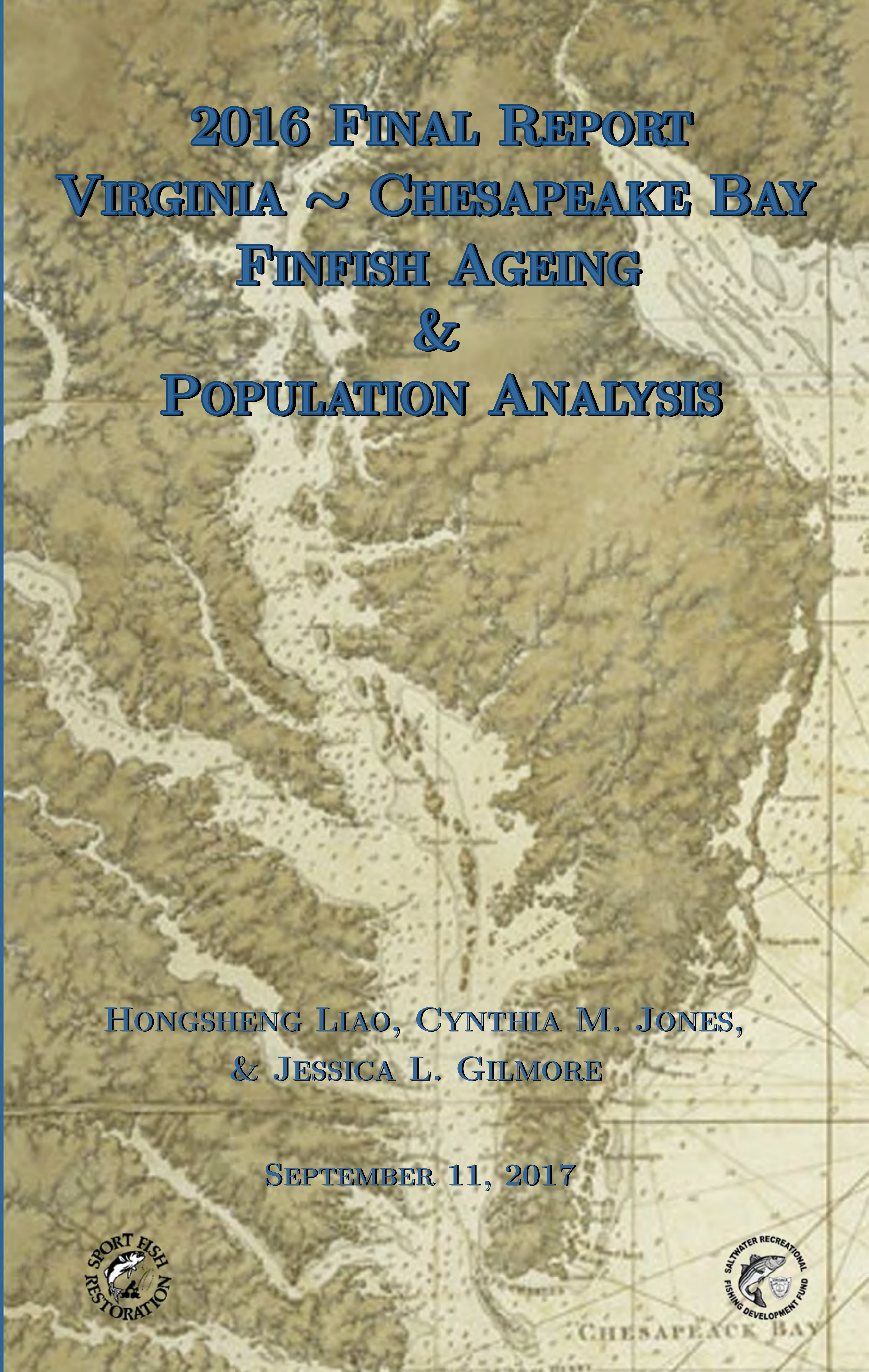
2016 Final Report
Virginia ~ Chesapeake Bay
Finfish Ageing
&
Population Analysis

Hongsheng Liao, Cynthia M. Jones,
& Jessica L. Gilmore

September 11, 2017

VMRC ~ ODU Age and Growth Laboratory
Center for Quantitative Fisheries Ecology
Old Dominion University
Norfolk, Virginia 23508



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Center for Quantitative Fisheries Ecology
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EXECUTIVE SUMMARY

In this report 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 2016. All fish were collected by the Virginia Marine Resources Commission's (VMRC) Stock Assessment Program and the Center for Quantitative Fisheries Ecology (CQFE) at Old Dominion University in 2016 and aged in 2017 at the Age and Growth Laboratory of CQFE. This report is broken into chapters, one for each of the 14 species. 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 = 880); summer flounder, *Paralichthys dentatus*, (n = 659); and tautog, *Tautoga onitis*, (n = 223). 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 = 346); black drum, *Pogonias cromis*, (n = 480); bluefish, *Pomatomus saltatrix*, (n = 424); cobia, *Rachycentron canadum*, (n = 255); red drum, *Sciaenops ocellatus*, (n = 19); sheepshead, *Archosargus probatocephalus*, (n = 130); Atlantic spadefish, *Chaetodipterus faber*, (n = 278); Spanish mackerel, *Scomberomorous maculates*, (n = 220); spot, *Leiostomus xanthurus*, (n = 248); spotted seatrout, *Cynoscion nebulosus*, (n = 239); and weakfish, *Cynoscion regalis*, (n = 284). In total, we made 10,860 age readings from scales, otoliths and opercula collected during 2016. A summary of the age ranges for all species aged is presented in Table 1.

In this report, we also 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.

To support environmental and wildlife agencies, and charities, we donated more than 2,436 pounds of dissected fish to the Salvation Army to feed the homeless, and the Wildlife Response, Inc., a local wildlife rescue agency which is responsible for saving injured animals found by the public.

In 2016, we continued to update our CQFE website, which can be accessed at http://odu.edu/sci/research/cqfe/. The website includes an electronic version of this document and our previous VMRC final reports from 2001 to 2015. The site also provides more detailed explanations of the methods and structures we use in age determination. In 2016, we posted three videos on the website, showing how to extract the otoliths of weakfish and tautog, and tautog opercula, respectively.

In order to share the VMRC/ODU data and findings with the stakeholders and other fisheries biologists, in 2016, we developed two more website applications (apps). One is called "Fish Growth Curve Predictor" (Click here to open the app). This app uses the length-at-age data to estimate the growth of each species, compare the growth between males and females, and between two years within each species. We have posted this app at the CQFE website. Another one is called "Data Sharing App" (Click here to open the app). This app allows users to download the database of each species collected from multiple years in Virginia. This app is not posted at the CQFE website, therefore, any potential users need to email their requests to VMRC (Contact person: Mr. Joe Cimino at Joe.Cimino@vmrc.virginia.gov). Once VMRC proves the requests, CQFE will email usernames and passwords to the requesters for using the app.

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 2016. The hard-parts and age readings include both scales and otoliths for striped bass and summer flounder, and both opercula and otoliths for tautog.

Species	Number of fish collected	Number of hard-parts	Number of fish aged	Number of readings	Minimum age	Maximum age
Atlantic Croaker	462	462	346	692	1	9
Black Drum	482	482	480	960	1	52
Bluefish	657	657	424	848	0	13
Cobia	257	255	255	510	2	12
Red Drum	19	19	19	38	0	29
Sheepshead	135	134	130	260	1	28
Spadefish	384	384	278	556	1	9
Spanish Mackerel	304	304	220	440	1	6
Spotted Seatrout	314	314	239	478	0	5
Spot	310	310	248	496	0	4
Striped Bass	1,182	1,496	880	2,400	2	23
Summer Flounder	849	1,050	659	1,726	1	11
Tautog	224	444	223	888	3	27
Weakfish	410	410	284	568	1	4
Totals	5,989	6,721	4,685	10,860		

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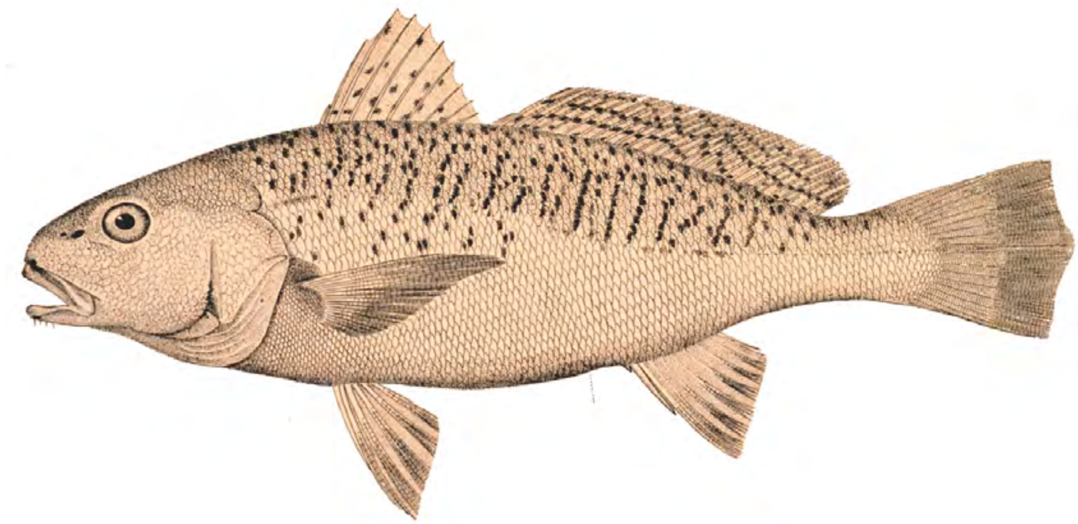
ACKNOWLEDGEMENTS

We thank James Black, Alicia Brown, Alex Gikakis, and Allison Roberts for their technical expertise in preparing otoliths, scales, and opercula for age determination. They all put in long hours processing "tons" of fish in our lab. A special note of appreciation is extended to Joe Cimino, Adam Kenyon, and their technicians at the VMRC, including Richard Hancock, Myra Thompson, and Chris Williams for their many efforts in this cooperative project. We would like also to thank our graduate students Kathleen Kirch and Mike Schmidtke for their help in processing fish whenever we were short of hands.

Chapter 1

ATLANTIC CROAKER
Micropogonias undulatus



1.1 INTRODUCTION

We aged a total of 346 Atlantic croaker, *Micropogonias undulatus*, collected by the VMRC's Biological Sampling Program for age and growth analysis in 2016. Croaker ages ranged from 1 to 9 years old with an average age of 4.1, a standard deviation of 1.1, and a standard error of 0.06. Nine age classes (1 to 9) were represented, comprising fish of the 2007 to 2015 year-classes. The sample was dominated by fish from the year-class of 2012 with 72%.

1.2 METHODS

1.2.1 Sample size for ageing

We estimated sample size for ageing croaker in 2016 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 2016; 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 2010 to 2014. θ_a, V_a, B_a, and CV were calculated using pooled age-length data of croaker collected from 2010 to 2014 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 2010 to 2014 catch. A_l is number of fish to be aged for length interval l in 2016.

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. (1994) with a few modifications. The left or right otolith was randomly selected and attached, distal side down, to a glass slide with clear Crystalbond™ 509 adhesive 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™ 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 CQFE 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. The number of annuli replaces "x" in our notation below, and is the initial "age" assignment of the fish.

Second, the thin-section is examined for translucent growth. If no translucent growth is visible beyond the last 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 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 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 annuli in the thin-section. 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 end of annulus deposition period, is interpreted as being toward the next age class.

For example, Atlantic croaker otolith annulus formation occurs between April and May (Barbieri et al. 1994a and b). A croaker captured between January 1 and May 31, before the end of the species' annulus deposition period, with three visible annuli and some translucent growth after the last annulus, would be assigned an age class of "x+(x+1)" or 3+(3+1), noted as 3+4. This is the same age-class assigned to a fish with four visible annuli captured after the end of May 31, the period of annulus deposition, which would be noted as 4+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. Each reader aged all of the otolith samples.

Due to discrepancy on identification of the first annulus of Atlantic croaker among Atlantic states, Atlantic States Marine Fisheries Commission (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 2016 (Figure 1.1) .

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

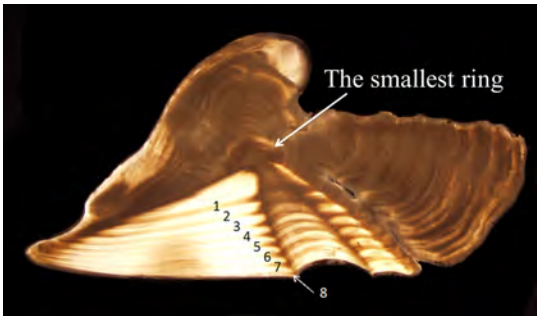


Figure 1.1: Otolith thin-sections of a 8 year-old croaker without counting the smallest ring and with the last annulus on the edge of the thin-section

fish. When the two readers disagreed, both readers sat down together and re-aged the fish, again without any knowledge of previously estimated ages or lengths, and assigned a final age to the fish. When the readers were unable to agree on a final age, the fish was excluded from further analysis.

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

1.2.5 Comparison tests

A symmetry test (Hoenig et al. 1995) and coefficient of variation (CV) analysis were used to detect any systematic difference and precision on age readings, respectively, for the following comparisons: 1) between the two readers in the current year, 2) within each reader in the current year, and 3) time-series bias between the current and previous years within each reader. The readings from the entire sample for the current year were used to examine the difference between two readers. A random sub-sample of 50 fish from the current year was selected for second readings to examine the difference within a reader. Fifty otoliths randomly selected from fish aged in 2003 were used to examine the time-series bias within each reader. A figure of 1:1 equivalence was used to illustrate those differences (Campana et al. 1995). All statistics analyses were performed in R 3.2.2 (R Core Team 2012).

1.3 RESULTS

1.3.1 Sample size

We estimated a sample size of 440 Atlantic croaker in 2016, ranging in length interval from 6 to 19 inches (Table 1.1). This sample size provided a range in (CV) for age composition approximately from the smallest (CV) of 8% for age 4 to the largest (CV) of 19% for age 7. In 2016, we randomly selected and aged 346 fish from 462 croaker collected by VMRC. We fell short in our over-all collections for this optimal length-class sampling estimate by 140 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 88% and a CV of 1.7% (test of symmetry: $\chi^2 = 3$, $df = 3$, $P = 0.3916$), and there was no significant difference between the first and second readings for Reader 2 with an agreement of 98% and a CV of 0.3% (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 96.24% and a CV of 0.6% (test of symmetry: $\chi^2 = 4.78$, $df = 4$, $P = 0.3109$) (Figure 1.2).

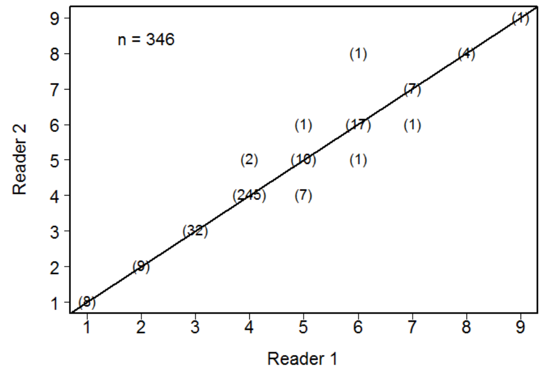


Figure 1.2: Between-reader comparison of otolith age estimates for Atlantic croaker collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2016.

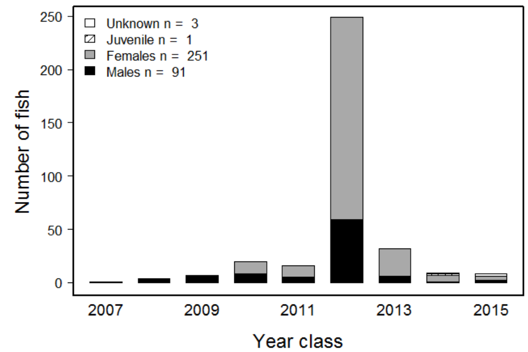


Figure 1.3: Year-class frequency distribution for Atlantic croaker collected for ageing in 2016. Distribution is broken down by sex. 'Unknown' is for gonads that were not available for examination or were not examined for sex during sampling.

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

1.3.3 Year class

Of the 346 fish aged with otoliths, 9 age classes (1 to 9) were represented (Table 1.2). The average age was 4.1 years, and the standard deviation and standard error were 1.1 and 0.06, respectively. Year-class data show that the fishery was comprised of 9 year-classes: fish from the 2007 to 2015 year-classes, with fish primarily from the year class of 2012 with 72%. The ratio of males to females was 1:2.76 in the sample collected (Figure 1.3).

1.3.4 Age-length key

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.

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Table 1.1: Number of Atlantic croaker collected and aged in each 1-inch length interval in 2016. 'Target' represents the sample size for ageing estimated for 2016, 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
6 - 6.99	5	11	5	0
7 - 7.99	8	14	8	0
8 - 8.99	7	21	8	0
9 - 9.99	20	63	20	0
10 - 10.99	31	100	52	0
11 - 11.99	55	79	79	0
12 - 12.99	101	92	92	9
13 - 13.99	74	54	54	20
14 - 14.99	58	26	26	32
15 - 15.99	37	2	2	35
16 - 16.99	21	0	0	21
17 - 17.99	13	0	0	13
18 - 18.99	5	0	0	5
19 - 19.99	5	0	0	5
Totals	440	462	346	140

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Table 1.2: The number of Atlantic croaker assigned to each total length-at-age category for 346 fish sampled for otolith age determination in Virginia during 2016.

	Age									
Interval	1	2	3	4	5	6	7	8	9	Totals
6 - 6.99	4	1	0	0	0	0	0	0	0	5
7 - 7.99	2	6	0	0	0	0	0	0	0	8
8 - 8.99	1	0	3	4	0	0	0	0	0	8
9 - 9.99	0	1	4	15	0	0	0	0	0	20
10 - 10.99	0	1	7	35	4	3	2	0	0	52
11 - 11.99	0	0	4	62	6	4	2	1	0	79
12 - 12.99	0	0	3	75	3	10	1	0	0	92
13 - 13.99	0	0	3	40	3	3	1	3	1	54
14 - 14.99	1	0	7	17	0	0	1	0	0	26
15 - 15.99	0	0	1	1	0	0	0	0	0	2
Totals	8	9	32	249	16	20	7	4	1	346

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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 2016.

	Age								
Interval	1	2	3	4	5	6	7	8	9
6 - 6.99	0.8	0.2	0	0	0	0	0	0	0
7 - 7.99	0.25	0.75	0	0	0	0	0	0	0
8 - 8.99	0.12	0	0.38	0.5	0	0	0	0	0
9 - 9.99	0	0.05	0.2	0.75	0	0	0	0	0
10 - 10.99	0	0.02	0.13	0.67	0.08	0.06	0.04	0	0
11 - 11.99	0	0	0.05	0.78	0.08	0.05	0.03	0.01	0
12 - 12.99	0	0	0.03	0.82	0.03	0.11	0.01	0	0
13 - 13.99	0	0	0.06	0.74	0.06	0.06	0.02	0.06	0.02
14 - 14.99	0.04	0	0.27	0.65	0	0	0.04	0	0
15 - 15.99	0	0	0.5	0.5	0	0	0	0	0

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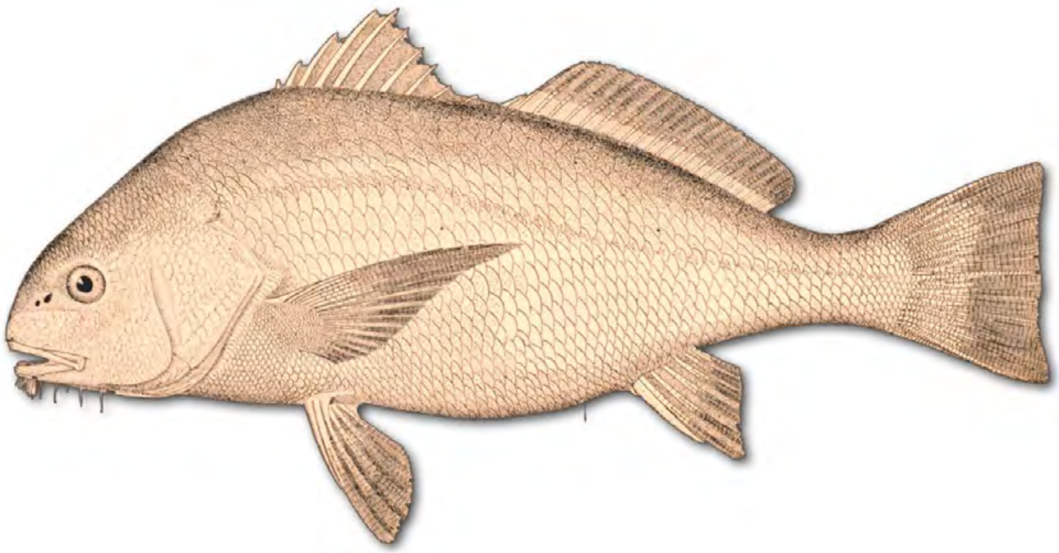
Chapter 2

BLACK DRUM *Pogonias cromis*



2.1 INTRODUCTION

We aged a total of 480 black drum, *Pogonias cromis*, collected by the VMRC's Biological Sampling Program for age and growth analysis in 2016. Black drum ages ranged from 1 to 52 years old with an average age of 12.6, a standard deviation of 7.1, and a standard error of 0.32. Thirty-seven age classes (1 to 23, 25 to 29, 31, 40 to 45, 47, and 52) were represented, comprising fish of the 1964, 1969, 1971 to 1976, 1985, 1987 to 1991, and 1993 to 2015 year-classes. The sample was dominated by fish from the year-classes of 2007 and 2006 with 17.5% and 16%, respectively.

2.2 METHODS

2.2.1 Handling of collections

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

2.2.2 Preparation

Otoliths were processed for age determination following the methods described in Bobko (1991) and Jones and Wells (1998). The left or right sagittal otolith was randomly selected and attached, distal side down, to a glass slide with Crystalbond™ 509 adhesive or embedded in epoxy. The otoliths were viewed by eye, and when necessary, under a stereo microscope to identify the location of the core, and the position of the core marked using a pencil across the otolith surface. At least one transverse cross-section (hereafter "thin-section") was then removed from the marked core of each otolith using a Buehler IsoMet™ low-speed saw equipped with two, three inch diameter, Norton Diamond Grinding Wheels, separated by a stainless steel spacer of 0.5 mm (diameter 2.5"). The position of the marked core fell within the 0.5 mm space between the blades, such that the core was included in the removed thin-section. Otolith thin-sections were placed on labeled glass slides and covered with a thin layer of Flo-texx mounting medium that not only fixed the sections to the slide, but more importantly, provided enhanced contrast and greater readability by increasing light transmission through the sections.

Click here to obtain the protocol at the CQFE website on how to prepare otolith thin-section for ageing black drum.

2.2.3 Readings

The CQFE 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. The number of annuli replaces "x" in our notation below, and is the initial "age" assignment of the fish.

Second, the thin-section is examined for translucent growth. If no translucent growth is visible beyond the last 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 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 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 annuli in the thin-section. 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 end of annulus deposition period, is interpreted as being toward the next age class.

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 captured between January 1 and June 30, before the end of the species' annulus deposition period, with three visible annuli and some translucent growth after the last annulus, would be assigned an age class of "x+(x+1)" or 3+(3+1), noted as 3+4. This is the same age-class assigned to a fish with four visible annuli captured after the end of May 31, the period of annulus deposition, which would be noted as 4+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 2.1). Each reader aged all of the otolith samples.

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.

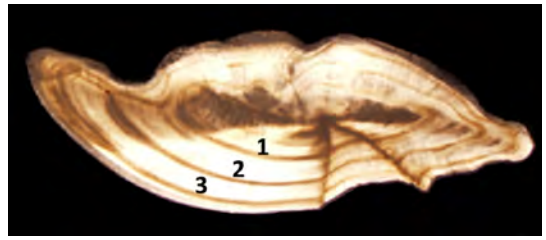


Figure 2.1: Otolith thin-section of a 3 year-old black drum

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

2.2.4 Comparison tests

A symmetry test (Hoenig et al. 1995) and coefficient of variation (*CV*) analysis were used to detect any systematic difference and precision on age readings, respectively, for the following comparisons: 1) between the two readers in the current year, 2) within each reader in the current year, and 3) time-series bias between the current and previous years within each reader. The readings from the entire sample for the current year were used to examine the difference between two readers. A random subsample of 50 fish from the current year was selected for second readings to examine the difference within a reader. Fifty otoliths randomly selected from fish aged in 2003 were used to examine the time-series bias within each reader. A figure of 1:1 equivalence was used to illustrate those differences (Campana et al. 1995). All statistics analyses were performed in R 3.2.2 (R Core

Team 2012).

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 86% and a *CV* of 0.7% (test of symmetry: $\chi^2 = 7$, $df = 6$, $P = 0.3208$), and there was no significant difference between the first and second readings for Reader 2 with an agreement of 94% and a *CV* of 0.3% (test of symmetry: $\chi^2 = 3$, $df = 3$, $P = 0.3916$). There was no evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 90.83% and a *CV* of 0.7% (test of symmetry: $\chi^2 = 28$, $df = 25$, $P = 0.3079$) (Figure 2.2).

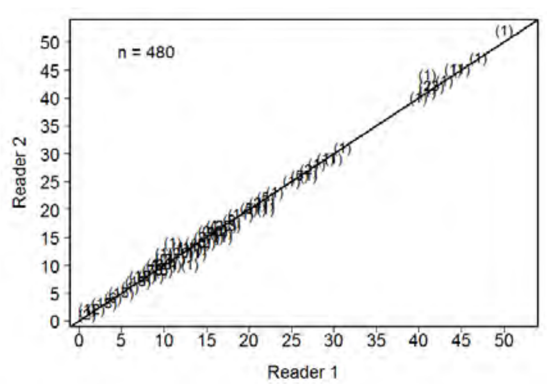


Figure 2.2: Between-reader comparison of otolith age estimates for black drum collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2016.

There was no time-series bias for both readers. Reader 1 had an agreement of 84% with ages of fish aged in 2000 with a *CV* of 0.5% (test of symmetry: $\chi^2 = 8$, $df = 8$, $P = 0.4335$). Reader 2 had an agreement of 80%with a *CV* of 0.7% (test of symmetry: $\chi^2 = 7.33$, $df = 8$, $P = 0.5011$).

2.3.2 Year class

Of the 480 fish aged with otoliths, 37 age classes (1 to 23, 25 to 29, 31, 40 to 45, 47, and 52) were represented (Table 2.1). The average age was 12.6 years, and the standard deviation and standard error were 7.1 and 0.32, respectively. Year-class data show that the fishery was comprised of 37 year-classes: fish from the 1964, 1969, 1971 to 1976, 1985, 1987 to 1991, and 1993 to 2015 year-classes, with fish primarily from the year classes of 2007 and 2006 with 17.5% and 16%, respectively. The ratio of males to females was 1:0.34 in the sample collected (Figure 2.3).

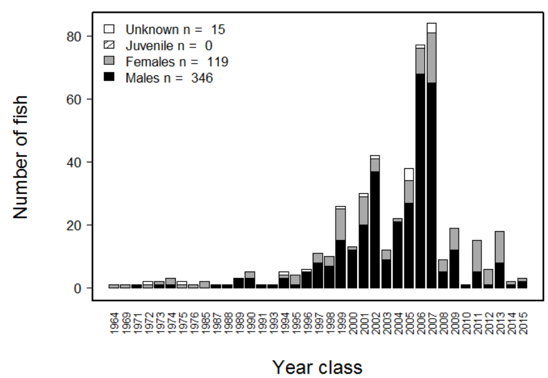


Figure 2.3: Year-class frequency distribution for black drum collected for ageing in 2016. Distribution is broken down by sex. 'Unknown' represents gonads that were not available for examination or were not examined for sex during sampling.

2.3.3 Age-length key

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.

2.4 REFERENCES

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Bobko, S. J. 1991. Age, growth, and reproduction of black drum, Pogonias cromis, in Virginia. M.S. thesis. Old Dominion University, Norfolk, VA.

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Hoenig, J.M., M.J. Morgan, and C.A. Brown. 1995. Analysing differences between two age determination methods by tests of symmetry. Can. J. Fish. Aquat. Sci. 52:364-368.

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Table 2.1: The number of black drum assigned to each total length (inch)-at-age category for 480 fish sampled for otolith age determination in Virginia during 2016.

	Age																																				
Interval	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	25	26	27	28	29	31	40	41	42	43	44	45	47	52	Totals
16 - 16.99	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
17 - 17.99	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
18 - 18.99	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
20 - 20.99	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
21 - 21.99	0	1	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8
22 - 22.99	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5
23 - 23.99	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
24 - 24.99	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
25 - 25.99	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
26 - 26.99	0	0	1	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5
27 - 27.99	0	0	0	1	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5
28 - 28.99	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4
29 - 29.99	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
30 - 30.99	0	0	0	0	3	0	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7
31 - 31.99	0	0	0	0	0	0	4	1	6	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12
32 - 32.99	0	0	0	0	0	0	6	3	15	7	3	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	36
33 - 33.99	0	0	0	0	0	0	4	1	30	16	5	1	3	3	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	64
34 - 34.99	0	0	0	0	0	0	3	1	20	17	7	4	1	5	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	60
35 - 35.99	0	0	0	0	0	0	0	1	12	24	17	5	0	12	5	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	79
36 - 36.99	0	0	0	0	0	0	0	0	1	9	4	9	3	10	11	4	1	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	56
37 - 37.99	0	0	0	0	0	0	0	0	0	3	2	2	4	9	5	2	7	2	2	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	39
38 - 38.99	0	0	0	0	0	0	0	0	0	0	0	1	0	1	2	3	8	0	2	1	2	2	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	23
39 - 39.99	0	0	0	0	0	0	0	0	0	0	0	0	1	1	3	1	7	4	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	19
40 - 40.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	4	1	2	1	1	0	2	1	0	0	0	0	0	0	0	0	0	0	0	14
41 - 41.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	1	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	6
42 - 42.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	1	1	1	1	0	0	0	0	0	1	0	0	0	0	7
43 - 43.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	2	0	0	0	0	0	4
44 - 44.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	1	3
45 - 45.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0	0	0	3
46 - 46.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	2
47 - 47.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	0	0	3
48 - 48.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
Totals	3	2	18	6	15	1	19	9	84	77	38	22	12	42	30	13	26	10	11	6	4	5	1	1	5	3	1	1	2	1	2	3	2	2	1	1	1	480

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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 2016.

	Age																																			
Interval	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	25	26	27	28	29	31	40	41	42	43	44	45	47	52
16 - 16.99	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17 - 17.99	0.5	0.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18 - 18.99	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20 - 20.99	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
21 - 21.99	0	0.12	0.88	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
22 - 22.99	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
23 - 23.99	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
24 - 24.99	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
25 - 25.99	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
26 - 26.99	0	0	0.2	0.4	0.4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
27 - 27.99	0	0	0	0.2	0.8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
28 - 28.99	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
29 - 29.99	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
30 - 30.99	0	0	0	0	0.43	0	0.29	0.29	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
31 - 31.99	0	0	0	0	0	0	0.33	0.08	0.5	0.08	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
32 - 32.99	0	0	0	0	0	0	0.17	0.08	0.42	0.19	0.08	0	0	0.03	0.03	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
33 - 33.99	0	0	0	0	0	0	0.06	0.02	0.47	0.25	0.08	0.02	0.05	0.05	0	0	0.02	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
34 - 34.99	0	0	0	0	0	0	0.05	0.02	0.33	0.28	0.12	0.07	0.02	0.08	0.02	0	0	0.02	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
35 - 35.99	0	0	0	0	0	0	0	0.01	0.15	0.3	0.22	0.06	0	0.15	0.06	0.04	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
36 - 36.99	0	0	0	0	0	0	0	0	0.02	0.16	0.07	0.16	0.05	0.18	0.2	0.07	0.02	0.04	0.04	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
37 - 37.99	0	0	0	0	0	0	0	0	0	0.08	0.05	0.05	0.1	0.23	0.13	0.05	0.18	0.05	0.05	0	0	0.03	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
38 - 38.99	0	0	0	0	0	0	0	0	0	0	0	0.04	0	0.04	0.09	0.13	0.35	0	0.09	0.04	0.09	0.09	0	0	0	0	0	0.04	0	0	0	0	0	0	0	0	0
39 - 39.99	0	0	0	0	0	0	0	0	0	0	0	0	0.05	0.05	0.16	0.05	0.37	0.21	0.05	0.05	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
40 - 40.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.07	0	0.07	0	0.29	0.07	0.14	0.07	0.07	0	0.14	0.07	0	0	0	0	0	0	0	0	0	0	0
41 - 41.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.17	0.17	0	0.17	0	0.17	0	0	0.17	0	0	0	0.17	0	0	0	0	0	0	0	0
42 - 42.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.14	0	0	0	0	0.14	0	0	0	0.14	0.14	0.14	0.14	0	0	0	0	0	0.14	0	0	0	0
43 - 43.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.25	0	0	0	0	0	0.25	0	0	0	0	0	0.5	0	0	0	0	0
44 - 44.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.33	0	0	0	0	0	0	0	0	0	0.33	0	0.33
45 - 45.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.33	0	0.33	0.33	0	0	0
46 - 46.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.5	0.5	0	0	0	0	0	0	0
47 - 47.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.33	0.33	0	0.33	0	0	0
48 - 48.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0

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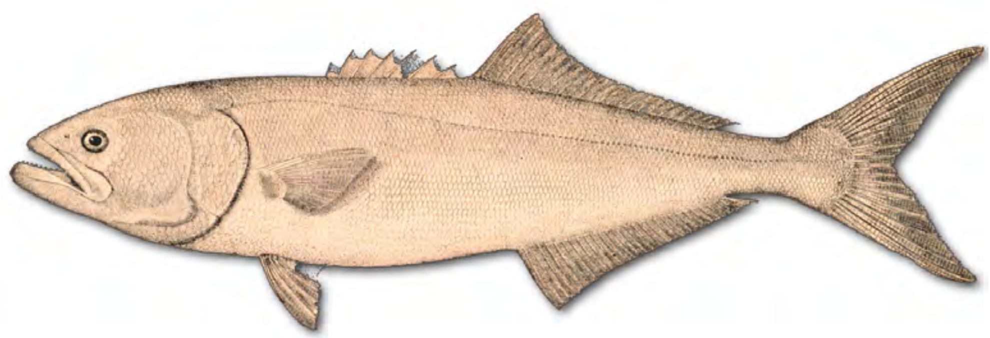
Chapter 3

BLUEFISH *Pomatomus saltatrix*



3.1 INTRODUCTION

We aged a total of 424 bluefish, *Pomatomus saltatrix*, collected by the VMRC's Biological Sampling Program for age and growth analysis in 2016. Bluefish ages ranged from 0 to 13 years old with an average age of 3.1, a standard deviation of 2.5, and a standard error of 0.12. Thirteen age classes (0 to 11, and 13) were represented, comprising fish of the 2003, and 2005 to 2016 year-classes. The sample was dominated by fish from the year-classes of 2015 and 2014 with 31.6% and 21.2%, respectively.

3.2 METHODS

3.2.1 Sample size for ageing

We estimated sample size for ageing bluefish in 2016 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 2016; 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 2010 to 2014. θ_a, V_a, B_a, and CV were calculated using pooled age-length data of bluefish collected from 2010 to 2014 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 2010 to 2014 catch. A_l is number of fish to be aged for length interval l in 2016. Based on VMRC's request in 2010, we used 1-cm length interval for bluefish, which differed from other species (1-inch).

3.2.2 Handling of collections

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

3.2.3 Preparation

We used our thin-section and bake technique to process bluefish sagittal otoliths (hereafter, referred to as "otoliths") for age determination (Robillard et al. 2009). Otolith preparation began by randomly selecting either the right or left otolith. Each whole otolith was placed in a ceramic "Coors" spot plate well and baked in a Thermolyne 1400 furnace at 400 °C. Baking time was dependent on the otolith's size and gauged by color, with a light caramel color desired. Once a suitable color was achieved the baked otolith was embedded in epoxy resin with its distal surface orientated downwards. The otoliths were viewed by eye and, when necessary, under a stereo microscope to identify the location of the core. Then, the position of the core was marked using a permanent marker across the epoxy resin surface. At least one transverse cross-section (hereafter, referred to as "thin-section") was then removed from the marked core of each otolith using a Buehler IsoMet^{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 CQFE 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. The number of annuli replaces "x" in our notation below, and is the initial "age" assignment of the fish.

Second, the thin-section is examined for translucent growth. If no translucent growth is visible beyond the last 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 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 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 annuli in the thin-section. 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 end of annulus deposition period, is interpreted as being toward the next age class.

For example, bluefish otolith deposition occurs between March and June (Robillard et al. 2009). A bluefish captured between January 1 and May 31, before the end of the species' annulus deposition period, with three visible annuli and some translucent growth after the last annulus, would be assigned an age class of "x+(x+1)" or 3+(3+1), noted as 3+4. This is the same age-class assigned to a fish with four visible annuli captured after the end of May 31, the period of annulus deposition, which would be noted as 4+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.

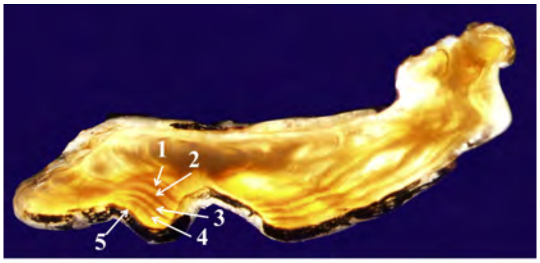


Figure 3.1: Otolith thin-section of a 5 year-old bluefish with the last annulus on the edge of the thin-section

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 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.

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

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 subsample of 50 fish from the current year was selected for second readings to examine the difference within a reader. Fifty otoliths randomly selected from fish aged in 2003 were used to examine the time-series bias within each reader. A figure of 1:1 equivalence was used to illustrate those differences (Campana et al. 1995). All statistics analyses were performed in R 3.2.2 (R Core Team 2012).

3.3 RESULTS

3.3.1 Sample size

We estimated a sample size of 441 bluefish in 2016, ranging in length interval from 14 to 97 centimeters (Table 3.1). This sample size provided a range in (*CV*) for age composition approximately from the smallest (*CV*) of 6% for age 1 and 2 to the largest (*CV*) of 21% for age 5 and 6. In 2016, we randomly selected and aged 424 fish from 657 bluefish collected by VMRC. We fell short in our over-all collections for this optimal length-class sampling estimate by 85 fish. We were not short any 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 not 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 94% and a *CV* of 3.2% (test of symmetry: $\chi^2 = 1$, $df = 2$, $P = 0.6065$), 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 90.8% and a *CV* of 3.5% (test of symmetry: $\chi^2 = 19.2$, $df = 11$, $P = 0.0577$) (Figure 3.2).

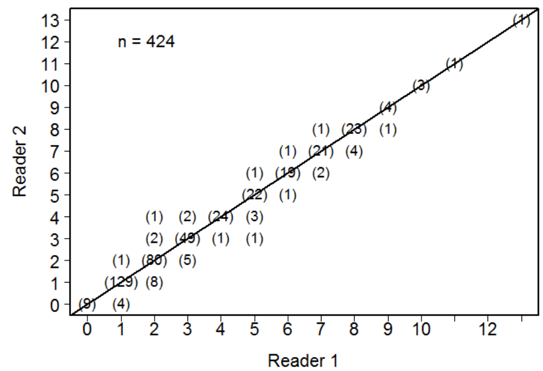


Figure 3.2: Between-reader comparison of otolith age estimates for bluefish collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2016.

There was no time-series bias for both readers. Reader 1 had an agreement of 94% with ages of fish aged in 2000 with a *CV* of 4.3% (test of symmetry: $\chi^2 = 3$, $df = 3$, $P = 0.3916$). Reader 2 had an agreement of 100% .

3.3.3 Year class

Of the 424 fish aged with otoliths, 13 age classes (0 to 11, and 13) were represented (Table 3.2). The average age was 3.1 years, and the standard deviation and standard error were 2.5 and 0.12, respectively. Year-class data show that the fishery was comprised of 13 year-classes: fish from the 2003, and 2005 to 2016 year-classes, with fish primarily from the year classes of 2015 and 2014 with 31.6% and 21.2%, respectively. The ratio of males to females was 1:1.9 in the sample collected (Figure 3.3).

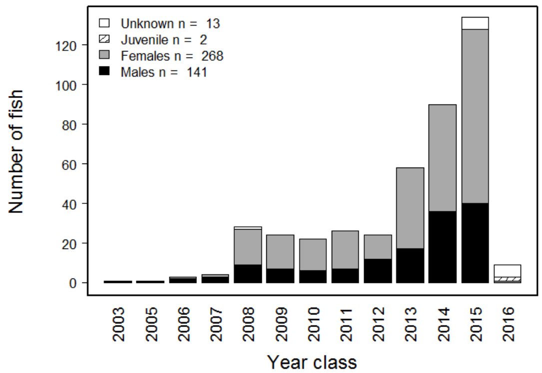


Figure 3.3: Year-class frequency distribution for bluefish collected for ageing in 2016. Distribution is broken down by sex. 'Unknown' represents gonads that were not available for examination or were not examined for sex during sampling.

3.3.4 Age-length key

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.

3.4 REFERENCES

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Quinn, T. J. II, and R. B. Deriso. 1999. Quantitative Fish Dynamics. Oxford Univeristy Press. New York.

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Table 3.1: Number of bluefish collected and aged in each 1-cm length interval in 2016. 'Target' represents the sample size for ageing estimated for 2016, and 'Need' represents number of fish shorted in each length interval compared to the optimum sample size for ageing and number of fish aged.

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Interval	Target	Collected	Aged	Need
14 - 14.99	5	0	0	5
15 - 15.99	5	0	0	5
16 - 16.99	5	0	0	5
17 - 17.99	5	1	1	4
18 - 18.99	5	1	1	4
19 - 19.99	5	1	1	4
20 - 20.99	5	8	6	0
21 - 21.99	5	6	6	0
22 - 22.99	5	8	6	0
23 - 23.99	5	16	6	0
24 - 24.99	5	11	5	0
25 - 25.99	5	13	6	0
26 - 26.99	5	11	6	0
27 - 27.99	5	8	6	0
28 - 28.99	5	16	6	0
29 - 29.99	5	22	6	0
30 - 30.99	5	12	6	0
31 - 31.99	5	9	6	0
32 - 32.99	5	19	6	0
33 - 33.99	5	17	5	0
34 - 34.99	5	15	6	0
35 - 35.99	5	11	6	0
36 - 36.99	7	10	8	0
37 - 37.99	7	17	8	0
38 - 38.99	6	19	6	0
39 - 39.99	7	8	8	0
40 - 40.99	7	12	9	0
41 - 41.99	6	12	6	0
42 - 42.99	8	12	8	0
43 - 43.99	7	16	8	0
44 - 44.99	5	16	6	0
45 - 45.99	8	9	8	0
46 - 46.99	6	15	6	0
47 - 47.99	6	12	6	0
48 - 48.99	6	12	6	0
49 - 49.99	5	11	6	0
50 - 50.99	5	16	6	0
51 - 51.99	5	8	6	0
52 - 52.99	5	9	6	0
53 - 53.99	5	11	6	0
54 - 54.99	5	6	6	0
55 - 55.99	5	7	6	0
56 - 56.99	5	3	3	2
57 - 57.99	5	8	7	0
58 - 58.99	5	2	2	3
59 - 59.99	5	12	8	0

(To continue)

Table 3.1 (Continued)

Interval	Target	Collected	Aged	Need
60 - 60.99	5	2	2	3
61 - 61.99	5	9	8	0
62 - 62.99	5	14	6	0
63 - 63.99	5	4	4	1
64 - 64.99	5	9	8	0
65 - 65.99	5	2	2	3
66 - 66.99	5	4	4	1
67 - 67.99	5	7	7	0
68 - 68.99	5	1	1	4
69 - 69.99	5	1	1	4
70 - 70.99	5	5	5	0
71 - 71.99	5	4	4	1
72 - 72.99	5	1	1	4
73 - 73.99	5	6	6	0
74 - 74.99	5	9	9	0
75 - 75.99	5	3	3	2
76 - 76.99	5	8	6	0
77 - 77.99	5	7	6	0
78 - 78.99	5	10	6	0
79 - 79.99	5	6	6	0
80 - 80.99	5	6	6	0
81 - 81.99	5	11	11	0
82 - 82.99	5	3	3	2
83 - 83.99	5	6	6	0
84 - 84.99	5	7	7	0
85 - 85.99	5	6	6	0
86 - 86.99	5	5	5	0
87 - 87.99	5	9	9	0
88 - 88.99	5	4	4	1
89 - 89.99	5	5	5	0
90 - 90.99	5	7	7	0
91 - 91.99	5	3	3	2
92 - 92.99	5	2	2	3
93 - 93.99	5	0	0	5
94 - 94.99	5	2	2	3
95 - 95.99	5	0	0	5
96 - 96.99	5	1	1	4
97 - 97.99	5	0	0	5
Totals	441	657	424	85

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Table 3.2: The number of bluefish assigned to each total length (cm)-at-age category for 424 fish sampled for otolith age determination in Virginia during 2016.

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

(To continue)

Table 3.2 (Continued)

	Age													
Interval	0	1	2	3	4	5	6	7	8	9	10	11	13	Totals
58 - 58.99	0	0	0	2	0	0	0	0	0	0	0	0	0	2
59 - 59.99	0	0	3	4	1	0	0	0	0	0	0	0	0	8
60 - 60.99	0	0	1	1	0	0	0	0	0	0	0	0	0	2
61 - 61.99	0	0	2	5	1	0	0	0	0	0	0	0	0	8
62 - 62.99	0	0	3	3	0	0	0	0	0	0	0	0	0	6
63 - 63.99	0	0	1	3	0	0	0	0	0	0	0	0	0	4
64 - 64.99	0	0	2	5	1	0	0	0	0	0	0	0	0	8
65 - 65.99	0	0	0	1	1	0	0	0	0	0	0	0	0	2
66 - 66.99	0	0	0	3	1	0	0	0	0	0	0	0	0	4
67 - 67.99	0	0	1	3	3	0	0	0	0	0	0	0	0	7
68 - 68.99	0	0	0	1	0	0	0	0	0	0	0	0	0	1
69 - 69.99	0	0	0	1	0	0	0	0	0	0	0	0	0	1
70 - 70.99	0	0	0	3	1	0	1	0	0	0	0	0	0	5
71 - 71.99	0	0	0	1	3	0	0	0	0	0	0	0	0	4
72 - 72.99	0	0	0	0	1	0	0	0	0	0	0	0	0	1
73 - 73.99	0	0	0	0	4	2	0	0	0	0	0	0	0	6
74 - 74.99	0	0	0	1	3	5	0	0	0	0	0	0	0	9
75 - 75.99	0	0	0	0	0	3	0	0	0	0	0	0	0	3
76 - 76.99	0	0	1	0	1	2	2	0	0	0	0	0	0	6
77 - 77.99	0	0	0	0	0	3	1	1	1	0	0	0	0	6
78 - 78.99	0	0	0	0	1	1	2	2	0	0	0	0	0	6
79 - 79.99	0	0	0	0	0	2	3	1	0	0	0	0	0	6
80 - 80.99	0	0	0	0	0	3	2	1	0	0	0	0	0	6
81 - 81.99	0	0	0	0	0	4	1	6	0	0	0	0	0	11
82 - 82.99	0	0	0	0	0	0	0	2	1	0	0	0	0	3
83 - 83.99	0	0	0	0	0	0	5	0	1	0	0	0	0	6
84 - 84.99	0	0	0	0	0	0	1	1	5	0	0	0	0	7
85 - 85.99	0	0	0	0	0	0	1	1	4	0	0	0	0	6
86 - 86.99	0	0	0	0	0	0	1	3	1	0	0	0	0	5
87 - 87.99	0	0	0	0	0	0	1	3	4	0	1	0	0	9
88 - 88.99	0	0	0	0	0	0	0	0	2	2	0	0	0	4
89 - 89.99	0	0	0	0	0	0	0	2	2	0	1	0	0	5
90 - 90.99	0	0	0	0	0	0	1	0	4	1	0	0	1	7
91 - 91.99	0	0	0	0	0	0	0	0	3	0	0	0	0	3
92 - 92.99	0	0	0	0	1	0	0	1	0	0	0	0	0	2
94 - 94.99	0	0	0	0	0	0	0	0	0	1	1	0	0	2
96 - 96.99	0	0	0	0	0	0	0	0	0	0	0	1	0	1
Totals	9	134	90	58	24	26	22	24	28	4	3	1	1	424

(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 2016.

	Age												
Interval	0	1	2	3	4	5	6	7	8	9	10	11	13
17 - 17.99	1	0	0	0	0	0	0	0	0	0	0	0	0
18 - 18.99	1	0	0	0	0	0	0	0	0	0	0	0	0
19 - 19.99	1	0	0	0	0	0	0	0	0	0	0	0	0
20 - 20.99	0.67	0.33	0	0	0	0	0	0	0	0	0	0	0
21 - 21.99	0	1	0	0	0	0	0	0	0	0	0	0	0
22 - 22.99	0.17	0.83	0	0	0	0	0	0	0	0	0	0	0
23 - 23.99	0	1	0	0	0	0	0	0	0	0	0	0	0
24 - 24.99	0.2	0.8	0	0	0	0	0	0	0	0	0	0	0
25 - 25.99	0	1	0	0	0	0	0	0	0	0	0	0	0
26 - 26.99	0	1	0	0	0	0	0	0	0	0	0	0	0
27 - 27.99	0	1	0	0	0	0	0	0	0	0	0	0	0
28 - 28.99	0	1	0	0	0	0	0	0	0	0	0	0	0
29 - 29.99	0	1	0	0	0	0	0	0	0	0	0	0	0
30 - 30.99	0	1	0	0	0	0	0	0	0	0	0	0	0
31 - 31.99	0	1	0	0	0	0	0	0	0	0	0	0	0
32 - 32.99	0	1	0	0	0	0	0	0	0	0	0	0	0
33 - 33.99	0	1	0	0	0	0	0	0	0	0	0	0	0
34 - 34.99	0	1	0	0	0	0	0	0	0	0	0	0	0
35 - 35.99	0	0.67	0.33	0	0	0	0	0	0	0	0	0	0
36 - 36.99	0	0.62	0.38	0	0	0	0	0	0	0	0	0	0
37 - 37.99	0	0.5	0.5	0	0	0	0	0	0	0	0	0	0
38 - 38.99	0	0.5	0.5	0	0	0	0	0	0	0	0	0	0
39 - 39.99	0	0.38	0.62	0	0	0	0	0	0	0	0	0	0
40 - 40.99	0	0.33	0.67	0	0	0	0	0	0	0	0	0	0
41 - 41.99	0	0.17	0.83	0	0	0	0	0	0	0	0	0	0
42 - 42.99	0	0.5	0.38	0.12	0	0	0	0	0	0	0	0	0
43 - 43.99	0	0.5	0.38	0.12	0	0	0	0	0	0	0	0	0
44 - 44.99	0	0.5	0.5	0	0	0	0	0	0	0	0	0	0
45 - 45.99	0	0.62	0.38	0	0	0	0	0	0	0	0	0	0
46 - 46.99	0	0.33	0.33	0.33	0	0	0	0	0	0	0	0	0
47 - 47.99	0	0.5	0.17	0.33	0	0	0	0	0	0	0	0	0
48 - 48.99	0	0.17	0.5	0.33	0	0	0	0	0	0	0	0	0
49 - 49.99	0	0.33	0.5	0.17	0	0	0	0	0	0	0	0	0
50 - 50.99	0	0	0.83	0.17	0	0	0	0	0	0	0	0	0
51 - 51.99	0	0.5	0.33	0.17	0	0	0	0	0	0	0	0	0
52 - 52.99	0	0.33	0.33	0.33	0	0	0	0	0	0	0	0	0
53 - 53.99	0	0	0.67	0.17	0	0.17	0	0	0	0	0	0	0
54 - 54.99	0	0	0.5	0.5	0	0	0	0	0	0	0	0	0
55 - 55.99	0	0	0.67	0.33	0	0	0	0	0	0	0	0	0
56 - 56.99	0	0	1	0	0	0	0	0	0	0	0	0	0
57 - 57.99	0	0	0.57	0.29	0.14	0	0	0	0	0	0	0	0
58 - 58.99	0	0	0	1	0	0	0	0	0	0	0	0	0
59 - 59.99	0	0	0.38	0.5	0.12	0	0	0	0	0	0	0	0
60 - 60.99	0	0	0.5	0.5	0	0	0	0	0	0	0	0	0
61 - 61.99	0	0	0.25	0.62	0.12	0	0	0	0	0	0	0	0
62 - 62.99	0	0	0.5	0.5	0	0	0	0	0	0	0	0	0

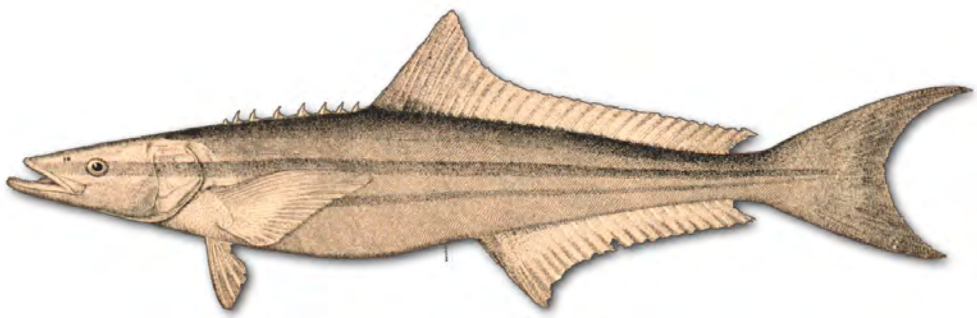
(To continue)

Table 3.3 (Continued)

	Age												
Interval	0	1	2	3	4	5	6	7	8	9	10	11	13
63 - 63.99	0	0	0.25	0.75	0	0	0	0	0	0	0	0	0
64 - 64.99	0	0	0.25	0.62	0.12	0	0	0	0	0	0	0	0
65 - 65.99	0	0	0	0.5	0.5	0	0	0	0	0	0	0	0
66 - 66.99	0	0	0	0.75	0.25	0	0	0	0	0	0	0	0
67 - 67.99	0	0	0.14	0.43	0.43	0	0	0	0	0	0	0	0
68 - 68.99	0	0	0	1	0	0	0	0	0	0	0	0	0
69 - 69.99	0	0	0	1	0	0	0	0	0	0	0	0	0
70 - 70.99	0	0	0	0.6	0.2	0	0.2	0	0	0	0	0	0
71 - 71.99	0	0	0	0.25	0.75	0	0	0	0	0	0	0	0
72 - 72.99	0	0	0	0	1	0	0	0	0	0	0	0	0
73 - 73.99	0	0	0	0	0.67	0.33	0	0	0	0	0	0	0
74 - 74.99	0	0	0	0.11	0.33	0.56	0	0	0	0	0	0	0
75 - 75.99	0	0	0	0	0	1	0	0	0	0	0	0	0
76 - 76.99	0	0	0.17	0	0.17	0.33	0.33	0	0	0	0	0	0
77 - 77.99	0	0	0	0	0	0.5	0.17	0.17	0.17	0	0	0	0
78 - 78.99	0	0	0	0	0.17	0.17	0.33	0.33	0	0	0	0	0
79 - 79.99	0	0	0	0	0	0.33	0.5	0.17	0	0	0	0	0
80 - 80.99	0	0	0	0	0	0.5	0.33	0.17	0	0	0	0	0
81 - 81.99	0	0	0	0	0	0.36	0.09	0.55	0	0	0	0	0
82 - 82.99	0	0	0	0	0	0	0	0.67	0.33	0	0	0	0
83 - 83.99	0	0	0	0	0	0	0.83	0	0.17	0	0	0	0
84 - 84.99	0	0	0	0	0	0	0.14	0.14	0.71	0	0	0	0
85 - 85.99	0	0	0	0	0	0	0.17	0.17	0.67	0	0	0	0
86 - 86.99	0	0	0	0	0	0	0.2	0.6	0.2	0	0	0	0
87 - 87.99	0	0	0	0	0	0	0.11	0.33	0.44	0	0.11	0	0
88 - 88.99	0	0	0	0	0	0	0	0	0.5	0.5	0	0	0
89 - 89.99	0	0	0	0	0	0	0	0.4	0.4	0	0.2	0	0
90 - 90.99	0	0	0	0	0	0	0.14	0	0.57	0.14	0	0	0.14
91 - 91.99	0	0	0	0	0	0	0	0	1	0	0	0	0
92 - 92.99	0	0	0	0	0.5	0	0	0.5	0	0	0	0	0
94 - 94.99	0	0	0	0	0	0	0	0	0	0.5	0.5	0	0
96 - 96.99	0	0	0	0	0	0	0	0	0	0	0	1	0

Chapter 4

COBIA *Rachycentron canadum*



4.1 INTRODUCTION

We aged a total of 255 cobia, *Rachycentron canadum*, collected by the VMRC's Biological Sampling Program for age and growth analysis in 2016. Cobia ages ranged from 2 to 12 years old with an average age of 5.1, a standard deviation of 1.4, and a standard error of 0.09. Ten age classes (2 to 9, and 11 to 12) were represented, comprising fish of the 2004 to 2005, and 2007 to 2014 year-classes. The sample was dominated by fish from the year-classes of 2012 and 2010 with 46.3% and 28.2%, respectively.

4.2 METHODS

4.2.1 Handling of collections

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

4.2.2 Preparation

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

Click here to obtain the protocol at the CQFE website on how to prepare otolith thin-section for ageing cobia.

4.2.3 Readings

The CQFE 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. The number of annuli replaces "x" in our notation below, and is the initial "age" assignment of the fish.

Second, the thin-section is examined for translucent growth. If no translucent growth is visible beyond the last 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 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 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 annuli in the thin-section. 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 end of annulus deposition period, is interpreted as being toward the next age class.

For example, cobia otolith annulus deposition occurs between June and July (Richards 1967 and modified by CQFE). A cobia captured between January 1 and July 31, before the end of the species' annulus deposition 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 July 31, the period of annulus deposition, which would be noted as 4+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 4.1). Each reader aged all of the otolith samples. 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.

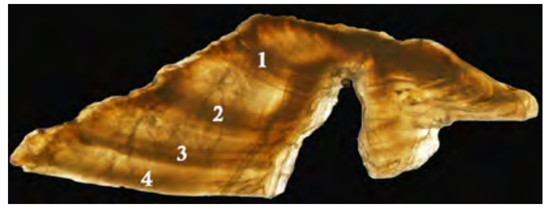


Figure 4.1: Otolith thin-section of a 4 year-old cobia.

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

4.2.4 Comparison tests

A symmetry test (Hoenig et al. 1995) and coefficient of variation (CV) analysis were used to detect any systematic difference and precision on age readings, respectively, for the following comparisons: 1) between the two readers in the current year, 2) within each reader in the current year, and 3) time-series bias between the current and previous years within each reader. The readings from the entire sample for the current year were used to examine the difference between two readers. A random subsample of 50 fish from the current year was selected for second readings to examine the difference within a reader. Fifty otoliths randomly selected from fish aged in 2003 were used to examine the time-series bias within each reader. A figure of 1:1 equivalence was used to illustrate those differences (Campana et al. 1995). All statistics analyses were performed in R 3.2.2 (R Core Team 2012).

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 0.7% (test of symmetry: $\chi^2 = 3$, $df = 3$, $P = 0.3916$), and there was no significant difference between the first and second readings for Reader 2 with an agreement of 98% and a *CV* of 0.2% (test of symmetry: $\chi^2 = 1$, $df = 1$, $P = 0.3173$). There was no evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 94.9% and a *CV* of 0.8% (test of symmetry: $\chi^2 = 4.11$, $df = 5$, $P = 0.5335$) (Figure 4.2).

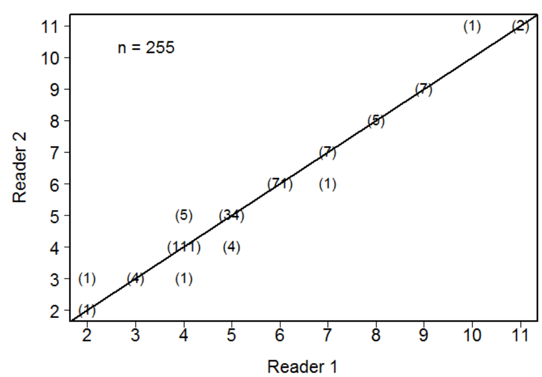


Figure 4.2: Between-reader comparison of otolith age estimates for cobia collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2016.

There was no time-series bias for both readers. Reader 1 had an agreement of 82% with ages of fish aged in 2000 with a *CV* of 1.9% (test of symmetry: $\chi^2 = 7$, $df = 8$, $P = 0.5366$). Reader 2 had an agreement of 82% with a *CV* of 1.7% (test of symmetry: $\chi^2 = 9$, $df = 7$, $P = 0.2527$).

4.3.2 Year class

Of the 255 fish aged with otoliths, 10 age classes (2 to 9, and 11 to 12) were represented (Table 4.1). The average age was 5.1 years, and the standard deviation and standard error were 1.4 and 0.09, respectively. Year-class data show that the fishery was comprised of 10 year-classes: fish from the 2004 to 2005, and 2007 to 2014 year-classes, with fish primarily from the year classes of 2012 and 2010 with 46.3% and 28.2%, respectively. The ratio of males to females was 1:1.36 in the sample collected (Figure 4.3).

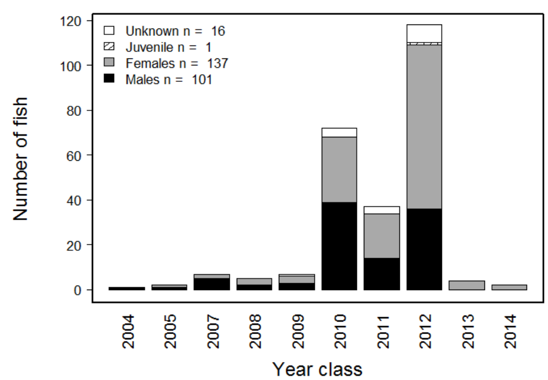


Figure 4.3: Year-class frequency distribution for cobia collected for ageing in 2016. Distribution is broken down by sex. 'Unknown' represents gonads that were not available for examination or were not examined for sex during sampling.

4.3.3 Age-length key

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

4.4 REFERENCES

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Hoenig, J.M., M.J. Morgan, and C.A. Brown. 1995. Analysing differences between two age determination methods by tests of symmetry. Can. J. Fish. Aquat. Sci. 52:364-368.

R Core Team (2012). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

Richards, C.E. 1967. Age, Growth, and Fecundity of the Cobia, Rachycentron canadum, from Chesapeake Bay and Adjacent Waters. Contribution No. 252, Virginia Institute of Marine Science, Gloucester Point, Virginia, 343-350.

Table 4.1: The number of cobia assigned to each total length (inch)-at-age category for 255 fish sampled for otolith age determination in Virginia during 2016.

	Age										
Interval	2	3	4	5	6	7	8	9	11	12	Totals
37 - 37.99	1	0	4	0	0	0	0	0	0	0	5
38 - 38.99	0	1	7	1	0	0	0	0	0	0	9
39 - 39.99	0	0	16	3	1	0	0	0	0	0	20
40 - 40.99	1	1	12	3	2	0	0	0	0	0	19
41 - 41.99	0	2	9	4	2	0	0	0	0	0	17
42 - 42.99	0	0	14	4	6	0	0	0	0	0	24
43 - 43.99	0	0	17	0	5	1	0	0	0	0	23
44 - 44.99	0	0	9	2	11	1	0	1	0	0	24
45 - 45.99	0	0	12	6	9	0	2	0	0	0	29
46 - 46.99	0	0	6	1	7	1	0	1	1	0	17
47 - 47.99	0	0	4	2	3	0	0	0	0	0	9
48 - 48.99	0	0	4	2	3	0	0	0	0	0	9
49 - 49.99	0	0	1	2	1	0	0	1	0	1	6
50 - 50.99	0	0	1	2	3	0	1	1	0	0	8
51 - 51.99	0	0	0	4	3	1	0	1	0	0	9
52 - 52.99	0	0	1	1	5	0	0	1	0	0	8
53 - 53.99	0	0	0	0	4	0	0	0	0	0	4
54 - 54.99	0	0	0	0	4	0	0	0	0	0	4
55 - 55.99	0	0	0	0	2	1	1	0	0	0	4
56 - 56.99	0	0	0	0	0	1	0	0	0	0	1
57 - 57.99	0	0	0	0	0	0	1	1	1	0	3
58 - 58.99	0	0	0	0	1	0	0	0	0	0	1
61 - 61.99	0	0	0	0	0	1	0	0	0	0	1
66 - 66.99	0	0	1	0	0	0	0	0	0	0	1
Totals	2	4	118	37	72	7	5	7	2	1	255

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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 2016.

	Age									
Interval	2	3	4	5	6	7	8	9	11	12
37 - 37.99	0.2	0	0.8	0	0	0	0	0	0	0
38 - 38.99	0	0.11	0.78	0.11	0	0	0	0	0	0
39 - 39.99	0	0	0.8	0.15	0.05	0	0	0	0	0
40 - 40.99	0.05	0.05	0.63	0.16	0.11	0	0	0	0	0
41 - 41.99	0	0.12	0.53	0.24	0.12	0	0	0	0	0
42 - 42.99	0	0	0.58	0.17	0.25	0	0	0	0	0
43 - 43.99	0	0	0.74	0	0.22	0.04	0	0	0	0
44 - 44.99	0	0	0.38	0.08	0.46	0.04	0	0.04	0	0
45 - 45.99	0	0	0.41	0.21	0.31	0	0.07	0	0	0
46 - 46.99	0	0	0.35	0.06	0.41	0.06	0	0.06	0.06	0
47 - 47.99	0	0	0.44	0.22	0.33	0	0	0	0	0
48 - 48.99	0	0	0.44	0.22	0.33	0	0	0	0	0
49 - 49.99	0	0	0.17	0.33	0.17	0	0	0.17	0	0.17
50 - 50.99	0	0	0.12	0.25	0.38	0	0.12	0.12	0	0
51 - 51.99	0	0	0	0.44	0.33	0.11	0	0.11	0	0
52 - 52.99	0	0	0.12	0.12	0.62	0	0	0.12	0	0
53 - 53.99	0	0	0	0	1	0	0	0	0	0
54 - 54.99	0	0	0	0	1	0	0	0	0	0
55 - 55.99	0	0	0	0	0.5	0.25	0.25	0	0	0
56 - 56.99	0	0	0	0	0	1	0	0	0	0
57 - 57.99	0	0	0	0	0	0	0.33	0.33	0.33	0
58 - 58.99	0	0	0	0	1	0	0	0	0	0
61 - 61.99	0	0	0	0	0	1	0	0	0	0
66 - 66.99	0	0	1	0	0	0	0	0	0	0

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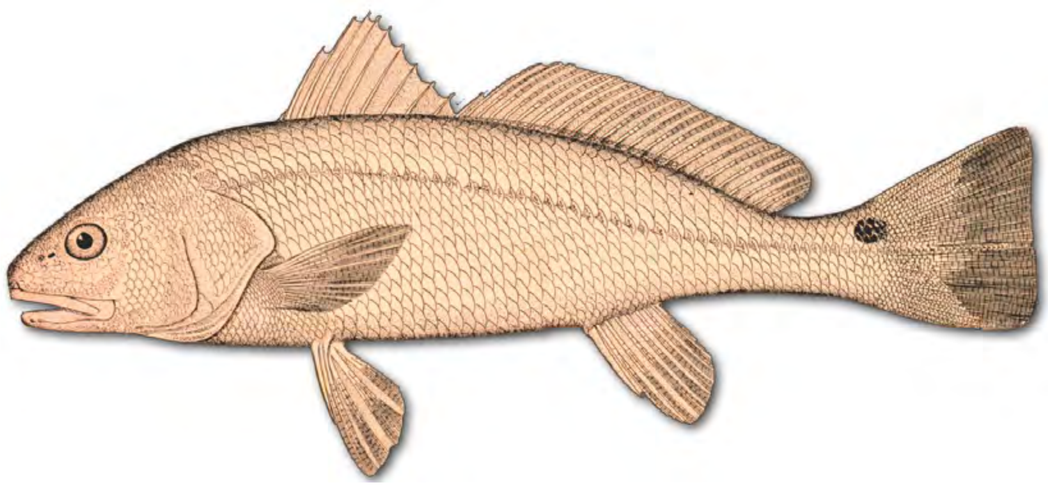
Chapter 5

RED DRUM *Sciaenops ocellatus*



5.1 INTRODUCTION

We aged a total of 19 red drum, *Sciaenops ocellatus*, collected by the VMRC's Biological Sampling Program for age and growth analysis in 2016. Red drum ages ranged from 0 to 29 years old with an average age of 1.7, a standard deviation of 6.6, and a standard error of 1.51. Three age classes (0 to 1, and 29) were represented, comprising fish of the 1987, and 2015 to 2016 year-classes. The sample was dominated by fish from the year-class of 2016 with 73.7%.

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. (1993) and Jones and Wells (1998) for red drum. The left or right sagittal otolith was randomly selected and attached, distal side down, to a glass slide with Crystalbond™ 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™ 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 CQFE 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. The number of annuli replaces "x" in our notation below, and is the initial "age" assignment of the fish.

Second, the thin-section is examined for translucent growth. If no translucent growth is visible beyond the last 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 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 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 annuli in the thin-section. 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 end of annulus deposition period, is interpreted as being toward the next age class.

For example, red drum annulus formation occurs between March and June (Ross et al. 1995 and modified by CQFE). A red drum captured between January 1 and June 30, before the end of the species' annulus deposition 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 deposition, which would be noted as 4+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 5.1). Each reader aged all of the otolith samples.

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.

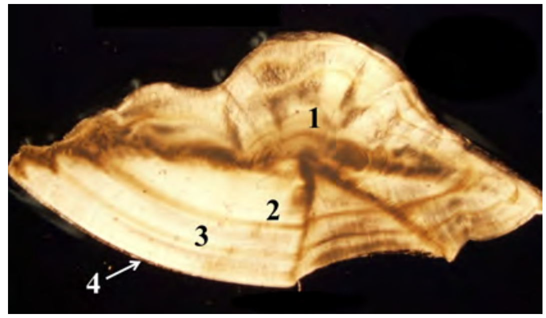


Figure 5.1: Otolith thin-section of a 4 year-old red drum with the last annulus on the edge of the thin-section

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

5.2.4 Comparison tests

A symmetry test (Hoenig et al. 1995) and coefficient of variation (CV) analysis were used to detect any systematic difference and precision on age readings, respectively, for the following comparisons: 1) between the two readers in the current year, 2) within each reader in the current year, and 3) time-series bias between the current and previous years within each reader. The readings from the entire sample for the current year were used to examine the difference between two readers. 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 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 3.2.2 (R Core Team 2012).

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 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 5.2).

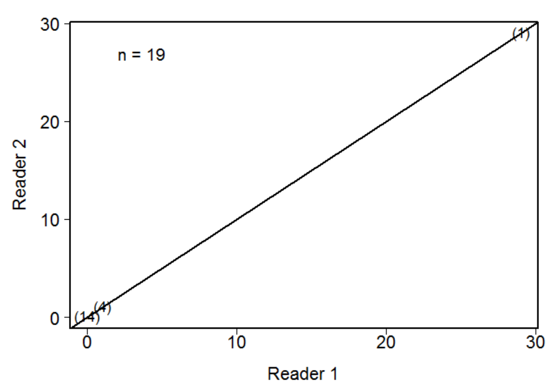


Figure 5.2: Between-reader comparison of otolith age estimates for red drum collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2016.

There was no time-series bias for both readers. Reader 1 had an agreement of 98% with ages of fish aged in 2000 with a *CV* of 0.1% (test of symmetry: $\chi^2 = 1$, $df = 1$, $P = 0.3173$). Reader 2 had an agreement of 100% .

5.3.2 Year class

Of the 19 fish aged with otoliths, 3 age classes (0 to 1, and 29) were represented (Table 5.1). The average age was 1.7 years, and the standard deviation and standard error were 6.6 and 1.51, respectively. Year-class data show that the fishery was comprised of 3 year-classes: fish from the 1987, and 2015 to 2016 year-classes, with fish primarily from the year class of 2016 with 73.7%. The ratio of males to females was 1:0.25 in the sample collected (Figure 5.3).

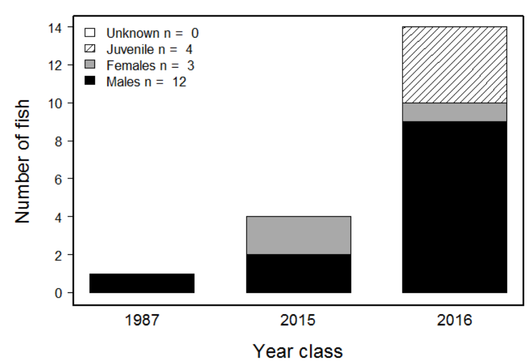


Figure 5.3: Year-class frequency distribution for red drum collected for ageing in 2016. Distribution is broken down by sex. 'Unknown' represents gonads that were not available for examination or were not examined for sex during sampling.

5.3.3 Age-length key

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

5.4 REFERENCES

Campana, S.E., M.C. Annand, and J.I. McMillan. 1995. Graphical and statistical methods for determining the consistency of age determinations. Trans.

Am. Fish. Soc. 124:131-138.

Hoenig, J.M., M.J. Morgan, and C.A. Brown. 1995. Analyzing differences between two age determination methods by tests of symmetry. Can. J. Fish. Aquat. Sci. 52:364-368.

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Ross, J., Stevens, T., Vaughan, D. 1995. Age, Growth, Mortality, and Reproductive Biology of Red Drums in North Carolina Waters. Trans. Amer. Fish. Soc. 124:37-54.

Table 5.1: The number of red drum assigned to each total length (inch)-at-age category for 19 fish sampled for otolith age determination in Virginia during 2016.

	Age			
Interval	0	1	29	Totals
15 - 15.99	1	0	0	1
17 - 17.99	0	1	0	1
18 - 18.99	8	0	0	8
19 - 19.99	4	0	0	4
20 - 20.99	1	1	0	2
24 - 24.99	0	1	0	1
25 - 25.99	0	1	0	1
45 - 45.99	0	0	1	1
Totals	14	4	1	19

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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 2016.

	Age		
Interval	0	1	29
15 - 15.99	1	0	0
17 - 17.99	0	1	0
18 - 18.99	1	0	0
19 - 19.99	1	0	0
20 - 20.99	0.5	0.5	0
24 - 24.99	0	1	0
25 - 25.99	0	1	0
45 - 45.99	0	0	1

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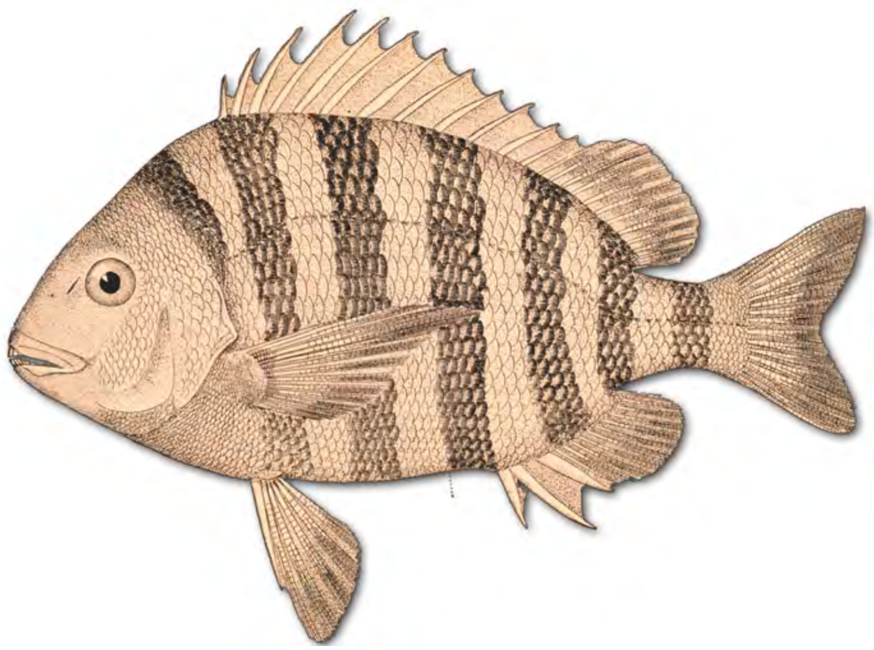
Chapter 6

SHEEPSHEAD *Archosargus probatocephalus*



6.1 INTRODUCTION

We aged a total of 130 sheepshead, *Archosargus probatocephalus*, collected by the VMRC's Biological Sampling Program for age and growth analysis in 2016. Sheepshead ages ranged from 1 to 28 years old with an average age of 9.9, a standard deviation of 7, and a standard error of 0.61. Twenty-one age classes (1 to 6, 8 to 11, 13, 15 to 16, 18 to 22, 24 to 25, and 28) were represented, comprising fish of the 1988, 1991 to 1992, 1994 to 1998, 2000 to 2001, 2003, 2005 to 2008, and 2010 to 2015 year-classes. The sample was dominated by fish from the year-class of 2011 with 46.2%.

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 et al. (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 CQFE 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. The number of annuli replaces "x" in our notation below, and is the initial "age" assignment of the fish.

Second, the thin-section is examined for translucent growth. If no translucent growth is visible beyond the last 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 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 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 annuli in the thin-section. 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 end of annulus deposition period, is interpreted as being toward the next age class.

For example, sheepshead otolith annulus formation occurs between May and June (Ballenger 2011). A sheepshead captured between January 1 and July 31, before the end of the species' annulus deposition period, with three visible annuli and some translucent growth after the last annulus, would be assigned an age class of "x+(x+1)" or 3+(3+1), noted as 3+4. This is the same age-class assigned to a fish with four visible annuli captured after the end of May 31, the period of annulus deposition, which would be noted as 4+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 6.1). Each reader aged all of the otolith samples. 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.

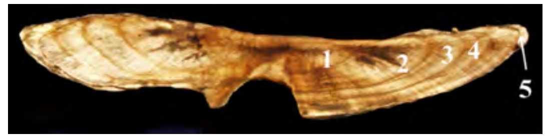


Figure 6.1: Otolith thin-section of a 5 year-old sheepshead

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

6.2.4 Comparison tests

A symmetry test (Hoenig et al. 1995) and coefficient of variation (CV) analysis were used to detect any systematic difference and precision on age readings, respectively, for the following comparisons: 1) between the two readers in the current year, 2) within each reader in the current year, and 3) time-series bias between the current and previous years within each reader. The readings from the entire sample for the current year were used to examine the difference between two readers. A random subsample of 50 fish from the current year was selected for second readings to examine the difference within a reader. Fifty otoliths randomly selected from fish aged in 2003 were used to examine the time-series bias within each reader. A figure of 1:1 equivalence was used to illustrate those differences (Campana et al. 1995). All statistics analyses were performed in R 3.2.2 (R Core Team 2012).

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 0.8% (test of symmetry: $\chi^2 = 5$, *df* = 6, $P = 0.5438$), and there was no significant difference between the first and second readings for Reader 2 with an agreement of 92% and a *CV* of 0.6% (test of symmetry: $\chi^2 = 4$, *df* = 4, $P = 0.406$). There was no evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 92.31% and a *CV* of 0.6% (test of symmetry: $\chi^2 = 8$, *df* = 8, $P = 0.4335$) (Figure 6.2).

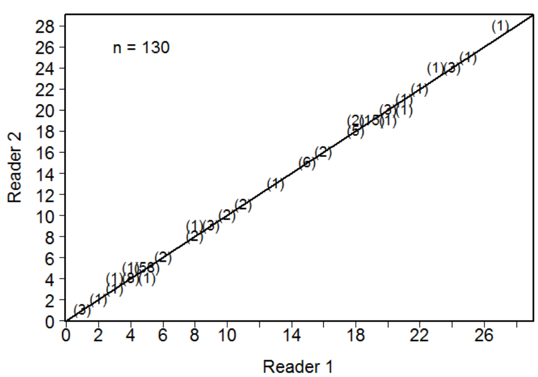


Figure 6.2: Between-reader comparison of otolith age estimates for sheepshead collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2016.

There was no time-series bias for both readers. Reader 1 had an agreement of 96% with ages of fish aged in 2008 with a *CV* of 0.2% (test of symmetry: $\chi^2 = 2$, *df* = 2, $P = 0.3679$). Reader 2 had an agreement of 96% with a *CV* of 0.2% (test of symmetry: $\chi^2 = 2$, *df* = 2, $P = 0.3679$).

6.3.2 Year class

Of the 130 fish aged with otoliths, 21 age classes (1 to 6, 8 to 11, 13, 15 to 16, 18 to 22, 24 to 25, and 28) were represented (Table 6.1). The average age was 9.9 years, and the standard deviation and standard error were 7 and 0.61, respectively. Year-class data show that the fishery was comprised of 21 year-classes: fish from the 1988, 1991 to 1992, 1994 to 1998, 2000 to 2001, 2003, 2005 to 2008, and 2010 to 2015 year-classes, with fish primarily from the year class of 2011 with 46.2%. The ratio of males to females was 1:1.03 in the sample collected (Figure 6.3).

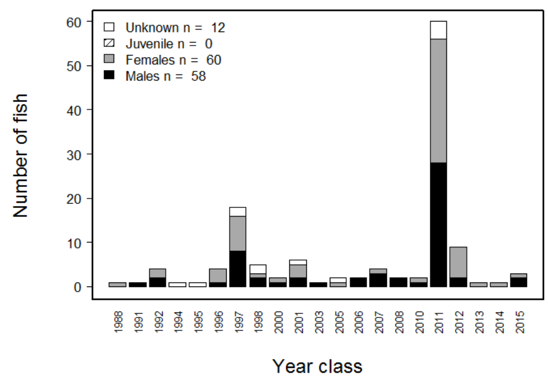


Figure 6.3: Year-class frequency distribution for sheepshead collected for ageing in 2016. Distribution is broken down by sex. 'Unknown' represents gonads that were not available for examination or were not examined for sex during sampling.

6.3.3 Age-length key

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

6.4 REFERENCES

Campana, S.E., M.C. Annand, and J.I. McMillan. 1995. Graphical and statistical methods for determining the consistency of age determinations. Trans. Am. Fish. Soc. 124:131-138.

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Hoenig, J.M., M.J. Morgan, and C.A. Brown. 1995. Analyzing differences between two age determination methods by tests of symmetry. Can. J. Fish. Aquat. Sci. 52:364-368.

R Core Team (2012). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

Table 6.1: The number of sheepshead assigned to each total length (inch)-at-age category for 130 fish sampled for otolith age determination in Virginia during 2016.

	Age																				
Interval	1	2	3	4	5	6	8	9	10	11	13	15	16	18	19	20	21	22	24	25	28	Totals
10 - 10.99	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
11 - 11.99	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
13 - 13.99	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
15 - 15.99	0	0	0	1	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5
16 - 16.99	0	0	1	3	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10
17 - 17.99	0	0	0	0	18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	18
18 - 18.99	0	0	0	4	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	13
19 - 19.99	0	0	0	0	15	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	17
20 - 20.99	0	0	0	1	5	1	1	2	1	0	0	0	0	0	0	0	0	0	0	0	0	11
21 - 21.99	0	0	0	0	3	1	1	0	0	2	1	0	0	1	0	0	0	0	1	0	0	10
22 - 22.99	0	0	0	0	0	0	0	0	0	0	0	2	0	0	6	1	1	0	1	1	0	12
23 - 23.99	0	0	0	0	0	0	0	1	0	0	0	3	2	3	6	2	0	0	2	0	0	19
24 - 24.99	0	0	0	0	0	0	0	0	0	0	0	1	0	0	4	0	0	1	0	0	1	7
25 - 25.99	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	2
26 - 26.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	2
Totals	3	1	1	9	60	2	2	4	2	2	1	6	2	5	18	4	1	1	4	1	1	130

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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 2016.

	Age																			
Interval	1	2	3	4	5	6	8	9	10	11	13	15	16	18	19	20	21	22	24	25	28
10 - 10.99	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11 - 11.99	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13 - 13.99	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15 - 15.99	0	0	0	0.2	0.8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16 - 16.99	0	0	0.1	0.3	0.6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
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	0	0	0.31	0.69	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19 - 19.99	0	0	0	0	0.88	0	0	0.06	0.06	0	0	0	0	0	0	0	0	0	0	0	0
20 - 20.99	0	0	0	0.09	0.45	0.09	0.09	0.18	0.09	0	0	0	0	0	0	0	0	0	0	0	0
21 - 21.99	0	0	0	0	0.3	0.1	0.1	0	0	0.2	0.1	0	0	0.1	0	0	0	0	0.1	0	0
22 - 22.99	0	0	0	0	0	0	0	0	0	0	0	0.17	0	0	0.5	0.08	0.08	0	0.08	0.08	0
23 - 23.99	0	0	0	0	0	0	0	0.05	0	0	0	0.16	0.11	0.16	0.32	0.11	0	0	0.11	0	0
24 - 24.99	0	0	0	0	0	0	0	0	0	0	0	0.14	0	0	0.57	0	0	0.14	0	0	0.14
25 - 25.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0.5	0	0.5	0	0	0	0	0
26 - 26.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0

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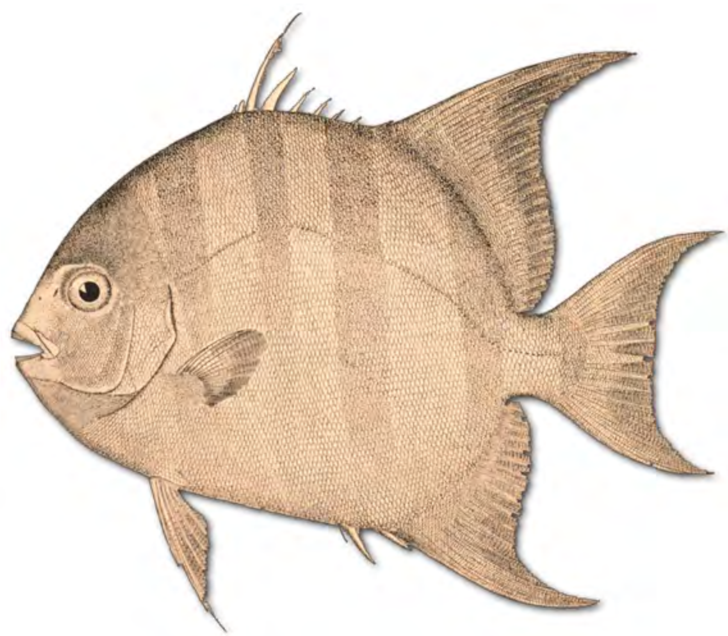
Chapter 7

ATLANTIC SPADEFISH
Chaetodipterus faber



7.1 INTRODUCTION

We aged a total of 278 spadefish, *Chaetodipterus faber*, collected by the VMRC's Biological Sampling Program for age and growth analysis in 2016. Spadefish ages ranged from 1 to 9 years old with an average age of 3.3, a standard deviation of 1.7, and a standard error of 0.1. Nine age classes (1 to 9) were represented, comprising fish of the 2007 to 2015 year-classes. The sample was dominated by fish from the year-classes of 2012 and 2015 with 37.4% and 21.2%, respectively.

7.2 METHODS

7.2.1 Sample size for ageing

We estimated sample size for ageing spadefish in 2016 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:

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

where A is the sample size for ageing spadefish in 2016; 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 2010 to 2014. θ_a, V_a, B_a, and CV were calculated using pooled age-length data of spadefish collected from 2010 to 2014 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 2010 to 2014 catch. A_l is number of fish to be aged for length interval l in 2016.

7.2.2 Handling of collections

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

7.2.3 Preparation

We used our thin-section and bake technique to process spadefish sagittal otoliths (hereafter, referred to as "otoliths") for age determination. Otolith preparation began by randomly selecting either the right or left otolith. Each whole otolith was placed in a ceramic "Coors" spot plate well and baked in a Thermolyne 1400 furnace at 400 °C. Baking time was dependent on the otolith's size and gauged by color, with a light caramel color desired. Once a suitable color was achieved the baked otolith was embedded in epoxy resin with its distal surface orientated downwards and allowed to harden overnight. The otoliths were viewed by eye and, when necessary, under a stereo microscope to identify the location of the core, and the position of the core was marked using a permanent marker across the epoxy resin surface. At least one transverse cross-section (hereafter, referred to as "thin-section") was then removed from the marked core of each otolith using a Buehler IsoMet™ 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 CQFE 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. The number of annuli replaces "x" in our notation below, and is the initial "age" assignment of the fish.

Second, the thin-section is examined for translucent growth. If no translucent growth is visible beyond the last 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 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 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 annuli in the thin-section. 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 end of annulus deposition period, is interpreted as being toward the next age class.

For example, spadefish otolith annulus formation occurs between December and July (Hayse 1989 and modified by CQFE). A spadefish captured between January 1 and July 31, before the end of the species' annulus deposition 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 July 31, the period of annulus deposition, which would be noted as 4+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 7.1). Each reader aged all of the otolith samples. All samples were aged in chronological order, based on collection date, without knowledge of

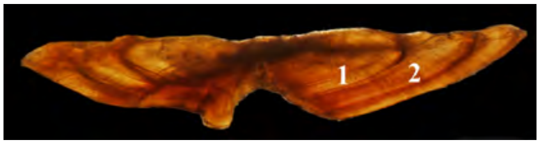


Figure 7.1: Otolith thin-section of a 2 year-old spadefish

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.

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

7.2.5 Comparison tests

A symmetry test (Hoenig et al. 1995) and coefficient of variation (CV) analysis were used to detect any systematic difference and precision on age readings, respectively, for the following comparisons: 1) between the two readers in the current year, 2) within each reader in the current year, and 3) time-series bias between the current and previous years within each reader. The readings from the entire sample for the current year were used to examine the difference between two readers. A random subsample of 50 fish from the current year was selected for second readings to examine the difference within a reader. Fifty otoliths randomly selected from fish aged in 2003 were used to examine the time-series bias within each reader. A figure of 1:1 equivalence was used to illustrate those differences (Campana et al. 1995). All statistics analyses were performed in R 3.2.2 (R Core Team 2012).

7.3 RESULTS

7.3.1 Sample size

We estimated a sample size of 278 spadefish in 2016, ranging in length interval from 4 to 22 inches (Table 7.1). This sample size provided a range in (CV) for age composition approximately from the smallest (CV) of 6% for age 2 to the largest (CV) of 22% for age 1. In 2016, we randomly selected and aged 278 fish from 384 spadefish collected by VMRC. We fell short in our overall collections for this optimal length-class sampling estimate by 10 fish. We were not short any fish from the major length intervals (The interval requires 10 or more fish), as a result, the precision for the estimates of major age groups would not be influenced significantly.

7.3.2 Reading precision

Both readers had high self-precision. Specifically, there was no significant difference between the first and second readings for Reader 1 with an agreement of 92% and a CV of 2.1% (test of symmetry: $\chi^2 = 2$, $df = 3$, $P = 0.5724$), and there was no significant difference between the first and second readings for Reader 2 with an agreement of 100% . There was no evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 89.57% and a CV of 2.5% (test of symmetry: $\chi^2 = 11.13$, $df = 7$, $P = 0.1329$) (Figure 7.2).

There was no time-series bias for both readers. Reader 1 had an agreement of 88% with ages of fish aged in 2003 with a CV of 1.9% (test of symmetry: $\chi^2 = 6$, $df = 6$, $P = 0.4232$). Reader 2 had an agreement of 96% with a CV of 1.1% (test of symmetry: $\chi^2 = 2$, $df = 2$, $P = 0.3679$).

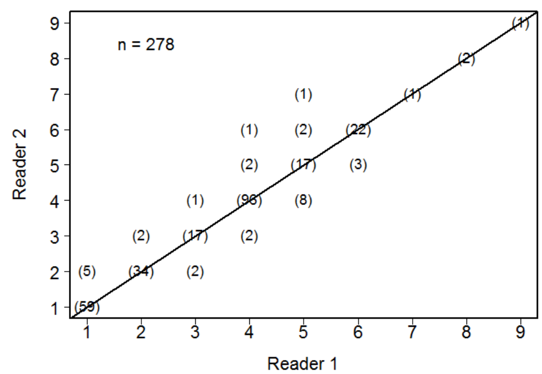


Figure 7.2: Between-reader comparison of otolith age estimates for spadefish collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2016.

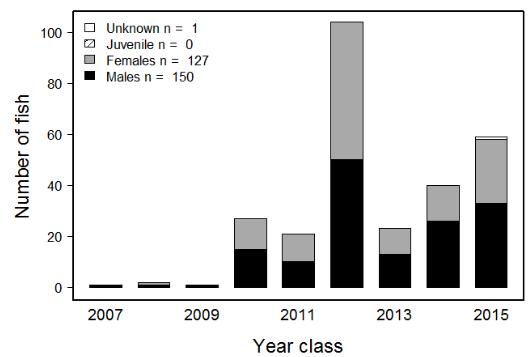


Figure 7.3: Year-class frequency distribution for spadefish collected for ageing in 2016. Distribution is broken down by sex. 'Unknown' represents gonads that were not available for examination or were not examined for sex during sampling.

7.3.3 Year class

Of the 278 fish aged with otoliths, 9 age classes (1 to 9) were represented (Table 7.2). The average age was 3.3 years, and the standard deviation and standard error were 1.7 and 0.1, respectively. Year-class data show that the fishery was comprised of 9 year-classes: fish from the 2007 to 2015 year-classes, with fish primarily from the year classes of 2012 and 2015 with 37.4% and 21.2%, respectively. The ratio of males to females was 1:0.85 in the sample collected (Figure 7.3).

7.3.4 Age-length key

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.

7.4 REFERENCES

Campana, S.E., M.C. Annand, and J.I. McMillan. 1995. Graphical and statistical methods for determining the consistency of age determinations. Trans. Am. Fish. Soc. 124:131-138.

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Quinn, T. J. II, and R. B. Deriso. 1999. Quantitative Fish Dynamics. Oxford Univeristy Press. New York.

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Table 7.1: Number of Atlantic spadefish collected and aged in each 1-inch length interval in 2016. 'Target' represents the sample size for ageing estimated for 2016, 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	7	6	0
5 - 5.99	8	15	8	0
6 - 6.99	32	38	32	0
7 - 7.99	42	52	42	0
8 - 8.99	29	36	29	0
9 - 9.99	22	29	22	0
10 - 10.99	15	18	16	0
11 - 11.99	13	17	14	0
12 - 12.99	18	26	18	0
13 - 13.99	16	37	16	0
14 - 14.99	13	24	14	0
15 - 15.99	13	19	13	0
16 - 16.99	9	21	9	0
17 - 17.99	15	17	16	0
18 - 18.99	8	13	8	0
19 - 19.99	5	10	10	0
20 - 20.99	5	4	4	1
21 - 21.99	5	1	1	4
22 - 22.99	5	0	0	5
Totals	278	384	278	10

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Table 7.2: The number of Atlantic spadefish assigned to each total length-at-age category for 278 fish sampled for otolith age determination in Virginia during 2016.

	Age									
Interval	1	2	3	4	5	6	7	8	9	Totals
4 - 4.99	6	0	0	0	0	0	0	0	0	6
5 - 5.99	8	0	0	0	0	0	0	0	0	8
6 - 6.99	31	1	0	0	0	0	0	0	0	32
7 - 7.99	13	23	4	2	0	0	0	0	0	42
8 - 8.99	1	13	7	8	0	0	0	0	0	29
9 - 9.99	0	3	3	16	0	0	0	0	0	22
10 - 10.99	0	0	2	14	0	0	0	0	0	16
11 - 11.99	0	0	2	10	2	0	0	0	0	14
12 - 12.99	0	0	3	13	2	0	0	0	0	18
13 - 13.99	0	0	1	15	0	0	0	0	0	16
14 - 14.99	0	0	0	11	2	1	0	0	0	14
15 - 15.99	0	0	0	9	1	3	0	0	0	13
16 - 16.99	0	0	0	2	3	4	0	0	0	9
17 - 17.99	0	0	1	3	4	8	0	0	0	16
18 - 18.99	0	0	0	0	3	5	0	0	0	8
19 - 19.99	0	0	0	1	2	4	0	2	1	10
20 - 20.99	0	0	0	0	2	1	1	0	0	4
21 - 21.99	0	0	0	0	0	1	0	0	0	1
Totals	59	40	23	104	21	27	1	2	1	278

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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 2016.

	Age								
Interval	1	2	3	4	5	6	7	8	9
4 - 4.99	1	0	0	0	0	0	0	0	0
5 - 5.99	1	0	0	0	0	0	0	0	0
6 - 6.99	0.97	0.03	0	0	0	0	0	0	0
7 - 7.99	0.31	0.55	0.1	0.05	0	0	0	0	0
8 - 8.99	0.03	0.45	0.24	0.28	0	0	0	0	0
9 - 9.99	0	0.14	0.14	0.73	0	0	0	0	0
10 - 10.99	0	0	0.12	0.88	0	0	0	0	0
11 - 11.99	0	0	0.14	0.71	0.14	0	0	0	0
12 - 12.99	0	0	0.17	0.72	0.11	0	0	0	0
13 - 13.99	0	0	0.06	0.94	0	0	0	0	0
14 - 14.99	0	0	0	0.79	0.14	0.07	0	0	0
15 - 15.99	0	0	0	0.69	0.08	0.23	0	0	0
16 - 16.99	0	0	0	0.22	0.33	0.44	0	0	0
17 - 17.99	0	0	0.06	0.19	0.25	0.5	0	0	0
18 - 18.99	0	0	0	0	0.38	0.62	0	0	0
19 - 19.99	0	0	0	0.1	0.2	0.4	0	0.2	0.1
20 - 20.99	0	0	0	0	0.5	0.25	0.25	0	0
21 - 21.99	0	0	0	0	0	1	0	0	0

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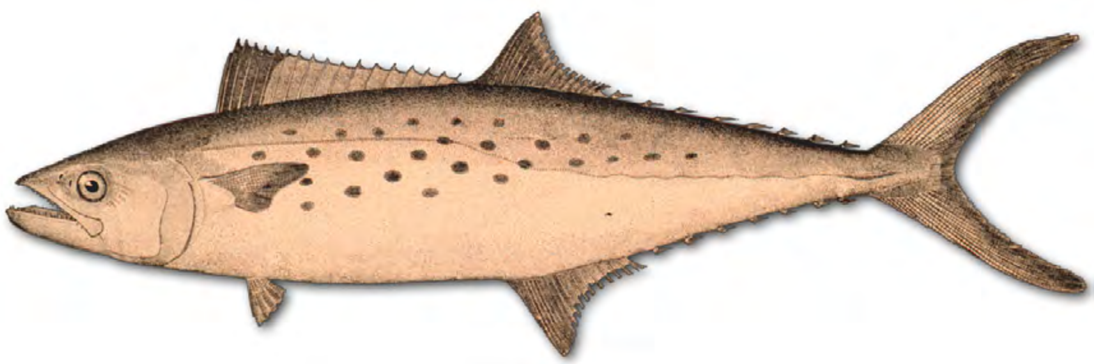
Chapter 8

SPANISH MACKEREL
Scomberomorous maculatus



8.1 INTRODUCTION

We aged a total of 220 Spanish mackerel, *Scomberomorous maculatus*, collected by the VMRC's Biological Sampling Program for age and growth analysis in 2016. Spanish mackerel ages ranged from 1 to 6 years old with an average age of 1.5, a standard deviation of 0.9, and a standard error of 0.06. Six age classes (1 to 6) were represented, comprising fish of the 2010 to 2015 year-classes. The sample was dominated by fish from the year-class of 2015 with 65.9%.

8.2 METHODS

8.2.1 Sample size for ageing

We estimated sample size for ageing Spanish mackerel in 2016 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:

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

where A is the sample size for ageing Spanish mackerel in 2016; 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 2010 to 2014. θ_a, V_a, B_a, and CV were calculated using pooled age-length data of Spanish mackerel collected from 2010 to 2014 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 2010 to 2014 catch. A_l is number of fish to be aged for length interval l in 2016.

8.2.2 Handling of collections

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

8.2.3 Preparation

Sagittal otoliths, hereafter, referred to as "otolith", were processed for age determination. The left or right otolith was randomly selected and embedded, distal side down, in epoxy resin and allowed to harden overnight. The otoliths were viewed by eye, and when necessary, under a stereo microscope to identify the location of the core, and the position of the core marked using a permanent marker across the epoxy resin surface. At least one transverse cross-section (hereafter "thin-section") was then removed from the marked core of each otolith using a Buehler IsoMet^{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 CQFE 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. The number of annuli replaces "x" in our notation below, and is the initial "age" assignment of the fish.

Second, the thin-section is examined for translucent growth. If no translucent growth is visible beyond the last 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 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 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 annuli in the thin-section. 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 end of annulus deposition period, is interpreted as being toward the next age class.

For example, Spanish mackerel annulus formation occurs between May and June (Schmidt et al. 1993). A Spanish mackerel captured between January 1 and June 30, before the end of the species' annulus deposition period, with three visible annuli and some translucent growth after the last annulus, would be assigned an age class of "x+(x+1)" or 3+(3+1), noted as 3+4. This is the same age-class assigned to a fish with four visible annuli captured after the end of May 31, the period of annulus deposition, which would be noted as 4+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 8.1). Each reader aged all of the otolith samples. 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

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

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.

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

8.2.5 Comparison tests

A symmetry test (Hoenig et al. 1995) and coefficient of variation (CV) analysis were used to detect any systematic difference and precision on age readings, respectively, for the following comparisons: 1) between the two readers in the current year, 2) within each reader in the current year, and 3) time-series bias between the current and previous years within each reader. The readings from the entire sample for the current year were used to examine the difference between two readers. A random subsample of 50 fish from the current year was selected for second readings to examine the difference within a reader. Fifty otoliths randomly selected from fish aged in 2003 were used to examine the time-series bias within each reader. A figure of 1:1 equivalence was used to illustrate those differences (Campana et al. 1995). All statistics analyses were performed in R 3.2.2 (R Core Team 2012).

8.3 RESULTS

8.3.1 Sample size

We estimated a sample size of 239 Spanish mackerel in 2016, ranging in length interval from 12 to 30 inches (Table 8.1). This sample size provided a range in (CV) for age composition approximately from the smallest (CV) of 4% for age 1 to the largest (CV) of 18% for age 3. In 2016, we randomly selected and aged 220 fish from 304 Spanish mackerel collected by VMRC. We fell short in our over-all collections for this optimal length-class sampling estimate by 31 fish. We were short 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

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 0.9% (test of symmetry: $\chi^2 = 1$, $df = 1$, $P = 0.3173$), and there was no significant difference between the first and second readings for Reader 2 with an agreement of 98% and a CV of 0.6% (test of symmetry: $\chi^2 = 1$, $df = 1$, $P = 0.3173$). There was no evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 94.09% and a CV of 2.1% (test of symmetry: $\chi^2 = 7$, $df = 3$, $P = 0.0719$) (Figure 8.2).

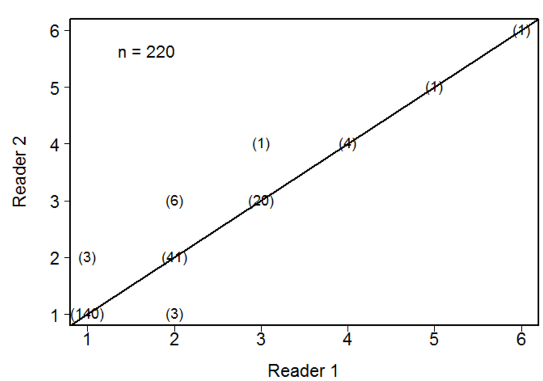


Figure 8.2: Between-reader comparison of otolith age estimates for Spanish mackerel collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2016.

There was no time-series bias for both readers. Reader 1 had an agreement of 94% with fish aged in 2003 with a *CV* of 2.2% (test of symmetry: $\chi^2 = 3$, $df = 2$, $P = 0.2231$). Reader 2 had an agreement of 98% with a *CV* of 0.9% (test of symmetry: $\chi^2 = 1$, $df = 1$, $P = 0.3173$).

8.3.3 Year class

Of the 220 fish aged with otoliths, 6 age classes (1 to 6) were represented (Table 8.2). The average age was 1.5 years, and the standard deviation and standard error were 0.9 and 0.06, respectively. Year-class data show that the fishery was comprised of 6 year-classes: fish from the 2010 to 2015 year-classes, with fish primarily from the year class of 2015 with 65.9%. The ratio of males to females was 1:3.06 in the sample collected (Figure 8.3).

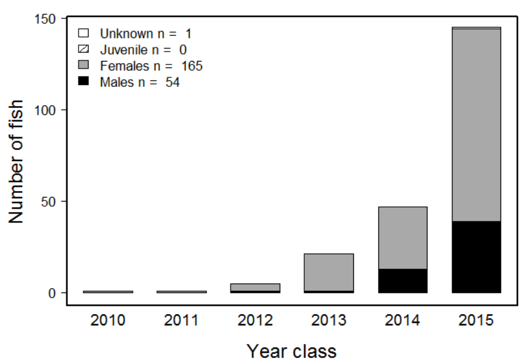


Figure 8.3: Year-class frequency distribution for Spanish mackerel collected for ageing in 2016. Distribution is broken down by sex. 'Unknown' represents gonads that were not available for examination or were not examined for sex during sampling.

8.3.4 Age-length key

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.

8.4 REFERENCES

Campana, S.E., M.C. Annand, and J.I. McMillan. 1995. Graphical and statistical methods for etermining the consistency of age terminations. Trans. Am. Fish. Soc. 124:131-138.

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Table 8.1: Number of Spanish mackerel collected and aged in each 1-inch length interval in 2016. 'Target' represents the sample size for ageing estimated for 2016, 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	35	20	0
15 - 15.99	34	59	34	0
16 - 16.99	39	51	40	0
17 - 17.99	34	40	33	1
18 - 18.99	19	25	20	0
19 - 19.99	16	28	16	0
20 - 20.99	12	14	12	0
21 - 21.99	12	17	12	0
22 - 22.99	7	9	8	0
23 - 23.99	5	7	6	0
24 - 24.99	5	6	6	0
25 - 25.99	5	4	4	1
26 - 26.99	5	8	8	0
27 - 27.99	5	0	0	5
28 - 28.99	5	0	0	5
29 - 29.99	5	0	0	5
30 - 30.99	5	0	0	5
Totals	239	304	220	31

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Table 8.2: The number of Spanish mackerel assigned to each total length-at-age category for 220 fish sampled for otolith age determination in Virginia during 2016.

	Age						
Interval	1	2	3	4	5	6	Totals
13 - 13.99	1	0	0	0	0	0	1
14 - 14.99	20	0	0	0	0	0	20
15 - 15.99	34	0	0	0	0	0	34
16 - 16.99	40	0	0	0	0	0	40
17 - 17.99	32	1	0	0	0	0	33
18 - 18.99	10	10	0	0	0	0	20
19 - 19.99	6	10	0	0	0	0	16
20 - 20.99	2	8	1	1	0	0	12
21 - 21.99	0	12	0	0	0	0	12
22 - 22.99	0	3	5	0	0	0	8
23 - 23.99	0	2	4	0	0	0	6
24 - 24.99	0	1	5	0	0	0	6
25 - 25.99	0	0	2	2	0	0	4
26 - 26.99	0	0	4	2	1	1	8
Totals	145	47	21	5	1	1	220

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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 2016.

	Age					
Interval	1	2	3	4	5	6
13 - 13.99	1	0	0	0	0	0
14 - 14.99	1	0	0	0	0	0
15 - 15.99	1	0	0	0	0	0
16 - 16.99	1	0	0	0	0	0
17 - 17.99	0.97	0.03	0	0	0	0
18 - 18.99	0.5	0.5	0	0	0	0
19 - 19.99	0.38	0.62	0	0	0	0
20 - 20.99	0.17	0.67	0.08	0.08	0	0
21 - 21.99	0	1	0	0	0	0
22 - 22.99	0	0.38	0.62	0	0	0
23 - 23.99	0	0.33	0.67	0	0	0
24 - 24.99	0	0.17	0.83	0	0	0
25 - 25.99	0	0	0.5	0.5	0	0
26 - 26.99	0	0	0.5	0.25	0.12	0.12

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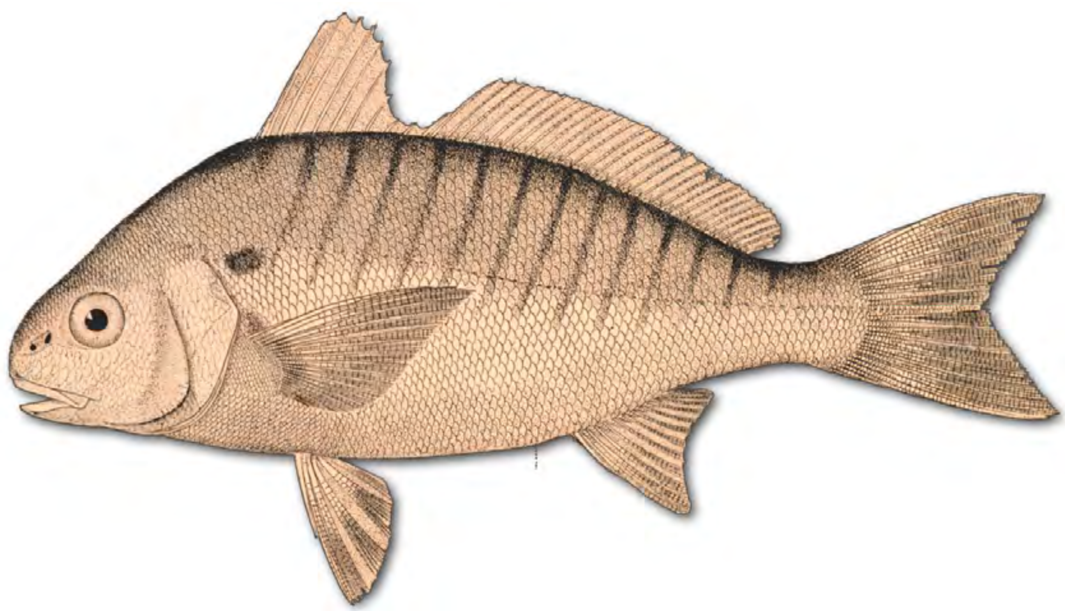
Chapter 9

SPOT *Leiostomus xanthurus*



9.1 INTRODUCTION

We aged a total of 248 spot, *Leiostomus xanthurus*, collected by the VMRC's Biological Sampling Program for age and growth analysis in 2016. spot ages ranged from 0 to 4 years old with an average age of 1.1, a standard deviation of 0.6, and a standard error of 0.04. Five age classes (0 to 4) were represented, comprising fish of the 2012 to 2016 year-classes. The sample was dominated by fish from the year-class of 2015 with 87.1%.

9.2 METHODS

9.2.1 Sample size for ageing

We estimated sample size for ageing spot in 2016 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 2016; 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 2010 to 2014. θ_a, V_a, B_a, and CV were calculated using pooled age-length data of spot collected from 2010 to 2014 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 2010 to 2014 catch. A_l is number of fish to be aged for length interval l in 2016.

9.2.2 Handling of collections

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

9.2.3 Preparation

Sagittal otoliths, hereafter, referred to as "otoliths", were processed for age determination following the methods described in Barbieri et al. (1994) with a few modifications. The left or right otolith was randomly selected and embedded (distal side down) in epoxy resin and allowed to harden overnight. The otoliths were viewed by eye and, when necessary, under a stereo microscope to identify the location of the core, and the position of the core was marked using a permanent marker across the epoxy resin surface. At least one transverse cross-section (hereafter, referred to as "thin-section") was then removed from the marked core of each otolith using a Buehler IsoMet^{TM} low-speed saw equipped with two, 3-inch diameter, Norton diamond grinding wheels (hereafter, referred to as "blades"), separated by a stainless steel spacer of 0.5 mm (diameter 2.5"). Thin-sections were placed on labeled glass slides and covered with a thin layer of Flo-texx mounting medium that not only fixed the sections to the slide, but more importantly, provided enhanced contrast and greater readability by increasing light transmission through the thin-sections.

Click here to obtain the protocol at the CQFE website on how to prepare otolith thin-section for ageing spot.

9.2.4 Readings

The CQFE 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. The number of annuli replaces "x" in our notation below, and is the initial "age" assignment of the fish.

Second, the thin-section is examined for translucent growth. If no translucent growth is visible beyond the last 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 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 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 annuli in the thin-section. 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 end of annulus deposition period, is interpreted as being toward the next age class.

For example, spot otolith annulus formation occurs between May and July (Piner and Jones 2004). A spot captured between January 1 and May 31, before the end of the species' annulus deposition period, with three visible annuli and some translucent growth after the last annulus, would be assigned an age class of "x+(x+1)" or 3+(3+1), noted as 3+4. This is the same age-class assigned to a fish with four visible annuli captured after the end of May 31, the period of annulus deposition, which would be noted as 4+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 9.1). Each reader aged all of the otolith samples. Due to discrepancy on identification of the first annulus of spot among Atlantic states, Atlantic States Marine Fisheries Commission (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 2016.

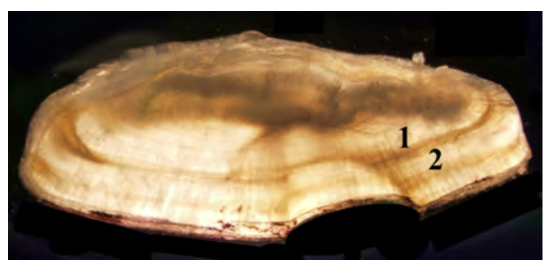


Figure 9.1: Otolith thin-section of a 2 year-old spot

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.

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

9.2.5 Comparison tests

A symmetry test (Hoenig et al. 1995) and coefficient of variation (CV) analysis were used to detect any systematic difference and precision on age readings, respectively, for the following comparisons: 1) between the two readers in the current year, 2) within each reader in the current year, and 3) time-series bias between the current and previous years within each reader. The readings from the entire sample for the current year were used to examine the difference between two readers. A random subsample of 50 fish from the current year was selected for second readings to examine the difference within a reader. Fifty otoliths randomly selected from fish aged in 2003 were used to examine the time-series bias within each reader. A figure of 1:1 equivalence was used to illustrate those differences (Campana et al. 1995). All statistics analyses were performed in R 3.2.2 (R Core Team 2012).

9.3 RESULTS

9.3.1 Sample size

We estimated a sample size of 256 spot in 2016, ranging in length interval from 5 to 12 inches (Table 9.1). This sample size provided a range in (CV) for age composition approximately from the smallest (CV) of 5% for age 1 to the largest (CV) of 8% for age 2. In 2016, we randomly selected and aged 248 fish from 310 spot collected by VMRC. We fell short in our over-all collections for this optimal length-class sampling estimate by 14 fish. We were not short any fish from the major length intervals (The interval requires 10 or more fish), as a result, the precision for the estimates of major age groups would not be influenced significantly.

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 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 99.19% and a CV of 0.4% (test of symmetry: $\chi^2 = 0$, $df = 1$, $P = 1$) (Figure 9.2).

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

9.3.3 Year class

Of the 248 fish aged with otoliths, 5 age classes (0 to 4) were represented (Table 9.2). The average age was 1.1 years, and

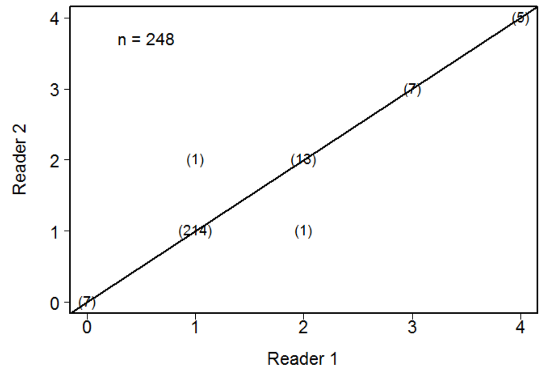


Figure 9.2: Between-reader comparison of otolith age estimates for spot collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2016.

the standard deviation and standard error were 0.6 and 0.04, respectively. Year-class data show that the fishery was comprised of 5 year-classes: fish from the 2012 to 2016 year-classes, with fish primarily from the year class of 2015 with 87.1%. The ratio of males to females was 1:5.97 in the sample collected (Figure 9.3).

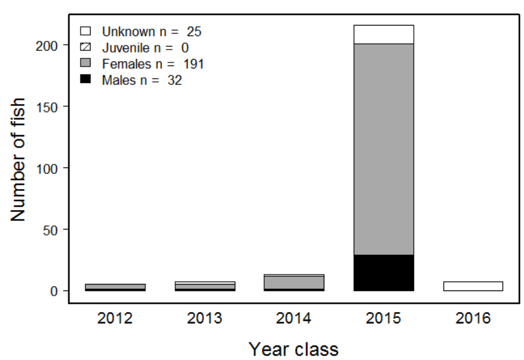


Figure 9.3: Year-class frequency distribution for spot collected for ageing in 2016. Distribution is broken down by sex. 'Unknown' is for gonads that were not available for examination or were not examined for sex during sampling.

9.3.4 Age-length key

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.

9.4 REFERENCES

Campana, S.E., M.C. Annand, and J.I. McMillan. 1995. Graphical and statistical methods for determining the consistency of age determinations. Trans. Am. Fish. Soc. 124:131-138.

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Table 9.1: Number of spot collected and aged in each 1-inch length interval in 2016. 'Target' represents the sample size for ageing estimated for 2016, and 'Need' represents number of fish shorted in each length interval compared to the optimum sample size for ageing and number of fish aged.

Interval	Target	Collected	Aged	Need
5 - 5.99	6	7	6	0
6 - 6.99	6	8	6	0
7 - 7.99	31	50	32	0
8 - 8.99	57	86	58	0
9 - 9.99	81	95	82	0
10 - 10.99	61	64	64	0
11 - 11.99	9	0	0	9
12 - 12.99	5	0	0	5
Totals	256	310	248	14

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Table 9.2: The number of spot assigned to each total length-at-age category for 248 fish sampled for otolith age determination in Virginia during 2016.

	Age					
Interval	0	1	2	3	4	Totals
5 - 5.99	6	0	0	0	0	6
6 - 6.99	1	5	0	0	0	6
7 - 7.99	0	32	0	0	0	32
8 - 8.99	0	51	5	2	0	58
9 - 9.99	0	70	3	5	4	82
10 - 10.99	0	58	5	0	1	64
Totals	7	216	13	7	5	248

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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 2016.

	Age				
Interval	0	1	2	3	4
5 - 5.99	1	0	0	0	0
6 - 6.99	0.17	0.83	0	0	0
7 - 7.99	0	1	0	0	0
8 - 8.99	0	0.88	0.09	0.03	0
9 - 9.99	0	0.85	0.04	0.06	0.05
10 - 10.99	0	0.91	0.08	0	0.02

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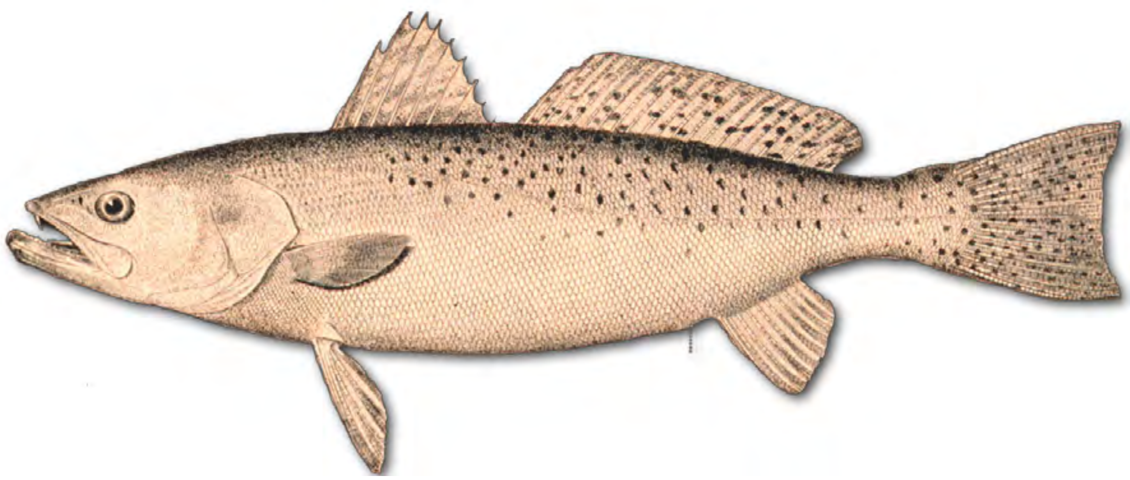
Chapter 10

SPOTTED SEATROUT *Cynoscion nebulosus*



10.1 INTRODUCTION

We aged a total of 239 spotted seatrout, *Cynoscion nebulosus*, collected by the VMRC's Biological Sampling Program for age and growth analysis in 2016. 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 0.06. Six age classes (0 to 5) were represented, comprising fish of the 2011 to 2016 year-classes. The sample was dominated by fish from the year-classes of 2015 and 2014 with 46.4% and 26.4%, respectively.

10.2 METHODS

10.2.1 Sample size for ageing

We estimated sample size for ageing spotted seatrout in 2016 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 2016; 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 2010 to 2014. θ_a, V_a, B_a, and CV were calculated using pooled age-length data of spotted seatrout collected from 2010 to 2014 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 2010 to 2014 catch. A_l is number of fish to be aged for length interval l in 2016.

10.2.2 Handling of collections

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

10.2.3 Preparation

Sagittal otoliths, hereafter, referred to as "otoliths", were processed for age determination. The left or right otolith was randomly selected and attached, distal side down, to a glass slide with clear Crystalbond^{TM} 509 adhesive. The otoliths were viewed by eye and, when necessary, under a stereo microscope to identify the location of the core, and the position of the core was marked using a pencil across the otolith surface. At least one transverse cross-section (hereafter, referred to as "thin-section") was then removed from the marked core of each otolith using a Buehler IsoMet^{TM} low-speed saw equipped with two, 3-inch diameter, Norton diamond grinding wheels (hereafter, referred to as "blades"), separated by a stainless steel spacer of 0.5 mm (diameter 2.5"). Thin-sections were placed on labeled glass slides and covered with a thin layer of Flo-texx mounting medium that not only fixed the sections to the slide, but more importantly, provided enhanced contrast and greater readability by increasing light transmission through the thin-sections.

Click here to obtain the protocol at the CQFE website on how to prepare otolith thin-section for ageing spotted seatrout.

10.2.4 Readings

The CQFE 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. The number of annuli replaces "x" in our notation below, and is the initial "age" assignment of the fish.

Second, the thin-section is examined for translucent growth. If no translucent growth is visible beyond the last 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 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 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 annuli in the thin-section. 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 end of annulus deposition period, is interpreted as being toward the next age class.

For example, spotted seatrout otolith annulus formation occurs between March and May (Ihde and Chittenden 2003). A spotted seatrout captured between January 1 and May 31, before the end of the species' annulus deposition period, with three visible annuli and some translucent growth after the last annulus, would be assigned an age class of "x+(x+1)" or 3+(3+1), noted as 3+4. This is the same age-class assigned to a fish with four visible annuli captured after the end of May 31, the period of annulus deposition, which would be noted as 4+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 10.1). Each reader aged all of the otolith samples. All samples were aged in chronological order, based on collection date, without knowledge of

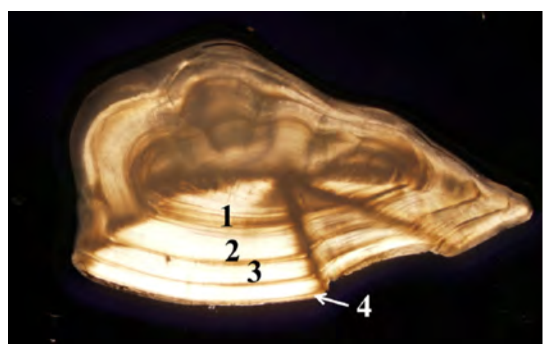


Figure 10.1: Otolith thin-section of a 4 year-old spotted seatrout with the last annulus on the edge of the thin-section

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.

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

10.2.5 Comparison tests

A symmetry test (Hoenig et al. 1995) and coefficient of variation (*CV*) analysis were used to detect any systematic difference and precision on age readings, respectively, for the following comparisons: 1) between the two readers in the current year, 2) within each reader in the current year, and 3) time-series bias between the current and previous years within each reader. The readings from the entire sample for the current year were used to examine the difference between two readers. A random subsample of 50 fish from the current year was selected for second readings to examine the difference within a reader. Fifty otoliths randomly selected from fish aged in 2003 were used to examine the time-series bias within each reader. A figure of 1:1 equivalence was used to illustrate those differences (Campana et al. 1995). All statistics analyses were performed in R 3.2.2 (R Core Team 2012).

10.3 RESULTS

10.3.1 Sample size

We estimated a sample size of 268 spotted seatrout in 2016, ranging in length interval from 8 to 33 inches (Table 10.1). This sample size provided a range in (*CV*) for age composition approximately from the smallest (*CV*) of 6% for age 1 to the largest (*CV*) of 23% for age 4. In 2016, we randomly selected and aged 239 fish from 314 spotted seatrout collected by VMRC. We fell short in our over-all collections for this optimal length-class sampling estimate by 43 fish. We were not short any fish from the major length intervals (The interval requires 10 or more fish), as a result, the precision for the estimates of major age groups would not be influenced significantly.

10.3.2 Reading precision

Both readers had high self-precision. Specifically, there was no significant difference between the first and second readings for Reader 1 with an agreement of 98% and a *CV* of 0.9% (test of symmetry: $\chi^2 = 1$, $df = 1$, $P = 0.3173$), and there was no significant difference between the first and second readings for Reader 2 with an agreement of 100%. There was no evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 99.58% and a *CV* of 0.2% (test of symmetry: $\chi^2 = 1$, $df = 1$, $P = 0.3173$) (Figure 10.2).

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

10.3.3 Year class

Of the 239 fish aged with otoliths, 6 age classes (0 to 5) were represented (Table

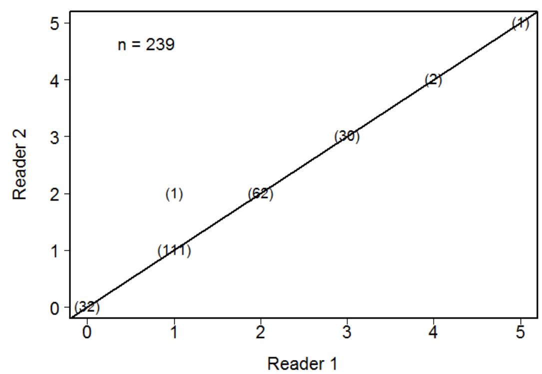


Figure 10.2: Between-reader comparison of otolith age estimates for spotted seatrout collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2016.

10.2). The average age was 1.4 years, and the standard deviation and standard error were 0.9 and 0.06, respectively. Year-class data show that the fishery was comprised of 6 year-classes: fish from the 2011 to 2016 year-classes, with fish primarily from the year classes of 2015 and 2014 with 46.4% and 26.4%, respectively. The ratio of males to females was 1:1.78 in the sample collected (Figure 10.3).

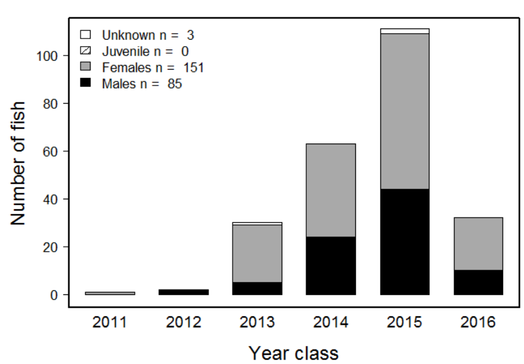


Figure 10.3: Year-class frequency distribution for spotted seatrout collected for ageing in 2016. Distribution is broken down by sex. 'Unknown' represents gonads that were not available for examination or were not examined for sex during sampling.

10.3.4 Age-length key

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.

10.4 REFERENCES

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Table 10.1: Number of spotted seatrout collected and aged in each 1-inch length interval in 2016. 'Target' represents the sample size for ageing estimated for 2016, and 'Need' represents number of fish shorted in each length interval compared to the optimum sample size for ageing and number of fish aged.

Interval	Target	Collected	Aged	Need
8 - 8.99	5	2	2	3
9 - 9.99	5	1	1	4
10 - 10.99	5	2	2	3
11 - 11.99	5	0	0	5
12 - 12.99	16	24	22	0
13 - 13.99	12	13	12	0
14 - 14.99	12	17	12	0
15 - 15.99	18	32	19	0
16 - 16.99	25	42	25	0
17 - 17.99	26	41	27	0
18 - 18.99	21	30	21	0
19 - 19.99	21	27	21	0
20 - 20.99	17	24	18	0
21 - 21.99	9	10	10	0
22 - 22.99	9	10	10	0
23 - 23.99	8	10	8	0
24 - 24.99	7	8	8	0
25 - 25.99	6	8	8	0
26 - 26.99	5	5	5	0
27 - 27.99	6	6	6	0
28 - 28.99	5	2	2	3
29 - 29.99	5	0	0	5
30 - 30.99	5	0	0	5
31 - 31.99	5	0	0	5
32 - 32.99	5	0	0	5
33 - 33.99	5	0	0	5
Totals	268	314	239	43

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Table 10.2: The number of spotted seatrout assigned to each total length-at-age category for 239 fish sampled for otolith age determination in Virginia during 2016.

	Age						
Interval	0	1	2	3	4	5	Totals
8 - 8.99	2	0	0	0	0	0	2
9 - 9.99	0	1	0	0	0	0	1
10 - 10.99	2	0	0	0	0	0	2
12 - 12.99	21	1	0	0	0	0	22
13 - 13.99	7	5	0	0	0	0	12
14 - 14.99	0	12	0	0	0	0	12
15 - 15.99	0	19	0	0	0	0	19
16 - 16.99	0	23	2	0	0	0	25
17 - 17.99	0	21	6	0	0	0	27
18 - 18.99	0	13	8	0	0	0	21
19 - 19.99	0	12	9	0	0	0	21
20 - 20.99	0	4	14	0	0	0	18
21 - 21.99	0	0	5	4	1	0	10
22 - 22.99	0	0	10	0	0	0	10
23 - 23.99	0	0	6	2	0	0	8
24 - 24.99	0	0	2	6	0	0	8
25 - 25.99	0	0	1	7	0	0	8
26 - 26.99	0	0	0	4	0	1	5
27 - 27.99	0	0	0	5	1	0	6
28 - 28.99	0	0	0	2	0	0	2
Totals	32	111	63	30	2	1	239

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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 2016.

	Age					
Interval	0	1	2	3	4	5
8 - 8.99	1	0	0	0	0	0
9 - 9.99	0	1	0	0	0	0
10 - 10.99	1	0	0	0	0	0
12 - 12.99	0.95	0.05	0	0	0	0
13 - 13.99	0.58	0.42	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	0.92	0.08	0	0	0
17 - 17.99	0	0.78	0.22	0	0	0
18 - 18.99	0	0.62	0.38	0	0	0
19 - 19.99	0	0.57	0.43	0	0	0
20 - 20.99	0	0.22	0.78	0	0	0
21 - 21.99	0	0	0.5	0.4	0.1	0
22 - 22.99	0	0	1	0	0	0
23 - 23.99	0	0	0.75	0.25	0	0
24 - 24.99	0	0	0.25	0.75	0	0
25 - 25.99	0	0	0.12	0.88	0	0
26 - 26.99	0	0	0	0.8	0	0.2
27 - 27.99	0	0	0	0.83	0.17	0
28 - 28.99	0	0	0	1	0	0

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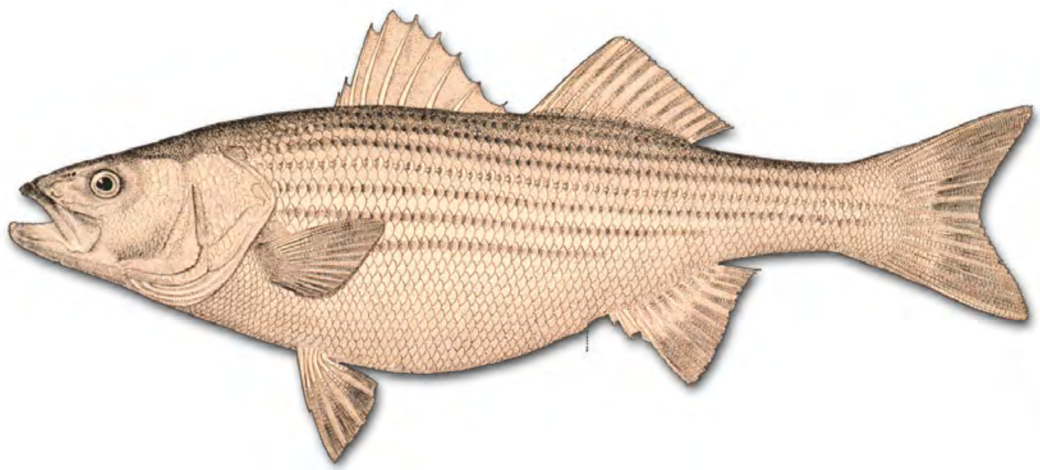
Chapter 11

STRIPED BASS *Morone saxatilis*



11.1 INTRODUCTION

We aged a total of 880 striped bass, *Morone saxatilis*, using their scales collected by the VMRC's Biological Sampling Program in 2016. Of 880 aged fish, 581 and 299 fish were collected in Chesapeake Bay (bay fish) and Virginia waters of the Atlantic Ocean (ocean fish), respectively. The average bay fish age was 8.3 years with a standard deviation of 4 and a standard error of 0.17. Twenty-two age classes (2 to 23) were represented in the bay fish, comprising fish from the 1993 to 2014 year classes. The bay fish sample in 2016 was dominated by the year class of 2011 with 27%. The average ocean fish age was 12.3 years with a standard deviation of 2.8 and a standard error of 0.16. Fifteen age classes (8 to 20, and 22 to 23) were represented in the ocean fish, comprising fish from the 1993 to 1994, and 1996 to 2008 year classes. The ocean fish sample in 2016 was dominated by the year classes of 2005, 2003, 2007, and 2004 with 19%, 15%, 13%, and 13%, respectively. We also aged a total of 319 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 2016, 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 2016; 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 2010 to 2014. θ_a, V_a, B_a, and CV were calculated using pooled age-length data of striped bass collected from 2010 to 2014 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 2010 to 2014 catch. A_l is number of fish to be aged for length interval l in 2016.

11.2.2 Handling of collection

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

11.2.3 Preparation

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 spadefish sagittal otoliths (hereafter, referred to as "otoliths") for age determination. Otolith preparation began by randomly selecting either the right or left otolith. Each whole otolith was placed in a ceramic "Coors" spot plate well and baked in a Thermolyne 1400 furnace at 400 °C. Baking time was dependent on the otolith's size and gauged by color, with a light caramel color desired. Once a suitable color was achieved the baked otolith was embedded in epoxy resin with its distal surface orientated downwards and allowed to harden overnight. The otoliths were viewed by eye and, when necessary, under a stereo microscope to identify the location of the core, and the position of the core was marked using a permanent marker across the epoxy resin surface. At least one transverse cross-section (hereafter, referred to as "thin-section") was then removed from the marked core of each otolith using a Buehler IsoMet™ low-speed saw equipped with two, 3-inch diameter, Norton diamond grinding wheels (hereafter, referred to as "blades"), separated by a stainless steel spacer of 0.5 mm (diameter 2.5"). The otolith was positioned so the blades straddled each side of the otolith focus. It was crucial that this cut be perpendicular to the long axis of the otolith. Failure to do so resulted in broadening and distorted winter growth zones. A proper cut resulted in annuli that were clearly defined and delineated. Once cut, thin-sections were placed on labeled glass slides and covered with a thin layer of Flo-texx mounting medium that not only fixed the sections to the slide, but more importantly, provided enhanced contrast and greater readability by increasing light transmission through the thin-section.

Click here to obtain the protocol at the CQFE website on how to prepare otolith thin-section for ageing striped bass.

11.2.4 Readings

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

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

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

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

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

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

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

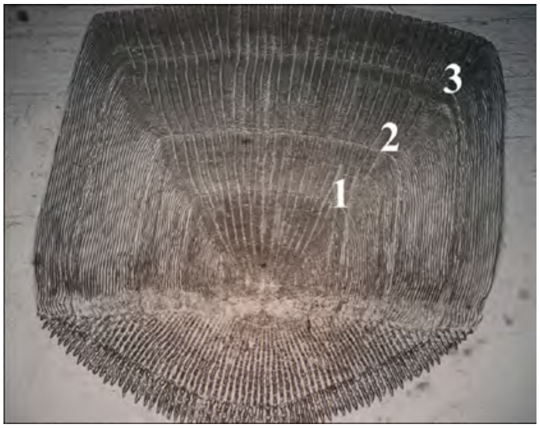


Figure 11.1: Scale impression of a 3 year-old striped bass.

remain consistent throughout the posterior field and rejoin the posterior/anterior interface on the opposite side of the focus. Annuli can also be observed in the anterior lateral field of the scale. Here the annuli typically reveal a pattern of discontinuous and suddenly breaking segmented circuli. This event can also be distinguished by the presence of concentric white lines, which are typically associated with the disruption of circuli.

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

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

Otoliths

All thin-sections were aged by two different readers using a Nikon SMZ1000 stereo microscope under transmitted light and darkfield 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 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

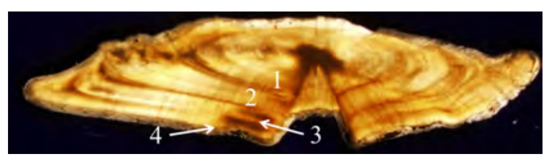


Figure 11.2: Otolith thin-section of a 4 year-old striped bass with the last annulus on the edge of the thin-section

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 subsample of 50 fish from the current year was selected for second readings to examine the difference within a reader. Fifty otoliths randomly selected from fish aged in 2000 were used to examine the time-series bias within each reader. A figure of 1:1 equivalence was used to illustrate those differences (Campana et al. 1995). All statistics analyses were performed in R 3.2.2 (R Core Team 2012).

11.3 RESULTS

11.3.1 Sample size

We estimated a sample size of 559 bay striped bass in 2016, ranging in length interval from 17 to 51 inches (Table 11.1). This sample size provided a range in *CV* for age composition approximately from the smallest *CV* of 11% for age 7 and 10 to the largest *CV* of 25% for age 14 of the bay fish. We randomly selected and aged 581 fish from 788 striped bass collected by VMRC in Chesapeake Bay in 2016. We fell short in our over-all collections for this optimal length-class sampling estimate by 50 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 482 ocean striped bass in 2016, ranging in length interval from 26 to 56 inches (Table 11.2). This sample size provided a range in *CV* for age composition approximately from the smallest *CV* of 9% for age 10 to the largest *CV* of 25% for age 15 of the ocean fish. We aged 299 of 389 (The rest of fish were either without scales or over-collected for certain length interval(s)) striped bass collected by VMRC in Virginia waters of the Atlantic Ocean in 2016. We fell short in our overall collections for this optimal length-class sampling estimate by 219 fish. We were short many fish from in the major length intervals (The interval requires 10 or more fish), as a result, the precision for the estimates of major age groups would definitely be influenced significantly. Therefore, precaution should be used when developing ALK using these age data.

11.3.2 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 56% (1 year or less agreement of 94%) and a *CV* of 3.9% (test of symmetry: $\chi^2 = 14.33$, $df = 11$, $P = 0.2151$), and there was no significant difference between the first and second readings for Reader 2 with an agreement of 62% (1 year or less agreement of 84%) and a *CV* of 4.6% (test of symmetry: $\chi^2 = 9.67$, $df = 12$, $P = 0.6452$). There was an evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 44% (1 year or less agreement of 80%) and a *CV* of 6.2% (test of symmetry: $\chi^2 = 238.21$, $df = 63$, $P < 0.0001$) (Figure 11.3).

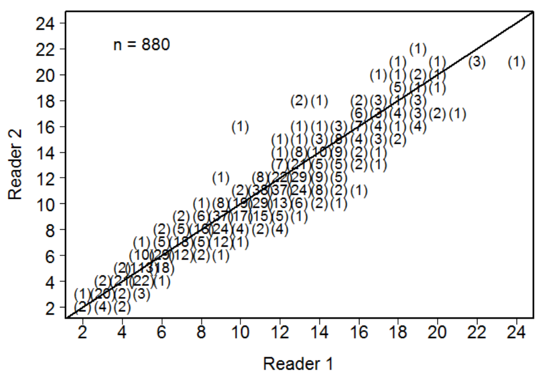


Figure 11.3: Between-reader comparison of scale age estimates for striped bass collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2016.

There was no time-series bias for both readers. Reader 1 had an agreement of 53% (1 year or less agreement of 90%) with ages of fish aged in 2000 with a *CV* of 6.4% (test of symmetry: $\chi^2 = 10.14$, $df = 13$, $P = 0.6822$). Reader 2 had an agreement of 70% (1 year or less agreement of 93%) with a *CV* of 4.1% (test of symmetry: $\chi^2 = 14.67$, $df = 9$, $P = 0.1005$).

Of the 581 bay striped bass aged with scales, 22 age classes (2 to 23) were represented (Table 11.3). The average age for the sample was 8.3 years. The standard deviation and standard error were 4 and 0.17, respectively. Year-class data (Figure 11.4) indicates that recruitment into the fishery in Chesapeake Bay begins at age 2, which corresponds to the 2014 year-class for striped bass caught in 2016. Striped bass in the sample in 2016 was dominated by the year class of 2011 with 27%. The sex ratio of male to female was 1:1.33 for the bay fish.

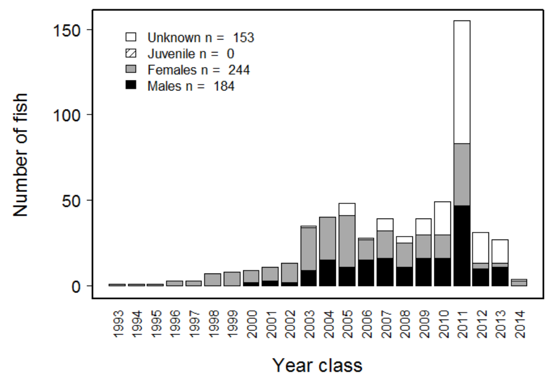


Figure 11.4: Year-class frequency distribution for striped bass collected in Chesapeake Bay, Virginia for ageing in 2016. Distribution is broken down by sex and estimated using scale ages. 'Unknown' represents the fish gonads that were not available for examination or were not examined for sex during sampling.

Of the 299 ocean striped bass aged with scales, 15 age classes (8 to 20, and 22 to 23) were represented (Table 11.4). The average age for the sample was 12.3 years. The standard deviation and standard error were 2.8 and 0.16, respectively. Year-class data (Figure 11.5) indicates that recruitment into the fishery in Virginia waters of Atlantic ocean begins at age 8, which corresponds to the 2008 year-class for striped bass caught in 2016. Striped bass in the sample in 2016 was dominated by the year classes of 2005, 2003, 2007, and 2004 with 19%, 15%, 13%, and 13%, respectively. The sex ratio of male to female was 1:13.85

for the ocean fish.

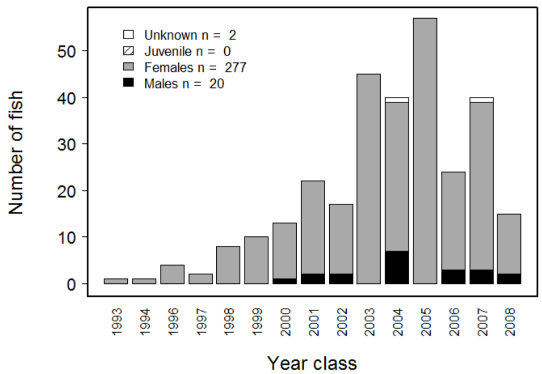


Figure 11.5: Year-class frequency distribution for striped bass collected in Virginia waters of the Atlantic Ocean for ageing in 2016. Distribution is broken down by sex and estimated using scale ages. 'Unknown' represents the fish gonads that were not available for examination or were not examined for sex during sampling.

11.3.3 Otoliths

Both readers had high self-precision. Specifically, there was no significant difference between the first and second readings for Reader 1 with an agreement of 88% and a *CV* of 0.9% (test of symmetry: $\chi^2 = 6$, *df* = 5, $P = 0.3062$), and there was no significant difference between the first and second readings for Reader 2 with an agreement of 94% and a *CV* of 0.3% (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% (1 year or less agreement of 99%) and a *CV* of 0.7% (test of symmetry: $\chi^2 = 28.13$, $df = 21$, $P = 0.1364$) (Figure 11.6).

There was no time-series bias for both readers. Reader 1 had an agreement of 85% with ages of fish aged in 2003 with a *CV* of 1.3% (test of symmetry: $\chi^2 = 7$, $df = 6$, $P = 0.3208$). Reader 2 had an agreement of 68% with a *CV* of 2.9% (test of symmetry:

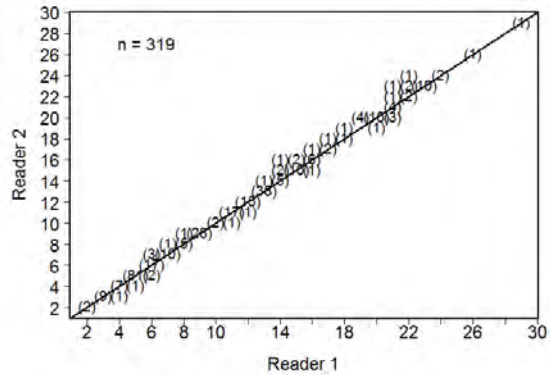


Figure 11.6: Between-reader comparison of otolith age estimates for striped bass collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2016.

$\chi^2 = 16.33$, $df = 9$, $P = 0.0602$).

Of the 319 striped bass aged with otoliths, 25 age classes (2 to 24, 26, and 29) were represented (Table 11.5). The average age for the sample was 10.5 years. The standard deviation and standard error were 5.9 and 0.33, respectively.

11.3.4 Comparison of scale and otolith ages

We aged 319 striped bass using scales and otoliths. There was an evidence of systematic disagreement between otolith and scale ages (test of symmetry: $\chi^2 = 119.74$, $df = 57$, $P < 0.0001$) with an average *CV* of 6.2%. There was an agreement of 49% between scale and otoliths ages whereas scales were assigned a lower and higher age than otoliths for 40% and 12% of the fish, respectively (Figure 11.7). There was also an evidence of bias between otolith and scale ages using an age bias plot (Figure 11.8), with scale generally assigned higher ages for younger fish and lower ages for older fish than otolith age estimates.

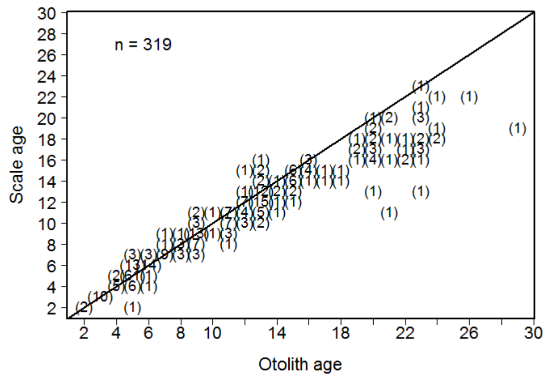


Figure 11.7: Comparison of scale and otolith age estimates for striped bass collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2016.

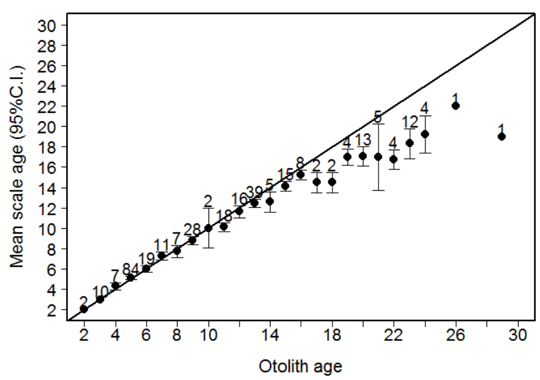


Figure 11.8: Age-bias plot for striped bass scale and otolith age estimates in 2016.

11.3.5 Age-Length-Key (ALK)

We developed an age-length-key for both bay (Table 11.6) and ocean fish (Table 11.7) 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.

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 (Liao et al. 2013; Secor et al. 1995). We will continue to compare the age estimates between otoliths and scales.

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Table 11.1: Number of bay striped bass collected and aged in each 1-inch length interval in 2016. 'Target' represents the sample size for ageing estimated for 2016, 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	0	0	5
18 - 18.99	9	23	13	0
19 - 19.99	21	38	24	0
20 - 20.99	23	49	29	0
21 - 21.99	27	47	33	0
22 - 22.99	28	43	33	0
23 - 23.99	29	66	40	0
24 - 24.99	30	42	30	0
25 - 25.99	27	47	29	0
26 - 26.99	27	42	29	0
27 - 27.99	23	32	24	0
28 - 28.99	18	35	19	0
29 - 29.99	15	32	17	0
30 - 30.99	14	29	14	0
31 - 31.99	16	17	17	0
32 - 32.99	19	18	18	1
33 - 33.99	20	19	18	2
34 - 34.99	23	20	20	3
35 - 35.99	26	23	23	3
36 - 36.99	32	18	18	14
37 - 37.99	30	22	22	8
38 - 38.99	16	22	22	0
39 - 39.99	11	21	15	0
40 - 40.99	10	14	11	0
41 - 41.99	8	12	10	0
42 - 42.99	8	12	8	0
43 - 43.99	8	12	12	0
44 - 44.99	6	12	12	0
45 - 45.99	5	7	7	0
46 - 46.99	5	8	8	0
47 - 47.99	5	4	4	1
48 - 48.99	5	2	2	3
49 - 49.99	5	0	0	5
51 - 51.99	5	0	0	5
Totals	559	788	581	50

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Table 11.2: Number of ocean striped bass collected and aged in each 1-inch length interval in 2016. 'Target' represents the sample size for ageing estimated for 2016, 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
26 - 26.99	5	0	0	5
27 - 27.99	5	0	0	5
28 - 28.99	10	0	0	10
29 - 29.99	23	0	0	23
30 - 30.99	26	0	0	26
31 - 31.99	27	0	0	27
32 - 32.99	28	3	3	25
33 - 33.99	34	7	7	27
34 - 34.99	33	6	6	27
35 - 35.99	43	22	22	21
36 - 36.99	47	42	42	5
37 - 37.99	47	63	62	0
38 - 38.99	33	44	43	0
39 - 39.99	23	30	23	0
40 - 40.99	17	32	17	0
41 - 41.99	13	29	14	0
42 - 42.99	11	33	12	0
43 - 43.99	9	21	10	0
44 - 44.99	7	17	8	0
45 - 45.99	6	15	6	0
46 - 46.99	5	7	7	0
47 - 47.99	5	8	8	0
48 - 48.99	5	8	7	0
49 - 49.99	5	0	0	5
50 - 50.99	5	2	2	3
51 - 51.99	5	0	0	5
56 - 56.99	5	0	0	5
Totals	482	389	299	219

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Table 11.3: The number of striped bass assigned to each total length-at-age category for 581 fish sampled for scale age determination in Chesapeake Bay, Virginia during 2016.

	Age																					
Interval	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	Totals
18 - 18.99	0	6	3	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	13
19 - 19.99	1	7	6	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	24
20 - 20.99	0	3	7	17	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	29
21 - 21.99	1	7	6	16	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	33
22 - 22.99	1	1	5	19	6	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	33
23 - 23.99	0	1	0	20	14	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	40
24 - 24.99	1	2	2	17	2	5	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	30
25 - 25.99	0	0	2	19	5	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	29
26 - 26.99	0	0	0	11	6	6	4	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	29
27 - 27.99	0	0	0	7	4	6	2	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	24
28 - 28.99	0	0	0	5	5	1	3	3	1	0	1	0	0	0	0	0	0	0	0	0	0	0	19
29 - 29.99	0	0	0	3	1	2	2	5	1	3	0	0	0	0	0	0	0	0	0	0	0	0	17
30 - 30.99	0	0	0	5	0	2	3	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	14
31 - 31.99	0	0	0	1	1	1	3	5	2	1	3	0	0	0	0	0	0	0	0	0	0	0	17
32 - 32.99	0	0	0	1	0	0	3	3	1	5	1	2	1	0	1	0	0	0	0	0	0	0	18
33 - 33.99	0	0	0	0	1	3	2	3	2	1	3	2	0	0	1	0	0	0	0	0	0	0	18
34 - 34.99	0	0	0	0	0	3	2	3	4	4	0	2	0	2	0	0	0	0	0	0	0	0	20
35 - 35.99	0	0	0	0	0	0	0	5	3	5	2	3	3	1	0	1	0	0	0	0	0	0	23
36 - 36.99	0	0	0	0	0	0	1	3	2	4	3	3	1	1	0	0	0	0	0	0	0	0	18
37 - 37.99	0	0	0	0	0	0	3	2	4	3	5	3	1	1	0	0	0	0	0	0	0	0	22
38 - 38.99	0	0	0	0	0	0	0	1	2	10	6	3	0	0	0	0	0	0	0	0	0	0	22
39 - 39.99	0	0	0	0	0	0	0	0	2	5	3	4	1	0	0	0	0	0	0	0	0	0	15
40 - 40.99	0	0	0	0	0	0	0	0	0	5	2	2	2	0	0	0	0	0	0	0	0	0	11
41 - 41.99	0	0	0	0	0	0	0	0	1	1	5	3	0	0	0	0	0	0	0	0	0	0	10
42 - 42.99	0	0	0	0	0	0	0	0	0	0	2	3	0	2	0	1	0	0	0	0	0	0	8
43 - 43.99	0	0	0	0	0	0	0	0	0	0	1	4	2	0	0	3	1	0	1	0	0	0	12
44 - 44.99	0	0	0	0	0	0	0	0	0	0	1	1	1	2	3	2	2	0	0	0	0	0	12
45 - 45.99	0	0	0	0	0	0	0	0	0	0	1	0	1	1	1	0	2	1	0	0	0	0	7
46 - 46.99	0	0	0	0	0	0	0	0	0	0	0	0	0	1	3	1	1	0	2	0	0	0	8
47 - 47.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1	1	4
48 - 48.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	2
Totals	4	27	31	155	49	39	29	39	28	48	40	35	13	11	9	8	7	3	3	1	1	1	581

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Table 11.4: The number of striped bass assigned to each total length-at-age category for 299 fish sampled for scale age determination in Virginia waters of Atlantic ocean during 2016.

	Age															
Interval	8	9	10	11	12	13	14	15	16	17	18	19	20	22	23	Totals
32 - 32.99	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	3
33 - 33.99	1	2	1	0	2	0	0	1	0	0	0	0	0	0	0	7
34 - 34.99	2	3	1	0	0	0	0	0	0	0	0	0	0	0	0	6
35 - 35.99	3	10	1	4	1	2	0	1	0	0	0	0	0	0	0	22
36 - 36.99	3	9	9	8	8	3	2	0	0	0	0	0	0	0	0	42
37 - 37.99	5	4	8	16	13	10	4	1	0	1	0	0	0	0	0	62
38 - 38.99	0	10	3	16	6	7	1	0	0	0	0	0	0	0	0	43
39 - 39.99	0	1	0	5	5	9	2	0	1	0	0	0	0	0	0	23
40 - 40.99	1	0	1	5	3	5	0	1	1	0	0	0	0	0	0	17
41 - 41.99	0	0	0	1	1	8	3	0	0	1	0	0	0	0	0	14
42 - 42.99	0	0	0	1	0	1	2	4	2	2	0	0	0	0	0	12
43 - 43.99	0	0	0	0	0	0	0	4	3	2	1	0	0	0	0	10
44 - 44.99	0	0	0	0	0	0	1	3	2	0	0	1	1	0	0	8
45 - 45.99	0	0	0	0	0	0	1	0	1	1	3	0	0	0	0	6
46 - 46.99	0	0	0	0	0	0	0	4	1	0	2	0	0	0	0	7
47 - 47.99	0	0	0	0	0	0	1	2	1	2	0	1	1	0	0	8
48 - 48.99	0	0	0	0	0	0	0	1	1	1	1	0	1	1	1	7
50 - 50.99	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	2
Totals	15	40	24	57	40	45	17	22	13	10	8	2	4	1	1	299

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Table 11.5: The number of striped bass assigned to each total length-at-age category for 319 fish sampled for otolith age determination in Chesapeake Bay and Virginia waters of Atlantic Ocean during 2016.

	Age																									
Interval	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	26	29	Totals
18 - 18.99	0	3	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7
19 - 19.99	0	2	1	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8
20 - 20.99	0	2	3	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12
21 - 21.99	1	1	2	6	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	14
22 - 22.99	1	1	0	15	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	18
23 - 23.99	0	1	0	16	3	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	22
24 - 24.99	0	0	0	10	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12
25 - 25.99	0	0	0	7	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11
26 - 26.99	0	0	1	4	1	2	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11
27 - 27.99	0	0	0	5	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8
28 - 28.99	0	0	0	2	4	0	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9
29 - 29.99	0	0	0	2	0	1	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	6
30 - 30.99	0	0	0	1	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4
31 - 31.99	0	0	0	0	0	1	0	3	0	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	0	0	2	1	2	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	8
33 - 33.99	0	0	0	0	1	0	0	5	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	8
34 - 34.99	0	0	0	0	0	2	0	4	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	8
35 - 35.99	0	0	0	0	0	0	0	3	1	4	3	1	0	0	1	0	0	1	0	0	0	0	0	0	0	14
36 - 36.99	0	0	0	0	0	0	0	4	0	2	0	3	0	2	0	0	0	0	0	0	0	0	0	0	0	11
37 - 37.99	0	0	0	0	0	0	2	1	0	1	2	4	0	1	0	1	0	0	0	1	0	1	0	0	0	14
38 - 38.99	0	0	0	0	0	0	0	1	0	6	2	4	1	0	0	0	0	0	0	0	0	0	0	0	0	14
39 - 39.99	0	0	0	0	0	0	0	0	0	0	3	5	0	1	0	0	0	0	1	0	0	0	0	0	0	10
40 - 40.99	0	0	0	0	0	0	0	0	0	1	0	6	0	2	0	0	0	0	0	0	0	0	0	0	0	9
41 - 41.99	0	0	0	0	0	0	0	0	0	1	2	4	2	1	0	0	0	0	0	0	0	0	0	0	0	10
42 - 42.99	0	0	0	0	0	0	0	0	0	0	1	3	1	2	1	0	0	0	1	0	0	0	0	0	0	9
43 - 43.99	0	0	0	0	0	0	0	0	0	0	0	2	0	2	1	0	0	1	1	2	0	1	0	0	0	10
44 - 44.99	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	1	2	1	3	1	2	2	0	0	0	14
45 - 45.99	0	0	0	0	0	0	0	0	0	0	0	1	0	2	2	0	0	0	1	0	0	3	0	0	1	10
46 - 46.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	1	3	0	1	2	0	0	0	9
47 - 47.99	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	1	2	2	0	0	7
48 - 48.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	3	0	0	0	1	1	0	6
50 - 50.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	2
Totals	2	10	7	84	19	11	7	28	2	18	16	39	5	15	8	2	2	4	13	5	4	12	4	1	1	319

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Table 11.6: 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 2016.

	Age																					
Interval	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
18 - 18.99	0	0.46	0.23	0.31	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19 - 19.99	0.04	0.29	0.25	0.42	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20 - 20.99	0	0.1	0.24	0.59	0.07	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
21 - 21.99	0.03	0.21	0.18	0.48	0.06	0.03	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
22 - 22.99	0.03	0.03	0.15	0.58	0.18	0.03	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
23 - 23.99	0	0.02	0	0.5	0.35	0.12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
24 - 24.99	0.03	0.07	0.07	0.57	0.07	0.17	0.03	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
25 - 25.99	0	0	0.07	0.66	0.17	0.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
26 - 26.99	0	0	0	0.38	0.21	0.21	0.14	0	0.07	0	0	0	0	0	0	0	0	0	0	0	0	0
27 - 27.99	0	0	0	0.29	0.17	0.25	0.08	0.21	0	0	0	0	0	0	0	0	0	0	0	0	0	0
28 - 28.99	0	0	0	0.26	0.26	0.05	0.16	0.16	0.05	0	0.05	0	0	0	0	0	0	0	0	0	0	0
29 - 29.99	0	0	0	0.18	0.06	0.12	0.12	0.29	0.06	0.18	0	0	0	0	0	0	0	0	0	0	0	0
30 - 30.99	0	0	0	0.36	0	0.14	0.21	0.07	0.07	0.07	0.07	0	0	0	0	0	0	0	0	0	0	0
31 - 31.99	0	0	0	0.06	0.06	0.06	0.18	0.29	0.12	0.06	0.18	0	0	0	0	0	0	0	0	0	0	0
32 - 32.99	0	0	0	0.06	0	0	0.17	0.17	0.06	0.28	0.06	0.11	0.06	0	0.06	0	0	0	0	0	0	0
33 - 33.99	0	0	0	0	0.06	0.17	0.11	0.17	0.11	0.06	0.17	0.11	0	0	0.06	0	0	0	0	0	0	0
34 - 34.99	0	0	0	0	0	0.15	0.1	0.15	0.2	0.2	0	0.1	0	0.1	0	0	0	0	0	0	0	0
35 - 35.99	0	0	0	0	0	0	0	0.22	0.13	0.22	0.09	0.13	0.13	0.04	0	0.04	0	0	0	0	0	0
36 - 36.99	0	0	0	0	0	0	0.06	0.17	0.11	0.22	0.17	0.17	0.06	0.06	0	0	0	0	0	0	0	0
37 - 37.99	0	0	0	0	0	0	0.14	0.09	0.18	0.14	0.23	0.14	0.05	0.05	0	0	0	0	0	0	0	0
38 - 38.99	0	0	0	0	0	0	0	0.05	0.09	0.45	0.27	0.14	0	0	0	0	0	0	0	0	0	0
39 - 39.99	0	0	0	0	0	0	0	0	0.13	0.33	0.2	0.27	0.07	0	0	0	0	0	0	0	0	0
40 - 40.99	0	0	0	0	0	0	0	0	0	0.45	0.18	0.18	0.18	0	0	0	0	0	0	0	0	0
41 - 41.99	0	0	0	0	0	0	0	0	0.1	0.1	0.5	0.3	0	0	0	0	0	0	0	0	0	0
42 - 42.99	0	0	0	0	0	0	0	0	0	0	0.25	0.38	0	0.25	0	0.12	0	0	0	0	0	0
43 - 43.99	0	0	0	0	0	0	0	0	0	0	0.08	0.33	0.17	0	0	0.25	0.08	0	0.08	0	0	0
44 - 44.99	0	0	0	0	0	0	0	0	0	0	0.08	0.08	0.08	0.17	0.25	0.17	0.17	0	0	0	0	0
45 - 45.99	0	0	0	0	0	0	0	0	0	0	0.14	0	0.14	0.14	0.14	0	0.29	0.14	0	0	0	0
46 - 46.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0.12	0.38	0.12	0.12	0	0.25	0	0	0
47 - 47.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.25	0	0	0.25	0.25	0.25
48 - 48.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0

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Table 11.7: Age-Length key, as proportion-at-age in each 1-inch length interval, based on scale ages for striped bass sampled in Virginia waters of the Atlantic Ocean during 2016.

	Age														
Interval	8	9	10	11	12	13	14	15	16	17	18	19	20	22	23
32 - 32.99	0	0.33	0	0.33	0.33	0	0	0	0	0	0	0	0	0	0
33 - 33.99	0.14	0.29	0.14	0	0.29	0	0	0.14	0	0	0	0	0	0	0
34 - 34.99	0.33	0.5	0.17	0	0	0	0	0	0	0	0	0	0	0	0
35 - 35.99	0.14	0.45	0.05	0.18	0.05	0.09	0	0.05	0	0	0	0	0	0	0
36 - 36.99	0.07	0.21	0.21	0.19	0.19	0.07	0.05	0	0	0	0	0	0	0	0
37 - 37.99	0.08	0.06	0.13	0.26	0.21	0.16	0.06	0.02	0	0.02	0	0	0	0	0
38 - 38.99	0	0.23	0.07	0.37	0.14	0.16	0.02	0	0	0	0	0	0	0	0
39 - 39.99	0	0.04	0	0.22	0.22	0.39	0.09	0	0.04	0	0	0	0	0	0
40 - 40.99	0.06	0	0.06	0.29	0.18	0.29	0	0.06	0.06	0	0	0	0	0	0
41 - 41.99	0	0	0	0.07	0.07	0.57	0.21	0	0	0.07	0	0	0	0	0
42 - 42.99	0	0	0	0.08	0	0.08	0.17	0.33	0.17	0.17	0	0	0	0	0
43 - 43.99	0	0	0	0	0	0	0	0.4	0.3	0.2	0.1	0	0	0	0
44 - 44.99	0	0	0	0	0	0	0.12	0.38	0.25	0	0	0.12	0.12	0	0
45 - 45.99	0	0	0	0	0	0	0.17	0	0.17	0.17	0.5	0	0	0	0
46 - 46.99	0	0	0	0	0	0	0	0.57	0.14	0	0.29	0	0	0	0
47 - 47.99	0	0	0	0	0	0	0.12	0.25	0.12	0.25	0	0.12	0.12	0	0
48 - 48.99	0	0	0	0	0	0	0	0.14	0.14	0.14	0.14	0	0.14	0.14	0.14
50 - 50.99	0	0	0	0	0	0	0	0	0	0	0.5	0	0.5	0	0

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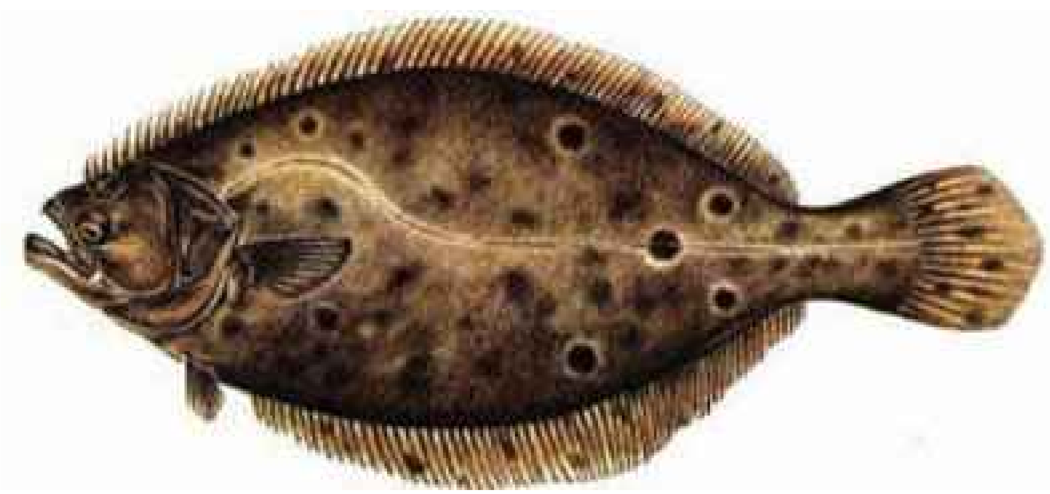
Chapter 12

SUMMER FLOUNDER *Paralichthys dentatus*



12.1 INTRODUCTION

We aged a total of 659 summer flounder,*Paralichthys dentatus*, using their scales collected by the VMRC's Biological Sampling Program in 2016. Of 659 aged fish, 270 and 389 fish were collected in Chesapeake Bay (bay fish) and Virginia waters of the Atlantic Ocean (ocean fish), respectively. The average bay fish age was 3.3 years with a standard deviation of 1.6 and a standard error of 0.1. Nine age classes (1 to 9) were represented in the bay fish, comprising fish from the 2007 to 2015 year classes. The bay fish sample in 2016 was dominated by the year classes of 2012 and 2014 with 27% and 26%, respectively. The average ocean fish age was 5 years with a standard deviation of 2 and a standard error of 0.1. Eleven age classes (1 to 11) were represented in the ocean fish, comprising fish from the 2005 to 2015 year classes. The ocean fish sample in 2016 was dominated by the year classes of 2012, 2010, and 2013 with 17%, 17%, and 16%, respectively. We also aged a total of 204 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 2016, 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:

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

where A is the sample size for ageing summer flounder in 2016; 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 2010 to 2014. θ_a, V_a, B_a, and CV were calculated using pooled age-length data of summer flounder collected from 2010 to 2014 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 2010 to 2014 catch. A_l is number of fish to be aged for length interval l in 2016.

12.2.2 Handling of collection

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

12.2.3 Preparation

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 spadefish sagittal otoliths (hereafter, referred to as "otoliths") for age determination. Otolith preparation began by randomly selecting either the right or left otolith. Each whole otolith was placed in a ceramic "Coors" spot plate well and baked in a Thermolyne 1400 furnace at 400 °C. Baking time was dependent on the otolith's size and gauged by color, with a light caramel color desired. Once a suitable color was achieved the baked otolith was embedded in epoxy resin with its distal surface orientated downwards and allowed to harden overnight. The otoliths were viewed by eye and, when necessary, under a stereo microscope to identify the location of the core, and the position of the core was marked using a permanent marker across the epoxy resin surface. At least one transverse cross-section (hereafter, referred to as "thin-section") was then removed from the marked core of each otolith using a Buehler IsoMet™ low-speed saw equipped with two, 3-inch diameter, Norton diamond grinding wheels (hereafter, referred to as "blades"), separated by a stainless steel spacer of 0.5 mm (diameter 2.5"). The otolith was positioned so the blades straddled each side of the otolith focus. It was crucial that this cut be perpendicular to the long axis of the otolith. Failure to do so resulted in broadening and distorted winter growth zones. A proper cut resulted in annuli that were clearly defined and delineated. Once cut, thin-sections were placed on labeled glass slides and covered with a thin layer of Flo-texx mounting medium that not only fixed the sections to the slide, but more importantly, provided enhanced contrast and greater readability by increasing light transmission through the thin-section.

Click here to obtain the protocol at the CQFE website on how to prepare otolith thin-section for ageing summer flounder.

12.2.4 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 et al. 2000). 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 et al. 1999 and modified by CQFE), and age class assignment using these hard-parts is conducted in the same way as otoliths.

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

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

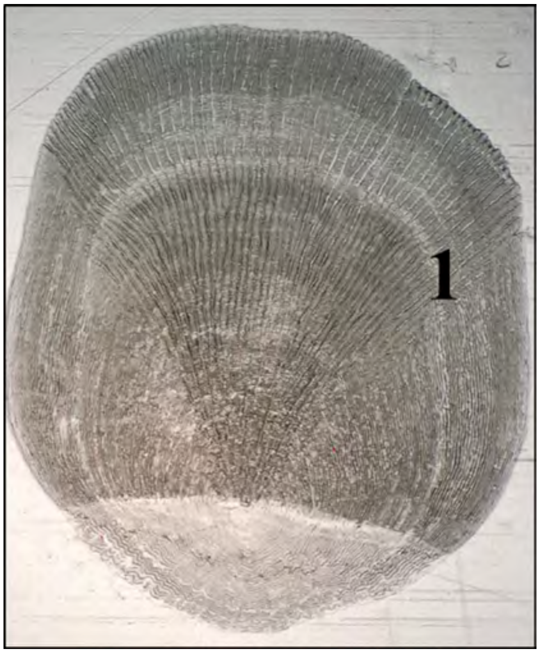


Figure 12.1: Scale impression of a 1 year-old summer flounder

year's growth. Typically annuli of the first three years can be observed transversing this interface as dark bands. These bands remain consistent throughout the posterior field and rejoin the posterior/anterior interface on the opposite side of the focus. Annuli can also be observed in the anterior lateral field of the scale. Here the annuli typically reveal a pattern of discontinuous and suddenly breaking segmented circuli. This event can also be distinguished by the presence of concentric white lines, which are typically associated with the disruption of circuli.

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

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

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 conven-

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

tion 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.

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 subsample of 50 fish from the current year was selected for second readings to examine the difference within a reader. Fifty otoliths randomly selected from fish aged in 2000 were used to examine the time-series bias within each reader. A figure of 1:1 equivalence was used to illustrate those differences (Campana et al. 1995). All statistics analyses were performed in R 3.2.2 (R Core Team 2012).

12.3 RESULTS

12.3.1 Sample size

We estimated a sample size of 317 bay summer flounder in 2016, ranging in length interval 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 21% for age 6 of the bay fish. We randomly selected and aged 270 fish from 337 summer flounder collected by VMRC in Chesapeake Bay in 2016. We fell short in our over-all collections for this optimal length-class sampling estimate by 60 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 388 ocean summer flounder in 2016, ranging in length interval 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 3 and 4 to the largest *CV* of 23% for age 8 of the ocean fish. We randomly selected and aged 389 fish from 510 summer flounder collected by VMRC in Virginia waters of the Atlantic Ocean in 2016. We fell short in our over-all collections for this optimal length-class sampling estimate by 18 fish. We were not short any fish from the major length intervals (The interval requires 10 or more fish), as a result, the precision for the estimates of major age groups would not be influenced significantly.

12.3.2 Scales

Both readers had moderate self-precision. Specifically, there was no significant difference between the first and second readings for Reader 1 with an agreement of 62% (1 year or less agreement of 96%) and a *CV* of 6.7% (test of symmetry: $\chi^2 = 5$, $df = 7$, $P = 0.66$), and there was no significant difference between the first and second readings for Reader 2 with an agreement of 60% (1 year or less agreement of 94%) and a *CV* of 8.3% (test of symmetry: $\chi^2 = 15.2$, $df = 9$, $P = 0.0856$). There was an evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 67% (1 year or less agreement of 96%) and a *CV* of 6.4% (test of symmetry: $\chi^2 = 34$, $df = 21$, $P = 0.0362$) (Figure 12.3).

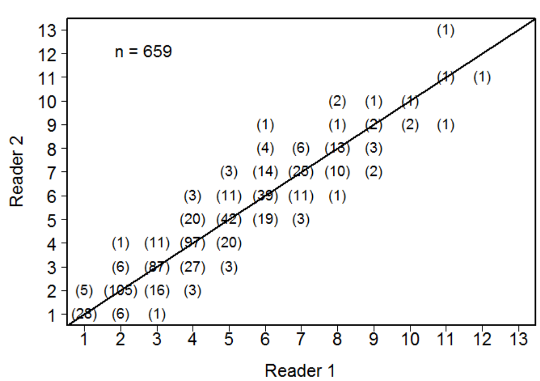


Figure 12.3: Between-reader comparison of scale age estimates for summer flounder collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2016.

There was no time-series bias for both readers. Reader 1 had an agreement of 82% (1 year or less agreement of 98%) with ages of fish aged in 2000 with a *CV* of 4.7% (test of symmetry: $\chi^2 = 4.2$, $df = 5$, $P = 0.521$). Reader 2 had an agreement of 76% (1 year or less agreement of 100%) with a *CV* of 5.1% (test of symmetry: $\chi^2 = 6$, $df = 5$, $P = 0.3062$).

Of the 270 bay summer flounder aged with scales, 9 age classes (1 to 9) were represented (Table 12.3). The average age for the sample was 3.3 years. The standard deviation and standard error were 1.6 and 0.1, respectively. Year-class data (Figure 12.4) indicates that recruitment into the fishery in Chesapeake Bay begins at age 1, which corresponds to the 2015 year-class for summer flounder caught in 2016. Summer flounder in the sample in 2016 was dominated by the year classes of 2012 and 2014 with 27% and 26%, respectively. The sex ratio of male to female was 1:35.5 for the bay fish.

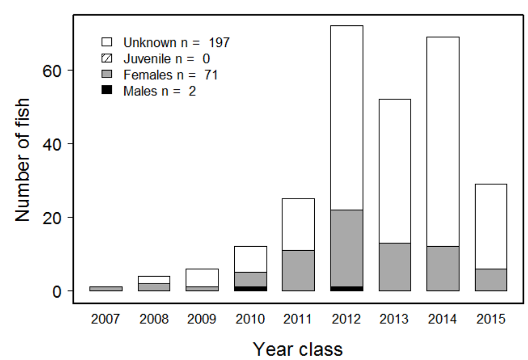


Figure 12.4: Year-class frequency distribution for summer flounder collected in Chesapeake Bay, Virginia for ageing in 2016. Distribution is broken down by sex and estimated using scale ages. 'Unknown' represents gonads that were not available for examination or were not examined for sex during sampling.

Of the 389 ocean summer flounder aged with scales, 11 age classes (1 to 11) were represented (Table 12.4). The average age for the sample was 5 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 1, which corresponds to the 2015 year-class for summer flounder caught in 2016. Summer flounder in the sample in 2016 was dominated by the year classes of 2012, 2010, and 2013 with 17%, 17%, and 16%, respectively. The sex ratio of male to female was 1:1.55 for the ocean fish.

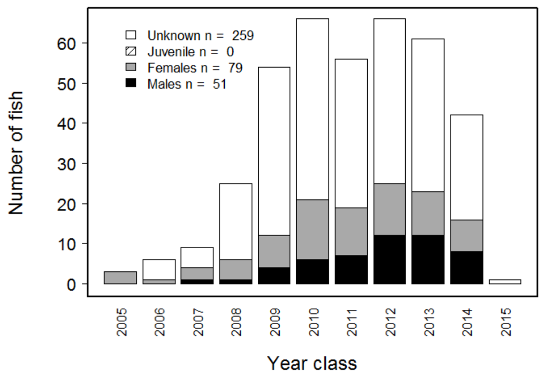


Figure 12.5: Year-class frequency distribution for summer flounder collected in Virginia waters of the Atlantic Ocean for ageing in 2016. Distribution is broken down by sex and estimated using scale ages. 'Unknown' represents gonads that were not available for examination or were not examined for sex during sampling.

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 90% and a *CV* of 2% (test of symmetry: $\chi^2 = 5$, $df = 4$, $P = 0.2873$), and there was no significant difference between the first and second readings for Reader 2 with an agreement of 98% and a *CV* of 0.3% (test of symmetry: $\chi^2 = 1$, $df = 1$, $P = 0.3173$). There was no evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 94% (1 year or less agreement of 100%) and a *CV* of 1.1% (test of symmetry: $\chi^2 = 7.33$, $df = 6$, $P = 0.2911$) (Figure 12.6).

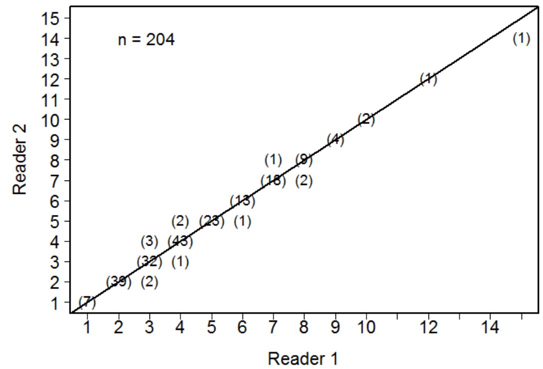


Figure 12.6: Between-reader comparison of otolith age estimates for summer flounder collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2016.

There was no time-series bias for both readers. Reader 1 had an agreement of 88% with ages of fish aged in 2003 with a *CV* of 4% (test of symmetry: $\chi^2 = 6$, $df = 3$, $P = 0.1116$). Reader 2 had an agreement of 92% with a *CV* of 1.5% (test of symmetry: $\chi^2 = 4$, $df = 4$, $P = 0.406$).

Of the 204 summer flounder aged with otoliths, 12 age classes (1 to 10, 12, and 15) were represented (Table 12.5). The average age for the sample was 4.3 years. The standard deviation and standard error were 2.2 and 0.15, respectively.

12.3.4 Comparison of scale and otolith ages

We aged 204 summer flounder using scales and otoliths. There was an evidence of systematic disagreement between otolith and scale ages (test of symmetry: $\chi^2 = 34.03$, $df = 22$, $P = 0.0488$) with an average *CV* of 9.6%. There was an agreement of 54% between scale and otoliths ages whereas scales were assigned a lower and higher age than otoliths for 15% and 30% of the fish, respectively (Figure 12.7). There was also an evidence of bias between otolith and scale ages using an age bias plot(Figure 12.8), with scale generally assigned higher ages for younger fish and lower ages for older fish than otolith age estimates.

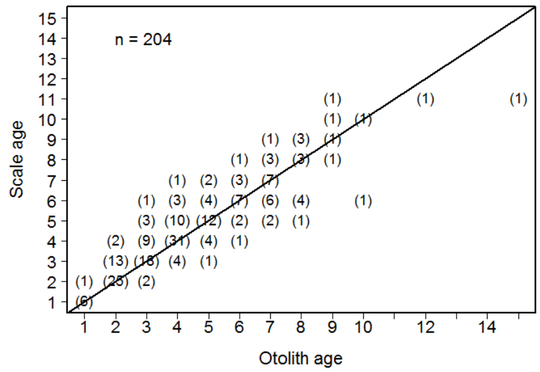


Figure 12.7: Comparison of scale and otolith age estimates for summer flounder collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2016.

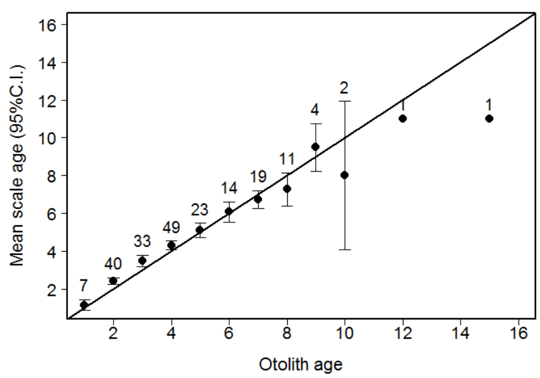


Figure 12.8: Age-bias plot for summer flounder scale and otolith age estimates in 2016.

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.

12.4 REFERENCES

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Table 12.1: Number of bay summer flounder collected and aged in each 1-inch length interval in 2016. 'Target' represents the sample size for ageing estimated for 2016, 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	5	5	0
14 - 14.99	56	94	56	0
15 - 15.99	47	55	48	0
16 - 16.99	37	43	39	0
17 - 17.99	33	39	34	0
18 - 18.99	26	33	27	0
19 - 19.99	23	32	25	0
20 - 20.99	18	24	24	0
21 - 21.99	18	8	8	10
22 - 22.99	11	1	1	10
23 - 23.99	8	2	2	6
24 - 24.99	5	1	1	4
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	317	337	270	60

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Table 12.2: Number of ocean summer flounder collected and aged in each 1-inch length interval in 2016. 'Target' represents the sample size for ageing estimated for 2016, 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	1	1	4
14 - 14.99	33	41	34	0
15 - 15.99	52	62	52	0
16 - 16.99	53	56	53	0
17 - 17.99	43	54	44	0
18 - 18.99	31	43	32	0
19 - 19.99	22	38	22	0
20 - 20.99	21	28	22	0
21 - 21.99	16	25	16	0
22 - 22.99	20	32	20	0
23 - 23.99	19	29	20	0
24 - 24.99	16	26	18	0
25 - 25.99	12	22	17	0
26 - 26.99	10	18	11	0
27 - 27.99	9	14	11	0
28 - 28.99	6	14	9	0
29 - 29.99	5	6	6	0
30 - 30.99	5	1	1	4
31 - 31.99	5	0	0	5
32 - 32.99	5	0	0	5
Totals	388	510	389	18

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Table 12.3: The number of summer flounder assigned to each total length-at-age category for 270 fish sampled for scale age determination in Chesapeake Bay, Virginia during 2016.

	Age									
Interval	1	2	3	4	5	6	7	8	9	Totals
13 - 13.99	1	1	2	1	0	0	0	0	0	5
14 - 14.99	22	17	8	8	1	0	0	0	0	56
15 - 15.99	4	21	17	2	2	2	0	0	0	48
16 - 16.99	2	17	8	9	0	2	1	0	0	39
17 - 17.99	0	10	5	13	5	1	0	0	0	34
18 - 18.99	0	1	4	13	7	0	2	0	0	27
19 - 19.99	0	1	2	15	1	3	1	2	0	25
20 - 20.99	0	1	5	9	7	1	1	0	0	24
21 - 21.99	0	0	1	2	1	2	1	1	0	8
22 - 22.99	0	0	0	0	1	0	0	0	0	1
23 - 23.99	0	0	0	0	0	1	0	1	0	2
24 - 24.99	0	0	0	0	0	0	0	0	1	1
Totals	29	69	52	72	25	12	6	4	1	270

(Go back to text)

Table 12.4: The number of summer flounder assigned to each total length-at-age category for 389 fish sampled for scale age determination in Virginia waters of Atlantic ocean during 2016.

	Age											
Interval	1	2	3	4	5	6	7	8	9	10	11	Totals
13 - 13.99	0	1	0	0	0	0	0	0	0	0	0	1
14 - 14.99	1	13	8	9	1	2	0	0	0	0	0	34
15 - 15.99	0	16	20	6	6	3	1	0	0	0	0	52
16 - 16.99	0	8	15	10	10	8	2	0	0	0	0	53
17 - 17.99	0	1	8	7	7	9	7	5	0	0	0	44
18 - 18.99	0	1	3	10	9	3	5	1	0	0	0	32
19 - 19.99	0	1	1	5	3	1	8	3	0	0	0	22
20 - 20.99	0	0	3	8	2	7	2	0	0	0	0	22
21 - 21.99	0	1	1	5	2	6	0	1	0	0	0	16
22 - 22.99	0	0	1	4	4	3	4	3	0	1	0	20
23 - 23.99	0	0	1	1	4	5	5	2	2	0	0	20
24 - 24.99	0	0	0	0	5	5	3	2	1	2	0	18
25 - 25.99	0	0	0	1	2	6	5	2	1	0	0	17
26 - 26.99	0	0	0	0	1	5	4	0	1	0	0	11
27 - 27.99	0	0	0	0	0	1	3	3	1	2	1	11
28 - 28.99	0	0	0	0	0	2	4	1	0	0	2	9
29 - 29.99	0	0	0	0	0	0	1	2	3	0	0	6
30 - 30.99	0	0	0	0	0	0	0	0	0	1	0	1
Totals	1	42	61	66	56	66	54	25	9	6	3	389

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Table 12.5: The number of summer flounder assigned to each total length-at-age category for 204 fish sampled for otolith age determination in Chesapeake Bay and Virginia waters of Atlantic Ocean during 2016.

	Age												
Interval	1	2	3	4	5	6	7	8	9	10	12	15	Totals
13 - 13.99	0	0	1	0	0	0	0	0	0	0	0	0	1
14 - 14.99	5	12	1	3	0	0	0	0	0	0	0	0	21
15 - 15.99	0	14	5	3	0	0	0	1	0	0	0	0	23
16 - 16.99	2	8	11	5	1	0	0	0	0	0	0	0	27
17 - 17.99	0	1	4	8	1	0	2	1	0	0	0	0	17
18 - 18.99	0	2	2	7	4	0	3	1	0	0	0	0	19
19 - 19.99	0	2	1	10	1	1	2	1	0	0	0	0	18
20 - 20.99	0	1	4	6	4	1	0	1	0	0	0	0	17
21 - 21.99	0	0	3	4	1	1	0	2	0	1	0	0	12
22 - 22.99	0	0	0	0	3	2	0	0	0	0	0	0	5
23 - 23.99	0	0	1	2	3	1	2	2	0	0	0	0	11
24 - 24.99	0	0	0	0	4	3	0	1	1	1	0	0	10
25 - 25.99	0	0	0	1	1	3	2	1	0	0	0	0	8
26 - 26.99	0	0	0	0	0	1	2	0	0	0	0	0	3
27 - 27.99	0	0	0	0	0	0	3	0	1	0	0	1	5
28 - 28.99	0	0	0	0	0	1	2	0	2	0	1	0	6
29 - 29.99	0	0	0	0	0	0	1	0	0	0	0	0	1
Totals	7	40	33	49	23	14	19	11	4	2	1	1	204

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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 2016.

	Age								
Interval	1	2	3	4	5	6	7	8	9
13 - 13.99	0.2	0.2	0.4	0.2	0	0	0	0	0
14 - 14.99	0.39	0.3	0.14	0.14	0.02	0	0	0	0
15 - 15.99	0.08	0.44	0.35	0.04	0.04	0.04	0	0	0
16 - 16.99	0.05	0.44	0.21	0.23	0	0.05	0.03	0	0
17 - 17.99	0	0.29	0.15	0.38	0.15	0.03	0	0	0
18 - 18.99	0	0.04	0.15	0.48	0.26	0	0.07	0	0
19 - 19.99	0	0.04	0.08	0.6	0.04	0.12	0.04	0.08	0
20 - 20.99	0	0.04	0.21	0.38	0.29	0.04	0.04	0	0
21 - 21.99	0	0	0.12	0.25	0.12	0.25	0.12	0.12	0
22 - 22.99	0	0	0	0	1	0	0	0	0
23 - 23.99	0	0	0	0	0	0.5	0	0.5	0
24 - 24.99	0	0	0	0	0	0	0	0	1

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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 2016.

	Age										
Interval	1	2	3	4	5	6	7	8	9	10	11
13 - 13.99	0	1	0	0	0	0	0	0	0	0	0
14 - 14.99	0.03	0.38	0.24	0.26	0.03	0.06	0	0	0	0	0
15 - 15.99	0	0.31	0.38	0.12	0.12	0.06	0.02	0	0	0	0
16 - 16.99	0	0.15	0.28	0.19	0.19	0.15	0.04	0	0	0	0
17 - 17.99	0	0.02	0.18	0.16	0.16	0.2	0.16	0.11	0	0	0
18 - 18.99	0	0.03	0.09	0.31	0.28	0.09	0.16	0.03	0	0	0
19 - 19.99	0	0.05	0.05	0.23	0.14	0.05	0.36	0.14	0	0	0
20 - 20.99	0	0	0.14	0.36	0.09	0.32	0.09	0	0	0	0
21 - 21.99	0	0.06	0.06	0.31	0.12	0.38	0	0.06	0	0	0
22 - 22.99	0	0	0.05	0.2	0.2	0.15	0.2	0.15	0	0.05	0
23 - 23.99	0	0	0.05	0.05	0.2	0.25	0.25	0.1	0.1	0	0
24 - 24.99	0	0	0	0	0.28	0.28	0.17	0.11	0.06	0.11	0
25 - 25.99	0	0	0	0.06	0.12	0.35	0.29	0.12	0.06	0	0
26 - 26.99	0	0	0	0	0.09	0.45	0.36	0	0.09	0	0
27 - 27.99	0	0	0	0	0	0.09	0.27	0.27	0.09	0.18	0.09
28 - 28.99	0	0	0	0	0	0.22	0.44	0.11	0	0	0.22
29 - 29.99	0	0	0	0	0	0	0.17	0.33	0.5	0	0
30 - 30.99	0	0	0	0	0	0	0	0	0	1	0

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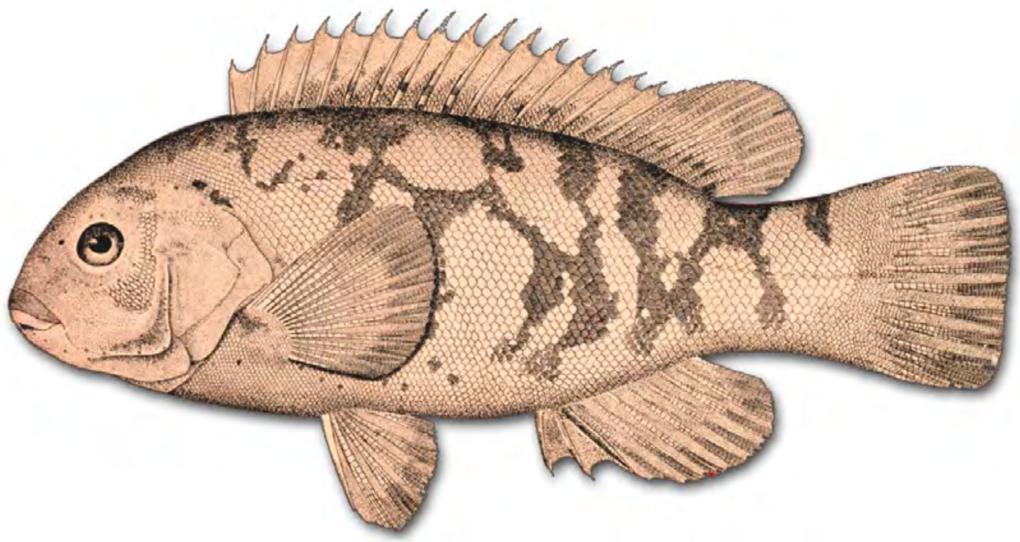
Chapter 13

TAUTOG *Tautoga onitis*



13.1 INTRODUCTION

We aged a total of 223 tautog, *Tautoga onitis*, using their opercula collected by the VMRC's Biological Sampling Program in 2016. Of 223 aged fish, 178 and 45 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 6 years with a standard deviation of 1.7 and a standard error of 0.13. Nine age classes (3 to 10, and 14) were represented in the bay fish, comprising fish from the 2002, and 2006 to 2013 year classes. The bay fish sample in 2016 was dominated by the year classes of 2011 and 2009 with 26% and 25%, respectively. The average age for the ocean fish was 9.8 years with a standard deviation of 6 and a standard error of 0.89. Sixteen age classes (3, 5 to 10, 13 to 16, 18, 20 to 21, 23, and 27) were represented in the ocean fish, comprising fish from the 1989, 1993, 1995 to 1996, 1998, 2000 to 2003, 2006 to 2011, and 2013 year classes. The ocean fish sample in 2016 was dominated by the year classes of 2009, 2011, 2008, and 2010 with 20%, 16%, 16%, and 13%, respectively. We also aged a total of 221 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 2016, 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 2016; 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 2010 to 2014. θ_a, V_a, B_a, and CV were calculated using pooled age-length data of tautog collected from 2010 to 2014 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 2010 to 2014 catch. A_l is number of fish to be aged for length interval l in 2016.

13.2.2 Handling of collection

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

13.2.3 Preparation

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 spadefish sagittal otoliths (hereafter, referred to as "otoliths") for age determination. Otolith preparation began by randomly selecting either the right or left otolith. Each whole otolith was placed in a ceramic "Coors" spot plate well and baked in a Thermolyne 1400 furnace at 400 °C. Baking time was dependent on the otolith's size and gauged by color, with a light caramel color desired. Once a suitable color was achieved the baked otolith was embedded in epoxy resin with its distal surface orientated downwards and allowed to harden overnight. The otoliths were viewed by eye and, when necessary, under a stereo microscope to identify the location of the core, and the position of the core was marked using a permanent marker across the epoxy resin surface. At least one transverse cross-section (hereafter, referred to as "thin-section") was then removed from the marked core of each otolith using a Buehler IsoMet™ low-speed saw equipped with two, 3-inch diameter, Norton diamond grinding wheels (hereafter, referred to as "blades"), separated by a stainless steel spacer of 0.5 mm (diameter 2.5"). The otolith was positioned so the blades straddled each side of the otolith focus. It was crucial that this cut be perpendicular to the long axis of the otolith. Failure to do so resulted in broadening and distorted winter growth zones. A proper cut resulted in annuli that were clearly defined and delineated. Once cut, thin-sections were placed on labeled glass slides and covered with a thin layer of Flo-texx mounting medium that not only fixed the sections to the slide, but more importantly, provided enhanced contrast and greater readability by increasing light transmission through the thin-section.

Click here to obtain the protocol at the CQFE website on how to prepare otolith thin-section for ageing tautog.

13.2.4 Readings

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

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

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

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

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

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

Opercula

All prepared opercula were aged by two different readers in chronological order based on collection date, without knowledge of previously estimated ages or the specimen lengths. Opercula were aged on a light table with no magnification (Figure 13.1).

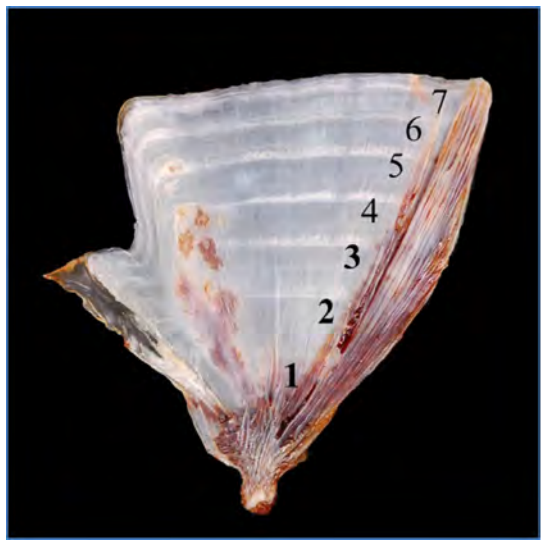


Figure 13.1: Operculum of a 7 year-old tautog

Otoliths

All otolith thin-sections were aged by two different readers using a Nikon SMZ1000 stereo microscope under transmitted light and dark-field polarization at between 8 and 20 times magnification (Figure 13.2). Each reader aged all of the otolith samples.

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

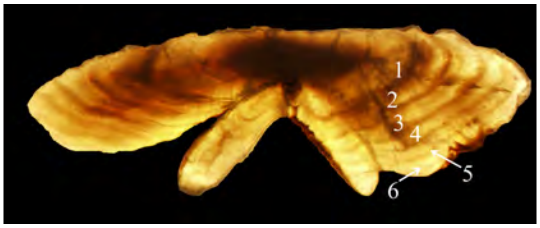


Figure 13.2: Otolith thin-section of 6 year-old tautog

13.2.5 Comparison Tests

A symmetry test (Hoenig et al. 1995) and coefficient of variation (CV) analysis were used to detect any systematic difference and precision on age readings, respectively, for following comparisons: 1) between the two readers in the current year; 2) within each reader in the current year; 3) time-series bias between the current and previous years within each reader; and 4) between operculum and otoliths ages. The readings from the entire sample for the current year were used to examine the difference between two readers. A random sub-sample of 50 fish from the current year was selected for second readings to examine the difference within a reader.Fifty otoliths randomly selected from fish aged in 2000 were used to examine the time-series bias within each reader. A figure of 1:1 equivalence was used to illustrate those differences (Campana et al. 1995). All statistics analyses were performed in R 3.2.2 (R Core Team 2012).

13.3 RESULTS

13.3.1 Sample size

We estimated a sample size of 410 bay tautog in 2016, ranging in length interval from 12 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 23% for age 2 of the bay fish. We aged all 178 tautog collected by VMRC in Chesapeake Bay in 2016. We fell short in our over-all collections for this optimal length-class sampling estimate by 232 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 420 ocean tautog in 2016, ranging in length interval from 11 to 31 inches (Table 13.2). This sample size provided a range in CV for age composition approximately from the smallest CV of 10% for age 5 to the largest CV of 25% for age 11 of the ocean fish. We aged all 45 tautog collected by VMRC in Virginia waters of the Atlantic Ocean in 2016. We fell short in our over-all collections for this optimal length-class sampling estimate by 375 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 60% (1 year or less agreement of 98%) and a CV of 4.2% (test of symmetry: $\chi^2 = 7.2$, $df = 10$, $P = 0.7064$), and there was no significant difference between the first and second readings for Reader 2 with an agreement of 74% (1 year or less agreement of 92%) and a CV of 2.9% (test of symmetry: $\chi^2 = 13$, $df = 11$, $P = 0.2933$). There was no evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 62% (1 year or less agreement of 92%) and a *CV* of 5.1% (test of symmetry: $\chi^2 = 29.17$, $df = 26$, $P = 0.3032$) (Figure 13.3).

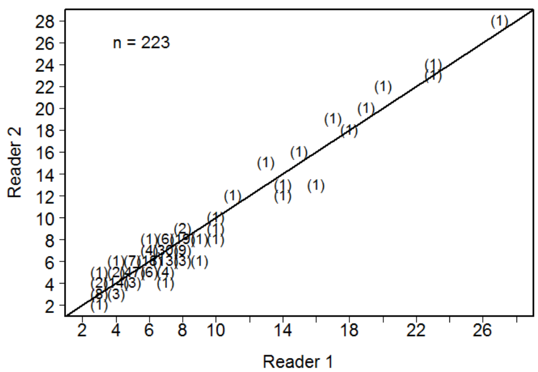


Figure 13.3: Between-reader comparison of operculum age estimates for tautog collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2016.

There was no time-series bias for both readers. Reader 1 had an agreement of 66% (1 year or less agreement of 94%) with ages of fish aged in 2000 with a *CV* of 5.4% (test of symmetry: $\chi^2 = 9$, $df = 10$, $P = 0.5321$). Reader 2 had an agreement of 66% (1 year or less agreement of 92%) with a *CV* of 5.5% (test of symmetry: $\chi^2 = 11$, $df = 12$, $P = 0.5289$).

Of the 178 bay tautog aged with opercula, 9 age classes (3 to 10, and 14) were represented (Table 13.3). The average age for the sample was 6 years. The standard deviation and standard error were 1.7 and 0.13, respectively. Year-class data (Figure 13.4) indicates that recruitment into the fishery in Chesapeake Bay begins at age 3, which corresponds to the 2013 year-class for tautog caught in 2016. Tautog in the sample in 2016 was dominated by the year classes of 2011 and 2009 with 26% and 25%, respectively. The sex ratio of male to female was 1:1.37 for the bay fish.

Of the 45 ocean tautog aged with opercula,

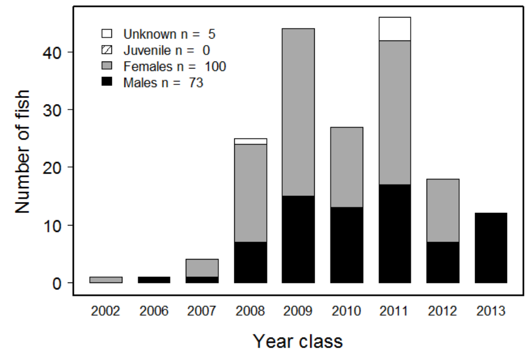


Figure 13.4: Year-class frequency distribution for tautog collected in Chesapeake Bay, Virginia for ageing in 2016. Distribution is broken down by sex and estimated using operculum ages. 'Unknown' represents the fish gonads that were not available for examination or were not examined for sex during sampling.

16 age classes (3, 5 to 10, 13 to 16, 18, 20 to 21, 23, and 27) were represented (Table 13.4). The average age for the sample was 9.8 years. The standard deviation and standard error were 6 and 0.89, respectively. Year-class data (Figure 13.5) indicates that recruitment into the fishery in Virginia waters of Atlantic ocean begins at age 3, which corresponds to the 2013 year-class for tautog caught in 2016. Tautog in the sample in 2016 was dominated by the year classes of 2009, 2011, 2008, and 2010 with 20%, 16%, 16%, and 13%, respectively. The sex ratio of male to female was 1:1.37 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 Reader 1 with an agreement of 86% and a *CV* of 1% (test of symmetry: $\chi^2 = 7$, $df = 6$, $P = 0.3208$), and there was no significant difference between the first and second readings for Reader 2 with an agreement of 90% and a *CV* of 1% (test of symmetry: $\chi^2 = 5$, $df = 4$, $P = 0.2873$). There was no ev-

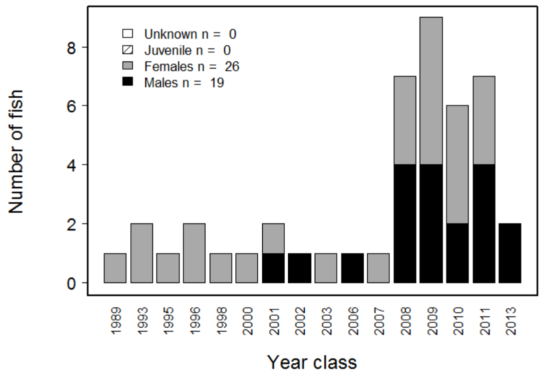


Figure 13.5: Year-class frequency distribution for tautog collected in Virginia waters of the Atlantic Ocean for ageing in 2016. Distribution is broken down by sex and estimated using operculum ages. 'Unknown' represents the fish gonads that were not available for examination or were not examined for sex during sampling.

idence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 90% (1 year or less agreement of 99%) and a *CV* of 0.9% (test of symmetry: $\chi^2 = 16.2$, $df = 16$, $P = 0.4391$) (Figure 13.6). There was no time-series bias for both read-

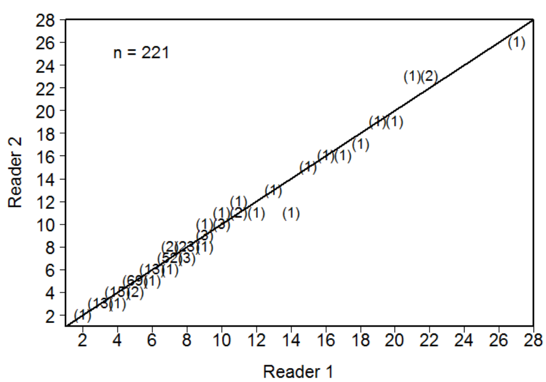


Figure 13.6: Between-reader comparison of otolith age estimates for tautog collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2016.

ers. Reader 1 had an agreement of 94% with ages of fish aged in 2003 with a *CV* of 0.6% (test of symmetry: $\chi^2 = 3$, $df = 2$, $P = 0.2231$). Reader 2 had an agreement of 96% with a *CV* of 0.5% (test of symmetry: $\chi^2 = 2$, $df = 1$, $P = 0.1573$).

Of the 221 tautog aged with otoliths, 21 age classes (2 to 16, 18 to 20, 22 to 23, and 26) were represented (Table 13.5). The average age for the sample was 6.8 years. The standard deviation and standard error were 3.5 and 0.24, respectively.

13.3.4 Comparison of operculum and otolith ages

We aged 221 tautog using opercula and otoliths. There was no evidence of systematic disagreement between otolith and operculum ages (test of symmetry: $\chi^2 = 37.36$, $df = 27$, $P = 0.0885$) with an average *CV* of 5.5%. There was an agreement of 62% between operculum and otoliths ages whereas opercula were assigned a lower and higher age than otoliths for 16% and 21% 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.

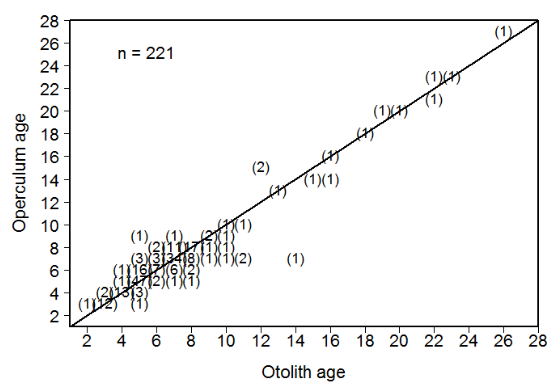


Figure 13.7: Comparison of operculum and otolith age estimates for tautog collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2016.

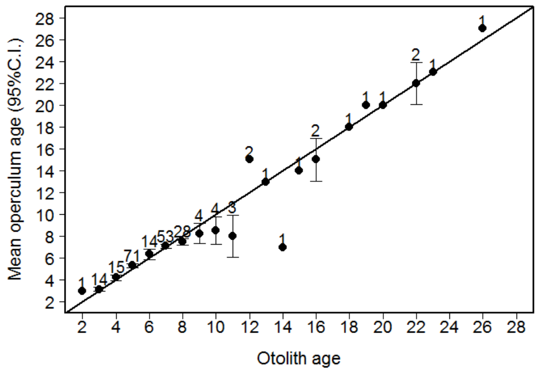


Figure 13.8: Age-bias plot for tautog operculum and otolith age estimates in 2016.

13.3.5 Age-Length-Key (ALK)

We developed an age-length-key for both bay (Table 13.6) and ocean fish (Table 13.7) using operculum ages, separately. The ALK can be used in the conversion of numbers-at-length in the estimated catch to numbers-at-age using operculum ages. The table is based on VMRC's stratified sampling of landings by total length inch intervals.

13.4 REFERENCES

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Table 13.1: Number of bay tautog collected and aged in each 1-inch length interval in 2016. 'Target' represents the sample size for ageing estimated for 2016, 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	20	0	0	20
14 - 14.99	56	19	19	37
15 - 15.99	82	49	49	33
16 - 16.99	79	48	48	31
17 - 17.99	60	35	35	25
18 - 18.99	43	14	14	29
19 - 19.99	28	8	8	20
20 - 20.99	12	2	2	10
21 - 21.99	5	1	1	4
22 - 22.99	5	2	2	3
23 - 23.99	5	0	0	5
24 - 24.99	5	0	0	5
26 - 26.99	5	0	0	5
Totals	410	178	178	232

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Table 13.2: Number of ocean tautog collected and aged in each 1-inch length interval in 2016. 'Target' represents the sample size for ageing estimated for 2016, and 'Need' represents number of fish shorted in each length interval compared to the optimum sample size for ageing and number of fish aged.

Interval	Target	Collected	Aged	Need
11 - 11.99	5	0	0	5
13 - 13.99	10	0	0	10
14 - 14.99	31	0	0	31
15 - 15.99	66	4	4	62
16 - 16.99	77	4	4	73
17 - 17.99	36	8	8	28
18 - 18.99	31	1	1	30
19 - 19.99	26	6	6	20
20 - 20.99	23	2	2	21
21 - 21.99	13	4	4	9
22 - 22.99	16	3	3	13
23 - 23.99	25	1	1	24
24 - 24.99	13	3	3	10
25 - 25.99	8	3	3	5
26 - 26.99	13	2	2	11
27 - 27.99	5	2	2	3
28 - 28.99	5	1	1	4
29 - 29.99	7	0	0	7
30 - 30.99	5	1	1	4
31 - 31.99	5	0	0	5
Totals	420	45	45	375

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Table 13.3: The number of tautog assigned to each total length-at-age category for 178 fish sampled for operculum age determination in Chesapeake Bay, Virginia during 2016.

	Age									
Interval	3	4	5	6	7	8	9	10	14	Totals
14 - 14.99	7	5	3	4	0	0	0	0	0	19
15 - 15.99	4	6	22	4	10	2	1	0	0	49
16 - 16.99	1	6	10	8	14	9	0	0	0	48
17 - 17.99	0	1	7	8	10	8	1	0	0	35
18 - 18.99	0	0	3	2	5	3	1	0	0	14
19 - 19.99	0	0	1	1	4	1	1	0	0	8
20 - 20.99	0	0	0	0	1	1	0	0	0	2
21 - 21.99	0	0	0	0	0	1	0	0	0	1
22 - 22.99	0	0	0	0	0	0	0	1	1	2
Totals	12	18	46	27	44	25	4	1	1	178

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Table 13.4: The number of tautog assigned to each total length-at-age category for 45 fish sampled for operculum age determination in Virginia waters of Atlantic ocean during 2016.

	Age																
Interval	3	5	6	7	8	9	10	13	14	15	16	18	20	21	23	27	Totals
15 - 15.99	1	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	4
16 - 16.99	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	4
17 - 17.99	0	5	2	1	0	0	0	0	0	0	0	0	0	0	0	0	8
18 - 18.99	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1
19 - 19.99	0	0	1	4	1	0	0	0	0	0	0	0	0	0	0	0	6
20 - 20.99	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	2
21 - 21.99	0	0	0	1	3	0	0	0	0	0	0	0	0	0	0	0	4
22 - 22.99	0	0	0	1	1	0	0	0	1	0	0	0	0	0	0	0	3
23 - 23.99	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1
24 - 24.99	0	0	0	0	0	0	1	0	0	0	0	0	1	1	0	0	3
25 - 25.99	0	0	0	0	0	0	0	1	0	1	0	0	1	0	0	0	3
26 - 26.99	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	2
27 - 27.99	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	2
28 - 28.99	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1
30 - 30.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
Totals	2	7	6	9	7	1	1	1	1	2	1	1	2	1	2	1	45

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Table 13.5: The number of tautog assigned to each total length-at-age category for 221 fish sampled for otolith age determination in Chesapeake Bay and Virginia waters of Atlantic Ocean during 2016.

	Age																				
Interval	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	18	19	20	22	23	26	Totals
14 - 14.99	1	8	3	5	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	19
15 - 15.99	0	5	6	27	2	9	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	52
16 - 16.99	0	1	4	18	6	18	3	0	1	1	0	0	0	0	0	0	0	0	0	0	0	52
17 - 17.99	0	0	2	17	4	11	8	0	0	0	0	0	1	0	0	0	0	0	0	0	0	43
18 - 18.99	0	0	0	3	2	5	4	1	0	0	0	0	0	0	0	0	0	0	0	0	0	15
19 - 19.99	0	0	0	1	0	5	5	1	1	0	0	0	0	0	0	0	0	0	0	0	0	13
20 - 20.99	0	0	0	0	0	2	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	4
21 - 21.99	0	0	0	0	0	1	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5
22 - 22.99	0	0	0	0	0	0	1	1	0	1	0	0	0	1	1	0	0	0	0	0	0	5
23 - 23.99	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
24 - 24.99	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	1	0	0	3
25 - 25.99	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	1	0	0	0	0	3
26 - 26.99	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	2
27 - 27.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	2
28 - 28.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1
30 - 30.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
Totals	1	14	15	71	14	53	28	4	4	3	2	1	1	1	2	1	1	1	2	1	1	221

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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 2016.

	Age								
Interval	3	4	5	6	7	8	9	10	14
14 - 14.99	0.37	0.26	0.16	0.21	0	0	0	0	0
15 - 15.99	0.08	0.12	0.45	0.08	0.2	0.04	0.02	0	0
16 - 16.99	0.02	0.12	0.21	0.17	0.29	0.19	0	0	0
17 - 17.99	0	0.03	0.2	0.23	0.29	0.23	0.03	0	0
18 - 18.99	0	0	0.21	0.14	0.36	0.21	0.07	0	0
19 - 19.99	0	0	0.12	0.12	0.5	0.12	0.12	0	0
20 - 20.99	0	0	0	0	0.5	0.5	0	0	0
21 - 21.99	0	0	0	0	0	1	0	0	0
22 - 22.99	0	0	0	0	0	0	0	0.5	0.5

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Table 13.7: Age-Length key, as proportion-at-age in each 1-inch length interval, based on operculum ages for tautog sampled in Virginia waters of the Atlantic Ocean during 2016.

	Age															
Interval	3	5	6	7	8	9	10	13	14	15	16	18	20	21	23	27
15 - 15.99	0.25	0.25	0.5	0	0	0	0	0	0	0	0	0	0	0	0	0
16 - 16.99	0.25	0.25	0.25	0.25	0	0	0	0	0	0	0	0	0	0	0	0
17 - 17.99	0	0.62	0.25	0.12	0	0	0	0	0	0	0	0	0	0	0	0
18 - 18.99	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
19 - 19.99	0	0	0.17	0.67	0.17	0	0	0	0	0	0	0	0	0	0	0
20 - 20.99	0	0	0	0	0.5	0.5	0	0	0	0	0	0	0	0	0	0
21 - 21.99	0	0	0	0.25	0.75	0	0	0	0	0	0	0	0	0	0	0
22 - 22.99	0	0	0	0.33	0.33	0	0	0	0.33	0	0	0	0	0	0	0
23 - 23.99	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
24 - 24.99	0	0	0	0	0	0	0.33	0	0	0	0	0	0.33	0.33	0	0
25 - 25.99	0	0	0	0	0	0	0	0.33	0	0.33	0	0	0.33	0	0	0
26 - 26.99	0	0	0	0	0	0	0	0	0	0.5	0	0	0	0	0	0.5
27 - 27.99	0	0	0	0	0	0	0	0	0	0	0.5	0	0	0	0.5	0
28 - 28.99	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
30 - 30.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0

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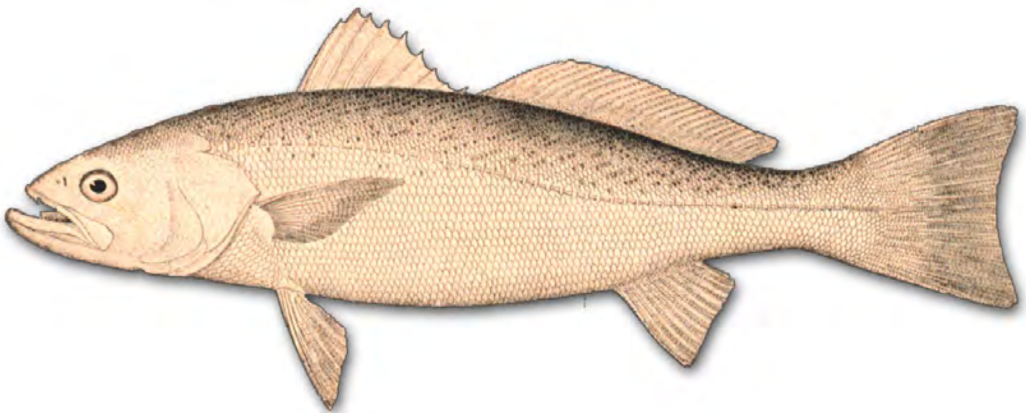
Chapter 14

WEAKFISH *Cynoscion regalis*



14.1 INTRODUCTION

We aged a total of 284 weakfish, *Cynoscion regalis*, collected by the VMRC's Biological Sampling Program for age and growth analysis in 2016. The weakfish ages ranged from 1 to 4 years old with an average age of 2.2, a standard deviation of 0.7, and a standard error of 0.04. Four age classes (1 to 4) were represented, comprising fish of the 2012 to 2015 year-classes. The sample was dominated by fish from the year-class of 2014 with 58.5%.

14.2 METHODS

14.2.1 Sample size for ageing

We estimated sample size for ageing weakfish in 2016 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 2016; 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 2010 to 2014. θ_a, V_a, B_a, and CV were calculated using pooled age-length data of weakfish collected from 2010 to 2014 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 2010 to 2014 catch. A_l is number of fish to be aged for length interval l in 2016.

14.2.2 Handling of collections

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

14.2.3 Preparation

Sagittal otoliths, hereafter, referred to as "otoliths", were processed for age determination following the methods described in Lowerre-Barbieri et al. (1994) with a few modifications. The left or right otolith was randomly selected and attached, distal side down, to a glass slide with clear Crystalbond™ 509 adhesive. The otoliths were viewed by eye and, when necessary, under a stereo microscope to identify the location of the core, and the position of the core was marked using a pencil across the otolith surface. At least one transverse cross-section (hereafter, referred to as "thin-section") was then removed from the marked core of each otolith using a Buehler IsoMet™ low-speed saw equipped with two, 3-inch diameter, Norton diamond grinding wheels (hereafter, referred to as "blades"), separated by a stainless steel spacer of 0.5 mm (diameter 2.5"). Thin-sections were placed on labeled glass slides and covered with a thin layer of Flo-texx mounting medium that not only fixed the sections to the slide, but more importantly, provided enhanced contrast and greater readability by increasing light transmission through the thin-sections.

Click here to obtain the protocol at the CQFE website on how to prepare otolith thin-section for ageing weakfish.

14.2.4 Readings

The CQFE 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. The number of annuli replaces "x" in our notation below, and is the initial "age" assignment of the fish.

Second, the thin-section is examined for translucent growth. If no translucent growth is visible beyond the last 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 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 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 annuli in the thin-section. 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 end of annulus deposition period, is interpreted as being toward the next age class.

For example, weakfish annulus formation occurs between April and July (Lowerre-Barbieri et al. 1994 and modified by CQFE). A weakfish captured between January 1 and July 31, before the end of the species' annulus deposition 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 July 31, the period of annulus deposition, which would be noted as 4+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 14.1). Each reader aged all of the otolith samples. 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

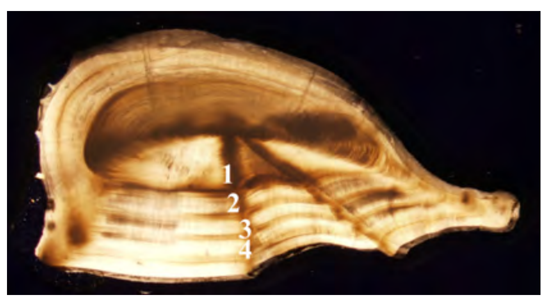


Figure 14.1: Otolith thin-section of 4 year-old weakfish

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.

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

14.2.5 Comparison tests

A symmetry test (Hoenig et al. 1995) and coefficient of variation (*CV*) analysis were used to detect any systematic difference and precision on age readings, respectively, for the following comparisons: 1) between the two readers in the current year, 2) within each reader in the current year, and 3) time-series bias between the current and previous years within each reader. The readings from the entire sample for the current year were used to examine the difference between two readers. A random subsample of 50 fish from the current year was selected for second readings to examine the difference within a reader. Fifty otoliths randomly selected from fish aged in 2003 were used to examine the time-series bias within each reader. A figure of 1:1 equivalence was used to illustrate those differences (Campana et al. 1995). All statistics analyses were performed in R 3.2.2 (R Core Team 2012).

14.3 RESULTS

14.3.1 Sample size

We estimated a sample size of 349 for ageing weakfish in 2016, ranging in length interval from 6 to 35 inches (Table 14.1). This sample size provided a range in (*CV*) for age composition approximately from the smallest (*CV*) of 6% for age 2 to the largest (*CV*) of 21% for age 4. In 2016, we randomly selected and aged 284 fish from 410 weakfish collected by VMRC. We fell short in our over-all collections for this optimal length-class sampling estimate by 75 fish. We were not short any fish from the major length intervals (The interval requires 10 or more fish), as a result, the precision for the estimates of major age groups would not be influenced significantly.

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 98% and a *CV* of 0.9% (test of symmetry: $\chi^2 = 1$, $df = 1$, $P = 0.3173$), and there was no significant difference between the first and second readings for Reader 2 with an agreement of 100%. There was no evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 100% (Figure 14.2).

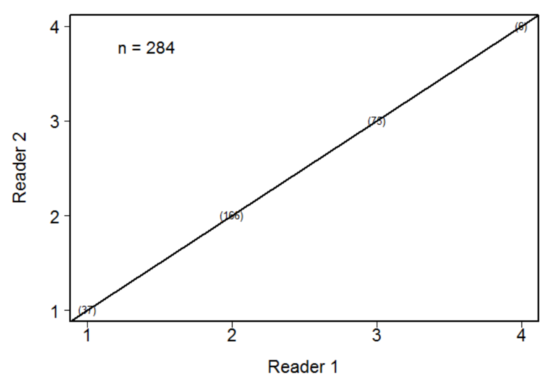


Figure 14.2: Between-reader comparison of otolith age estimates for weakfish collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2016.

There was no time-series bias for both readers. Reader 1 had an agreement of 100% with ages of fish aged in 2003. Reader 2 had an agreement of 98% with a *CV* of 0.2% (test of symmetry: $\chi^2 = 1$, $df = 1$, $P = 0.3173$).

14.3.3 Year class

Of the 284 fish aged with otoliths, 4 age classes (1 to 4) were represented (Table 14.2). The average age was 2.2 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 4 year-classes: fish from the 2012 to 2015 year-classes, with fish primarily from the year-class of 2014 with 58.5%. The ratio of males to females was 1:4.34 in the sample collected (Figure 14.3).

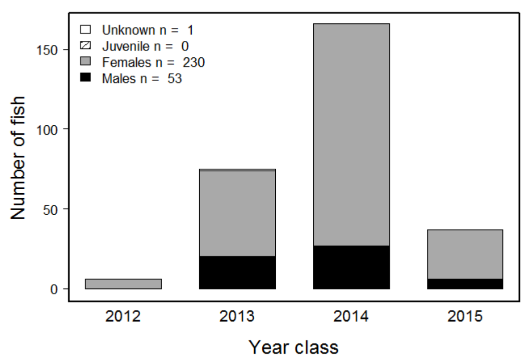


Figure 14.3: Year-class frequency distribution for weakfish collected for ageing in 2016. Distribution is broken down by sex. 'Unknown' represents gonads that were not available for examination or were not examined for sex during sampling.

14.3.4 Age-length key

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.

14.4 REFERENCES

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Table 14.1: Number of weakfish collected and aged in each 1-inch length interval in 2016. 'Target' represents the sample size for ageing estimated for 2016, 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
6 - 6.99	5	0	0	5
7 - 7.99	5	2	2	3
8 - 8.99	5	7	7	0
9 - 9.99	34	54	34	0
10 - 10.99	67	86	68	0
11 - 11.99	47	50	48	0
12 - 12.99	30	38	30	0
13 - 13.99	22	46	22	0
14 - 14.99	16	46	16	0
15 - 15.99	18	42	18	0
16 - 16.99	14	18	18	0
17 - 17.99	9	11	11	0
18 - 18.99	7	7	7	0
19 - 19.99	5	2	2	3
20 - 20.99	5	0	0	5
21 - 21.99	5	0	0	5
22 - 22.99	5	1	1	4
23 - 23.99	5	0	0	5
24 - 24.99	5	0	0	5
25 - 25.99	5	0	0	5
26 - 26.99	5	0	0	5
27 - 27.99	5	0	0	5
28 - 28.99	5	0	0	5
29 - 29.99	5	0	0	5
31 - 31.99	5	0	0	5
34 - 34.99	5	0	0	5
35 - 35.99	5	0	0	5
Totals	349	410	284	75

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Table 14.2: The number of weakfish assigned to each total length-at-age category for 284 fish sampled for otolith age determination in Virginia during 2016.

	Age				
Interval	1	2	3	4	Totals
7 - 7.99	2	0	0	0	2
8 - 8.99	5	2	0	0	7
9 - 9.99	5	28	1	0	34
10 - 10.99	11	55	2	0	68
11 - 11.99	7	29	12	0	48
12 - 12.99	4	13	13	0	30
13 - 13.99	3	9	10	0	22
14 - 14.99	0	5	10	1	16
15 - 15.99	0	9	9	0	18
16 - 16.99	0	7	8	3	18
17 - 17.99	0	5	5	1	11
18 - 18.99	0	2	4	1	7
19 - 19.99	0	1	1	0	2
22 - 22.99	0	1	0	0	1
Totals	37	166	75	6	284

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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 2016.

	Age			
Interval	1	2	3	4
7 - 7.99	1	0	0	0
8 - 8.99	0.71	0.29	0	0
9 - 9.99	0.15	0.82	0.03	0
10 - 10.99	0.16	0.81	0.03	0
11 - 11.99	0.15	0.6	0.25	0
12 - 12.99	0.13	0.43	0.43	0
13 - 13.99	0.14	0.41	0.45	0
14 - 14.99	0	0.31	0.62	0.06
15 - 15.99	0	0.5	0.5	0
16 - 16.99	0	0.39	0.44	0.17
17 - 17.99	0	0.45	0.45	0.09
18 - 18.99	0	0.29	0.57	0.14
19 - 19.99	0	0.5	0.5	0
22 - 22.99	0	1	0	0

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