 - 19.99	0	0	0	0	0	0	0	2	0	0	0	0	0	2
20 - 20.99	0	0	0	0	1	0	0	0	0	0	0	0	1	2
24 - 24.99	0	0	0	0	0	0	0	0	0	0	0	1	0	1
Totals	2	1	11	48	18	8	3	7	2	1	1	2	1	105

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Table 13.4: The number of Tautog assigned to each total length-at-age category for 4 fish sampled for operculum age determination in Virginia waters of Atlantic ocean during 2020.

	Age				
Interval	5	6	8	12	Totals
16 - 16.99	1	0	0	0	1
17 - 17.99	0	1	0	0	1
20 - 20.99	0	0	1	0	1
24 - 24.99	0	0	0	1	1
Totals	1	1	1	1	4

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Table 13.5: The number of Tautog assigned to each total length-at-age category for 109 fish sampled for otolith age determination in Chesapeake Bay and Virginia waters of Atlantic Ocean during 2020.

						Age							
Interval	1	3	4	5	6	7	8	9	10	11	13	17	Totals
8 - 8.99	1	0	0	0	0	0	0	0	0	0	0	0	1
9 - 9.99	1	0	0	0	0	0	0	0	0	0	0	0	1
13 - 13.99	0	0	0	1	0	0	0	0	0	0	0	0	1
14 - 14.99	0	0	3	15	2	1	0	0	0	0	0	0	21
15 - 15.99	0	1	6	17	4	1	0	0	0	1	0	0	30
16 - 16.99	0	0	1	10	8	3	1	3	1	0	0	0	27
17 - 17.99	0	1	0	6	4	2	1	2	0	0	0	0	16
18 - 18.99	0	0	0	1	0	1	1	0	1	1	0	0	5
19 - 19.99	0	0	0	0	0	0	1	1	0	0	0	0	2
20 - 20.99	0	0	0	0	0	1	0	0	1	1	0	0	3
24 - 24.99	0	0	0	0	0	0	0	0	0	0	1	1	2
Totals	2	2	10	50	18	9	4	6	3	3	1	1	109

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Table 13.6: Age-Length key, as proportion-at-age in each 1-inch length interval, based on operculum ages for Tautog sampled in Chesapeake Bay, Virginia during 2020.

	Age												
Interval	1	3	4	5	6	7	8	9	10	11	12	13	14
8 - 8.99	1	0	0	0	0	0	0	0	0	0	0	0	0
9 - 9.99	1	0	0	0	0	0	0	0	0	0	0	0	0
13 - 13.99	0	0	0	1	0	0	0	0	0	0	0	0	0
14 - 14.99	0	0	0.14	0.71	0.1	0.05	0	0	0	0	0	0	0
15 - 15.99	0	0.03	0.1	0.67	0.1	0.03	0.03	0	0	0.03	0	0	0
16 - 16.99	0	0	0.12	0.23	0.31	0.15	0.08	0.04	0.08	0	0	0	0
17 - 17.99	0	0	0.13	0.33	0.27	0.07	0	0.2	0	0	0	0	0
18 - 18.99	0	0	0	0.2	0	0.2	0	0.2	0	0	0.2	0.2	0
19 - 19.99	0	0	0	0	0	0	0	1	0	0	0	0	0
20 - 20.99	0	0	0	0	0.5	0	0	0	0	0	0	0	0.5
24 - 24.99	0	0	0	0	0	0	0	0	0	0	0	1	0

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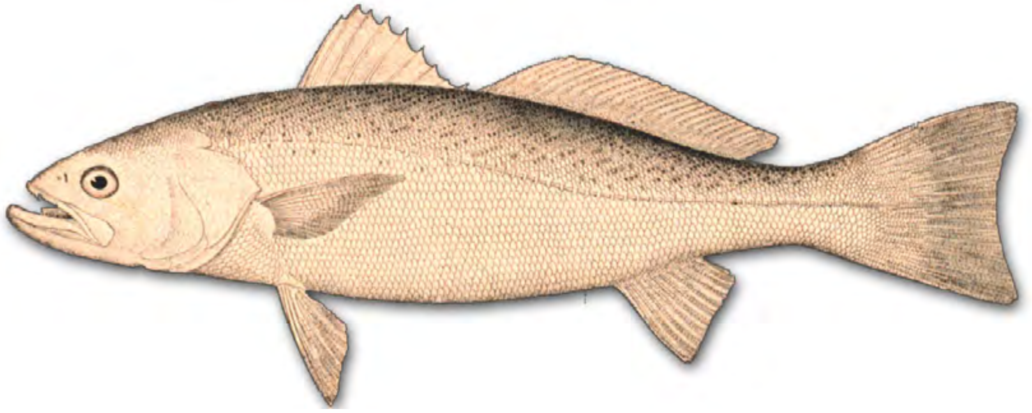
CHAPTER 14

WEAKFISH *Cynoscion regalis*



14.1 INTRODUCTION

We aged a total of 246 Weakfish, *Cynoscion regalis*, collected by the VMRC's Biological Sampling Program for age and growth analysis in 2020. The Weakfish ages ranged from 0 to 5 years old with an average age of 2.3, a standard deviation of 0.7, and a standard error of 0.04. Six age classes (0 to 5) were represented, comprising fish of the 2015 to 2020 year-classes. The sample was dominated by fish from the year-classes of 2017 and 2018 with 36.6% and 50.8%, respectively.

14.2 METHODS

14.2.1 Sample size for ageing

We estimated sample size for ageing Weakfish in 2020 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:

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

where A is the sample size for ageing Weakfish in 2020; θ_a stands for the proportion of age a fish in a catch. V_a and B_a represent variance components within and between length intervals for age a, respectively; CV is the coefficient of variation; L was the total number of Weakfish used by VMRC to estimate length distribution of the catches from 2014 to 2018. θ_a, V_a, B_a, and CV were calculated using pooled age-length data of Weakfish collected from 2014 to 2018 and using equations in Quinn and Deriso (1999). For simplicity, the equations are not listed here. The equation (14.1) indicates that the more fish that are aged, the smaller the CV (or higher precision) that will be obtained. Therefore, the criterion to age A (number) of fish is that A should be a number above which there is only a 1% CV reduction for the most major age in catch by aging 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 fish aged in the lab between 2014 and 2018. A_l is number of fish to be aged for length interval l in 2020.

14.2.2 Handling of collections

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

14.2.3 Preparation

Sagittal otoliths, hereafter, referred to as "otoliths", were processed for age determination following the methods described in Lowerre-Barbieri et al. (1994) with a few modifications. The left or right otolith was randomly selected and attached, distal side down, to a glass slide with clear Crystalbond$^{\text{TM}}$ 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$^{\text{TM}}$ 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 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).

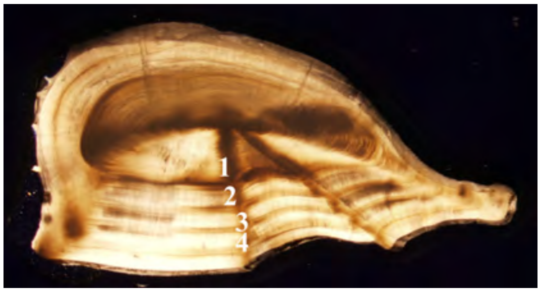


Figure 14.1: Otolith thin-section of 4 year-old Weakfish

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, within the reader, for the following comparisons: 1) in the current year and 2) time-series bias between the current and previous years. The readings from the entire sample for the current year were used to examine the difference and precision in the current. A random sub-sample of 50 fish from the current year was selected for second readings of the reader. Fifty otoliths randomly selected from the fish aged in 2003 were used to examine the time-series bias within the reader. All statistics analyses were performed in R 3.6.1 (R Core Team 2019).

14.3 RESULTS

14.3.1 Sample size

We estimated a sample size of 345 for ageing Weakfish in 2020, ranging in length intervals 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 0.06% for Age 2 to the largest (CV) of 0.18% for Age 4. In 2020, we aged 246 of 264 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 over-all collections for this optimal length-class sampling estimate by 114 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.

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 100%. There was no evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 99.59% and a *CV* of 0.08% (test of symmetry: $\chi^2 = 1$, $df = 1$, $P = 0.3173$) (Figure 14.2).

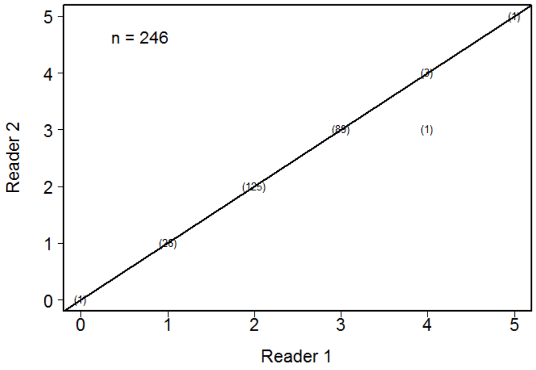


Figure 14.2: Between-reader comparison of otolith age estimates for Weakfish collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2020.

There was no time-series bias for either reader. Reader 1 had an agreement of 96% with ages of fish aged in 2003 with a *CV* of 0.57% (test of symmetry: $\chi^2 = 2$, $df = 2$, $P = 0.3679$), and Reader 2 had an agreement of 98% with a *CV* of 0.19% (test of symmetry: $\chi^2 = 1$, $df = 1$, $P = 0.3173$).

14.3.3 Year class

Of the 246 fish aged with otoliths, 6 age classes (0 to 5) were represented (Table 14.2). The average age was 2.3 years, and the standard deviation and standard error were 0.7 and 0.04, respectively. Year-class data show that the fishery was comprised of 6 year-classes: fish from the 2015 to 2020 year-classes, with fish primarily from the year-classes of 2017 and 2018 with 36.6% and 50.8%, respectively.

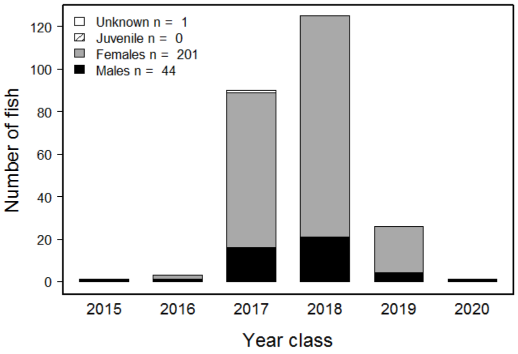


Figure 14.3: Year-class frequency distribution for Weakfish collected for ageing in 2020. Distribution is broken down by sex. 'Unknown' represents gonads that were not available for examination or were not examined for sex during sampling.

The ratio of males to females was 1:4.57 in the sample collected (Figure 14.3).

14.3.4 Age-length-key (ALK)

We developed an age-length-key (Table 14.3) 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 2020. 'Target' represents the sample size for ageing estimated for 2020, 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	0	0	5
8 - 8.99	8	13	13	0
9 - 9.99	31	34	34	0
10 - 10.99	57	34	34	23
11 - 11.99	43	34	34	9
12 - 12.99	32	35	32	0
13 - 13.99	22	27	22	0
14 - 14.99	15	19	17	0
15 - 15.99	18	24	18	0
16 - 16.99	14	16	14	0
17 - 17.99	9	8	8	1
18 - 18.99	6	9	9	0
19 - 19.99	5	7	7	0
20 - 20.99	5	1	1	4
21 - 21.99	5	0	0	5
22 - 22.99	5	0	0	5
23 - 23.99	5	0	0	5
24 - 24.99	5	0	0	5
25 - 25.99	5	0	0	5
26 - 26.99	5	0	0	5
27 - 27.99	5	0	0	5
28 - 28.99	5	1	1	4
29 - 29.99	5	1	1	4
30 - 30.99	5	1	1	4
31 - 31.99	5	0	0	5
33 - 33.99	5	0	0	5
34 - 34.99	5	0	0	5
Totals	345	264	246	114

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Table 14.2: The number of Weakfish assigned to each total length-at-age category for 246 fish sampled for otolith age determination in Virginia during 2020.

	Age						
Interval	0	1	2	3	4	5	Totals
8 - 8.99	1	9	3	0	0	0	13
9 - 9.99	0	7	27	0	0	0	34
10 - 10.99	0	6	26	2	0	0	34
11 - 11.99	0	3	27	4	0	0	34
12 - 12.99	0	1	18	13	0	0	32
13 - 13.99	0	0	10	12	0	0	22
14 - 14.99	0	0	5	12	0	0	17
15 - 15.99	0	0	3	13	2	0	18
16 - 16.99	0	0	0	14	0	0	14
17 - 17.99	0	0	1	6	1	0	8
18 - 18.99	0	0	2	7	0	0	9
19 - 19.99	0	0	1	6	0	0	7
20 - 20.99	0	0	0	1	0	0	1
28 - 28.99	0	0	0	0	0	1	1
29 - 29.99	0	0	1	0	0	0	1
30 - 30.99	0	0	1	0	0	0	1
Totals	1	26	125	90	3	1	246

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Table 14.3: 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 2020.

	Age					
Interval	0	1	2	3	4	5
8 - 8.99	0.08	0.69	0.23	0	0	0
9 - 9.99	0	0.21	0.79	0	0	0
10 - 10.99	0	0.18	0.76	0.06	0	0
11 - 11.99	0	0.09	0.79	0.12	0	0
12 - 12.99	0	0.03	0.56	0.41	0	0
13 - 13.99	0	0	0.45	0.55	0	0
14 - 14.99	0	0	0.29	0.71	0	0
15 - 15.99	0	0	0.17	0.72	0.11	0
16 - 16.99	0	0	0	1	0	0
17 - 17.99	0	0	0.12	0.75	0.12	0
18 - 18.99	0	0	0.22	0.78	0	0
19 - 19.99	0	0	0.14	0.86	0	0
20 - 20.99	0	0	0	1	0	0
28 - 28.99	0	0	0	0	0	1
29 - 29.99	0	0	1	0	0	0
30 - 30.99	0	0	1	0	0	0

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