2010 Final Report Virginia ~ Chesapeake Bay Finfish Ageing & Population Analysis

Hongsheng Liao, Cynthia M. Jones & James R. Davies

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Finfish Ageing for Virginia Catches and Application of Virtual Population Analysis to Provide Management Advice

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Funded by contract No. F-126-R-8 from the Virginia Saltwater Recreational Development Fund through the Virginia Marine Resources Commission

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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 2010. 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 2010 and aged in 2011 at the Ageing 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 = 923); summer flounder, *Paralichthys dentatus*, (n = 816); and tautog, *Tautoga onitis*, (n = 186). Scales and otoliths were used to age summer flounder and striped bass, 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 = 451); black drum, *Pogonias cromis,* (n = 92); bluefish, *Pomatomus saltatrix*, (n = 401); cobia, *Rachycentron canadum*, (n = 109); red drum, *Sciaenops ocellatus,* (n = 57); Sheepshead, *Archosargus probatocephalus*, (n = 70); spadefish, *Chaetodipterus faber*, (n = 263); Spanish mackerel, *Scomberomorous maculates*, (n = 225); spot, *Leiostomus xanthurus*, (n = 277); spotted seatrout, *Cynoscion nebulosus*, (n = 229); and weakfish, *Cynoscion regalis*, (n = 260). In total, we made 8,718 age readings from scales, otoliths and opercula collected during 2010. A summary of the age ranges for all species aged is presented in Table I.

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 3,666 pounds of dissected fish to Wildlife Response, Inc., a local wildlife rescue agency which is responsible for saving injured animals found by the public and to the Salvation Army.

In 2010, we continued to upgrade our Age & Growth Laboratory website, which can be accessed at http://www.odu.edu/fish.The website includes an electronic version of this document and our previous VMRC final reports- from 1999 to 2009. The site also provides more detailed explanations of the methods and structures we use in age determination.

Table I. 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 2010. The hard-parts and age readings include only scales for striped bass and summer flounder, and only opercula for tautog. Numbers of otoliths aged for these species can be found in their chapters.

Species	Number of fish collected	Number of hard-parts	Number of fish aged	Number of readings	Minimum age	Maximum age
Atlantic Croaker	718	718	451	902	1	13
Black Drum	92	92	92	184	3	51
Bluefish	715	715	401	802	0	12
Cobia	111	111	109	218	2	12
Red Drum	57	57	57	114	1	21
Sheepshead	77	76	70	140	2	28
Spadefish	339	338	263	526	1	12
Spanish Mackerel	364	364	225	450	1	8
Spot	371	369	277	554	0	4
Spotted Seatrout	253	253	229	458	0	6
Striped Bass	1331	1325	923	1846	2	21
Summer Flounder	1259	1219	816	1632	1	12
Tautog	187	187	186	372	2	16
Weakfish	379	379	260	520	0	14
Totals	6255	6203	4359	8718		

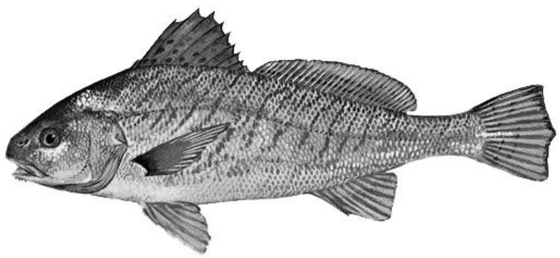
* Age readings don't include those for the estimates of reader-self and time-series precision. Please see details in each chapter.

Acknowledgements

We thank Jessica Gilmore, Brandy Thompson, Megan Matthews, Rebekah Joyce, and David Franklin, and for their technical expertise in preparing otoliths, scales, and opercula for age determination. They all put in long hours processing "tons" of fish in our lab. We are specifically thankful for Dr. William Persons' III hard work on our *Species Updates* and web page. A special note of appreciation is extended to Joe Grist and Joe Cimino 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 Ph. D. students Stacy Beharry, Jason Ferguson, and Renee Reilly and post-doc Jason Schaffler for their help in processing fish whenever we were short of hands.

The image on the front cover is an otolith thin-section from 5 year-old spot.

Chapter 1
Atlantic Croaker



Micropogonias undulatus

INTRODUCTION

We aged a total of 451 Atlantic croaker, *Micropogonias undulatus*, collected by the VMRC's Biological Sampling Program for age and growth analysis in 2010. The croaker ages ranged from 1 to 13 years old with an average age of 5.3, and standard deviation of 2.3, and a standard error of 0.11. Thirteen age classes (1 to 13) were represented, comprising fish from the 1997 to 2009 year-classes. Fish from the 2006 year-class dominated the sample with 32%, followed by 2002 (15%) and 2004 (15%).

METHODS

Sample size for ageing — We estimated sample size for ageing croaker in 2010 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:

$$A = \frac{V_a}{\theta_a^2 CV^2 - B_a / L}, \qquad (1)$$

where A is the sample size for ageing croaker in 2010; 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 is a subsample from a catch and used to estimate length distribution in the catch. θ_a, V_a, B_a, and CV were calculated using pooled age-length data of croaker collected from 2004 to 2008 and using equations in Quinn and Deriso (1999). For simplicity, the equations are not listed here. L was the total number of croaker used by VMRC to estimate length distribution of the catches from 2004 to 2008. The equation (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 achieved by aging an additional 100 or more fish.

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.

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. 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 0.4 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 adhered the sections to the slide, but more importantly, provided enhanced contrast and greater readability by increasing light transmission through the thin-sections.

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, and is the initial "age" assignment of the fish.

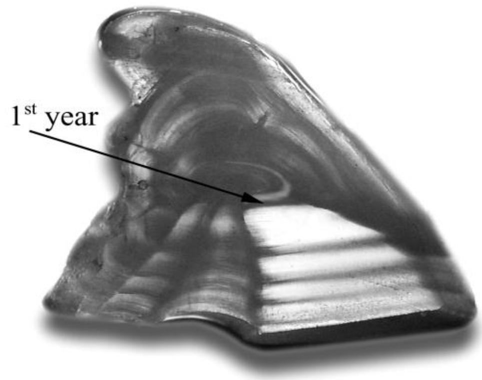
Second, the thin-section is examined for translucent growth. If no translucent growth is visible beyond the last annulus, the otolith is called "even" and no modification of the assigned age is made. The initial assigned age, then, is the age class of the fish. Any growth beyond the last annulus can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

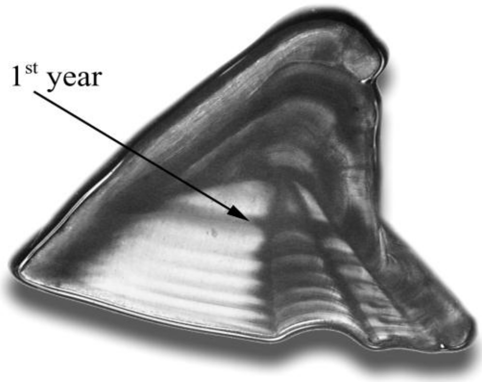
If the fish is captured between January 1 and the end of the species-specific annulus deposition period, it is assigned an age class notation of "x + (x+1)". Thus, any growth beyond the last annulus, after its "birthday" but before the end of annulus deposition period, is interpreted as being toward the next age class.

For example, Atlantic croaker annulus formation occurs between the months of April and May (Barbieri et al. 1994). 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. In addition to the CQFE system of ageing, the ageing criteria reported in Barbieri et al. (1994) were used in age determination, particularly regarding the location of the first annulus (Figure 1).



(a)



(b)

Figure 1. Otolith cross-sections of a) a 5 year old croaker with a small 1st annulus, and b) a 6 year old croaker with a large 1st annulus.

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 2009.

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.

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 and figures were made using R (R Development Core Team 2009).

RESULTS

We estimated a sample size of 487 for ageing Atlantic croaker in 2010, ranging in length interval from 6 to 25 inches (Table 1). This sample size provided a range in CV for age composition approximately from the smallest CV of 9% for age 7 to the largest CV of 24% for age 11 fish. In 2010, we randomly selected and aged 451 fish from 718 croaker collected by VMRC. We fell short in our over-all collections for this optimal length-class sampling estimate by

40 fish. However, the significant shortage were primarily from the very large length intervals, therefore, the precision for the estimates of major age groups (from age 4, 6, and 8) would not be influenced significantly.

The measurement of reader self-precision was very high for both readers. There is 100% agreement between the first and second readings for Reader 1. There is no significant difference between the first and second readings for Reader 2 with an agreement of 98% and a CV of 0.4% (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 99% and a CV of 0.1% (Figure 2).

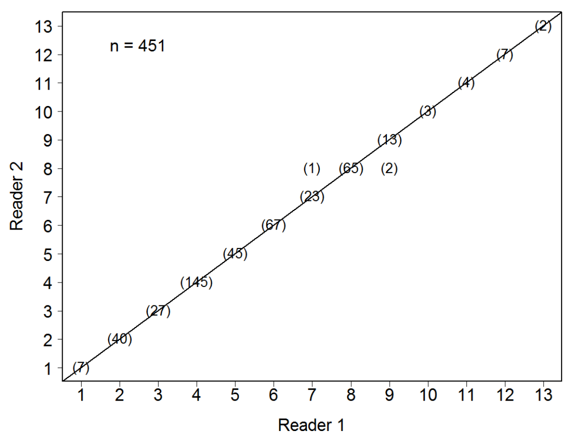


Figure 2. Between-reader comparison of otolith age estimates for Atlantic croaker collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2010.

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

Of the 451 fish aged with otoliths, 13 age classes (1 to 13) were represented (Table 2). The average age was 5.3 years, and the standard deviation and standard error were 2.3 and 0.11, respectively.

Year-class data show that the fishery was comprised of 13 year-classes: fish from the 1997 to 2009 year-classes, with fish primarily from the 2006 year-class (32%), followed by the 2002 (15%) and 2004 (15%). The ratio of males to females was 1:1.84 in the sample collected (Figure 3).

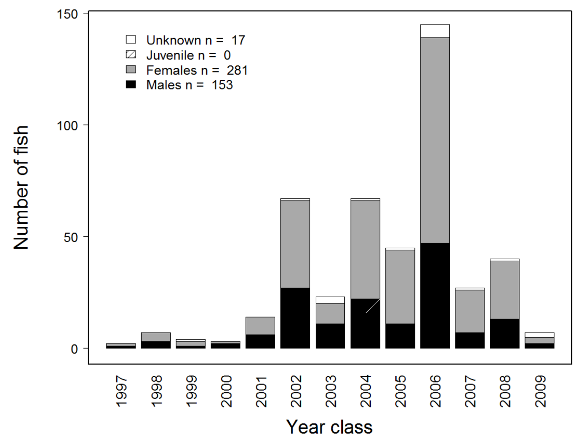


Figure 3. Year-class frequency distribution for Atlantic croaker collected for ageing in 2010. Distribution is broken down by sex. "Unknown" is for the fish either whose gonads were not available for examination or those were not examined for sex during sampling.

Age-Length-Key — We developed an age-length-key (Table 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. Number of Atlantic croaker collected and aged in each 1-inch length interval in 2010. "Target" represents the sample size for ageing estimated for 2010, 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	16	6	0
8 - 8.99	5	19	6	0
9 - 9.99	18	60	18	0
10 - 10.99	29	71	30	0
11 - 11.99	52	68	52	0
12 - 12.99	101	131	101	0
13 - 13.99	71	141	72	0
14 - 14.99	60	87	60	0
15 - 15.99	51	70	51	0
16 - 16.99	34	23	23	11
17 - 17.99	27	24	24	3
18 - 18.99	9	6	6	3
19 - 19.99	5	2	2	3
20 - 20.99	5	0	0	5
21 - 21.99	5	0	0	5
25 - 25.99	5	0	0	5
Totals	487	718	451	40

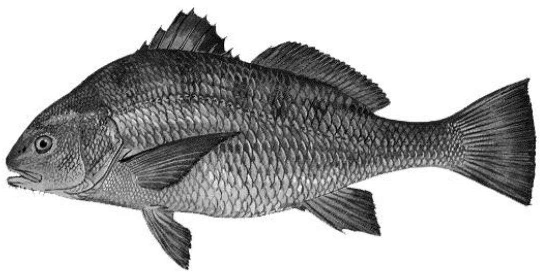
Table 2. The number of Atlantic croaker assigned to each total length-at-age category for 451 fish sampled for otolith age determination in Virginia during 2010.

	Age													
Interval	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**Totals**
7 - 7.99	6	0	0	0	0	0	0	0	0	0	0	0	0	6
8 - 8.99	1	4	1	0	0	0	0	0	0	0	0	0	0	6
9 - 9.99	0	5	5	5	3	0	0	0	0	0	0	0	0	18
10 - 10.99	0	12	4	12	1	1	0	0	0	0	0	0	0	30
11 - 11.99	0	10	6	25	4	7	0	0	0	0	0	0	0	52
12 - 12.99	0	9	3	55	13	14	3	3	1	0	0	0	0	101
13 - 13.99	0	0	7	26	8	14	9	7	1	0	0	0	0	72
14 - 14.99	0	0	1	14	7	11	4	15	4	2	0	2	0	60
15 - 15.99	0	0	0	7	5	11	5	19	0	1	1	1	1	51
16 - 16.99	0	0	0	0	3	6	0	6	5	0	1	1	1	23
17 - 17.99	0	0	0	1	1	3	1	14	3	0	1	0	0	24
18 - 18.99	0	0	0	0	0	0	1	3	0	0	1	1	0	6
19 - 19.99	0	0	0	0	0	0	0	0	0	0	0	2	0	2
Totals	7	40	27	145	45	67	23	67	14	3	4	7	2	451

Table 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 2010.

	Age												
Interval	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
7 - 7.99	1	0	0	0	0	0	0	0	0	0	0	0	0
8 - 8.99	0.167	0.667	0.167	0	0	0	0	0	0	0	0	0	0
9 - 9.99	0	0.278	0.278	0.278	0.167	0	0	0	0	0	0	0	0
10 - 10.99	0	0.4	0.133	0.4	0.033	0.033	0	0	0	0	0	0	0
11 - 11.99	0	0.192	0.115	0.481	0.077	0.135	0	0	0	0	0	0	0
12 - 12.99	0	0.089	0.03	0.545	0.129	0.139	0.03	0.03	0.01	0	0	0	0
13 - 13.99	0	0	0.097	0.361	0.111	0.194	0.125	0.097	0.014	0	0	0	0
14 - 14.99	0	0	0.017	0.233	0.117	0.183	0.067	0.25	0.067	0.033	0	0.033	0
15 - 15.99	0	0	0	0.137	0.098	0.216	0.098	0.373	0	0.02	0.02	0.02	0.02
16 - 16.99	0	0	0	0	0.13	0.261	0	0.261	0.217	0	0.043	0.043	0.043
17 - 17.99	0	0	0	0.042	0.042	0.125	0.042	0.583	0.125	0	0.042	0	0
18 - 18.99	0	0	0	0	0	0	0.167	0.5	0	0	0.167	0.167	0
19 - 19.99	0	0	0	0	0	0	0	0	0	0	0	1	0

Chapter 2
Black Drum



Pogonias cromis

INTRODUCTION

A total of 92 black drum, *Pogonias cromis*, were collected by the VMRC's Biological Sampling Program for age and growth analysis in 2010. The average age of the sample was 12.8 years, with a standard deviation of 10.9 and a standard error of 1.14. Twenty-six age classes were represented with the youngest age of 3 and the oldest age of 51 years, comprising fish from the earliest year-class of 1959 to the most recent year-class of 2007.

METHODS

Handling of collection — Sagittal otoliths (hereafter, refer to as "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.

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. 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 0.4mm (diameter 2.5"). The position of the marked core fell within the 0.4mm 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 adhered the sections to the slide, but more importantly, provided enhanced contrast and greater readability by increasing light transmission through the sections.

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, 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, black drum otolith deposition occurs from May through June (Beckman et al. 1990; Jones and Wells 1997). A black drum 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.

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

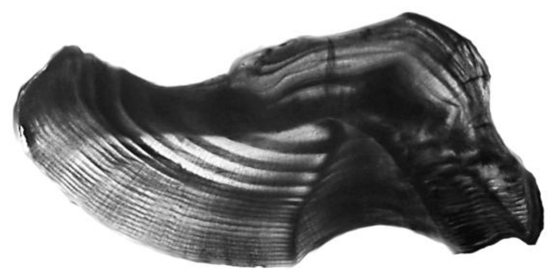


Figure 1. Otolith thin-section from a 20 year-old black drum.

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 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.

Comparison Tests — A symmetry test (Hoenig et al. 1995) and coefficient of variation (CV) analysis were used to detect any systematic difference and precision on age readings, respectively, for following comparisons: 1) between the two readers in the current year, 2) within each reader in the current year, and 3) time-series bias between the current and previous years within each reader. The readings from the entire sample for the current year were used to examine the difference between two readers. A random sub-sample of 50 fish from the current year was selected for second readings to examine the difference within a reader. Fifty otoliths randomly selected from fish aged in 2000 were used to examine the time-series bias within each reader. A figure of 1:1 equivalence was used to illustrate those differences (Campana et al. 1995). All statistics analyses and figures were made using R (R Development Core Team 2009).

RESULTS

The measurement of reader self-precision was very high for both readers. Both readers had a CV of 0.1% and an agreement of 97% (test of symmetry: $\chi^2 = 1$, df = 1, P = 0.3173) between the first and second readings. There was no evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 96.7% and a CV of 0.1% (test of symmetry: $\chi^2 = 3$, df = 3, P = 0.3916) (Figure 2).

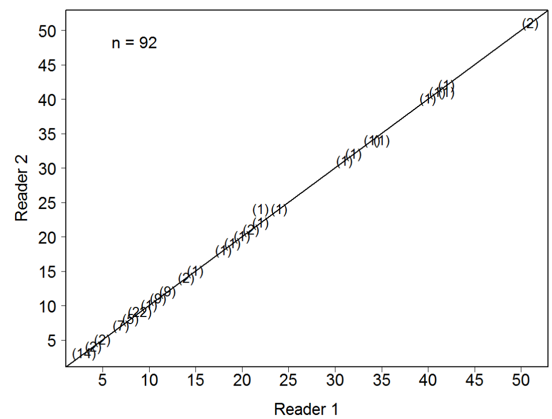


Figure 2. Between-reader comparison of otolith age estimates for black drum collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2010.

Reader 1 had an agreement of 86% with ages of fish aged in 2000 with a CV of 0.3% (test of symmetry: $\chi^2 = 7$, df = 7, P = 0.4289). Reader 2 had an agreement of 90% with ages of fish aged in 2000 with a CV of 0.2% (test of symmetry: $\chi^2 = 5$, df = 4, P = 0.2873).

Of the 92 fish aged with otoliths, 26 age classes were represented (Table 1). The average age of the sample was 12.8 years, with a standard deviation of 10.9 and a standard error of 1.14. The youngest fish was a 3 year old and the oldest fish was 51 years old, representing the year-classes as early as 1959 and as late as 2007 (Figure 3).

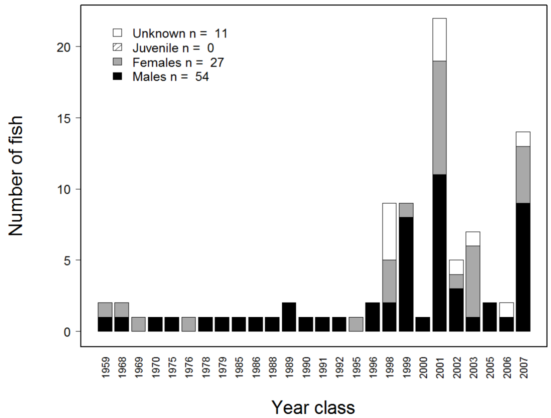


Figure 3. Year-class frequency distribution for black drum collected for ageing in 2010. Distribution is broken down by sex. "Unknown" is for the fish either whose gonads were not available for examination or those were not examined for sex during sampling.

Age-Length-Key — We present an age-length-key (Table 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.

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Table 1. The number of black drum assigned to each total length (inch)-at-age category for 92 fish sampled for otolith age determination in Virginia during 2010.

	Age												
Interval	**3**	**4**	**5**	**7**	**8**	**9**	**10**	**11**	**12**	**14**	**15**	**18**	**19**
21 - 21.99	3	0	0	0	0	0	0	0	0	0	0	0	0
22 - 22.99	2	0	0	0	0	0	0	0	0	0	0	0	0
23 - 23.99	4	0	0	0	0	0	0	0	0	0	0	0	0
24 - 24.99	3	0	0	0	0	0	0	0	0	0	0	0	0
25 - 25.99	2	0	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	0	1	0	0	0	0	0	0	0	0	0	0
29 - 29.99	0	1	0	0	0	1	0	0	0	0	0	0	0
31 - 31.99	0	0	0	0	1	2	0	0	0	0	0	0	0
32 - 32.99	0	0	0	4	1	1	0	0	0	0	0	0	0
33 - 33.99	0	0	0	0	1	2	0	0	0	0	0	0	0
34 - 34.99	0	0	0	2	2	6	1	1	0	0	0	0	0
35 - 35.99	0	0	0	0	0	4	0	4	1	0	0	0	0
36 - 36.99	0	0	1	1	0	3	0	2	3	0	0	0	0
37 - 37.99	0	0	0	0	0	3	0	1	5	1	0	0	0
38 - 38.99	0	0	0	0	0	0	0	1	0	1	1	0	0
40 - 40.99	0	0	0	0	0	0	0	0	0	0	0	0	1
41 - 41.99	0	0	0	0	0	0	0	0	0	0	0	1	0
42 - 42.99	0	0	0	0	0	0	0	0	0	0	0	0	0
43 - 43.99	0	0	0	0	0	0	0	0	0	0	0	0	0
44 - 44.99	0	0	0	0	0	0	0	0	0	0	0	0	0
45 - 45.99	0	0	0	0	0	0	0	0	0	0	0	0	0
46 - 46.99	0	0	0	0	0	0	0	0	0	0	0	0	0
47 - 47.99	0	0	0	0	0	0	0	0	0	0	0	0	0
49 - 49.99	0	0	0	0	0	0	0	0	0	0	0	0	0
Totals	14	2	2	7	5	22	1	9	9	2	1	1	1

Table 1. (continued)

	Age													
Interval	**20**	**21**	**22**	**24**	**25**	**31**	**32**	**34**	**35**	**40**	**41**	**42**	**51**	**Totals**
21 - 21.99	0	0	0	0	0	0	0	0	0	0	0	0	0	3
22 - 22.99	0	0	0	0	0	0	0	0	0	0	0	0	0	2
23 - 23.99	0	0	0	0	0	0	0	0	0	0	0	0	0	4
24 - 24.99	0	0	0	0	0	0	0	0	0	0	0	0	0	3
25 - 25.99	0	0	0	0	0	0	0	0	0	0	0	0	0	2
27 - 27.99	0	0	0	0	0	0	0	0	0	0	0	0	0	1
28 - 28.99	0	0	0	0	0	0	0	0	0	0	0	0	0	1
29 - 29.99	0	0	0	0	0	0	0	0	0	0	0	0	0	2
31 - 31.99	0	0	0	0	0	0	0	0	0	0	0	0	0	3
32 - 32.99	0	0	0	0	0	0	0	0	0	0	0	0	0	6
33 - 33.99	0	0	0	0	0	0	0	0	0	0	0	0	0	3
34 - 34.99	0	0	0	0	0	0	0	0	0	0	0	0	0	12
35 - 35.99	0	0	0	0	0	0	0	0	0	0	0	0	0	9
36 - 36.99	0	0	0	0	0	0	0	0	0	0	0	0	0	10
37 - 37.99	0	0	0	0	0	0	0	0	0	0	0	0	0	10
38 - 38.99	0	0	0	0	0	0	0	0	0	0	0	0	0	3
40 - 40.99	0	0	1	0	0	0	0	0	0	0	0	0	0	2
41 - 41.99	0	0	0	1	0	0	0	0	0	0	0	0	0	2
42 - 42.99	1	2	0	0	0	0	0	0	0	0	0	0	0	3
43 - 43.99	0	0	0	0	1	0	1	0	0	0	0	1	1	4
44 - 44.99	0	0	0	0	0	0	0	1	0	0	1	0	0	2
45 - 45.99	0	0	0	0	0	0	0	0	1	0	0	0	0	1
46 - 46.99	0	0	0	0	0	1	0	0	0	0	0	0	0	1
47 - 47.99	0	0	0	0	0	0	0	0	0	1	0	0	0	1
49 - 49.99	0	0	0	0	0	0	0	0	0	0	0	1	1	2
Totals	1	2	1	1	1	1	1	1	1	1	1	2	2	92

Table 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 2010.

	Age								
Interval	**3**	**4**	**5**	**7**	**8**	**9**	**10**	**11**	**12**
21 - 21.99	1	0	0	0	0	0	0	0	0
22 - 22.99	1	0	0	0	0	0	0	0	0
23 - 23.99	1	0	0	0	0	0	0	0	0
24 - 24.99	1	0	0	0	0	0	0	0	0
25 - 25.99	1	0	0	0	0	0	0	0	0
27 - 27.99	0	1	0	0	0	0	0	0	0
28 - 28.99	0	0	1	0	0	0	0	0	0
29 - 29.99	0	0.5	0	0	0	0.5	0	0	0
31 - 31.99	0	0	0	0	0.333	0.667	0	0	0
32 - 32.99	0	0	0	0.667	0.167	0.167	0	0	0
33 - 33.99	0	0	0	0	0.333	0.667	0	0	0
34 - 34.99	0	0	0	0.167	0.167	0.5	0.083	0.083	0
35 - 35.99	0	0	0	0	0	0.444	0	0.444	0.111
36 - 36.99	0	0	0.1	0.1	0	0.3	0	0.2	0.3
37 - 37.99	0	0	0	0	0	0.3	0	0.1	0.5
38 - 38.99	0	0	0	0	0	0	0	0.333	0
40 - 40.99	0	0	0	0	0	0	0	0	0
41 - 41.99	0	0	0	0	0	0	0	0	0
42 - 42.99	0	0	0	0	0	0	0	0	0
43 - 43.99	0	0	0	0	0	0	0	0	0
44 - 44.99	0	0	0	0	0	0	0	0	0
45 - 45.99	0	0	0	0	0	0	0	0	0
46 - 46.99	0	0	0	0	0	0	0	0	0
47 - 47.99	0	0	0	0	0	0	0	0	0
49 - 49.99	0	0	0	0	0	0	0	0	0

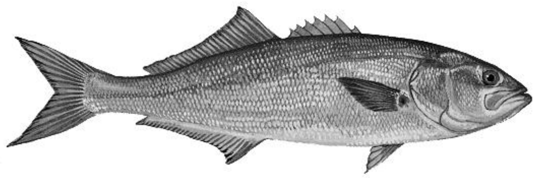
Table 2. (continued)

	Age								
Interval	**14**	**15**	**18**	**19**	**20**	**21**	**22**	**24**	**25**
21 - 21.99	0	0	0	0	0	0	0	0	0
22 - 22.99	0	0	0	0	0	0	0	0	0
23 - 23.99	0	0	0	0	0	0	0	0	0
24 - 24.99	0	0	0	0	0	0	0	0	0
25 - 25.99	0	0	0	0	0	0	0	0	0
27 - 27.99	0	0	0	0	0	0	0	0	0
28 - 28.99	0	0	0	0	0	0	0	0	0
29 - 29.99	0	0	0	0	0	0	0	0	0
31 - 31.99	0	0	0	0	0	0	0	0	0
32 - 32.99	0	0	0	0	0	0	0	0	0
33 - 33.99	0	0	0	0	0	0	0	0	0
34 - 34.99	0	0	0	0	0	0	0	0	0
35 - 35.99	0	0	0	0	0	0	0	0	0
36 - 36.99	0	0	0	0	0	0	0	0	0
37 - 37.99	0.1	0	0	0	0	0	0	0	0
38 - 38.99	0.333	0.333	0	0	0	0	0	0	0
40 - 40.99	0	0	0	0.5	0	0	0.5	0	0
41 - 41.99	0	0	0.5	0	0	0	0	0.5	0
42 - 42.99	0	0	0	0	0.333	0.667	0	0	0
43 - 43.99	0	0	0	0	0	0	0	0	0.25
44 - 44.99	0	0	0	0	0	0	0	0	0
45 - 45.99	0	0	0	0	0	0	0	0	0
46 - 46.99	0	0	0	0	0	0	0	0	0
47 - 47.99	0	0	0	0	0	0	0	0	0
49 - 49.99	0	0	0	0	0	0	0	0	0

Table 2. (continued)

	Age							
Interval	**31**	**32**	**34**	**35**	**40**	**41**	**42**	**51**
21 - 21.99	0	0	0	0	0	0	0	0
22 - 22.99	0	0	0	0	0	0	0	0
23 - 23.99	0	0	0	0	0	0	0	0
24 - 24.99	0	0	0	0	0	0	0	0
25 - 25.99	0	0	0	0	0	0	0	0
27 - 27.99	0	0	0	0	0	0	0	0
28 - 28.99	0	0	0	0	0	0	0	0
29 - 29.99	0	0	0	0	0	0	0	0
31 - 31.99	0	0	0	0	0	0	0	0
32 - 32.99	0	0	0	0	0	0	0	0
33 - 33.99	0	0	0	0	0	0	0	0
34 - 34.99	0	0	0	0	0	0	0	0
35 - 35.99	0	0	0	0	0	0	0	0
36 - 36.99	0	0	0	0	0	0	0	0
37 - 37.99	0	0	0	0	0	0	0	0
38 - 38.99	0	0	0	0	0	0	0	0
40 - 40.99	0	0	0	0	0	0	0	0
41 - 41.99	0	0	0	0	0	0	0	0
42 - 42.99	0	0	0	0	0	0	0	0
43 - 43.99	0	0.25	0	0	0	0	0.25	0.25
44 - 44.99	0	0	0.5	0	0	0.5	0	0
45 - 45.99	0	0	0	1	0	0	0	0
46 - 46.99	1	0	0	0	0	0	0	0
47 - 47.99	0	0	0	0	1	0	0	0
49 - 49.99	0	0	0	0	0	0	0.5	0.5

Chapter 3
Bluefish



Pomatomus saltatrix

INTRODUCTION

We aged a total of 401 bluefish, *Pomatomus saltatrix*, collected by the VMRC's Biological Sampling Program for age and growth analysis in 2010. The bluefish ages ranged from 0 to 8, and 12 years old with an average age of 2.6, and standard deviation of 2, and a standard error of 0.1. Ten age classes represented fish of the 1998, and 2002 through 2010 year-classes. Fish from the 2008 year-class dominated the sample with 41%, followed by 2009 (27%).

METHODS

Sample size for ageing — We estimated sample size for ageing bluefish in 2010 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:

$$A = \frac{V_a}{\theta_a^2 CV^2 - B_a / L}, \qquad (1)$$

where A is the sample size for ageing bluefish in 2010; 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 coefficient of variation; L is a subsample from a catch and is used to estimate length distribution in the catch. θ_a, V_a, B_a, and CV were calculated using pooled age-length data of bluefish collected from 2004 to 2008 and using equations in Quinn and Deriso (1999). For simplicity, the equations are not listed here. L was the total number of bluefish used by VMRC to estimate length distribution of the catches from 2004 to 2008. The equation (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 achieved by aging an additional 100 or more fish. Based on VMRC's request in 2010, we used 1-cm length interval for bluefish, which differed from other species (1-inch).

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.

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 otolith was mounted with clear, Crystalbond™ 509 adhesive onto a standard microscope slide with its distal surface orientated upwards. 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, 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 0.4 mm (diameter 2.5”). The otolith was positioned so that the blades straddled each side of the otolith focus marked by pencil. It was crucial that this cut be perpendicular to the long axis of the otolith. Failure to do so resulted in “broadening” and distortion of winter growth zones. A proper cut resulted in annuli that were clearly defined and delineated. Once cut, the thin-section was placed into a ceramic “Coors” spot plate well and baked in a Thermolyne 1400 furnace at 400^{o}C. Baking time was dependent on the thin-section’s size and gauged by color, with a light caramel color desired. Once a suitable color was reached the baked thin-section was placed on a labeled glass slide and covered with a thin layer of Flo-texx® mounting medium that not only adhered the sections to the slide, but more importantly, provided enhanced contrast and greater readability by increasing light transmission through the sections.

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, and is the initial “age” assignment of the fish.

Second, the otolith thin-section is examined for translucent growth. If no translucent growth is visible beyond the last opaque 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 counted 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 opaque band deposition period, is interpreted as being toward the next age class.

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

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 1).

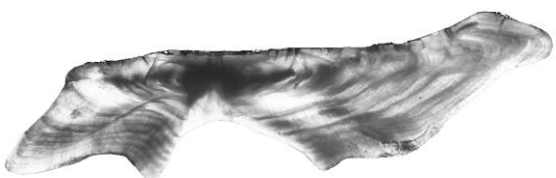


Figure 1. Otolith thin-section from a 8 year-old female bluefish with a total length of 850 mm.

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 4-7years 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.

Comparison Tests — A symmetry test (Hoenig et al. 1995) and coefficient of variation (CV) analysis were used to detect any systematic difference and precision on age readings, respectively, for the following comparisons: 1) between the two readers in the current year, 2) within each reader in the current year, and 3) time-series bias between the current and previous years within each reader. The readings from the entire sample for the current year were used to examine the difference between two readers. A random sub-sample of 50 fish from the current year was selected for second readings to examine the difference within a reader. Fifty otoliths randomly selected from fish aged in 2000 were used to examine the time-series bias within each reader. A figure of 1:1 equivalence was used to illustrate those differences (Campana et al. 1995). All statistics analyses and figures were made using R (R Development Core Team 2009).

RESULTS

We estimated a sample size of 445 for ageing bluefish in 2010, ranging in length interval from 18 to 91 cm (Table 1). This sample size provided a range in CV for age composition approximately from the smallest CV of 4% for age 2 and the largest CV of 25% for age 7 fish. In 2010, we randomly selected and aged 401 fish from the 711 bluefish collected by VMRC. We fell short in our over-all collections for this optimal length-class sampling estimate by 71 fish. Because those fish mainly fell within the very large and small length intervals (Table 1), the precision for the estimates of major age groups (Age 1 and 2) would not be influenced significantly.

The measurement of reader self-precision was very good for Reader 1 with a CV of 1.3% smaller than 2009 (2.9%) ($\chi^2 = 2$, df = 2, P = 0.3679). The measurement of reader self-precision was good for Reader 2 with a CV of 4.3% smaller than 2009 (7.7%) ($\chi^2 = 2$, df = 3, P = 0.5724). There was no evidence of systematic disagreement between Reader 1 and Reader 2 in 2010 (CV = 2.1%, test of symmetry: χ^2 = 12.29, df = 10, P = 0.2664) (Figure 2).

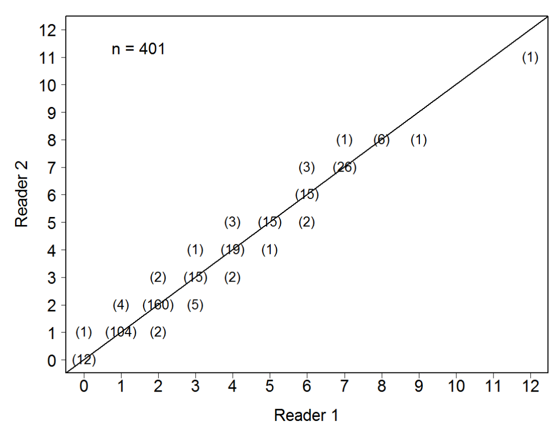


Figure 2. Between-reader comparison of otolith age estimates for bluefish collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2010.

There is no time-series bias for both readers. Reader 1 had an agreement of 90% with ages of fish aged in 2000 with a CV of 4.6% (test of symmetry: $\chi^2 = 3$, df

= 4, P = 0.5578). Reader 2 had an agreement of 94% with ages of fish aged in 2000 with a CV of 2.8% (test of symmetry: $\chi^2 = 3$, df = 2, $P = 0.2231$).

Of the 401 fish aged, 10 age classes were represented (Table 2). The average age for the sample was 2.6 years, and the standard deviation and standard error were 2 and 0.1, respectively.

Year-class data indicates that recruitment into the fishery began at age 0, which corresponded to the 2010 year-class for bluefish caught in 2010. One and two-year-old fish were the dominant year-classes in the 2009 sample (Figure 3).

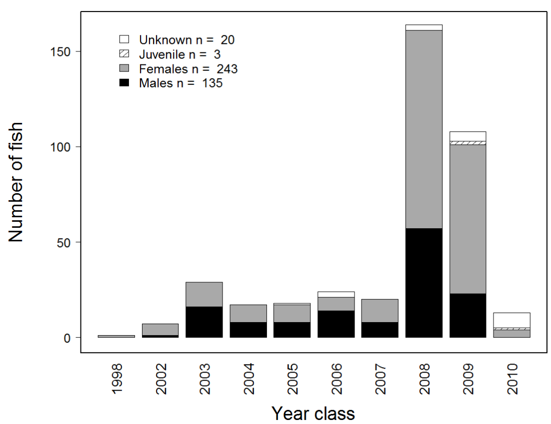


Figure 3. Year-class frequency distribution for bluefish collected for ageing in 2010. Distribution is broken down by sex. “Unknown” is for the fish either whose gonads were not available for examination or those were not examined for sex during sampling.

Age-Length-Key — We present an age-length-key (Table 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. Number of bluefish collected and aged in each 1-cm length interval in 2010. "Target" represent the sample size for ageing estimated for 2010, "Collected" represents number of fish with both total length and otoliths (4 fish without total length not included), and "Need" represents number of fish shorted in each length interval compared to the optimum sample size for ageing and number of fish aged.

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

Table 1. (continued)

Interval	Target	Collected	Aged	Need
53 - 53.99	5	7	6	0
54 - 54.99	5	9	6	0
55 - 55.99	5	3	3	2
56 - 56.99	5	5	5	0
57 - 57.99	5	4	4	1
58 - 58.99	5	2	2	3
59 - 59.99	5	6	6	0
60 - 60.99	5	4	4	1
61 - 61.99	5	3	3	2
62 - 62.99	5	4	4	1
63 - 63.99	5	3	3	2
64 - 64.99	5	2	2	3
65 - 65.99	5	2	2	3
66 - 66.99	5	2	2	3
67 - 67.99	5	4	4	1
68 - 68.99	5	3	3	2
69 - 69.99	5	2	2	3
70 - 70.99	5	3	3	2
71 - 71.99	5	0	0	5
72 - 72.99	5	6	5	0
73 - 73.99	5	5	5	0
74 - 74.99	5	3	3	2
75 - 75.99	5	5	5	0
76 - 76.99	5	8	5	0
77 - 77.99	5	9	5	0
78 - 78.99	5	16	5	0
79 - 79.99	5	17	5	0
80 - 80.99	5	19	5	0
81 - 81.99	5	16	5	0
82 - 82.99	5	15	6	0
83 - 83.99	5	15	6	0
84 - 84.99	5	15	6	0
85 - 85.99	5	9	5	0
86 - 86.99	5	5	5	0
87 - 87.99	5	7	7	0
88 - 88.99	5	1	1	4
89 - 89.99	5	0	0	5
90 - 90.99	5	1	1	4
91 - 91.99	5	0	0	5
Totals	445	711	401	71

Table 2. The number of bluefish assigned to each total length-at-age category for 401 fish sampled for otolith age determination in Virginia during 2010.

	Age										
Interval	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**12**	**Totals**
20 - 20.99	1	3	0	0	0	0	0	0	0	0	4
21 - 21.99	0	3	0	0	0	0	0	0	0	0	3
22 - 22.99	4	1	0	0	0	0	0	0	0	0	5
23 - 23.99	3	3	0	0	0	0	0	0	0	0	6
24 - 24.99	2	3	0	0	0	0	0	0	0	0	5
25 - 25.99	2	4	0	0	0	0	0	0	0	0	6
26 - 26.99	1	5	0	0	0	0	0	0	0	0	6
27 - 27.99	0	6	0	0	0	0	0	0	0	0	6
28 - 28.99	0	6	0	0	0	0	0	0	0	0	6
29 - 29.99	0	6	0	0	0	0	0	0	0	0	6
30 - 30.99	0	6	0	0	0	0	0	0	0	0	6
31 - 31.99	0	4	2	0	0	0	0	0	0	0	6
32 - 32.99	0	4	2	0	0	0	0	0	0	0	6
33 - 33.99	0	5	3	0	0	0	0	0	0	0	8
34 - 34.99	0	3	3	0	0	0	0	0	0	0	6
35 - 35.99	0	4	4	0	0	0	0	0	0	0	8
36 - 36.99	0	4	4	0	0	0	0	0	0	0	8
37 - 37.99	0	5	5	0	0	0	0	0	0	0	10
38 - 38.99	0	5	5	0	0	0	0	0	0	0	10
39 - 39.99	0	2	6	2	0	0	0	0	0	0	10
40 - 40.99	0	3	6	0	0	0	0	0	0	0	9
41 - 41.99	0	4	5	1	0	0	0	0	0	0	10
42 - 42.99	0	1	10	1	0	0	0	0	0	0	12
43 - 43.99	0	1	9	2	0	0	0	0	0	0	12
44 - 44.99	0	4	6	0	0	0	0	0	0	0	10
45 - 45.99	0	2	10	0	0	0	0	0	0	0	12
46 - 46.99	0	5	6	1	0	0	0	0	0	0	12
47 - 47.99	0	1	8	1	0	0	0	0	0	0	10
48 - 48.99	0	1	7	2	0	0	0	0	0	0	10
49 - 49.99	0	1	3	2	0	0	0	0	0	0	6
50 - 50.99	0	0	5	1	0	0	0	0	0	0	6
51 - 51.99	0	1	4	1	0	0	0	0	0	0	6
52 - 52.99	0	0	4	2	0	0	0	0	0	0	6

Table 2. (continued)

	Age										
Interval	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**12**	**Totals**
53 - 53.99	0	0	6	0	0	0	0	0	0	0	6
54 - 54.99	0	0	5	1	0	0	0	0	0	0	6
55 - 55.99	0	1	2	0	0	0	0	0	0	0	3
56 - 56.99	0	1	4	0	0	0	0	0	0	0	5
57 - 57.99	0	0	4	0	0	0	0	0	0	0	4
58 - 58.99	0	0	2	0	0	0	0	0	0	0	2
59 - 59.99	0	0	5	1	0	0	0	0	0	0	6
60 - 60.99	0	0	4	0	0	0	0	0	0	0	4
61 - 61.99	0	0	3	0	0	0	0	0	0	0	3
62 - 62.99	0	0	4	0	0	0	0	0	0	0	4
63 - 63.99	0	0	3	0	0	0	0	0	0	0	3
64 - 64.99	0	0	1	0	1	0	0	0	0	0	2
65 - 65.99	0	0	2	0	0	0	0	0	0	0	2
66 - 66.99	0	0	2	0	0	0	0	0	0	0	2
67 - 67.99	0	0	0	2	1	1	0	0	0	0	4
68 - 68.99	0	0	0	0	3	0	0	0	0	0	3
69 - 69.99	0	0	0	0	1	1	0	0	0	0	2
70 - 70.99	0	0	0	0	3	0	0	0	0	0	3
72 - 72.99	0	0	0	0	4	1	0	0	0	0	5
73 - 73.99	0	0	0	0	2	2	1	0	0	0	5
74 - 74.99	0	0	0	0	2	1	0	0	0	0	3
75 - 75.99	0	0	0	0	3	0	1	1	0	0	5
76 - 76.99	0	0	0	0	1	3	0	1	0	0	5
77 - 77.99	0	0	0	0	2	1	1	1	0	0	5
78 - 78.99	0	0	0	0	1	1	2	1	0	0	5
79 - 79.99	0	0	0	0	0	1	2	2	0	0	5
80 - 80.99	0	0	0	0	0	1	2	2	0	0	5
81 - 81.99	0	0	0	0	0	1	2	2	0	0	5
82 - 82.99	0	0	0	0	0	3	2	1	0	0	6
83 - 83.99	0	0	0	0	0	1	0	4	1	0	6
84 - 84.99	0	0	0	0	0	0	1	4	1	0	6
85 - 85.99	0	0	0	0	0	0	0	2	3	0	5
86 - 86.99	0	0	0	0	0	0	0	3	2	0	5
87 - 87.99	0	0	0	0	0	0	3	3	0	1	7
88 - 88.99	0	0	0	0	0	0	0	1	0	0	1
90 - 90.99	0	0	0	0	0	0	0	1	0	0	1
Totals	13	108	164	20	24	18	17	29	7	1	401

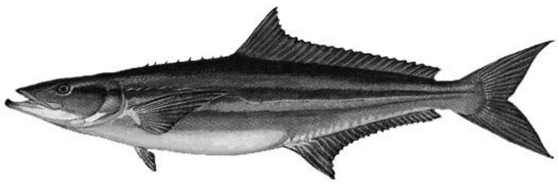
Table 3. Age-Length key, as proportion-at-age in each 1-cm length interval, based on otolith ages for bluefish sampled for age determination in Virginia during 2010.

	Age									
Interval	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**12**
20 - 20.99	0.25	0.75	0	0	0	0	0	0	0	0
21 - 21.99	0	1	0	0	0	0	0	0	0	0
22 - 22.99	0.8	0.2	0	0	0	0	0	0	0	0
23 - 23.99	0.5	0.5	0	0	0	0	0	0	0	0
24 - 24.99	0.4	0.6	0	0	0	0	0	0	0	0
25 - 25.99	0.333	0.667	0	0	0	0	0	0	0	0
26 - 26.99	0.167	0.833	0	0	0	0	0	0	0	0
27 - 27.99	0	1	0	0	0	0	0	0	0	0
28 - 28.99	0	1	0	0	0	0	0	0	0	0
29 - 29.99	0	1	0	0	0	0	0	0	0	0
30 - 30.99	0	1	0	0	0	0	0	0	0	0
31 - 31.99	0	0.667	0.333	0	0	0	0	0	0	0
32 - 32.99	0	0.667	0.333	0	0	0	0	0	0	0
33 - 33.99	0	0.625	0.375	0	0	0	0	0	0	0
34 - 34.99	0	0.5	0.5	0	0	0	0	0	0	0
35 - 35.99	0	0.5	0.5	0	0	0	0	0	0	0
36 - 36.99	0	0.5	0.5	0	0	0	0	0	0	0
37 - 37.99	0	0.5	0.5	0	0	0	0	0	0	0
38 - 38.99	0	0.5	0.5	0	0	0	0	0	0	0
39 - 39.99	0	0.2	0.6	0.2	0	0	0	0	0	0
40 - 40.99	0	0.333	0.667	0	0	0	0	0	0	0
41 - 41.99	0	0.4	0.5	0.1	0	0	0	0	0	0
42 - 42.99	0	0.083	0.833	0.083	0	0	0	0	0	0
43 - 43.99	0	0.083	0.75	0.167	0	0	0	0	0	0
44 - 44.99	0	0.4	0.6	0	0	0	0	0	0	0
45 - 45.99	0	0.167	0.833	0	0	0	0	0	0	0
46 - 46.99	0	0.417	0.5	0.083	0	0	0	0	0	0
47 - 47.99	0	0.1	0.8	0.1	0	0	0	0	0	0
48 - 48.99	0	0.1	0.7	0.2	0	0	0	0	0	0
49 - 49.99	0	0.167	0.5	0.333	0	0	0	0	0	0
50 - 50.99	0	0	0.833	0.167	0	0	0	0	0	0
51 - 51.99	0	0.167	0.667	0.167	0	0	0	0	0	0
52 - 52.99	0	0	0.667	0.333	0	0	0	0	0	0

Table 3. (continued)

	Age									
Interval	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**12**
53 - 53.99	0	0	1	0	0	0	0	0	0	0
54 - 54.99	0	0	0.833	0.167	0	0	0	0	0	0
55 - 55.99	0	0.333	0.667	0	0	0	0	0	0	0
56 - 56.99	0	0.2	0.8	0	0	0	0	0	0	0
57 - 57.99	0	0	1	0	0	0	0	0	0	0
58 - 58.99	0	0	1	0	0	0	0	0	0	0
59 - 59.99	0	0	0.833	0.167	0	0	0	0	0	0
60 - 60.99	0	0	1	0	0	0	0	0	0	0
61 - 61.99	0	0	1	0	0	0	0	0	0	0
62 - 62.99	0	0	1	0	0	0	0	0	0	0
63 - 63.99	0	0	1	0	0	0	0	0	0	0
64 - 64.99	0	0	0.5	0	0.5	0	0	0	0	0
65 - 65.99	0	0	1	0	0	0	0	0	0	0
66 - 66.99	0	0	1	0	0	0	0	0	0	0
67 - 67.99	0	0	0	0.5	0.25	0.25	0	0	0	0
68 - 68.99	0	0	0	0	1	0	0	0	0	0
69 - 69.99	0	0	0	0	0.5	0.5	0	0	0	0
70 - 70.99	0	0	0	0	1	0	0	0	0	0
72 - 72.99	0	0	0	0	0.8	0.2	0	0	0	0
73 - 73.99	0	0	0	0	0.4	0.4	0.2	0	0	0
74 - 74.99	0	0	0	0	0.667	0.333	0	0	0	0
75 - 75.99	0	0	0	0	0.6	0	0.2	0.2	0	0
76 - 76.99	0	0	0	0	0.2	0.6	0	0.2	0	0
77 - 77.99	0	0	0	0	0.4	0.2	0.2	0.2	0	0
78 - 78.99	0	0	0	0	0.2	0.2	0.4	0.2	0	0
79 - 79.99	0	0	0	0	0	0.2	0.4	0.4	0	0
80 - 80.99	0	0	0	0	0	0.2	0.4	0.4	0	0
81 - 81.99	0	0	0	0	0	0.2	0.4	0.4	0	0
82 - 82.99	0	0	0	0	0	0.5	0.333	0.167	0	0
83 - 83.99	0	0	0	0	0	0.167	0	0.667	0.167	0
84 - 84.99	0	0	0	0	0	0	0.167	0.667	0.167	0
85 - 85.99	0	0	0	0	0	0	0	0.4	0.6	0
86 - 86.99	0	0	0	0	0	0	0	0.6	0.4	0
87 - 87.99	0	0	0	0	0	0	0.429	0.429	0	0.143
88 - 88.99	0	0	0	0	0	0	0	1	0	0
90 - 90.99	0	0	0	0	0	0	0	1	0	0

Chapter 4
Cobia



Rachycentron canadum

INTRODUCTION

We aged a total of 109 cobia, *Rachycentron canadum*, collected by the VMRC's Biological Sampling Program for age and growth analysis in 2010. The average age of the sample was 5.2 years, with a standard deviation of 1.9 and a standard error of 0.18. Nine age classes were represented with the youngest age of 2 and the oldest age of 12 years (from age 2 to 9, and 12), comprising fish from the earliest year-class of 1998, and 2001 through the most recent year-class of 2008. The year class of 2007 (28%), 2004 (23%), and 2005 (20%) were dominant in the sample.

METHODS

Handling of collection — Sagittal otoliths (hereafter, referred to as "otoliths") were received by the Age & Growth Laboratory in labeled coin envelopes. Once in our hands, they were sorted based on date of capture, their envelope labels were verified against VMRC's collection data, and assigned unique Age and Growth Laboratory identification numbers. All otoliths were stored inside of protective Axygen 2.0ml microtubes within their original labeled coin envelopes.

Preparation — Due to their fragility, we used our embedding and thin-sectioning method to prepare cobia otoliths for age determination. To start, a series of 14 mm x 5 mm x 3 mm wells (Ladd Industries silicon rubber mold) were pre-filled to half-volume with Loctite® 349 adhesive and permitted to cure for 24 hours until solidified. Otoliths were placed distal-side up on the solidified base layer. The remaining volume in the well was filled with Loctite® 349. When all the wells were filled, and no bubbles remained within the wells, the silicon rubber mold was placed under a UV light to solidify overnight. Once dry, each embedded otolith was removed from the mold and mounted 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 an Ultra-Fine Point Sharpie® permanent marker. 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 (hereafter, "blades"), separated by a stainless steel spacer of 0.4mm (diameter 2.5"). The position of the marked core fell within the 0.4mm 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.

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, 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, cobia otolith deposition occurs during June (Franks et al. 1999). A cobia 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.

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 1).

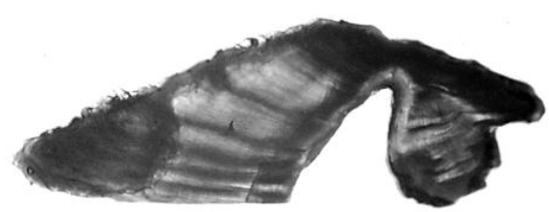


Figure 1. Otolith thin-section from a 1524 mm TL 6 year old cobia.

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.

Comparison Tests — A symmetry test (Hoenig et al. 1995) and coefficient of variation (CV) analysis were used to detect any systematic difference and precision on age readings, respectively, for the following comparisons: 1) between the two readers in the current year, 2) within each reader in the current year, and 3) time-series bias between the current and previous years within each reader. The readings from the entire sample for the current year were used to examine the difference between two readers. A random sub-sample of 50 fish from the current year was selected for second readings to examine the difference within a reader. Fifty otoliths randomly selected from fish aged in 2000 were used to examine the time-series bias within each reader. A figure of 1:1 equivalence was used to illustrate those differences (Campana et al. 1995). All statistics analyses and figures were made using R (R Development Core Team 2009).

RESULTS

The measurement of reader self-precision was very high for both readers. There is no significant difference between the first and second readings for Reader 1 with a CV of 0.7% and an agreement of 97% (test of symmetry: $\chi^2 = 1$, df = 1, $P = 0.3173$). There is no significant difference between the first and second readings for Reader 2 with a CV of 1% and an agreement of 93% (test of symmetry: $\chi^2 = 0$, df = 1, $P = 1$). There was no evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 89.91% and a CV of 1.4% (test of symmetry: $\chi^2 = 4.33$, df = 5, $P = 0.5025$) (Figure **2**).

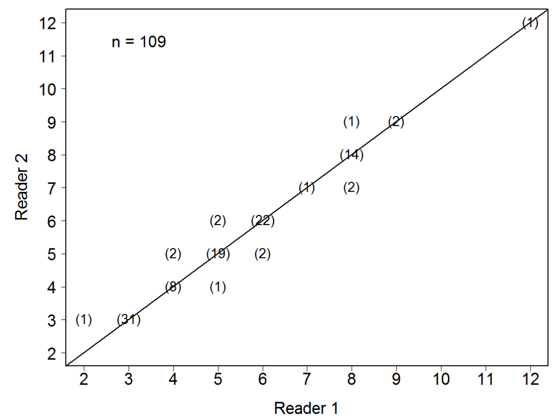


Figure 2. Between-reader comparison of otolith age estimates for cobia collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2010.

Reader 1 had an agreement of 80% with ages of fish aged in 2000 with a CV of 2% (test of symmetry: $\chi^2 = 7.33$, df = 7, $P = 0.395$). Reader 2 had an agreement of 86% with ages of fish aged in 2000 with a CV of 1.3% (test of symmetry: $\chi^2 = 5$, df = 5, $P = 0.4159$).

Of the 109 fish aged, 9 age classes were represented (from age 2 to 9, and 12) (**Table 1**). The average age of the sample was 5.2 years, and the standard deviation and standard error were 1.9 and 0.18, respectively.

Year-class data indicates that recruitment into the fishery begins at age 2, which corresponds to the 2008 year-class for cobia caught in 2010. The year class of 2007 (28%), 2004 (23%), and 2005 (20%) were dominant in the sample (Figure 3).

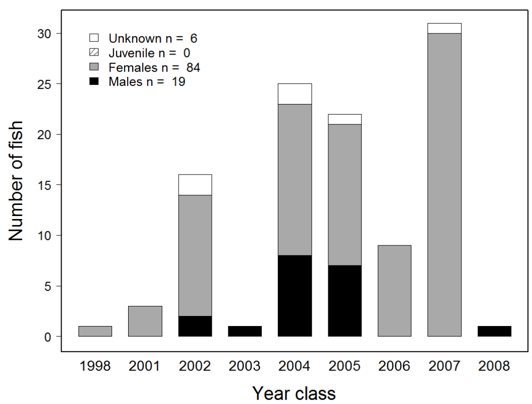


Figure 3. Year-class frequency distribution for cobia collected for ageing in 2010. Distribution is broken down by sex. “Unknown” is for the fish either whose gonads were not available for examination or those were not examined for sex during sampling.

Age-Length-Key — We present an age-length-key (**Table 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.

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Franks, J.S., J.R. Warren, and M.V. Buchanan. 1999. Age and growth of cobia, Rachycentron canadum, from the northeastern Gulf of Mexico. Fish. Bull. 97:459-471.

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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 Development Core Team. 2009. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org.

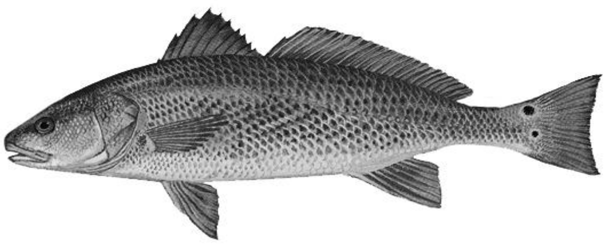
Table 1. The number of cobia assigned to each total length (inch)-at-age category for 109 fish sampled for otolith age determination in Virginia during 2010.

	Age									
Interval	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**12**	**Totals**
33 - 33.99	1	0	0	0	0	0	0	0	0	1
37 - 37.99	0	2	0	2	0	0	0	0	0	4
38 - 38.99	0	5	0	1	0	0	0	0	0	6
39 - 39.99	0	6	0	0	0	0	0	0	0	6
40 - 40.99	0	4	0	1	0	0	0	0	0	5
41 - 41.99	0	2	0	0	0	0	0	0	0	2
42 - 42.99	0	4	0	1	2	0	0	0	0	7
43 - 43.99	0	1	0	1	1	0	0	0	0	3
44 - 44.99	0	4	0	0	2	1	0	0	0	7
45 - 45.99	0	1	4	1	1	0	0	0	0	7
46 - 46.99	0	1	2	0	1	0	1	0	0	5
47 - 47.99	0	1	0	3	0	0	0	0	0	4
48 - 48.99	0	0	0	0	2	0	0	0	0	2
49 - 49.99	0	0	1	4	1	0	0	0	0	6
50 - 50.99	0	0	2	3	3	0	1	0	0	9
51 - 51.99	0	0	0	1	2	0	1	0	0	4
52 - 52.99	0	0	0	1	3	0	2	0	0	6
53 - 53.99	0	0	0	1	3	0	2	0	0	6
54 - 54.99	0	0	0	2	3	0	1	1	1	8
55 - 55.99	0	0	0	0	1	0	1	0	0	2
56 - 56.99	0	0	0	0	0	0	1	0	0	1
57 - 57.99	0	0	0	0	0	0	3	1	0	4
58 - 58.99	0	0	0	0	0	0	2	1	0	3
60 - 60.99	0	0	0	0	0	0	1	0	0	1
Totals	1	31	9	22	25	1	16	3	1	109

Table 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 2010.

	Age								
Interval	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**12**
33 - 33.99	1	0	0	0	0	0	0	0	0
37 - 37.99	0	0.5	0	0.5	0	0	0	0	0
38 - 38.99	0	0.833	0	0.167	0	0	0	0	0
39 - 39.99	0	1	0	0	0	0	0	0	0
40 - 40.99	0	0.8	0	0.2	0	0	0	0	0
41 - 41.99	0	1	0	0	0	0	0	0	0
42 - 42.99	0	0.571	0	0.143	0.286	0	0	0	0
43 - 43.99	0	0.333	0	0.333	0.333	0	0	0	0
44 - 44.99	0	0.571	0	0	0.286	0.143	0	0	0
45 - 45.99	0	0.143	0.571	0.143	0.143	0	0	0	0
46 - 46.99	0	0.2	0.4	0	0.2	0	0.2	0	0
47 - 47.99	0	0.25	0	0.75	0	0	0	0	0
48 - 48.99	0	0	0	0	1	0	0	0	0
49 - 49.99	0	0	0.167	0.667	0.167	0	0	0	0
50 - 50.99	0	0	0.222	0.333	0.333	0	0.111	0	0
51 - 51.99	0	0	0	0.25	0.5	0	0.25	0	0
52 - 52.99	0	0	0	0.167	0.5	0	0.333	0	0
53 - 53.99	0	0	0	0.167	0.5	0	0.333	0	0
54 - 54.99	0	0	0	0.25	0.375	0	0.125	0.125	0.125
55 - 55.99	0	0	0	0	0.5	0	0.5	0	0
56 - 56.99	0	0	0	0	0	0	1	0	0
57 - 57.99	0	0	0	0	0	0	0.75	0.25	0
58 - 58.99	0	0	0	0	0	0	0.667	0.333	0
60 - 60.99	0	0	0	0	0	0	1	0	0

Chapter 5
Red Drum
Sciaenops ocellatus



INTRODUCTION

We aged a total of 57 red drum, *Sciaenops ocellatus*, collected by the VMRC's Biological Sampling Program for age and growth analysis in 2010. The average age of the sample was 3.1 years, with a standard deviation of 3.1 and a standard error of 0.41. Six age classes were represented with the youngest age of 1 and the oldest age of 21 years (from age 1 to 3, 9, 13, and 21), comprising fish from the year-classes of 1989, 1997, 2001, and 2007 to 2009. The 2007 year-class was dominant in the sample in 2010 with 53%, followed by the 2008 (23%) and 2009 (18%) year-class.

METHODS

Handling of collection — Sagittal otoliths (hereafter, refer to as "otoliths") were received by the Age & Growth Laboratory in labeled coin envelopes. Once in our hands, they were sorted based on date of capture, their envelope labels were verified against VMRC's collection data, and assigned unique Age and Growth Laboratory identification numbers. All otoliths were stored dry in their original labeled coin envelopes.

Preparation — Otoliths were processed for age determination following the methods described in Ross et al. (1993) and Jones and Wells (1998) for black 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 (hereafter "blades"), separated by a stainless steel spacer of 0.4mm (diameter 2.5"). The position of the marked core fell within the 0.4mm 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 adhered the sections to the slide, but more importantly, provided enhanced contrast and greater readability by increasing light transmission through the sections.

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

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 1).

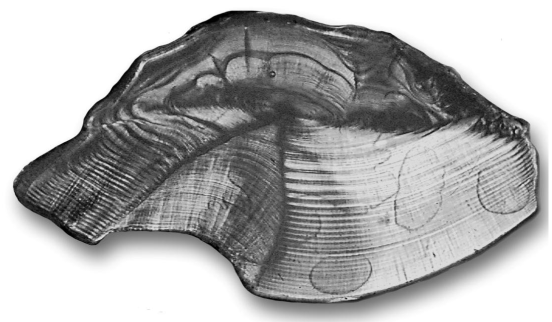


Figure 1. Otolith thin-section from 26 year old red drum.

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. Red drum year-class assignment was based on a January 1 annual birth date.

Comparison Tests — A symmetry test (Hoenig et al. 1995) and coefficient of variation (CV) analysis were used to detect any systematic difference and precision on age readings, respectively, for the following comparisons: 1) between the two readers in the current year, 2) within each reader in the current year, and 3) time-series bias between the current and previous years within each reader. The readings from the entire sample for the current year were used to examine the difference between two readers. A random sub-sample of 50 fish from the current year was selected for second readings to examine the difference within a reader. Fifty otoliths randomly selected from fish aged in 2000 were used to examine the time-series bias within each reader. A figure of 1:1 equivalence was used to illustrate those differences (Campana et al. 1995). All statistics analyses and figures were made using R (R Development Core Team 2009).

RESULTS

The measurement of reader self-precision was very high for both readers. Both readers had 100% agreement between their first and second readings. There was no evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 96.49% and a CV of 0.2% (test of symmetry $\chi^2 = 2$, df = 2, P = 0.3679) (Figure 2).

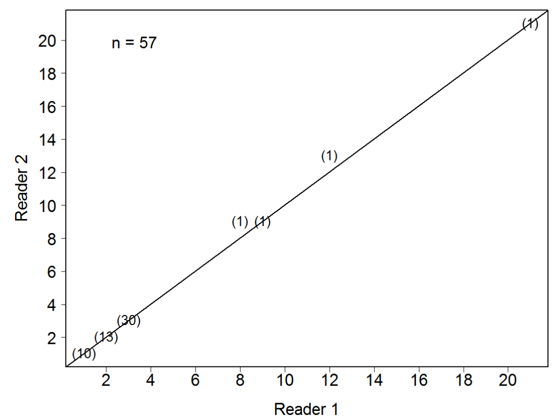


Figure 2. Between-reader comparison of otolith age estimates for red drum collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2010.

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% with ages of fish aged in 2000.

Of the 57 fish aged with otoliths, 6 age classes were represented (from age 1 to 3, 9, 13, and 21) (**Table 1**). The average age of the sample was 3.1 years, and the standard deviation and standard error were 3.1 and 0.41, respectively. The 2007 year-class was dominant in the sample in 2010 with 53%, followed by the 2008 (23%) and 2009 (18%) year-class. Indicative of the trend in the recreational fishing, very few older fish were collected in 2010 (Figure 3).

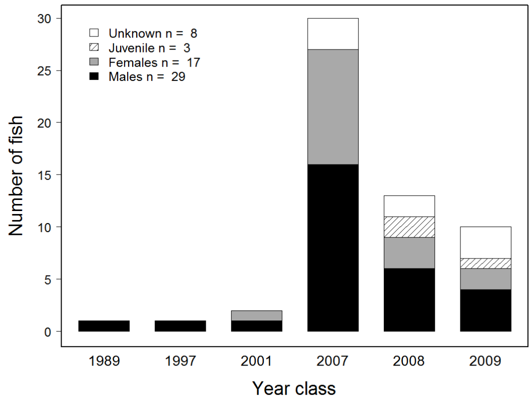


Figure 3. Year-class frequency distribution for red drum collected for ageing in 2010. Distribution is broken down by sex. “Unknown” is for the fish either whose gonads were not available for examination or those were not examined for sex during sampling.

Age-Length-Key — We present an age-length-key (**Table 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.

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.

Jones, C.J. and B.K. Wells. 1998. Age, growth, and mortality of black drum, *Pogonias cromis*, in the Chesapeake Bay region. Fish. Bull. 96:451-461.

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Ross, J. L., T. M. Stevens, and D. S. Vaughan. 1993. Age, growth, mortality, and reproductive biology of red drums in North Carolina waters. Transactions of the American Fisheries Society 124: 37-54.

Table 1. The number of red drum assigned to each total length (inch)-at-age category for 109 fish sampled for otolith age determination in Virginia during 2010.

	Age						
Interval	**1**	**2**	**3**	**9**	**13**	**21**	**Totals**
17 - 17.99	3	0	0	0	0	0	3
18 - 18.99	6	1	0	0	0	0	7
19 - 19.99	1	0	0	0	0	0	1
20 - 20.99	0	1	0	0	0	0	1
21 - 21.99	0	1	1	0	0	0	2
22 - 22.99	0	6	7	0	0	0	13
23 - 23.99	0	0	7	0	0	0	7
24 - 24.99	0	1	5	0	0	0	6
25 - 25.99	0	2	9	0	0	0	11
26 - 26.99	0	1	0	0	0	0	1
28 - 28.99	0	0	1	0	0	0	1
37 - 37.99	0	0	0	1	0	0	1
41 - 41.99	0	0	0	0	1	0	1
42 - 42.99	0	0	0	1	0	0	1
44 - 44.99	0	0	0	0	0	1	1
Totals	10	13	30	2	1	1	57

Table 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 2010.

	Age					
Interval	**1**	**2**	**3**	**9**	**13**	**21**
17 - 17.99	1	0	0	0	0	0
18 - 18.99	0.857	0.143	0	0	0	0
19 - 19.99	1	0	0	0	0	0
20 - 20.99	0	1	0	0	0	0
21 - 21.99	0	0.5	0.5	0	0	0
22 - 22.99	0	0.462	0.538	0	0	0
23 - 23.99	0	0	1	0	0	0
24 - 24.99	0	0.167	0.833	0	0	0
25 - 25.99	0	0.182	0.818	0	0	0
26 - 26.99	0	1	0	0	0	0
28 - 28.99	0	0	1	0	0	0
37 - 37.99	0	0	0	1	0	0
41 - 41.99	0	0	0	0	1	0
42 - 42.99	0	0	0	1	0	0
44 - 44.99	0	0	0	0	0	1

Chapter 6
Sheepshead

Archosargus probatocephalus

INTRODUCTION

We aged a total of 70 sheepshead, *Archosargus probatocephalus*, collected by the VMRC's Biological Sampling Program for age and growth analysis in 2010. The average age of the sample was 9.7 years, with a standard deviation of 6.6 and a standard error of 0.79. Nineteen age classes were represented with the youngest age of 2 and the oldest age of 28 years (from age 2 to 9, 12 to 14, 16 to 20, 22, 27, and 28), comprising fish from the year-class of 1982, 1983, 1988, 1990 to 1994, 1996 to 1998, and from 2001 to 2008. The year class of 2006 was dominant (19%) in the sample of 2010, followed by the 1997 (13%) and 2007 (13%) year-class.

METHODS

Handling of collection — Sagittal otoliths (hereafter, refer to as "otoliths") were received by the Age & Growth Laboratory in labeled coin envelopes. Once in our hands, they were sorted based on date of capture, their envelope labels were verified against VMRC's collection data, and assigned unique Age and Growth Laboratory identification numbers. All otoliths were stored dry in labeled coin envelopes.

Preparation — 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 0.4mm (diameter 2.5"). The position of the marked core fell within the 0.4mm 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 adhered the thin-sections to the slide, but more importantly, provided enhanced contrast and greater readability by increasing light transmission through the sections.

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, 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, sheepshead otolith deposition occurs between May to July (Ballenger et al, in review). A sheepshead 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 July 31, the period of annulus formation, 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 (Figure1).

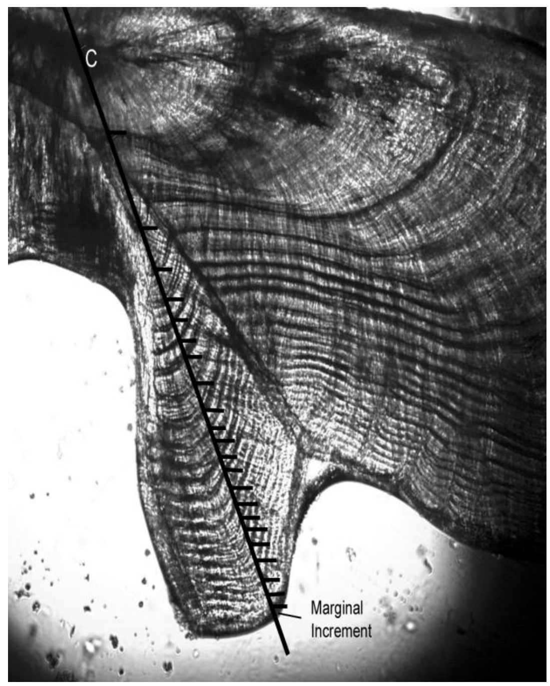

Figure1. Thin-sectioned otolith from a 22-yr old sheepshead showing the core (C) of the otolith, the measuring axis with annuli marked, and the marginal increment or growth on the edge of the otolith.

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. Sheepshead year-class assignment was based on a January 1 annual birth date.

Comparison Tests — A symmetry test (Hoenig et al. 1995) and coefficient of variation (CV) analysis were used to detect any systematic difference and precision on age readings, respectively, for the following comparisons: 1) between the two readers in the current year, 2) within each reader in the current year, and 3) time-series bias between the current and previous years within each reader. The readings from the entire sample for the current year were used to examine the difference between two readers. A random sub-sample of 50 fish from the current year was selected for second readings to examine the difference within a reader. Fifty otoliths randomly selected from fish aged in 2008 were used to examine the time-series bias within each reader. A figure of 1:1 equivalence was used to illustrate those differences (Campana et al. 1995). All statistics analyses and figures were made using R (R Development Core Team 2009).

RESULTS

The measurement of reader self-precision was fair for both readers. There is no significant difference between the first and second readings for Reader 1 with a CV of 2.4% and an agreement of 77% (test of symmetry: $\chi^2 = 7$, df = 7, $P = 0.43$). There is no significant difference between the first and second readings for Reader 2 with a CV of 2.5% and an agreement of 77% (test of symmetry: $\chi^2 = 7$, df = 7, $P = 0.43$). There was no evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 85% and a CV of 1.2% (test of symmetry: $\chi^2 = 8$, df = 9, $P = 0.5341$) (Figure 2).

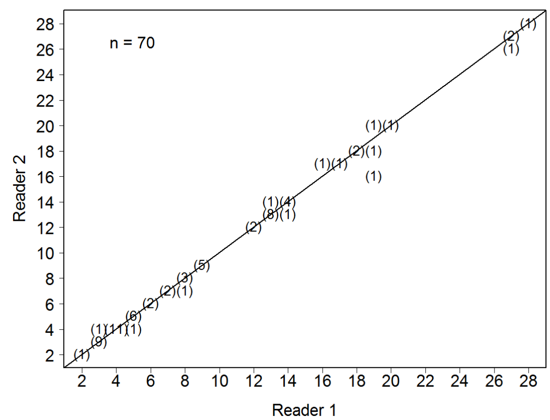

Figure 2. Between-reader comparison of otolith age estimates for cobia collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2010.

Reader 1 had an agreement of 92% with ages of fish aged in 2008 and a CV of 1.4% (test of symmetry: $\chi^2 = 4$, df = 4, $P = 0.41$). Reader 2 had an agreement of 92% with ages of fish aged in 2008 and a CV of 1.2% (test of symmetry: $\chi^2 = 4$, df = 4, $P = 0.41$).

Of the 70 fish aged with otoliths, 19 age classes were represented (from age 2 to 9, 12 to 14, 16 to 20, 22, 27, and 28) (Table 1). The average age of the sample was 9.7 years, and the standard deviation and standard error were 6.6 and 0.79, respectively. Year-class data indicate that the 2006 year-classes dominated the sample (19%), followed by the 1997 (13%) and 2007 (13%) year-class (Figure 3).

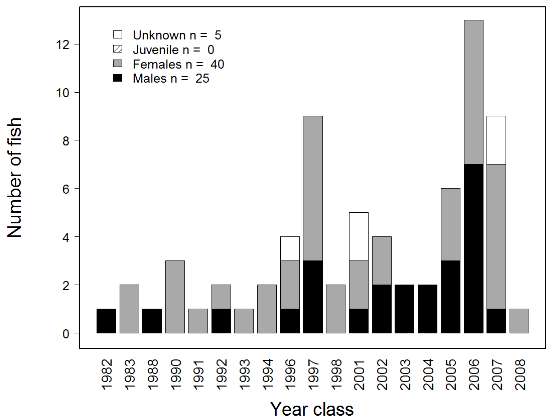

Figure 3. Year-class frequency distribution for sheepshead collected for ageing in 2010. Distribution is broken down by sex. "Unknown" is for the fish either whose gonads were not available for examination or those were not examined for sex during sampling.

Age-Length-Key — We present an age-length-key (Table 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.

REFERENCES

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Table 1. The number of Sheepshead assigned to each total length (inch)-at-age category for 69 fish sampled for otolith age determination in Virginia during 2010. One fish aged without total length is not included. Seven fish were not aged due to damaged otoliths.

	Age																		
Interval	2	3	4	5	6	7	8	9	12	13	14	16	17	18	19	20	22	27	28	Totals
13 - 13.99	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
14 - 14.99	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
15 - 15.99	0	1	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5
16 - 16.99	1	2	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6
17 - 17.99	0	1	3	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6
18 - 18.99	0	0	2	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	4
19 - 19.99	0	0	2	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	5
20 - 20.99	0	0	0	0	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	3
21 - 21.99	0	0	0	0	1	0	0	1	0	0	2	1	0	0	0	0	0	0	0	5
22 - 22.99	0	0	0	0	0	0	1	3	0	3	1	1	0	1	1	0	0	0	0	11
23 - 23.99	0	0	0	0	0	0	3	0	0	3	1	0	0	0	0	1	0	0	0	8
24 - 24.99	0	0	0	0	0	0	0	0	1	1	0	0	0	1	0	0	1	1	0	5
25 - 25.99	0	0	0	0	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0	3
26 - 26.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	1	3
Totals	1	9	13	6	2	2	4	5	2	9	4	2	1	2	1	3	1	1	1	69

Table 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 2010.

	Age																		
Interval	2	3	4	5	6	7	8	9	12	13	14	16	17	18	19	20	22	27	28
13 - 13.99	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14 - 14.99	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15 - 15.99	0	0.2	0.6	0.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16 - 16.99	0.167	0.333	0.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17 - 17.99	0	0.167	0.5	0.333	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18 - 18.99	0	0	0.5	0.25	0	0	0	0	0	0.25	0	0	0	0	0	0	0	0	0
19 - 19.99	0	0	0.4	0.4	0	0.2	0	0	0	0	0	0	0	0	0	0	0	0	0
20 - 20.99	0	0	0	0	0.333	0.333	0	0.333	0	0	0	0	0	0	0	0	0	0	0
21 - 21.99	0	0	0	0	0.2	0	0	0.2	0	0	0.4	0.2	0	0	0	0	0	0	0
22 - 22.99	0	0	0	0	0	0	0.091	0.273	0	0.273	0.091	0.091	0	0.091	0.091	0	0	0	0
23 - 23.99	0	0	0	0	0	0	0.375	0	0	0.375	0.125	0	0	0	0	0.125	0	0	0
24 - 24.99	0	0	0	0	0	0	0	0	0.2	0.2	0	0	0	0.2	0	0	0.2	0.2	0
25 - 25.99	0	0	0	0	0	0	0	0	0.333	0.333	0	0	0.333	0	0	0	0	0	0
26 - 26.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.667	0	0	0.333

Chapter 7
Atlantic Spadefish

Chaetodipterus faber

INTRODUCTION

We aged a total of 263 spadefish, *Chaetodipterus faber*, collected by the VMRC's Biological Sampling Program for age and growth analysis in 2010. The spadefish ages ranged from 1 to 12 years old with an average age of 3.1, and standard deviation of 1.4, and a standard error of 0.09. Ten age classes (1 to 9, and 12) were represented, comprising fish from the 1998, 2001 through 2009 year-classes. Fish from the 2007 year-class dominated the sample (40%), followed by 2008 (35%).

METHODS

Sample size for ageing — We estimated sample size for ageing spadefish in 2010 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:

$$A = \frac{V_a}{\theta_a^2 CV^2 - B_a / L}, \qquad (1)$$

where A is the sample size for ageing spadefish in 2010; 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 is a subsample from a catch and used to estimate length distribution in the catch. θ_a, V_a, B_a, and CV were calculated using pooled age-length data of spadefish collected from 2004 to 2008 and using equations in Quinn and Deriso (1999). For simplicity, the equations are not listed here. L was the total number of spadefish used by VMRC to estimate length distribution of the catches from 2004 to 2008. The equation (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 achieved by aging an additional 100 or more fish.

Handling of collection — Otoliths were received by the Age & Growth Laboratory in labeled coin envelopes. Once in our hands, they were sorted based on date of capture, their envelope labels were verified against VMRC's collection data, and assigned unique Age and Growth Laboratory identification numbers. All otoliths were stored dry inside of protective Axygen 2.0ml labeled microtubes within their original labeled coin envelopes.

Preparation Due to their fragility, small spadefish sagittal otoliths (hereafter, referred to as "otoliths") (less than 14 mm x 5 mm x 3 mm in all dimensions) were processed for age determination using an embedding and thin-sectioning technique. In order to increase the contrast of opaque and translucent regions in the otolith matrices, both small and large spadefish otoliths were baked either before or after sectioning, respectively. The right or left otolith was selected randomly from every fish.

For small spadefish otoliths, a series of 14 mm x 5 mm x 3 mm wells (Ladd Industries silicon rubber mold) were pre-filled to half-volume with Loctite® 349 adhesive, and permitted to cure for 24 hours until solidified.

The small, whole spadefish otoliths were placed in a ceramic "Coors" spot plate well and baked in a Thermolyne 1400 furnace at 400^{o}C. Baking time was otolith size-dependent and gauged by color, with a light caramel color desired. Once a suitable color was reached the baked otoliths could be individually placed into the pre-filled silicon rubber mold with Loctite® 349 adhesive.

The remaining volume of the wells were then filled with fresh, non-cured Loctite® 349 adhesive, at which point the small whole spadefish otoliths (baked) could be inserted into the wells on top of the solidified Loctite® 349 base, within a stable embedding atmosphere before sectioning. The otoliths were inserted into the fresh Loctite® 349 adhesive, proximal side up, with the long axis of the otolith exactly parallel with the long axis of the mold well. Once the otoliths were properly oriented within the Loctite® 349-filled wells, the mold was placed under UV light and left to solidify overnight. Once dry, each embedded otolith was removed from the mold and mounted with clear Crystalbond™ 509 onto a standard microscope slide. Once mounted, a small mark was made in permanent ink on the otolith-mold surface directly above the otolith focus, which was located using a stereo microscope under transmitted light. The embedded small spadefish otoliths could now be processed along with the larger spadefish otoliths.

Large spadefish otoliths were mounted directly with clear Crystalbond™ 509 adhesive onto a standard microscope slide with its distal surface orientated upwards. Once mounted, a small permanent-ink mark was placed on the otolith surface directly above the otolith focus, which was identified under a stereomicroscope in transmitted light. 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 0.4 mm (diameter 2.5"). The otolith was positioned so that the blades straddled each side of the otolith focus marked by pencil. It was crucial that this cut be perpendicular to the long axis of the otolith. Failure to do so resulted in "broadening" and distortion of winter growth zones. A proper cut resulted in annuli that were clearly defined and delineated. Once cut, the large otolith sections were placed into a ceramic "Coors" spot plate well and baked in a Thermolyne 1400 furnace at 400^{o}C until achieving the light caramel color desired. Once a suitable color was reached the baked thin-section was placed on a labeled glass slide and covered with a thin layer of Flo-texx mounting medium, which provided enhanced contrast and greater readability by increasing light transmission through the sections. Small otolith sections of quality were mounted with Flo-texx directly.

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, 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 when it deposits an 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 deposition occurs December through April (Hayse 1989). A spadefish 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 April 30, the period of annulus formation, 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 1).

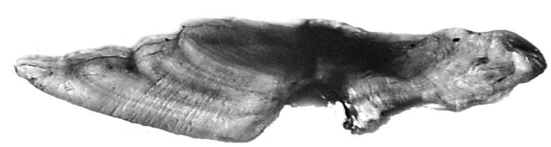

Figure 1. Sectioned otolith from a 3-year-old female spadefish.

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.

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 and figures were made using R (R Development Core Team 2009).

RESULTS

We estimated a sample size of 305 for ageing spadefish in 2010, ranging in length interval from 3 to 24 inches (Table 1). This sample size provided a range in CV for age composition approximately from the smallest CV of 5% for age 2 and the largest CV of 21% for age 0 and 5 fish. In 2010, we randomly selected and aged 263 fish from 338 spadefish collected by VMRC. We fell short in our over-all collections for this optimal length-class sampling estimate by 45 fish (Table 1). However, the significant shortage were primarily from the very small and large length intervals, therefore, the precision for the estimates of major age groups (from age 2 and 3) would not be influenced significantly.

Measurements of reader self-precision were good for both readers. Reader 1 had a CV of 4.1% and an agreement of 86% (test of symmetry: $\chi^2 = 2.2$, df = 3, P = 0.5319). Reader 2 had a CV of 4.5% and an agreement of 84% (test of symmetry: $\chi^2 = 2.67$, df = 3, P = 0.4459). There was no evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 89% (CV = 2.9%, test of symmetry: $\chi^2 = 11.48$, df = 7, P = 0.1188) (Figure 2).

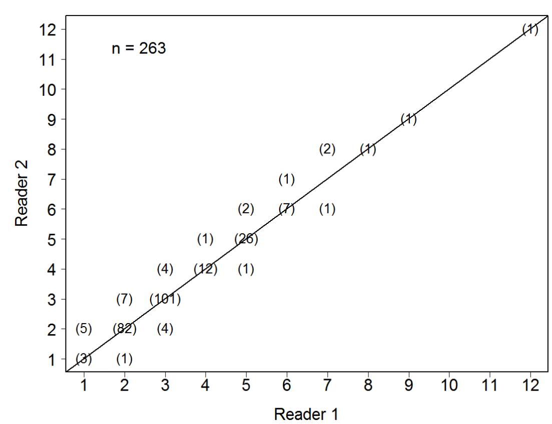

Figure 2. Between-reader comparison of otolith age estimates for spadefish collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2010.

There is no time-series bias for both readers. Reader 1 had an agreement of 82% with ages of fish aged in 2003 with a CV of 2.3% (test of symmetry: $\chi^2 = 7$, df = 7, P = 0.4289). Reader 2 had an agreement of 86% with ages of fish aged in 2003 with a CV of 2.4% (test of symmetry: $\chi^2 = 4.33$, df = 5, P = 0.5025).

Of the 263 fish aged, 10 age classes were represented (Table 2). The average age of the sample was 3.1 years, and the standard deviation and standard error were 1.4 and 0.09, respectively. Year-class data indicate that the 2007 year-class dominated the sample (40%), followed by 2008 (35%) (Figure 3).

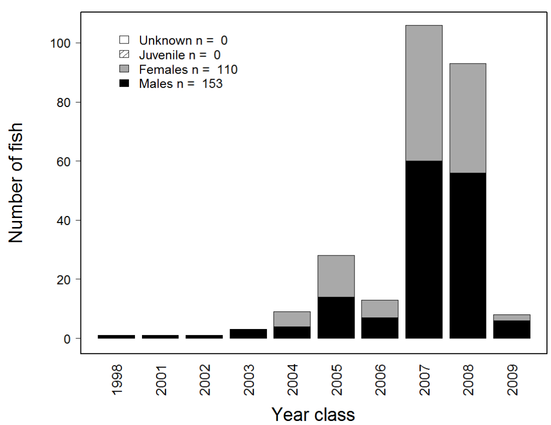

Figure 3. Year-class frequency distribution for spadefish collected for ageing in 2010. Distribution is broken down by sex. “Unknown” is for the fish either whose gonads were not available for examination or those were not examined for sex during sampling.

Age-Length-Key — We present an age-length-key (Table 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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Quinn, T. J. II, and R. B. Deriso. 1999. Quantitative Fish Dynamics. Oxford Univeristy Press. New York.

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Table 1. Number of spadefish collected and aged in each 1-inch length interval in 2010. "Target" represent the sample size for ageing estimated for 2010, "Collected" represents number of fish with both total length and otoliths, and "Need" represents number of fish shorted in each length interval compared to the optimum sample size for ageing and number of fish aged. One fish without otolith is not included.

Interval	Target	Collected	Aged	Need
3 - 3.99	5	0	0	5
4 - 4.99	5	0	0	5
5 - 5.99	16	3	3	13
6 - 6.99	32	40	32	0
7 - 7.99	47	59	47	0
8 - 8.99	36	45	36	0
9 - 9.99	29	33	29	0
10 - 10.99	18	23	18	0
11 - 11.99	15	24	15	0
12 - 12.99	15	19	15	0
13 - 13.99	14	14	14	0
14 - 14.99	8	16	8	0
15 - 15.99	9	13	9	0
16 - 16.99	6	15	6	0
17 - 17.99	10	13	10	0
18 - 18.99	8	11	11	0
19 - 19.99	6	5	5	1
20 - 20.99	6	3	3	3
21 - 21.99	5	2	2	3
22 - 22.99	5	0	0	5
23 - 23.99	5	0	0	5
24 - 24.99	5	0	0	5
Totals	305	338	263	45

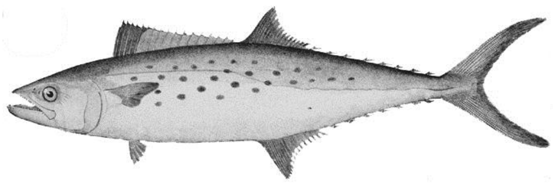
Table 2. The number of spadefish assigned to each total length-at-age category for 263 fish sampled for otolith age determination in Virginia during 2010.

	Age										
Interval	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**12**	**Totals**
5 - 5.99	0	3	0	0	0	0	0	0	0	0	3
6 - 6.99	2	23	7	0	0	0	0	0	0	0	32
7 - 7.99	4	30	13	0	0	0	0	0	0	0	47
8 - 8.99	2	20	14	0	0	0	0	0	0	0	36
9 - 9.99	0	9	20	0	0	0	0	0	0	0	29
10 - 10.99	0	4	14	0	0	0	0	0	0	0	18
11 - 11.99	0	1	14	0	0	0	0	0	0	0	15
12 - 12.99	0	1	10	4	0	0	0	0	0	0	15
13 - 13.99	0	2	10	1	1	0	0	0	0	0	14
14 - 14.99	0	0	3	1	4	0	0	0	0	0	8
15 - 15.99	0	0	0	3	6	0	0	0	0	0	9
16 - 16.99	0	0	1	1	4	0	0	0	0	0	6
17 - 17.99	0	0	0	2	4	4	0	0	0	0	10
18 - 18.99	0	0	0	0	7	2	1	0	1	0	11
19 - 19.99	0	0	0	1	2	2	0	0	0	0	5
20 - 20.99	0	0	0	0	0	1	1	1	0	0	3
21 - 21.99	0	0	0	0	0	0	1	0	0	1	2
Totals	8	93	106	13	28	9	3	1	1	1	263

Table 3. Age-Length key, as proportion-at-age in each 1-inch length interval, based on otolith ages for spadefish sampled for age determination in Virginia during 2010.

	Age									
Interval	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**12**
5 - 5.99	0	1	0	0	0	0	0	0	0	0
6 - 6.99	0.062	0.719	0.219	0	0	0	0	0	0	0
7 - 7.99	0.085	0.638	0.277	0	0	0	0	0	0	0
8 - 8.99	0.056	0.556	0.389	0	0	0	0	0	0	0
9 - 9.99	0	0.31	0.69	0	0	0	0	0	0	0
10 - 10.99	0	0.222	0.778	0	0	0	0	0	0	0
11 - 11.99	0	0.067	0.933	0	0	0	0	0	0	0
12 - 12.99	0	0.067	0.667	0.267	0	0	0	0	0	0
13 - 13.99	0	0.143	0.714	0.071	0.071	0	0	0	0	0
14 - 14.99	0	0	0.375	0.125	0.5	0	0	0	0	0
15 - 15.99	0	0	0	0.333	0.667	0	0	0	0	0
16 - 16.99	0	0	0.167	0.167	0.667	0	0	0	0	0
17 - 17.99	0	0	0	0.2	0.4	0.4	0	0	0	0
18 - 18.99	0	0	0	0	0.636	0.182	0.091	0	0.091	0
19 - 19.99	0	0	0	0.2	0.4	0.4	0	0	0	0
20 - 20.99	0	0	0	0	0	0.333	0.333	0.333	0	0
21 - 21.99	0	0	0	0	0	0	0.5	0	0	0.5

Chapter 8
Spanish Mackerel



Scomberomorous maculatus

INTRODUCTION

We aged a total of 225 Spanish mackerel, *Scomberomorous maculatus*, collected by the VMRC's Biological Sampling Program for age and growth analysis in 2010. The Spanish mackerel ages ranged from 1 to 8 years old with an average age of 1.6, and standard deviation of 1, and a standard error of 0.07. Six age classes (1 to 3, 5 and 6, and 8) were represented, comprising fish from the 2002, and 2004 and 2005, and 2007 through 2009 year-classes. Fish from the 2009 year-class dominated the sample (59%), followed with the year-class of 2008 (29%).

METHODS

Sample size for ageing — We estimated sample size for ageing Spanish mackerel in 2010 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:

$$A = \frac{V_a}{\theta_a^2 CV^2 - B_a / L}, \qquad (1)$$

where A is the sample size for ageing Spanish mackerel in 2010; 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 is a subsample from a catch and used to estimate length distribution in the catch. θ_a, V_a, B_a, and CV were calculated using pooled age-length data of Spanish mackerel collected from 2004 to 2008 and using equations in Quinn and Deriso (1999). For simplicity, the equations are not listed here. L was the total number of Spanish mackerel used by VMRC to estimate length distribution of the catches from 2004 to 2008. The equation (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 achieved by aging an additional 100 or more fish.

Handling of collection — All Sagittal otoliths (hereafter, referred to as "otoliths") and associated data were transferred to the Center for Quantitative Fisheries Ecology's Age and Growth Laboratory as they were collected. In the lab they were sorted by date of capture, their envelope labels verified against VMRC's collection data, and each fish was assigned a unique Age and Growth Laboratory identification number. All otoliths were stored dry inside of protective Axygen 2.0 ml microtubes within their original labeled coin envelopes.

Preparation Due to their fragility, we used our embedding and thin-sectioning method to prepare Spanish mackerel otoliths for age determination. To start, a series of 14 mm x 5 mm x 3 mm wells (Ladd Industries silicon rubber mold) were pre-filled to half-volume with Loctite® 349 adhesive, and permitted to cure for 24 hours until solidified. The remaining volume in the wells was then filled with fresh, non-cured Loctite® 349 adhesive, at which point the whole Spanish mackerel otoliths could be inserted into the wells on top of the solidified Loctite® 349 base, suspended within a stable embedding atmosphere before sectioning. The otoliths were inserted into the fresh Loctite® 349 adhesive, distal side up, with the long axis of the otolith exactly parallel with the long axis of the mold well. Once the otoliths were properly oriented within the Loctite® 349 filled wells, the mold was placed under UV light and left to solidify overnight. Once dry, each embedded otolith was removed from the mold and mounted with clear Crystalbond™ 509 onto a standard microscope slide. Once mounted, a small mark was made in permanent ink on the otolith-mold surface directly above the otolith focus, which was located using a stereo microscope under transmitted light. At least one transverse cross-section (hereafter, referred to as "thin-section") was then removed from 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 0.4 mm (diameter 2.5"). The otolith was positioned so that the blades straddled each side of the focus ink mark. The glass slide was adjusted to ensure that the blades were exactly perpendicular to the long axis of the otolith. The otolith thin-section was viewed under a stereo microscope to determine which side (cut surface) of the otolith was closer to the focus. The otolith thin-section was mounted best-side up onto a glass slide with Flo-texx® mounting medium, which provided enhanced contrast and greater readability by increasing light transmission through the sections.

Reading - 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, 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 otolith deposition occurs between May and June (Fable et al. 1987). A Spanish mackerel 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.

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. The first annulus on the thin-sections was often quite distant from the core, with subsequent annuli regularly spaced along the sulcal groove out towards the proximal (inner-face) edge of the otolith (Figure 1).

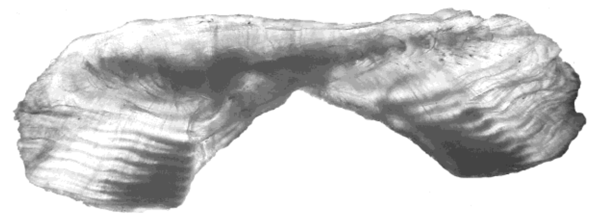


Figure 1. An eight year old Spanish mackerel otolith thin section from a 1 kg female.

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.

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 and figures were made using R (R Development Core Team 2009).

RESULTS

We estimated a sample size of 310 for ageing Spanish mackerel in 2010, ranging in length interval from 7 to 31 inches (Table 1). This sample size provided a range in CV for age composition approximately from the smallest CV of 4% for age 1 and the largest CV of 18% for age 0 and 3 fish. In 2010, we randomly selected and aged 225 fish from 364 Spanish mackerel collected by VMRC. We fell short in our over-all collections for this optimal length-class sampling estimate by 87 fish. However, these were primarily from the very small and large length intervals (Table 1), therefore, the precision for the estimates of major age group (age 1 and 2) would not be influenced significantly.

The measurement of reader self-precision was very good for Reader 1 with an agreement of 96% (CV = 1.5%, test of symmetry: $\chi^2 = 2$, df = 2, $P = 0.3679$). The measurement of reader self-precision was good for Reader 2 with an agreement of 90% (CV = 4.3%, test of symmetry: $\chi^2 = 2$, df = 2, $P = 0.3679$). There was no evidence of systematic disagreement between reader 1 and reader 2 (test of symmetry: $\chi^2 = 3.6$, df = 2, $P = 0.1653$). The average between-reader coefficient of variation (CV) was 1.7% with an agreement of 96% (Figure 2).

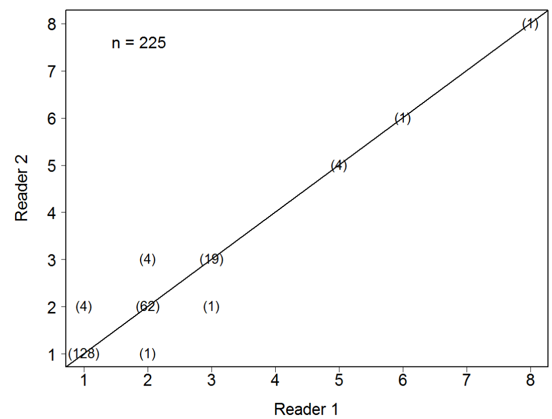


Figure 2. Between-reader comparison of otolith age estimates for Spanish mackerel collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2010.

There is no time-series bias for both readers. Reader 1 had an agreement of 92% with ages of fish aged in 2003 with a CV of 2.8% (test of symmetry: $\chi^2 = 4$, df = 3, $P = 0.2615$). Reader 2 had an agreement of 96% with ages of fish aged in 2003 with a CV of 1.3% (test of symmetry: $\chi^2 = 2$, df = 2, $P = 0.3679$).

Of the 225 Spanish mackerel aged, 6 age classes were represented (Table 2). The average age was 1.6 year old, and the standard deviation and standard error were 1 and 0.07, respectively. Year-class data show that the fishery was comprised of 6 year-classes, comprising fish from the 2002, 2004 and 2005, 2007 through 2009 year-classes, with 59% and 29% of fish from the 2009 and 2008 year-classes, respectively (Figure 3).

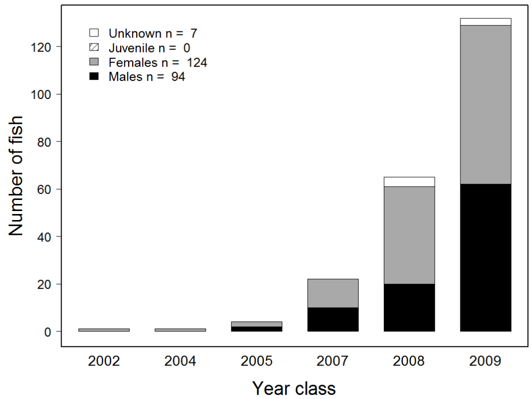


Figure 3. Year-class frequency distribution for Spanish mackerel collected for ageing in 2010. Distribution is broken down by sex. “Unknown” is for the fish gonads that were not available for examination or were not examined for sex during sampling.

Age-Length-Key — We present an age-length-key (Table 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. Number of Spanish mackerel collected and aged in each 1-inch length interval in 2010. "Target" represent the sample size for ageing estimated for 2010, "Collected" represents number of fish with both total length and otoliths, and "Need" represents number of fish shorted in each length interval compared to the optimum sample size for ageing and number of fish aged.

Interval	Target	Collected	Aged	Need
7 - 7.99	5	0	0	5
8 - 8.99	5	0	0	5
9 - 9.99	5	0	0	5
10 - 10.99	5	0	0	5
11 - 11.99	5	0	0	5
12 - 12.99	6	0	0	6
13 - 13.99	8	4	4	4
14 - 14.99	19	17	16	3
15 - 15.99	45	38	38	7
16 - 16.99	47	57	47	0
17 - 17.99	37	83	38	0
18 - 18.99	22	73	22	0
19 - 19.99	19	35	19	0
20 - 20.99	15	27	16	0
21 - 21.99	12	17	12	0
22 - 22.99	10	6	6	4
23 - 23.99	5	1	1	4
24 - 24.99	5	1	1	4
25 - 25.99	5	1	1	4
26 - 26.99	5	1	1	4
27 - 27.99	5	1	1	4
28 - 28.99	5	1	1	4
29 - 29.99	5	0	0	5
30 - 30.99	5	1	1	4
31 - 31.99	5	0	0	5
Totals	310	364	225	87

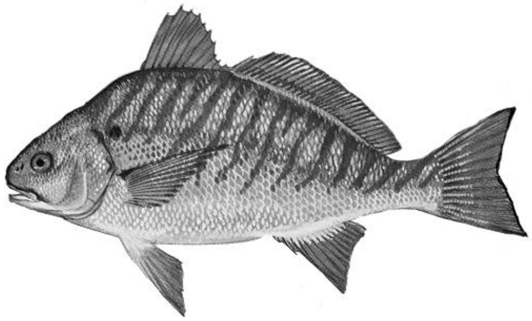
Table 2. The number of Spanish mackerel assigned to each total length-at-age category for 225 fish sampled for otolith age determination in Virginia during 2010.

	Age						
Interval	**1**	**2**	**3**	**5**	**6**	**8**	**Totals**
13 - 13.99	4	0	0	0	0	0	4
14 - 14.99	16	0	0	0	0	0	16
15 - 15.99	37	1	0	0	0	0	38
16 - 16.99	40	6	1	0	0	0	47
17 - 17.99	24	13	1	0	0	0	38
18 - 18.99	7	14	1	0	0	0	22
19 - 19.99	4	12	3	0	0	0	19
20 - 20.99	0	13	3	0	0	0	16
21 - 21.99	0	3	7	2	0	0	12
22 - 22.99	0	3	3	0	0	0	6
23 - 23.99	0	0	1	0	0	0	1
24 - 24.99	0	0	0	1	0	0	1
25 - 25.99	0	0	0	1	0	0	1
26 - 26.99	0	0	0	0	1	0	1
27 - 27.99	0	0	1	0	0	0	1
28 - 28.99	0	0	0	0	0	1	1
30 - 30.99	0	0	1	0	0	0	1
Totals	132	65	22	4	1	1	225

Table 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 2010.

	Age					
Interval	**1**	**2**	**3**	**5**	**6**	**8**
13 - 13.99	1	0	0	0	0	0
14 - 14.99	1	0	0	0	0	0
15 - 15.99	0.974	0.026	0	0	0	0
16 - 16.99	0.851	0.128	0.021	0	0	0
17 - 17.99	0.632	0.342	0.026	0	0	0
18 - 18.99	0.318	0.636	0.045	0	0	0
19 - 19.99	0.211	0.632	0.158	0	0	0
20 - 20.99	0	0.812	0.188	0	0	0
21 - 21.99	0	0.25	0.583	0.167	0	0
22 - 22.99	0	0.5	0.5	0	0	0
23 - 23.99	0	0	1	0	0	0
24 - 24.99	0	0	0	1	0	0
25 - 25.99	0	0	0	1	0	0
26 - 26.99	0	0	0	0	1	0
27 - 27.99	0	0	1	0	0	0
28 - 28.99	0	0	0	0	0	1
30 - 30.99	0	0	1	0	0	0

Chapter 9
Spot



Leiostomus xanthurus

INTRODUCTION

We aged a total of 277 spot, *Leiostomus xanthurus*, collected by the VMRC's Biological Sampling Program for age and growth analysis in 2010. The spot ages ranged from 0 to 4 years old with an average age of 1.6, and standard deviation of 0.6, and a standard error of 0.04. Five age classes (0 to 4) were represented, comprising fish from the 2006 to 2010 year-classes. Fish from the 2008 and 2009 year-classes dominated the sample with 53% and 43%, respectively.

METHODS

Sample size for ageing — We estimated sample size for ageing spot in 2010 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:

$$A = \frac{V_a}{\theta_a^2 CV^2 - B_a / L}, \qquad (1)$$

where A is the sample size for ageing spot in 2010; 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 is a subsample from a catch and used to estimate length distribution in the catch. θ_a, V_a, B_a, and CV were calculated using pooled age-length data of spot collected from 2004 to 2008 and using equations in Quinn and Deriso (1999). For simplicity, the equations are not listed here. L was the total number of spot used by VMRC to estimate length distribution of the catches from 2004 to 2008. The equation (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 achieved by aging an additional 100 or more fish.

Handling of collection — Otoliths were received by the Age & Growth Laboratory in labeled coin envelopes. Once in our hands, they were sorted based on date of capture, their envelope labels were verified against VMRC's collection data, and assigned unique Age and Growth Laboratory identification numbers. All otoliths were stored dry inside of protective Axygen 2.0 ml microtubes within their original labeled coin envelopes.

Preparation — Sagittal otoliths (hereafter, referred to as "otoliths") were processed for age determination following our thin-sectioning method, as described in Chapters 1, 2 and 5 for other sciaenids.

The left or right sagittal otolith was randomly selected and attached 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 marked using a pencil across the otolith surface. At least one transverse cross-section (hereafter, referred to as "thin-section") was then removed from 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 0.4 mm (diameter 2.5"). The position of the marked core fell within the 0.3 mm space between the blades, such that the core was included in the transverse removed cross-section. Otolith thin-sections were placed on labeled glass slides and covered with a thin layer of Flo-texx mounting medium that not only adhered the sections to the slide, but more importantly, provided enhanced contrast and greater readability by increasing light transmission through the sections.

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, 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 deposition occurs between May and July (Piner and Jones 2004). A spot 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 July 31, the period of annulus formation, 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 1).

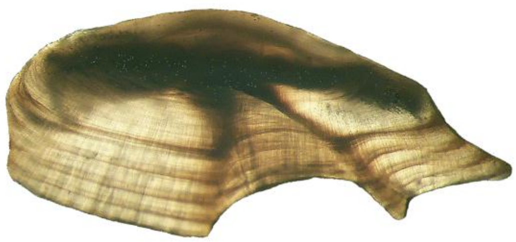


Figure 1. Sectioned otolith from a 5 year old spot.

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.

Comparison Tests — A symmetry test (Hoenig et al. 1995) and coefficient of variation (CV) analysis were used to detect any systematic difference and precision on age readings, respectively, for the following comparisons: 1) between the two readers in the current year, 2) within each reader in the current year, and 3) time-series bias between the current and previous years within each reader. The readings from the entire sample for the current year were used to examine the difference between two readers. A random sub-sample of 50 fish from the current year was selected for second readings to examine the difference within a reader. Fifty otoliths randomly selected from fish aged in 2000 were used to examine the time-series bias within each reader. A figure of 1:1 equivalence was used to illustrate those differences (Campana et al. 1995). All statistics analyses and figures were made using R (R Development Core Team 2009).

RESULTS

We estimated a sample size of 339 for ageing spot in 2010, ranging in length interval from 5 to 14 inches (Table 1). This sample size provided a range in CV for age composition approximately from the smallest CV of 6% for age 1 and 2, and the largest CV of 13% for age 3 fish. In 2010, we randomly selected and aged 277 fish from 369 Spot collected by VMRC. We fell short in our over-all collections for this optimal length-class sampling estimate by 65 fish. However, these were primarily from the very large length intervals (Table 1), therefore, the precision for the estimates of major age groups (age 1 and 2) would not be influenced significantly.

The measurement of reader self-precision was good for both readers. Reader 1 had 100% agreement with a CV of 0% (test of symmetry: $\chi^2 = 0$, df = 0, $P = 1$). Reader 2 had 100% agreement with a CV of 0% (test of symmetry: $\chi^2 = 0$, df = 0, $P = 1$). There was no evidence of systematic disagreement between Reader 1 and Reader 2 (test of symmetry: $\chi^2 = 0$, df = 0, $P = 1$). The average between-reader coefficient of variation (CV) of 0% was good with an agreement of 100% between two readers (Figure 2). There is no time-series bias for both readers. Reader 1 and

Reader 2 had an agreement of 100% with ages of fish aged in 2000.

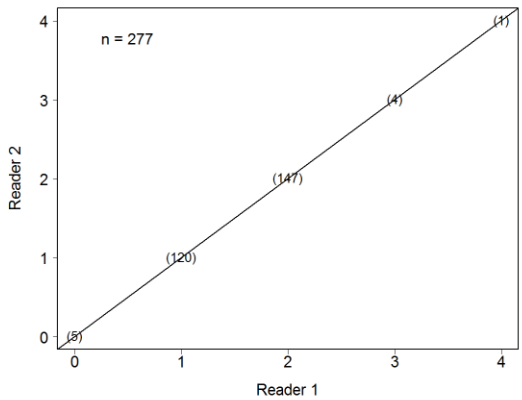


Figure 2. Between-reader comparison of otolith age estimates for spot collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2010.

Of the 277 fish aged with otoliths, 5 age classes were represented (Table 2). The average age for the sample was 1.6 years old, and 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, with fish spawned in both 2008 (53%) and 2009 (43%) dominating the catch (Figure 3).

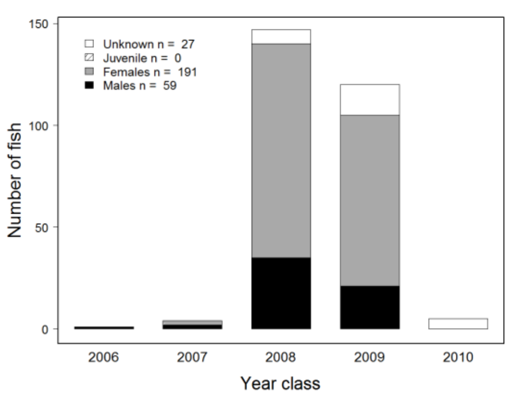


Figure 3. Year-class frequency distribution for spot collected for ageing in 2010. Distribution is broken down by sex. "Unknown" is for the fish gonads that were not available for examination or were not examined for sex during sampling.

Age-Length-Key — We present an age-length-key (Table 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. Number of spot collected and aged in each 1-inch length interval in 2010. "Target" represent the sample size for ageing estimated for 2010, "Collected" represents number of fish with both total length and otoliths, and "Need" represents number of fish shorted in each length interval compared to the optimum sample size for ageing and number of fish aged. Two fish without otoliths are not included.

Interval	Target	Collected	Aged	Need
5 - 5.99	5	7	6	0
6 - 6.99	5	12	6	0
7 - 7.99	36	42	36	0
8 - 8.99	62	106	62	0
9 - 9.99	91	117	92	0
10 - 10.99	72	82	72	0
11 - 11.99	41	3	3	38
12 - 12.99	17	0	0	17
13 - 13.99	5	0	0	5
14 - 14.99	5	0	0	5
Totals	339	369	277	65

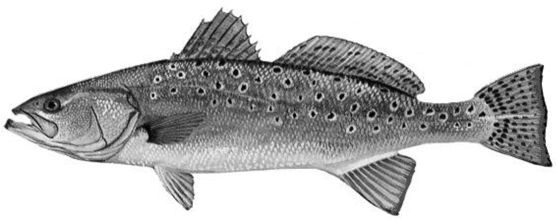
Table 2. The number of spot assigned to each total length-at-age category for 277 fish sampled for otolith age determination in Virginia during 2010.

	Age					
Intervals	**0**	**1**	**2**	**3**	**4**	**Totals**
5 - 5.99	3	3	0	0	0	6
6 - 6.99	2	4	0	0	0	6
7 - 7.99	0	23	13	0	0	36
8 - 8.99	0	19	42	1	0	62
9 - 9.99	0	22	69	1	0	92
10 - 10.99	0	49	21	2	0	72
11 - 11.99	0	0	2	0	1	3
Totals	5	120	147	4	1	277

Table 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 2010.

	Age				
Interval	**0**	**1**	**2**	**3**	**4**
5 - 5.99	0.5	0.5	0	0	0
6 - 6.99	0.333	0.667	0	0	0
7 - 7.99	0	0.639	0.361	0	0
8 - 8.99	0	0.306	0.677	0.016	0
9 - 9.99	0	0.239	0.75	0.011	0
10 - 10.99	0	0.681	0.292	0.028	0
11 - 11.99	0	0	0.667	0	0.333

Chapter 10
Spotted Seatrout



Cynoscion nebulosus

INTRODUCTION

We aged a total of 229 spotted seatrout, *Cynoscion nebulosus*, collected by the VMRC's Biological Sampling Program for age and growth analysis in 2010. The spotted seatrout ages ranged from 0 to 6 years old with an average age of 1.8, and standard deviation of 1.4, and a standard error of 0.09. Seven age classes (from 0 to 6) were represented, comprising fish from the 2004 through 2010 year-classes. Fish from the 2009 year-classes dominated the sample with 34%, followed by 2008 year-class (22%).

METHODS

Sample size for ageing — We estimated sample size for ageing spotted seatrout in 2010 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:

$$A = \frac{V_a}{\theta_a^2 CV^2 - B_a / L}, \qquad (1)$$

where A is the sample size for ageing spotted seatrout in 2010; 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 is a subsample from a catch and used to estimate length distribution in the catch. θ_a, V_a, B_a, and CV were calculated using pooled age-length data of spotted seatrout collected from 2004 to 2008 and using equations in Quinn and Deriso (1999). For simplicity, the equations are not listed here. L was the total number of spotted seatrout used by VMRC to estimate length distribution of the catches from 2004 to 2008. The equation (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 achieved by aging an additional 100 or more fish.

Handling of collection — Sagittal otoliths (hereafter, referred to as "otoliths") were received by the Age & Growth Laboratory in labeled coin envelopes. They 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 were stored dry inside of protective Axygen 2.0 ml microtubes inside of their original labeled coin envelopes.

Preparation — Because spotted seatrout otolith material is used for additional projects at the CQFE, preparation of these otoliths for age determination required modification of our thin-sectioning method, as introduced in Chapters 1, 2, 5, and 9 for other sciaenids. The left or right sagittal otolith was randomly selected and attached to a glass slide with clear silicone instead of clear Crystalbond™ 509 adhesive. This prevented contamination of the otolith by the Crystalbond™ 509 and allowed easy removal of the remaining otolith halves from the mounting slide after sectioning. Once mounted, 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, 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 0.4 mm (diameter 2.5"). The position of the marked core fell within the 0.3 mm space between the blades, such that the core was included in the removed transverse cross-section. Otolith thin-sections were placed on labeled glass slides and covered with a thin layer of Flo-texx mounting medium that not only adhered the sections to the slide, but more importantly, provided enhanced contrast and greater readability by increasing light transmission through the sections.

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, 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 deposition occurs between April and May

(Murphy and Taylor 1994). A spotted seatrout captured between January 1 and May 31, before the end of the species' annulus formation period, with three visible annuli and some translucent growth after the last annulus, would be assigned an age class of "x + (x+1)" or 3 + (3+1), noted as 3 + 4. This is the same age-class assigned to a fish with four visible annuli captured after the end of May 31, the period of annulus formation, which would be noted as 4 + 4.

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 1).

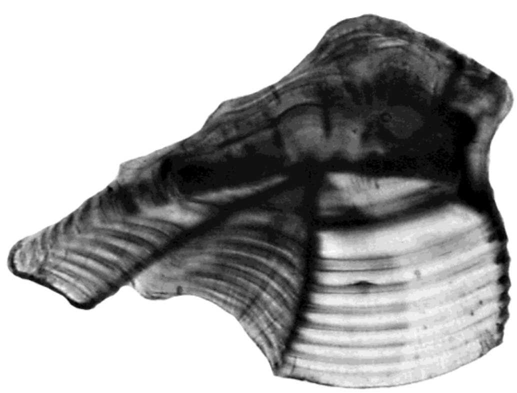


Figure 1. Sectioned otolith from an 8 year old, male spotted seatrout.

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.

Comparison Tests — A symmetry test (Hoenig et al. 1995) and coefficient of variation (CV) analysis were used to detect any systematic difference and precision on age readings, respectively, for the following comparisons: 1) between the two readers in the current year, 2) within each reader in the current year, and 3) time-series bias between the current and previous years within each reader. The readings from the entire sample for the current year were used to examine the difference between two readers. A random sub-sample of 50 fish from the current year was selected for second readings to examine the difference within a reader. Fifty otoliths randomly selected from fish aged in 2000 were used to examine the time-series bias within each reader. A figure of 1:1 equivalence was used to illustrate those differences (Campana et al. 1995). All statistics analyses and figures were made using R (R Development Core Team 2009).

RESULTS

We estimated a sample size of 282 for ageing spotted seatrout in 2010, ranging in length interval from 4 to 31 inches (Table 1). This sample size provided a range in CV for age composition approximately from the smallest CV of 5% for age 1 and the largest CV of 16% for age 3 fish. In 2010, we randomly selected and aged 229 fish from 253 spotted seatrout collected by VMRC. We fell short in our over-all collections for this optimal length-class sampling estimate by 63 fish. However, these were primarily from the small length intervals (Table 1), therefore, the precision for the major age group (age 1 and 2) would not be influenced significantly.

The measurement of reader self-precision was very high for both readers. Both readers had 100% agreement between their first and second readings. There was no evidence of systematic disagreement between Reader 1 and 2 with 98.7% agreement and a 0.3% CV (test of symmetry: $\chi^2 = 1$, df = 2, $P = 0.6065$) (Figure 2).

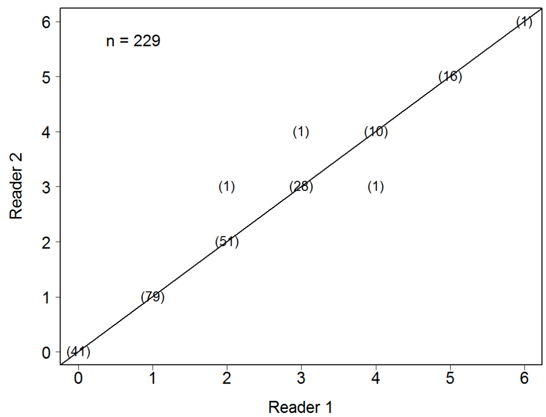


Figure 2. Between-reader comparison of otolith age estimates for spotted seatrout collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2010.

There is no time-series bias for both readers. Reader 1 and 2 had an agreement of 100% with ages of fish aged in 2000.

Of the 229 fish aged with otoliths, 7 age classes were represented (Table 2). The average age for the sample was 1.8 years old, and the standard deviation and standard error were 1.4 and 0.09, respectively. Year-class data show that the fishery was comprised of 7 year-classes, comprising fish from the 2004 through 2010 year-classes, with fish primarily from the 2009 (34%), followed by the 2008 (22%) year-classes (Figure 3).

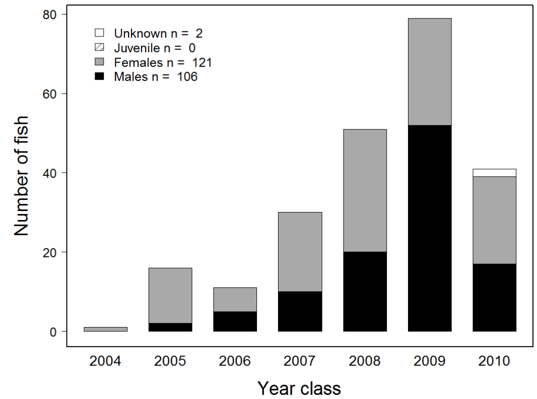


Figure 3. Year-class frequency distribution for spotted seatrout collected for ageing in 2010. Distribution is broken down by sex. “Unknown” is for the fish gonads that were not available for examination or were not examined for sex during sampling.

Age-Length-Key — We present an age-length-key (Table 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. Number of spotted trout collected and aged in each 1-inch length interval in 2010. "Target" represent the sample size for ageing estimated for 2010, "Collected" represents number of fish with both total length and otoliths, and "Need" represents number of fish shorted in each length interval compared to the optimum sample size for ageing and number of fish aged.

Interval	Target	Collected	Aged	Need
4 - 4.99	5	0	0	5
5 - 5.99	5	0	0	5
6 - 6.99	5	0	0	5
7 - 7.99	5	0	0	5
8 - 8.99	5	1	1	4
9 - 9.99	5	1	1	4
10 - 10.99	10	5	5	5
11 - 11.99	13	11	11	2
12 - 12.99	17	20	18	0
13 - 13.99	14	16	14	0
14 - 14.99	16	8	8	8
15 - 15.99	18	18	18	0
16 - 16.99	26	17	17	9
17 - 17.99	28	26	26	2
18 - 18.99	19	21	20	0
19 - 19.99	18	20	18	0
20 - 20.99	14	18	14	0
21 - 21.99	7	8	8	0
22 - 22.99	7	11	8	0
23 - 23.99	5	9	6	0
24 - 24.99	5	6	6	0
25 - 25.99	5	6	6	0
26 - 26.99	5	10	6	0
27 - 27.99	5	7	6	0
28 - 28.99	5	8	6	0
29 - 29.99	5	5	5	0
30 - 30.99	5	1	1	4
31 - 31.99	5	0	0	5
Totals	282	253	229	63

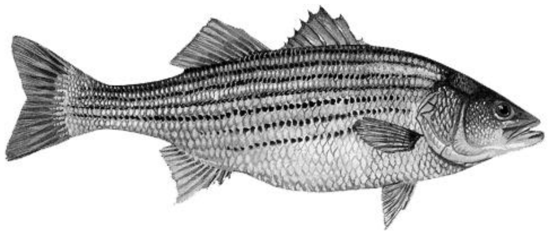
Table 2. The number of spotted seatrout assigned to each total length-at-age category for 229 fish sampled for otolith age determination in Virginia during 2010.

	Age							
Interval	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**Totals**
8 - 8.99	1	0	0	0	0	0	0	1
9 - 9.99	1	0	0	0	0	0	0	1
10 - 10.99	5	0	0	0	0	0	0	5
11 - 11.99	10	1	0	0	0	0	0	11
12 - 12.99	15	3	0	0	0	0	0	18
13 - 13.99	7	7	0	0	0	0	0	14
14 - 14.99	2	6	0	0	0	0	0	8
15 - 15.99	0	18	0	0	0	0	0	18
16 - 16.99	0	14	3	0	0	0	0	17
17 - 17.99	0	19	7	0	0	0	0	26
18 - 18.99	0	7	11	2	0	0	0	20
19 - 19.99	0	3	12	2	1	0	0	18
20 - 20.99	0	0	11	1	2	0	0	14
21 - 21.99	0	1	2	4	0	1	0	8
22 - 22.99	0	0	1	4	2	1	0	8
23 - 23.99	0	0	2	3	1	0	0	6
24 - 24.99	0	0	1	3	2	0	0	6
25 - 25.99	0	0	1	4	1	0	0	6
26 - 26.99	0	0	0	3	1	2	0	6
27 - 27.99	0	0	0	4	0	2	0	6
28 - 28.99	0	0	0	0	1	5	0	6
29 - 29.99	0	0	0	0	0	5	0	5
30 - 30.99	0	0	0	0	0	0	1	1
Totals	41	79	51	30	11	16	1	229

Table 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 2010.

	Age						
Interval	**0**	**1**	**2**	**3**	**4**	**5**	**6**
8 - 8.99	1	0	0	0	0	0	0
9 - 9.99	1	0	0	0	0	0	0
10 - 10.99	1	0	0	0	0	0	0
11 - 11.99	0.909	0.091	0	0	0	0	0
12 - 12.99	0.833	0.167	0	0	0	0	0
13 - 13.99	0.5	0.5	0	0	0	0	0
14 - 14.99	0.25	0.75	0	0	0	0	0
15 - 15.99	0	1	0	0	0	0	0
16 - 16.99	0	0.824	0.176	0	0	0	0
17 - 17.99	0	0.731	0.269	0	0	0	0
18 - 18.99	0	0.35	0.55	0.1	0	0	0
19 - 19.99	0	0.167	0.667	0.111	0.056	0	0
20 - 20.99	0	0	0.786	0.071	0.143	0	0
21 - 21.99	0	0.125	0.25	0.5	0	0.125	0
22 - 22.99	0	0	0.125	0.5	0.25	0.125	0
23 - 23.99	0	0	0.333	0.5	0.167	0	0
24 - 24.99	0	0	0.167	0.5	0.333	0	0
25 - 25.99	0	0	0.167	0.667	0.167	0	0
26 - 26.99	0	0	0	0.5	0.167	0.333	0
27 - 27.99	0	0	0	0.667	0	0.333	0
28 - 28.99	0	0	0	0	0.167	0.833	0
29 - 29.99	0	0	0	0	0	1	0
30 - 30.99	0	0	0	0	0	0	1

Chapter 11
Striped Bass



Morone saxatilis

INTRODUCTION

We aged a total of 923 striped bass, *Morone saxatilis*, using their scales collected by the VMRC's Biological Sampling Program in 2010. Of 923 aged fish, 586 and 337 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 8.6 years with a standard deviation of 3.1 and a standard error of 0.13. Nineteen age classes (from 2 to 17, 19 to 21) were represented in the bay fish, comprising fish from the 1989 to 1991, and 1993 through 2008 year classes. The year class of 2003 was dominant (27%) in the bay fish sample in 2010, followed by the year classes of 2001 (12%) and 2004 (10%). The average age for the ocean fish was 8.8 years with a standard deviation of 2.1 and a standard error of 0.11. Twelve age classes (from 5 to 15, and 18) were represented in the ocean fish, comprising fish from the 1992, and 1995 through 2005 year classes. The year class of 2003 (31%) was dominant in the ocean fish sample in 2010, followed by the year class of 2001 (26%). We also aged a total of 466 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 (please see details in Results).

METHODS

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 2010, 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:

$$A = \frac{V_a}{\theta_a^2 CV^2 - B_a / L}, \qquad (1)$$

where A is the sample size for ageing striped bass in 2010; 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 is a subsample from a catch and used to estimate length distribution in the catch. θ_a, V_a, B_a, and CV were calculated using pooled age-length data of striped bass collected from 2004 to 2008 and using equations in Quinn and Deriso (1999). For simplicity, the equations are not listed here. L was the total number of striped bass used by VMRC to estimate length distribution of the caches from 2004 to 2008. The equation (1) indicates that the more fish that are aged, the smaller the CV (or higher precision) that will be obtained. Therefore, the criterion to decide A (number of fish) is that A should be a number above which there is only a 1% CV reduction achieved by aging an additional 100 or more fish.

Handling of collection — Sagittal otoliths (hereafter, referred to as "otoliths") and scales were received by the Age & Growth Laboratory in labeled coin envelopes. Once in our hands, they 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.

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.

Otoliths — We used a thin-section and bake technique to process striped bass otoliths for age determination. Otolith preparation began by randomly selecting either the right or left otolith. The otolith was mounted 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, 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, three inch diameter, Norton® Diamond Grinding Wheels (hereafter, referred to as "blades"), separated by a stainless steel spacer of 0.4mm (diameter 2.5"). The otolith was positioned so that the blades straddled each side of the otolith focus pencil mark. It was crucial that this cut be perpendicular to the long axis of the otolith. Failure to do so resulted in "broadening" and distortion of winter growth zones. A proper cut resulted in annuli that were clearly defined and delineated. Once cut, the otolith thin-section was placed into a ceramic "Coors" spot plate well and baked in a Thermolyne 1400 furnace at 400^{o}C. Baking time was dependent on otolith size and gauged by color, with a light, caramel color desired. Once a suitable color was reached the baked thin-section was placed on a labeled glass slide and covered with a thin layer of Flo-texx® mounting medium, which provided enhanced contrast and greater readability by increasing light transmission through the sections.

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, 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 period of April through 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 1) with a standard Bell and Howell R-735 microfiche reader equipped with 20 and 29 mm lenses. Annuli on striped bass scales are identified based on two scale microstructure features, “crossing over” and circuli disruption. Primarily, “crossing over” in the lateral margins near the posterior/anterior interface of the scale is used to determine the origin of the annulus. Here compressed circuli (annulus) “cross over” the previously deposited circuli of the previous year’s growth. Typically annuli of the first three years can be observed transversing this interface as dark bands. These bands remain consistent throughout the posterior field and rejoin the posterior/anterior interface on the opposite side of the focus. Annuli can also be observed in the anterior lateral field of the scale. Here the annuli typically reveal a pattern of discontinuous and suddenly breaking segmented circuli. This event can also be distinguished by the presence of concentric white lines, which are typically associated with the disruption of circuli.

Figure 1. Scale impression of a 5-year-old male striped bass.

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

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

Otoliths – All thin-sections were aged by two different readers using a Nikon SMZ1000 stereo microscope under transmitted light and dark-field polarization at between 8 and 20 times magnification (Figure 2).

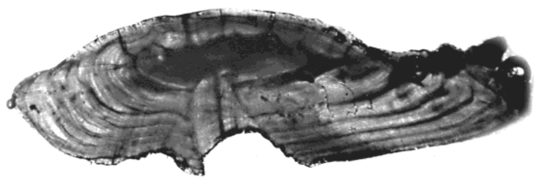


Figure 2. Otolith thin-section of a 5-year-old male striped bass.

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

Comparison Tests — A symmetry test (Hoenig et al. 1995) and coefficient of variation (CV) analysis were used to detect any systematic difference and precision on age readings, respectively, for following comparisons: 1) between the two readers in the current year; 2) within each reader in the current year; 3) time-series bias between the current and previous years within each reader; and 4) between scale and otoliths ages. The readings from the entire sample for the current year were used to examine the difference between two readers. A random sub-sample of 50 fish from the current year was selected for second readings to examine the difference within a reader. Fifty otoliths randomly selected from fish aged in 2000 were used to examine the time-series bias within each reader. A figure of 1:1 equivalence was used to illustrate those differences (Campana et al. 1995). All statistics analyses and figures were made using R (R Development Core Team 2009).

RESULTS

We estimated a sample size of 526 for ageing the bay striped bass in 2010, ranging in length interval from 15 to 53 inches (Table 1). This sample size provided a range in CV for age composition approximately from the smallest CV of 11% for age 9 and 10 to the largest CV of 21% for age 13 of the bay fish. We randomly selected and aged 586 fish from 981 striped bass collected by VMRC in Chesapeake Bay in 2010. We fell short in our over-all collections for this optimal length-class sampling estimate by 48 fish, mainly in the very small and large length intervals (Table 1), as a result, the precision for the estimates of the majority of age categories would not be influenced significantly.

We estimated a sample size of 488 for ageing the ocean striped bass in 2010, ranging in length interval from 21 to 52 inches (Table 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 6 of the ocean fish. We randomly selected and aged 337 fish from 344 striped bass collected by VMRC in Virginia waters of the Atlantic Ocean in 2010. We fell short in our overall collections for this optimal length-class sampling estimate by 223 fish, from among the small, medium, and large length intervals (Table 2), as a result, the precision for the estimates of all age groups would be influenced significantly.

Scales — The measurement of reader self-precision was very good for both readers. There is no significant difference between the first and second readings for Reader 1 with a CV of 2.8% and an one-year or less agreement of 96% (test of symmetry: $\chi^2 = 7.33$, df = 8, $P = 0.5011$). There is no significant difference between the first and second readings for Reader 2 with a CV of 3.5% and an one-year or less agreement of 96% (test of symmetry: $\chi^2 = 12.67$, df = 9, $P = 0.1783$). There was evidence of systematic disagreement between Reader 1 and Reader 2 with a CV of 5.1% (test of symmetry: $\chi^2 = 102$, df = 43, $P < 0.0001$) (Figure 3). The CV of 5.1% was fair and smaller than 2009 (5.6%). The between-reader agreement for scale for one year or less was 88% of all aged fish larger than 85% in 2009.

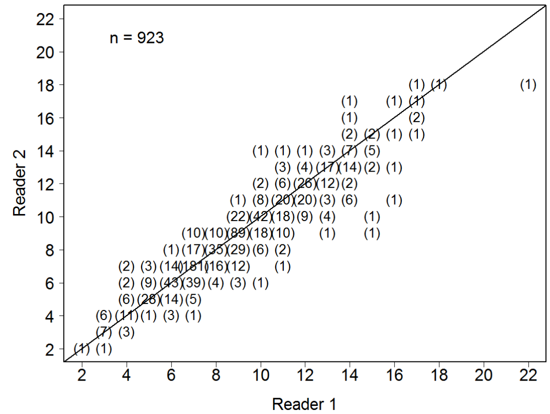


Figure 3. Between-reader comparison of scale age estimates for striped bass collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2010.

There is no time-series bias for both readers. 92% of the age readings by Reader 1 in 2010 had either an agreement with or one-year difference from those fish aged in 2000 with a CV of 6% (test of symmetry: $\chi^2 = 16.13$, df = 13, $P = 0.2420$). The age readings of 98% fish by Reader 2 in 2010 had either an agreement with or one-year different from those fish aged in 2000 with a CV of 3.5% (test of symmetry: $\chi^2 = 8.53$, df = 10, $P = 0.5769$).

Of the 586 bay striped bass aged with scales, 19 age classes (from 2 to 17, 19 to 21) were represented (Table 3). The average age for the sample was 8.6 years. The standard deviation and standard error were 3.1 and 0.13, respectively. Year-class data (Figure 4) indicates that recruitment into the fishery in Chesapeake Bay begins at age 2, which corresponds to the 2008 year-class for striped bass caught in 2010. The year class of 2003 (27%) striped bass was dominated in the sample in 2010, followed by the year-class of 2001 (12%)

and 2004 (10%). The sex ratio of male to female was 1:1.03 for the bay fish.

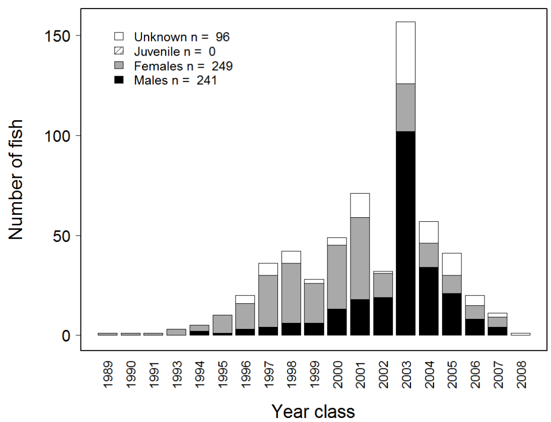


Figure 4. Year-class frequency distribution for striped bass collected in Chesapeake Bay, Virginia for ageing in 2010. Distribution is broken down by sex and estimated using scale ages. "Unknown" is for the fish gonads that were not available for examination or were not examined for sex during sampling.

Of the 337 ocean striped bass aged with scales, 12 age classes (from 5 to 15, and 18) were represented (Table 4). The average age for the sample was 8.8 years. The standard deviation and standard error were 2.1 and 0.11, respectively. Year-class data (Figure 5) indicates that recruitment into the fishery in Virginia waters of the Atlantic Ocean begins at age 5, which corresponds to the 2005 year-class for striped bass caught in 2010. The year class of 2003 (age 7) striped bass with 31% was dominated in the sample in 2010, followed by the year-class of 2001 (26%). The sex ratio of male to female was 1:1.52 for the ocean fish.

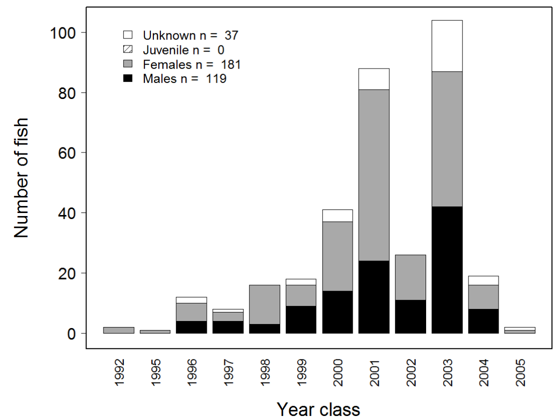


Figure 5. Year-class frequency distribution for striped bass collected in Virginia waters of the Atlantic Ocean for ageing in 2010. Distribution is broken down by sex and estimated using scale ages. "Unknown" is for the fish gonads that were not available for examination or were not examined for sex during sampling.

Otoliths — The measurement of reader self-precision was very good for both readers. There is no significant difference between the first and second readings for Reader 1 with a CV of 0.8% and an agreement of 92% (test of symmetry: $\chi^2 = 4$, df = 4, P = 0.4060). There is no significant difference between the first and second readings for Reader 2 with a CV of 1.3% and an agreement of 82% (test of symmetry: $\chi^2 = 4.33$, df = 6, P = 0.6317). There was an evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 84% and a CV of 1.3% (test of symmetry: $\chi^2 = 40.52$, df = 20, P = 0.0043) (Figure 6). In general, Reader 1 had a tendency to under-age one year than the final ages of 8 and 9 mainly due to missing reading one annulus at the edge of otolith sections.

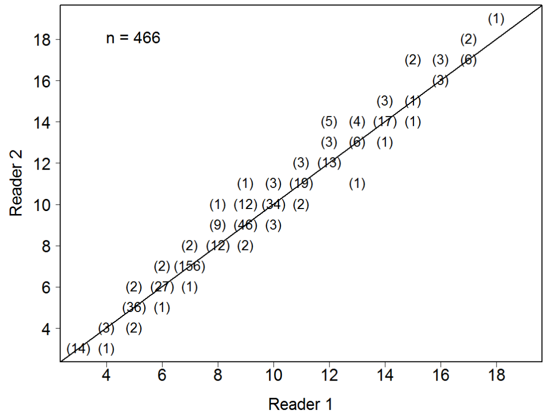


Figure 6. Between-reader comparison of otolith age estimates for striped bass collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2010.

There is no time-series bias for both readers. Reader 1 had an agreement of 80% with the fish aged in 2003 with a CV of 2.2% (test of symmetry: $\chi^2 = 12$, df = 8, $P = 0.1512$). Reader 2 had an agreement of 80% with the fish aged in 2003 with a CV of 2% (test of symmetry: $\chi^2 = 9.33$, df = 5, $P = 0.0965$).

Of 466 fish aged with otoliths, 16 age classes (3 to 17, 19) were represented for striped bass aged with otoliths. The average age for the sample was 8.6 years. The standard deviation and standard error were 3.1 and 0.14, respectively.

Comparison of Scale and Otolith Ages — We aged 464 striped bass using both their scales and otoliths. There was evidence of systematic disagreement between otolith and scale ages (test of symmetry: $\chi^2 = 81.68$, df = 36, $P < 0.0001$) with an average CV of 5.8%. There was an agreement of 54% between scale and otoliths ages whereas scales were assigned a lower and higher age than otoliths for 30% and 16% of the fish, respectively (Figure 7). There was also evidence of bias between otolith and scale ages using an age bias plot (Figure 8), with scale generally assigned higher ages for younger fish and lower ages for older fish than otolith age estimates.

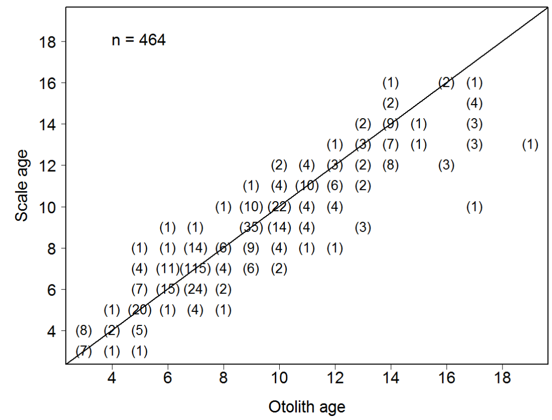


Figure 7. Comparison of scale and otolith age estimates for striped bass collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2010.

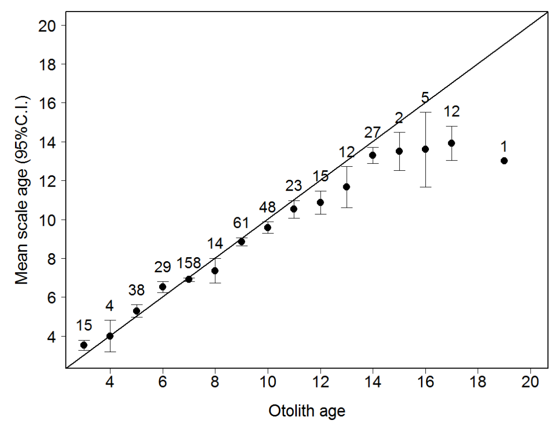


Figure 8. Age-bias plot for striped bass scale and otolith age estimates in 2010.

Age-Length-Key (ALK) — We developed an age-length-key for both bay (Table 5) and ocean fish (Table 6) 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.

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. We will continue to compare the age estimates between otoliths and scales.

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Table 1. Number of striped bass collected in the Chesapeake Bay, Virginia in 2010 and scale-aged in each 1-inch length interval. "Target" represents the sample size for ageing estimated for 2010, "Collected" represents number of fish with both total length and otoliths, and "Need" represents number of fish that were not obtained in each length interval compared to the optimum sample size for ageing and number of fish aged. Five fish without scales are not included.

Interval	Target	Collected	Aged	Need
15 - 15.99	5	0	0	5
17 - 17.99	5	0	0	5
18 - 18.99	7	9	7	0
19 - 19.99	15	37	21	0
20 - 20.99	14	57	28	0
21 - 21.99	21	44	27	0
22 - 22.99	23	76	35	0
23 - 23.99	28	59	33	0
24 - 24.99	29	67	29	0
25 - 25.99	24	67	29	0
26 - 26.99	22	69	32	0
27 - 27.99	21	47	28	0
28 - 28.99	17	32	18	0
29 - 29.99	13	23	16	0
30 - 30.99	12	30	13	0
31 - 31.99	15	28	18	0
32 - 32.99	19	30	18	1
33 - 33.99	20	23	20	0
34 - 34.99	25	25	25	0
35 - 35.99	28	28	28	0
36 - 36.99	39	44	38	1
37 - 37.99	30	63	42	0
38 - 38.99	13	33	24	0
39 - 39.99	11	26	14	0
40 - 40.99	9	27	14	0
41 - 41.99	5	11	6	0
42 - 42.99	6	9	6	0
43 - 43.99	5	8	8	0
44 - 44.99	5	4	4	1
45 - 45.99	5	3	3	2
46 - 46.99	5	2	2	3
47 - 47.99	5	0	0	5
48 - 48.99	5	0	0	5
49 - 49.99	5	0	0	5
50 - 50.99	5	0	0	5
52 - 52.99	5	0	0	5
53 - 53.99	5	0	0	5
Totals	526	981	586	48

Table 2. Number of striped bass collected in Virginia waters of the Atlantic Ocean in 2010 and scale-aged in each 1-inch length interval. “Target” represents the sample size for ageing estimated for 2010, "Collected" represents number of fish with both total length and otoliths, and “Need” represents number of fish that were not obtained in each length interval compared to the optimum sample size for ageing and number of fish aged. One fish without scales is not included.

Interval	Target	Collected	Aged	Need
21 - 21.99	5	0	0	5
26 - 26.99	5	0	0	5
27 - 27.99	5	3	3	2
28 - 28.99	16	26	26	0
29 - 29.99	25	43	43	0
30 - 30.99	24	38	37	0
31 - 31.99	24	48	46	0
32 - 32.99	26	35	35	0
33 - 33.99	37	37	35	2
34 - 34.99	42	32	30	12
35 - 35.99	46	23	23	23
36 - 36.99	50	15	15	35
37 - 37.99	60	17	17	43
38 - 38.99	25	13	13	12
39 - 39.99	20	6	6	14
40 - 40.99	15	2	2	13
41 - 41.99	8	2	2	6
42 - 42.99	5	0	0	5
43 - 43.99	5	2	2	3
44 - 44.99	5	0	0	5
45 - 45.99	5	1	1	4
46 - 46.99	5	0	0	5
47 - 47.99	5	0	0	5
48 - 48.99	5	0	0	5
49 - 49.99	5	0	0	5
50 - 50.99	5	1	1	4
51 - 51.99	5	0	0	5
52 - 52.99	5	0	0	5
Totals	488	344	337	223

Table 3. The number of striped bass assigned to each total length-at-age category for 586 fish sampled for scale age determination in Chesapeake Bay,Virginia during 2010.

	Age																			
Interval	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**19**	**20**	**21**	**Totals**
18 - 18.99	1	1	3	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7
19 - 19.99	0	5	4	2	5	5	0	0	0	0	0	0	0	0	0	0	0	0	0	21
20 - 20.99	0	1	2	3	7	14	1	0	0	0	0	0	0	0	0	0	0	0	0	28
21 - 21.99	0	2	1	3	2	17	2	0	0	0	0	0	0	0	0	0	0	0	0	27
22 - 22.99	0	1	3	9	4	13	4	1	0	0	0	0	0	0	0	0	0	0	0	35
23 - 23.99	0	0	4	6	9	14	0	0	0	0	0	0	0	0	0	0	0	0	0	33
24 - 24.99	0	1	1	8	4	9	5	0	1	0	0	0	0	0	0	0	0	0	0	29
25 - 25.99	0	0	0	5	6	13	1	2	2	0	0	0	0	0	0	0	0	0	0	29
26 - 26.99	0	0	2	1	7	16	2	3	1	0	0	0	0	0	0	0	0	0	0	32
27 - 27.99	0	0	0	2	5	17	3	1	0	0	0	0	0	0	0	0	0	0	0	28
28 - 28.99	0	0	0	0	3	11	2	1	0	0	1	0	0	0	0	0	0	0	0	18
29 - 29.99	0	0	0	1	2	7	4	2	0	0	0	0	0	0	0	0	0	0	0	16
30 - 30.99	0	0	0	0	1	5	0	2	4	0	0	0	0	1	0	0	0	0	0	13
31 - 31.99	0	0	0	0	0	7	2	5	2	0	1	0	1	0	0	0	0	0	0	18
32 - 32.99	0	0	0	0	1	2	1	9	2	2	1	0	0	0	0	0	0	0	0	18
33 - 33.99	0	0	0	0	0	3	1	9	1	1	3	1	1	0	0	0	0	0	0	20
34 - 34.99	0	0	0	0	0	2	1	10	4	5	1	1	1	0	0	0	0	0	0	25
35 - 35.99	0	0	0	0	0	1	1	7	8	5	3	3	0	0	0	0	0	0	0	28
36 - 36.99	0	0	0	0	0	1	1	8	12	2	4	6	3	1	0	0	0	0	0	38
37 - 37.99	0	0	0	0	0	0	1	7	7	8	8	7	2	1	1	0	0	0	0	42
38 - 38.99	0	0	0	0	0	0	0	4	4	1	10	4	1	0	0	0	0	0	0	24
39 - 39.99	0	0	0	0	0	0	0	0	0	3	3	5	3	0	0	0	0	0	0	14
40 - 40.99	0	0	0	0	0	0	0	0	1	0	4	2	2	4	1	0	0	0	0	14
41 - 41.99	0	0	0	0	0	0	0	0	0	0	1	2	1	1	1	0	0	0	0	6
42 - 42.99	0	0	0	0	0	0	0	0	0	0	0	1	2	1	1	1	0	0	0	6
43 - 43.99	0	0	0	0	0	0	0	0	0	1	1	3	2	0	0	1	0	0	0	8
44 - 44.99	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0	0	1	0	4
45 - 45.99	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	1	3
46 - 46.99	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	2
Totals	1	11	20	41	57	157	32	71	49	28	42	36	20	10	5	3	1	1	1	586

Table 4. The number of striped bass assigned to each total length-at-age category for 337 fish sampled for scale age determination in Virginia waters of the Atlantic Ocean during 2010.

	Age											
Interval	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**18**	**Totals**
27 - 27.99	0	1	1	0	0	1	0	0	0	0	0	0	3
28 - 28.99	1	5	15	0	1	2	2	0	0	0	0	0	26
29 - 29.99	0	7	25	4	6	0	1	0	0	0	0	0	43
30 - 30.99	0	4	22	3	4	3	0	0	1	0	0	0	37
31 - 31.99	1	2	20	6	10	6	0	0	0	1	0	0	46
32 - 32.99	0	0	12	2	11	5	2	1	1	1	0	0	35
33 - 33.99	0	0	3	5	17	6	3	1	0	0	0	0	35
34 - 34.99	0	0	4	5	12	6	2	1	0	0	0	0	30
35 - 35.99	0	0	1	1	14	4	0	1	0	2	0	0	23
36 - 36.99	0	0	1	0	4	2	2	6	0	0	0	0	15
37 - 37.99	0	0	0	0	6	2	2	3	1	3	0	0	17
38 - 38.99	0	0	0	0	2	3	3	2	3	0	0	0	13
39 - 39.99	0	0	0	0	1	0	1	1	1	2	0	0	6
40 - 40.99	0	0	0	0	0	0	0	0	1	0	1	0	2
41 - 41.99	0	0	0	0	0	1	0	0	0	1	0	0	2
43 - 43.99	0	0	0	0	0	0	0	0	0	2	0	0	2
45 - 45.99	0	0	0	0	0	0	0	0	0	0	0	1	1
50 - 50.99	0	0	0	0	0	0	0	0	0	0	0	1	1
Totals	2	19	104	26	88	41	18	16	8	12	1	2	337

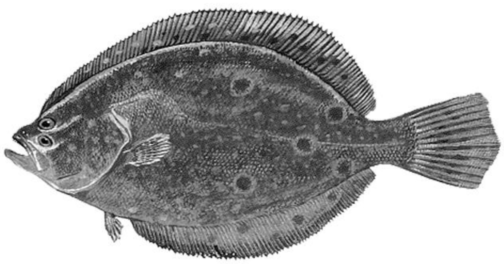
Table 5. Age-Length key, as proportion-at-age in each 1-inch length interval, based on scale ages for striped bass sampled in Chesapeake Bay, Virginia during 2010.

	Age																	
Interval	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**19**	**20**	**21**
18 - 18.99	0.143	0.143	0.429	0.143	0.143	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19 - 19.99	0	0.238	0.19	0.095	0.238	0.238	0	0	0	0	0	0	0	0	0	0	0	0	0
20 - 20.99	0	0.036	0.071	0.107	0.25	0.5	0.036	0	0	0	0	0	0	0	0	0	0	0	0
21 - 21.99	0	0.074	0.037	0.111	0.074	0.63	0.074	0	0	0	0	0	0	0	0	0	0	0	0
22 - 22.99	0	0.029	0.086	0.257	0.114	0.371	0.114	0.029	0	0	0	0	0	0	0	0	0	0	0
23 - 23.99	0	0	0.121	0.182	0.273	0.424	0	0	0	0	0	0	0	0	0	0	0	0	0
24 - 24.99	0	0.034	0.034	0.276	0.138	0.31	0.172	0	0.034	0	0	0	0	0	0	0	0	0	0
25 - 25.99	0	0	0	0.172	0.207	0.448	0.034	0.069	0.069	0	0	0	0	0	0	0	0	0	0
26 - 26.99	0	0	0.062	0.031	0.219	0.5	0.062	0.094	0.031	0	0	0	0	0	0	0	0	0	0
27 - 27.99	0	0	0	0.071	0.179	0.607	0.107	0.036	0	0	0	0	0	0	0	0	0	0	0
28 - 28.99	0	0	0	0	0.167	0.611	0.111	0.056	0	0	0.056	0	0	0	0	0	0	0	0
29 - 29.99	0	0	0	0.062	0.125	0.438	0.25	0.125	0	0	0	0	0	0	0	0	0	0	0
30 - 30.99	0	0	0	0	0.077	0.385	0	0.154	0.308	0	0	0	0	0.077	0	0	0	0	0
31 - 31.99	0	0	0	0	0	0.389	0.111	0.278	0.111	0	0.056	0	0.056	0	0	0	0	0	0
32 - 32.99	0	0	0	0	0.056	0.111	0.056	0.5	0.111	0.111	0.056	0	0	0	0	0	0	0	0
33 - 33.99	0	0	0	0	0	0.15	0.05	0.45	0.05	0.05	0.15	0.05	0.05	0	0	0	0	0	0
34 - 34.99	0	0	0	0	0	0.08	0.04	0.4	0.16	0.2	0.04	0.04	0.04	0	0	0	0	0	0
35 - 35.99	0	0	0	0	0	0.036	0.036	0.25	0.286	0.179	0.107	0.107	0	0	0	0	0	0	0
36 - 36.99	0	0	0	0	0	0.026	0.026	0.211	0.316	0.053	0.105	0.158	0.079	0.026	0	0	0	0	0
37 - 37.99	0	0	0	0	0	0	0.024	0.167	0.167	0.19	0.19	0.167	0.048	0.024	0.024	0	0	0	0
38 - 38.99	0	0	0	0	0	0	0	0.167	0.167	0.042	0.417	0.167	0.042	0	0	0	0	0	0
39 - 39.99	0	0	0	0	0	0	0	0	0	0.214	0.214	0.357	0.214	0	0	0	0	0	0
40 - 40.99	0	0	0	0	0	0	0	0	0.071	0	0.286	0.143	0.143	0.286	0.071	0	0	0	0
41 - 41.99	0	0	0	0	0	0	0	0	0	0	0.167	0.333	0.167	0.167	0.167	0	0	0	0
42 - 42.99	0	0	0	0	0	0	0	0	0	0	0	0.167	0.333	0.167	0.167	0.167	0	0	0
43 - 43.99	0	0	0	0	0	0	0	0	0	0.125	0.125	0.375	0.25	0	0	0.125	0	0	0
44 - 44.99	0	0	0	0	0	0	0	0	0	0	0.25	0.25	0	0	0.25	0	0	0.25	0
45 - 45.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0.333	0	0	0.333	0	0.333
46 - 46.99	0	0	0	0	0	0	0	0	0	0	0	0	0.5	0	0	0.5	0	0	0

Table 6. 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 2010.

	Age											
Interval	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**18**
27 - 27.99	0	0.333	0.333	0	0	0.333	0	0	0	0	0	0
28 - 28.99	0.038	0.192	0.577	0	0.038	0.077	0.077	0	0	0	0	0
29 - 29.99	0	0.163	0.581	0.093	0.14	0	0.023	0	0	0	0	0
30 - 30.99	0	0.108	0.595	0.081	0.108	0.081	0	0	0.027	0	0	0
31 - 31.99	0.022	0.043	0.435	0.13	0.217	0.13	0	0	0	0.022	0	0
32 - 32.99	0	0	0.343	0.057	0.314	0.143	0.057	0.029	0.029	0.029	0	0
33 - 33.99	0	0	0.086	0.143	0.486	0.171	0.086	0.029	0	0	0	0
34 - 34.99	0	0	0.133	0.167	0.4	0.2	0.067	0.033	0	0	0	0
35 - 35.99	0	0	0.043	0.043	0.609	0.174	0	0.043	0	0.087	0	0
36 - 36.99	0	0	0.067	0	0.267	0.133	0.133	0.4	0	0	0	0
37 - 37.99	0	0	0	0	0.353	0.118	0.118	0.176	0.059	0.176	0	0
38 - 38.99	0	0	0	0	0.154	0.231	0.231	0.154	0.231	0	0	0
39 - 39.99	0	0	0	0	0.167	0	0.167	0.167	0.167	0.333	0	0
40 - 40.99	0	0	0	0	0	0	0	0	0.5	0	0.5	0
41 - 41.99	0	0	0	0	0	0.5	0	0	0	0.5	0	0
43 - 43.99	0	0	0	0	0	0	0	0	0	1	0	0
45 - 45.99	0	0	0	0	0	0	0	0	0	0	0	1
50 - 50.99	0	0	0	0	0	0	0	0	0	0	0	1

Chapter 12
Summer Flounder



Paralichthys dentatus

INTRODUCTION

We aged a total of 816 summer flounder, *Paralichthys dentatus*, using their scales collected by the VMRC's Biological Sampling Program in 2010. Of 816 aged fish, 283 and 533 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 3.3 years with a standard deviation of 1.7 and a standard error of 0.1. Ten age classes (1 to 9, and 12) were represented in the bay fish, comprising fish from the 1998 and 2001 to 2009 year classes. The year class of 2008 (40%) dominated the bay sample in 2010, followed by the 2007 (24%) and 2006 (16%). The average age for the ocean fish was 4.4 years with a standard deviation of 1.9 and a standard error of 0.08. Twelve age classes (1 to 12) were represented in the ocean fish, comprising fish of the 1998 to 2009 year classes. The year class of 2007 (26%) was dominant in the ocean fish sample in 2010, followed by the year classes of 2006 (21%) and 2004 (19%). We also aged a total of 369 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 (please see details in Results).

METHODS

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 2010, respectively, using a two-stage random sampling method (Quinn and Deriso 1999) in order to increase precision in estimates of age composition from fish sampled efficiently and effectively. The basic equation is:

$$A = \frac{V_a}{\theta_a^2 CV^2 - B_a / L}, \qquad (1)$$

where A is the sample size for ageing summer flounder in 2010; 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 is a subsample from a catch and used to estimate length distribution in the catch. θ_a, V_a, B_a, and CV were calculated using pooled age-length data of summer flounder collected from 2004 to 2009 and using equations in Quinn and Deriso (1999). For simplicity, the equations are not listed here. L was the total number of summer flounder used by VMRC to estimate length distribution of the catches from 2004 to 2009. The equation (1) indicates that the more fish that are aged, the smaller the CV (or higher precision) that will be obtained. Therefore, the criterion to decide A (number of fish) is that A should be a number above which there is only a 1% CV reduction achieved by aging an additional 100 or more fish.

Handling of collection — Sagittal otoliths (hereafter, referred to as “otoliths”) and scales were received by the Age & Growth Laboratory in labeled coin envelopes. Once in our hands, they 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.

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 uniform size. We selected a range of five to ten 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:	12000 to 15000 psi
Temperature:	Room temperature
Time:	7 minutes

Otoliths – The left otoliths of summer flounder are symmetrical in relation to the otolith nucleus, while right otoliths are asymmetrical. The right sagittal otolith was mounted 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 (hereafter referred to as “blades”), separated by a stainless steel spacer of 0.4mm (diameter 2.5”). The otolith was positioned so that the blades straddled each side of the otolith focus mark. It was crucial that this cut be perpendicular to the long axis of the otolith. Failure to do so resulted in “broadening” and distortion of winter growth zones. A proper cut resulted in annuli that were clearly defined and delineated. Once cut, the otolith thin-section was placed into a ceramic “Coors” spot plate well and baked in a Thermolyne 1400 furnace at 400^{o}C. Baking time was dependent on otolith size and gauged by color, with a light caramel color desired. Once a suitable color was reached the baked thin-section was placed on a labeled glass slide and covered with a thin layer of Flo-texx® mounting medium, which provided enhanced contrast and greater readability by increasing light transmission through the sections.

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, 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. 1999). A summer flounder captured between January 1 and April 30, before the end of the species’ annulus formation period, with three visible annuli and some translucent growth after the last annulus, would be assigned an age class of “x + (x+1)” or 3 + (3+1), noted as 3 + 4. This is the same age-class assigned to a fish with four visible annuli captured after the end of April 30, the period of annulus formation, which would be noted as 4 + 4.

Summer flounder scales are also considered to have a deposition period of January through April (Bolz et al. 1999), 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, 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.

Scales - We determined fish age by viewing the acetate impressions of scales (Figure 1) with a standard Bell and Howell R-735 microfiche reader equipped with 20 and 29 mm lenses.

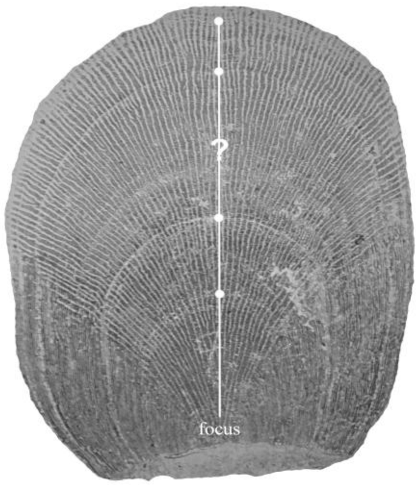


Figure 1. Scale impression of a 590 mm female summer flounder collected in November and aged as 4 years old with scales. The question mark is located at a possible "3rd" annulus.

Annuli on summer flounder scales are primarily identified by the presence of crossing over of circuli. Crossing over is most evident on the lateral margins near the posterior/anterior interface of the scale. Here compressed circuli (annulus) "cross over" the deposited circuli of the previous year's growth. Typically the annulus will protrude partially into the ctenii of the posterior field, but not always.

Following the annulus up into the anterior field of the scale reveals a pattern of discontinuous and suddenly breaking segmented circuli. This event can also be distinguished by the presence of concentric white lines, which are associated with the disruption of circuli. This pattern should be continuous throughout the entire anterior field of the scale. Locating the first annulus can be difficult due to latitudinal differences in growth rates and changes in the size of the first annulus due to a protracted spawning season. We consider the first annulus to be the first continuous crossing over event formed on the scale.

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 2).

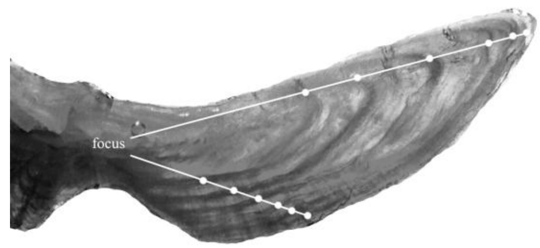


Figure 2. Otolith section from a 590 mm, 6-year-old female summer flounder collected in November. Same fish as Figure 1.

Summer flounder otoliths are composed of visually distinct summer and winter growth zones. By convention, an annulus is identified as the narrow opaque zone, or winter growth band. With sectioned otoliths, to be considered a true annulus, these growth bands must be rooted in the sulcus and able to be followed, 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 the reading path proceeds in a direction from the sulcus to the proximal surface. The first annulus is located directly below the focus and near the upper portion of the sulcal groove. The distance from the focus to the first year is moderate, with translucent zone deposition gradually becoming smaller as consecutive annuli are deposited towards the outer edge.

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; 3) time-series bias between the current and previous years within each reader; and 4) between scale and otoliths ages. The readings from the entire sample for the current year were used to examine the difference between two readers. A random sub-sample of 50 fish from the current year was selected for second readings to examine the difference within a reader. Fifty otoliths randomly selected from fish aged in 2000 were used to examine the time-series bias within each reader. A figure of 1:1 equivalence was used to illustrate those differences (Campana et al. 1995). All statistics analyses and figures were made using R (R Development Core Team 2009).

RESULTS

We estimated a sample size of 306 for ageing the bay summer flounder in 2010, ranging in length interval from 11 to 28 inches (Table 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 19% for age 5 of the bay fish. We randomly selected and aged 283 fish from 337 summer flounder collected by VMRC in Chesapeake Bay in 2010. We fell short in our over-all collections for this optimal length-class sampling estimate by 40 fish mainly in the small, median, and large length intervals (Table 1), as a result, the precision for the estimates of the majority of age categories would be influenced significantly.

We estimated a sample size of 467 for ageing the ocean summer flounder in 2010, ranging in length interval from 12 to 33 inches (Table 2). This sample size provided a range in CV for age composition approximately from the smallest CV of 8% for age 3 to the largest CV of 23% for age 8 of the ocean fish. We randomly selected and aged 533 fish from 882 summer flounder collected by VMRC in Virginia waters of the Atlantic Ocean in 2010. We fell short in our over-all collections for this optimal length-class sampling estimate by 28 fish mainly from the small and large length intervals (table 2), as a result, the precision for the estimates of all age groups would not be influenced significantly.

Scales — There is no significant difference between the first and second readings for Reader 1 with an agreement of 66% and a CV of 6.5% (test of symmetry: $\chi^2 = 6.47$, df = 7, $P = 0.4864$). There is no significant difference between the first and second readings for Reader 2 with an agreement of 70% and a CV of 5.7% (test of symmetry: $\chi^2 = 7$, df = 7, $P = 0.4289$). There was no evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 72% and a CV of 6.1% (test of symmetry: $\chi^2 = 34.74$, df = 25, $P = 0.0930$) (Figure 3).

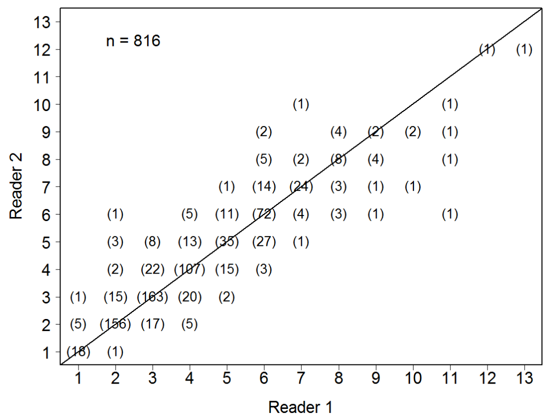


Figure 3. Between-reader comparison of scale age estimates for summer flounder collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2010.

There is a time-series bias for Reader 1. The age readings of 80% fish by Reader 1 in 2010 had an agreement with those fish aged in 2000 with a CV of 3.8% (test of symmetry: $\chi^2 = 10$, df $= 4$, $P = 0.0404$). There is time-series bias for Reader 2. The age readings of 82% fish by Reader 2 in 2010 had an agreement with those fish aged in 2000 with a CV of 3% (test of symmetry: $\chi^2 = 9$, df $= 3$, $P = 0.0293$). The time-series bias is due to that 7 and 6 fish were aged as 3 years old by Reader 1 and 2, respectively, in 2010 whereas those fish were aged as 4 years old in 2000 (Figure 4 and Figure 5). However, 4 of those fish were aged as 3 years old by using their otoliths in 2000. Therefore, the time-series bias indicates that we improved our scale-ageing in 2010.

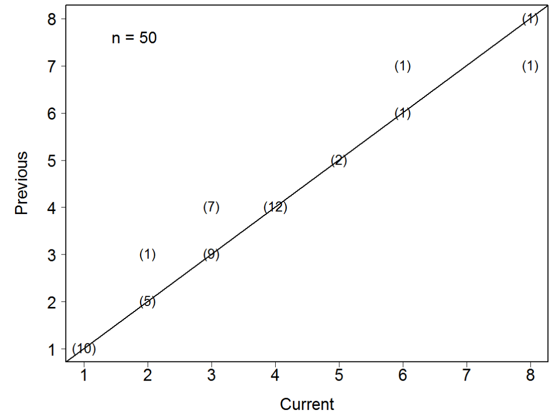


Figure 4. Time series precision of scale ages for Reader 1. "Previous" and "Current" represent that the same fish were aged in 2000 and 2010, respectively

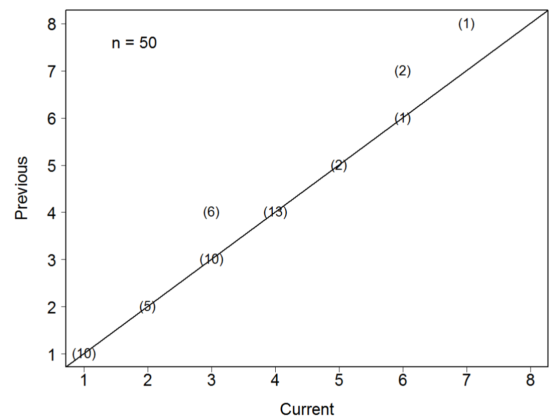


Figure 5. Time series precision of scale ages for Reader 2. "Previous" and "Current" represent that the same fish were aged in 2000 and 2010, respectively

Of the 283 bay summer flounder aged with scales, 10 age classes (1 to 9, 12) were represented (Table 3). The average age for the sample was 3.3 years. The standard deviation and standard error were 1.7 and 0.1, respectively. Year-class data indicates that recruitment into the fishery in Chesapeake Bay begins at age 1, which corresponds to the 2009 year-class for summer flounder caught in 2010. The year class of 2008 (40%) summer flounder dominated the sample in 2010, followed by the 2007 (24%) and 2006 (16%). The sex ratio of male to female was 1:8.69 for the bay fish (Figure 6).

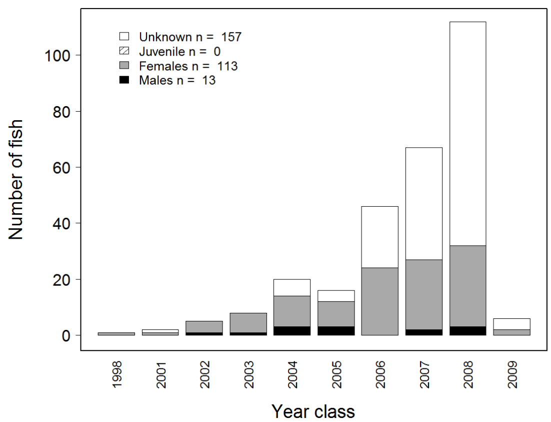


Figure 6. Year-class frequency distribution for summer flounder collected in Chesapeake Bay, Virginia for ageing in 2010. Distribution is broken down by sex and estimated using scale ages. "Unknown" is for the fish gonads that were not available for examination or were not examined for sex during sampling.

Of the 533 ocean summer flounder aged with scales, 12 age classes (1 to 12) were represented (Table 4). The average age for the sample was 4.4 years. The standard deviation and standard error were 1.9 and 0.08, respectively. Year-class data indicates that recruitment into the fishery in Virginia waters of the Atlantic Ocean begins at age 1, which corresponds to the 2009 year-class for summer flounder caught in 2010. The year class of 2007 (26%) summer flounder dominated the sample in 2010, followed by the year class of 2006 (21%), and 2004 (19%) (Figure 7). The sex ratio of male to female was 1:1.25 for the ocean fish.

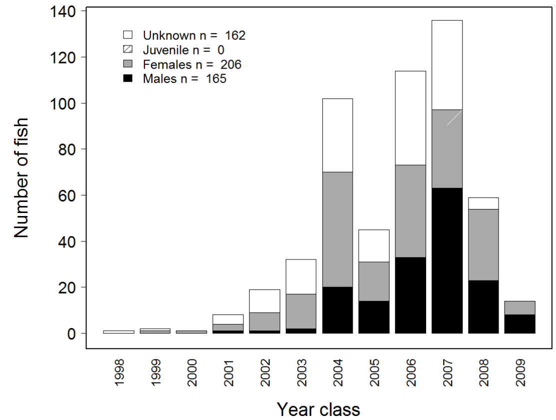


Figure 7. Year-class frequency distribution for summer flounder collected in Virginia waters of the Atlantic Ocean for ageing in 2010. Distribution is broken down by sex and estimated using scale ages. "Unknown" is for the fish gonads that were not available for examination or were not examined for sex during sampling.

Otoliths — The measurement of reader self-precision was very good for both readers. There is no significant difference between the first and second readings for Reader 1 and Reader 2. Reader 1 had a CV of 0.7% and an agreement of 96% (test of symmetry: $\chi^2 = 2$, df = 2, $P = 0.3679$). Reader 2 had a CV of 0.8% and an agreement of 94% (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 92% and a CV of 1.5% (test of symmetry: $\chi^2 = 10.63$, df = 10, $P = 0.3868$) (Figure 8).

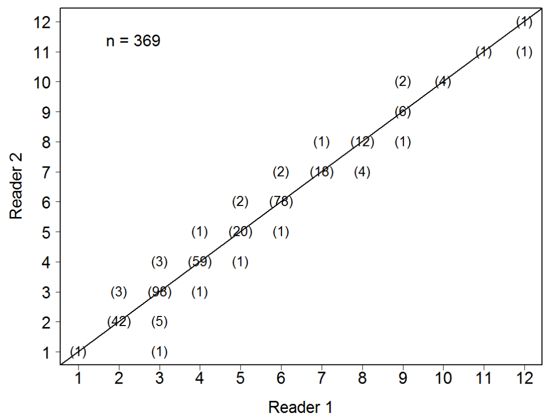


Figure 8. Between-reader comparison of otolith age estimates for summer flounder collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2010.

There is no time-series bias for both readers. Reader 1 had an agreement of 88% with the fish aged in 2000 with a CV of 2.8% (test of symmetry: $\chi^2 = 6$, df = 5, $P = 0.3062$). Reader 2 had an agreement of 92% with the fish aged in 2000 with a CV of 1.2% (test of symmetry: $\chi^2 = 2$, df = 3, $P = 0.5724$).

Of 369 fish aged with otoliths, 12 age classes (1 to 12) were represented for summer flounder. The average age for the sample was 4.6 years. The standard deviation and standard error were 2 and 0.1, respectively.

Comparison of Scale and Otolith Ages — We aged 369 summer flounder using scales and otoliths. There was no evidence of systematic disagreement between otolith and scale ages (test of symmetry: $\chi^2 = 35.61$, df = 24, $P = 0.0598$) with an average CV of 8%, indicating, once again, that we improved our scale-ageing in 2010. There was an agreement of 64% between scale and otolith ages. Scales were assigned a lower and higher age than otoliths for 22% and 14% of the fish, respectively (Figure 9). The 1:1 equivalence plot also indicated that there was no evidence of systematic disagreement between otolith and scale ages (Figure 10).

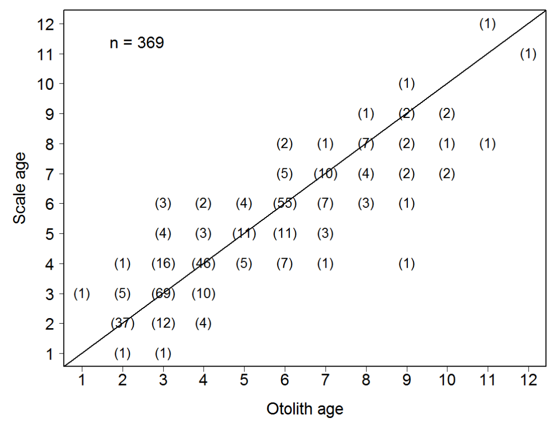


Figure 9. Comparison of scale and otolith age estimates for summer flounder collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2010.

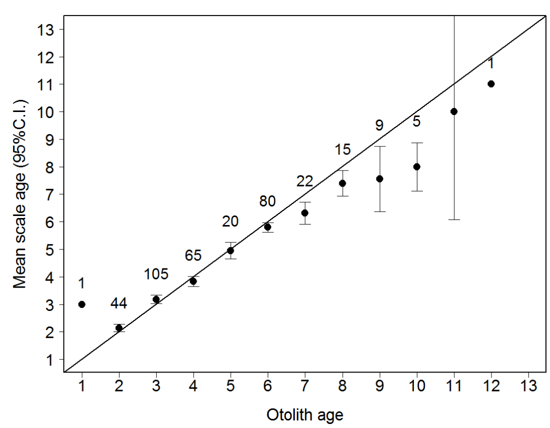


Figure 10. Age-bias plot for summer flounder scale and otolith age estimates in 2010.

Age-Length-Key (ALK) —We developed an ALK for both bay (Table 5) and ocean fish (Table 6) 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.

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Table 1. Number of summer flounder collected in the Chesapeake Bay, Virginia in 2010 and scale-aged in each 1-inch length interval. “Target” represents the sample size for ageing estimated for 2010, "Collected" represents number of fish with both total length and otoliths, and “Need” represents number of fish that were not obtained 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
12 - 12.99	5	0	0	5
13 - 13.99	9	2	2	7
14 - 14.99	52	65	52	0
15 - 15.99	50	48	48	2
16 - 16.99	38	37	37	1
17 - 17.99	31	34	31	0
18 - 18.99	22	33	22	0
19 - 19.99	18	37	23	0
20 - 20.99	14	27	20	0
21 - 21.99	15	21	16	0
22 - 22.99	11	17	16	0
23 - 23.99	11	7	7	4
24 - 24.99	5	4	4	1
25 - 25.99	5	3	3	2
26 - 26.99	5	1	1	4
27 - 27.99	5	1	1	4
28 - 28.99	5	0	0	5
Totals	306	337	283	40

Table 2. Number of summer flounder collected in Virginia waters of the Atlantic Ocean in 2010 and scale-aged in each 1-inch length interval. “Target” represents the sample size for ageing estimated for 2010, "Collected" represents number of fish with both total length and otoliths, and “Need” represents number of fish that were not obtained 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	12	9	9	3
14 - 14.99	41	69	48	0
15 - 15.99	69	93	73	0
16 - 16.99	68	114	78	0
17 - 17.99	53	119	68	0
18 - 18.99	39	79	45	0
19 - 19.99	26	59	30	0
20 - 20.99	24	38	32	0
21 - 21.99	16	48	21	0
22 - 22.99	19	75	31	0
23 - 23.99	19	75	32	0
24 - 24.99	17	53	23	0
25 - 25.99	15	25	17	0
26 - 26.99	9	9	9	0
27 - 27.99	5	6	6	0
28 - 28.99	5	6	6	0
29 - 29.99	5	4	4	1
30 - 30.99	5	0	0	5
31 - 31.99	5	0	0	5
32 - 32.99	5	1	1	4
33 - 33.99	5	0	0	5
Totals	467	882	533	28

Table 3. The number of summer flounder assigned to each total length-at-age category for 283 fish sampled for scale age determination in Chesapeake Bay,Virginia during 2010.

	Age										
Interval	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**12**	**Totals**
13 - 13.99	0	2	0	0	0	0	0	0	0	0	2
14 - 14.99	4	39	7	1	1	0	0	0	0	0	52
15 - 15.99	0	37	9	2	0	0	0	0	0	0	48
16 - 16.99	2	14	16	5	0	0	0	0	0	0	37
17 - 17.99	0	12	11	7	0	1	0	0	0	0	31
18 - 18.99	0	3	8	9	2	0	0	0	0	0	22
19 - 19.99	0	2	8	6	3	4	0	0	0	0	23
20 - 20.99	0	3	5	5	2	2	2	0	1	0	20
21 - 21.99	0	0	2	3	4	3	3	1	0	0	16
22 - 22.99	0	0	1	7	2	4	1	1	0	0	16
23 - 23.99	0	0	0	1	1	2	1	1	1	0	7
24 - 24.99	0	0	0	0	0	2	1	1	0	0	4
25 - 25.99	0	0	0	0	1	1	0	1	0	0	3
26 - 26.99	0	0	0	0	0	1	0	0	0	0	1
27 - 27.99	0	0	0	0	0	0	0	0	0	1	1
Totals	6	112	67	46	16	20	8	5	2	1	283

Table 4. The number of summer flounder assigned to each total length-at-age category for 533 fish sampled for scale age determination in Virginia waters of the Atlantic Ocean during 2010.

	Age												
Interval	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**Totals**
13 - 13.99	2	5	2	0	0	0	0	0	0	0	0	0	9
14 - 14.99	5	10	22	9	0	2	0	0	0	0	0	0	48
15 - 15.99	6	19	23	21	2	2	0	0	0	0	0	0	73
16 - 16.99	1	10	34	18	10	4	1	0	0	0	0	0	78
17 - 17.99	0	8	27	20	5	7	0	1	0	0	0	0	68
18 - 18.99	0	2	13	10	7	13	0	0	0	0	0	0	45
19 - 19.99	0	4	7	12	1	4	1	1	0	0	0	0	30
20 - 20.99	0	1	6	11	3	7	2	0	2	0	0	0	32
21 - 21.99	0	0	0	3	7	9	1	1	0	0	0	0	21
22 - 22.99	0	0	2	9	3	13	3	1	0	0	0	0	31
23 - 23.99	0	0	0	1	5	20	3	1	2	0	0	0	32
24 - 24.99	0	0	0	0	2	9	9	2	0	1	0	0	23
25 - 25.99	0	0	0	0	0	8	5	4	0	0	0	0	17
26 - 26.99	0	0	0	0	0	3	5	1	0	0	0	0	9
27 - 27.99	0	0	0	0	0	1	1	4	0	0	0	0	6
28 - 28.99	0	0	0	0	0	0	1	2	1	0	2	0	6
29 - 29.99	0	0	0	0	0	0	0	1	3	0	0	0	4
32 - 32.99	0	0	0	0	0	0	0	0	0	0	0	1	1
Totals	14	59	136	114	45	102	32	19	8	1	2	1	533

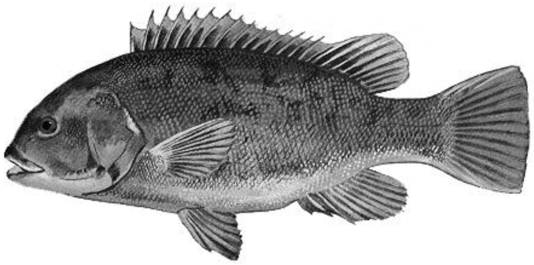
Table 5. Age-Length key, as proportion-at-age in each 1-inch length interval, based on scale ages for summer flounder sampled in Chesapeake Bay, Virginia during 2010.

	Age									
Interval	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**12**
13 - 13.99	0	1	0	0	0	0	0	0	0	0
14 - 14.99	0.077	0.75	0.135	0.019	0.019	0	0	0	0	0
15 - 15.99	0	0.771	0.188	0.042	0	0	0	0	0	0
16 - 16.99	0.054	0.378	0.432	0.135	0	0	0	0	0	0
17 - 17.99	0	0.387	0.355	0.226	0	0.032	0	0	0	0
18 - 18.99	0	0.136	0.364	0.409	0.091	0	0	0	0	0
19 - 19.99	0	0.087	0.348	0.261	0.13	0.174	0	0	0	0
20 - 20.99	0	0.15	0.25	0.25	0.1	0.1	0.1	0	0.05	0
21 - 21.99	0	0	0.125	0.188	0.25	0.188	0.188	0.062	0	0
22 - 22.99	0	0	0.062	0.438	0.125	0.25	0.062	0.062	0	0
23 - 23.99	0	0	0	0.143	0.143	0.286	0.143	0.143	0.143	0
24 - 24.99	0	0	0	0	0	0.5	0.25	0.25	0	0
25 - 25.99	0	0	0	0	0.333	0.333	0	0.333	0	0
26 - 26.99	0	0	0	0	0	1	0	0	0	0
27 - 27.99	0	0	0	0	0	0	0	0	0	1

Table 6. 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 2010.

	Age											
Interval	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
13 - 13.99	0.222	0.556	0.222	0	0	0	0	0	0	0	0	0
14 - 14.99	0.104	0.208	0.458	0.188	0	0.042	0	0	0	0	0	0
15 - 15.99	0.082	0.26	0.315	0.288	0.027	0.027	0	0	0	0	0	0
16 - 16.99	0.013	0.128	0.436	0.231	0.128	0.051	0.013	0	0	0	0	0
17 - 17.99	0	0.118	0.397	0.294	0.074	0.103	0	0.015	0	0	0	0
18 - 18.99	0	0.044	0.289	0.222	0.156	0.289	0	0	0	0	0	0
19 - 19.99	0	0.133	0.233	0.4	0.033	0.133	0.033	0.033	0	0	0	0
20 - 20.99	0	0.031	0.188	0.344	0.094	0.219	0.062	0	0.062	0	0	0
21 - 21.99	0	0	0	0.143	0.333	0.429	0.048	0.048	0	0	0	0
22 - 22.99	0	0	0.065	0.29	0.097	0.419	0.097	0.032	0	0	0	0
23 - 23.99	0	0	0	0.031	0.156	0.625	0.094	0.031	0.062	0	0	0
24 - 24.99	0	0	0	0	0.087	0.391	0.391	0.087	0	0.043	0	0
25 - 25.99	0	0	0	0	0	0.471	0.294	0.235	0	0	0	0
26 - 26.99	0	0	0	0	0	0.333	0.556	0.111	0	0	0	0
27 - 27.99	0	0	0	0	0	0.167	0.167	0.667	0	0	0	0
28 - 28.99	0	0	0	0	0	0	0.167	0.333	0.167	0	0.333	0
29 - 29.99	0	0	0	0	0	0	0	0.25	0.75	0	0	0
32 - 32.99	0	0	0	0	0	0	0	0	0	0	0	1

Chapter 13
Tautog



Tautoga onitis

INTRODUCTION

We aged a total of 186 tautog, *Tautoga onitis*, using their opercula collected by the VMRC's Biological Sampling Program in 2010. Of 186 aged fish, 165 and 21 fish were collected in Chesapeake Bay (bay fish) and Virginia waters of the Atlantic Ocean (ocean fish), respectively. The average age of the bay fish was 5.2 years with a standard deviation of 2.1 and a standard error of 0.16. Ten age classes (from 2 to 11) were represented in the bay fish, comprising fish from the 1999 to 2008 year classes. The year class of 2006 (24%) was dominant in the bay fish sample in 2010 followed by the year classes of 2004 (18%) and 2005 (17%). The average age for the ocean fish was 8.9 years with a standard deviation of 4 and a standard error of 0.87. Thirteen age classes (from 3 to 14, and 16 years old) were represented in the ocean fish, comprising fish from the 1994, 1996 through 2007 year classes.

We also aged a total of 181 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 (please see details in Results).

METHODS

Sample size for ageing — We estimated sample sizes for ageing tautog collected in both Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2010, respectively, using a two-stage random sampling method (Quinn and Deriso 1999) in order to increase precision in estimates of age composition from fish sampled efficiently and effectively. The basic equation is:

$$A = \frac{V_a}{\theta_a^2 CV^2 - B_a / L}, \qquad (1)$$

where A is the sample size for ageing tautog in 2010; 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 is a subsample from a catch and used to estimate length distribution in the catch. θ_a, V_a, B_a, and CV were calculated using pooled age-length data of tautog collected from 2004 to 2008 and using equations in Quinn and Deriso (1999). For simplicity, the equations are not listed here. L was the total number of tautog used by VMRC to estimate length distribution of the caches from 2004 to 2008. The equation (1) indicates that the more fish that are aged, the smaller the CV (or higher precision) that will be obtained. Therefore, the criterion to decide A (number of fish) is that A should be a number above which there is only a 1%

CV reduction achieved by aging an additional 100 or more fish.

Handling of collection — Sagittal otoliths (hereafter, refer to as "otoliths") and opercula were received by the Age & Growth Laboratory in labeled coin envelopes. Once in our hands, they 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.

Preparation —

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

Otoliths Due to their fragility, we used our embedding and thin-sectioning method to prepare tautog otoliths for age determination. To start, a series of 14 mm x 5 mm x 3 mm wells (Ladd Industries silicon rubber mold) were pre-filled to half-volume with Loctite® 349 adhesive and permitted to cure for 24 hours until solidified. The wells were then filled to capacity with fresh, non-cured Loctite® 349 adhesive, at which point the otoliths could be inserted into the wells, suspended within a stable embedding atmosphere before sectioning. Otoliths were baked before embedding in the Loctite® 349 adhesive to produce better contrast of opaque and translucent zones within the matrix. Each otolith was baked in a Thermolyne 1400 furnace at 400°C for one to two minutes until it turned a medium brown color (caramel). The baked otoliths were inserted into the fresh Loctite® 349 adhesive, distal side up, with the long axis of the otolith exactly parallel with the long axis of the mold. Once the otoliths were properly oriented, the mold was placed under UV light and left to solidify overnight. Once dry, each embedded otolith was removed from the mold and mounted 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, 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, three-inch diameter, Norton® Diamond Grinding Wheels (hereafter, referred to as "blades"), separated by a stainless steel spacer of 0.4mm (diameter 2.5"). The otolith was positioned so that the blades straddled each side of the focus marked by pencil. The glass slide was adjusted to ensure that the blades were exactly perpendicular to the long axis of the otolith. The otolith thin-section was viewed under a stereo microscope to determine which side (cut surface) of the otolith was closer to the focus. The otolith thin-section was mounted best-side up onto a glass slide with Flo-texx® mounting medium, which provided enhanced contrast and greater readability by increasing light transmission through the sections.

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, 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 July 31, 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 (prepared 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. Opercula were aged on a light table with no magnification (Figure 1).

Figure 1. Operculum from a 13 year-old male tautog.

All otolith thin-sections were aged by two different readers using a Nikon SMZ1000 stereo microscope under transmitted light and dark-field polarization at between 8 and 20 times magnification (Figure 2).

Figure 2. Otolith section from a 13 year-old male tautog. Same fish as Figure 1.

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.

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; 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 and figures were made using R (R Development Core Team 2009).

RESULTS

We estimated a sample size of 397 for ageing the bay tautog in 2010, ranging in length interval from 9 to 24 inches (Table 1). This sample size provided a range in CV for age composition approximately from the smallest CV of 8% for age 3 and 4 to the largest CV of 21% for age 1 of the bay fish. We aged all 165 tautog who had both total lengths and opercula collected by VMRC in Chesapeake Bay in 2010. We fell short in our over-all collections for this optimal length-class sampling estimate by 232 fish from among the small, medium, and large length intervals (Table 1), as a result, the precision for the estimates of all age groups would be influenced significantly.

We estimated a sample size of 452 for ageing the ocean tautog in 2010, ranging in length interval from 8 to 30 inches (Table 2). This sample size provided a range in CV for age composition approximately from the smallest CV of 9% for age 5 to the largest CV of 23% for age 9 of the ocean fish. We aged all 21 tautog who had both total lengths and opercula collected by VMRC in Virginia waters of the Atlantic Ocean in 2010. We fell short in our over-all collections for this optimal length-class sampling estimate by 431 fish from among the small, medium, and large length intervals (Table 2), as a result, the precision for the estimates of all age groups would be influenced significantly.

Opercula — The measurement of reader self-precision was good for both readers. There is no significant difference between the first and second readings for Reader 1 with a CV of 5.1% and an agreement of 68% (test of symmetry: $\chi^2 = 7.33$, df = 9, $P = 0.6025$). There is no significant difference between the first and second readings for Reader 2 with a CV of 4.5% an agreement of 72% (test of symmetry: $\chi^2 = 9.33$, df = 8, $P = 0.3150$). There was an evidence of systematic disagreement between Reader 1 and Reader 2 with a CV of 6.3% an agreement of 61% (test of symmetry: $\chi^2 = 33.94$, df = 21, $P = 0.0368$) (Figure 3).

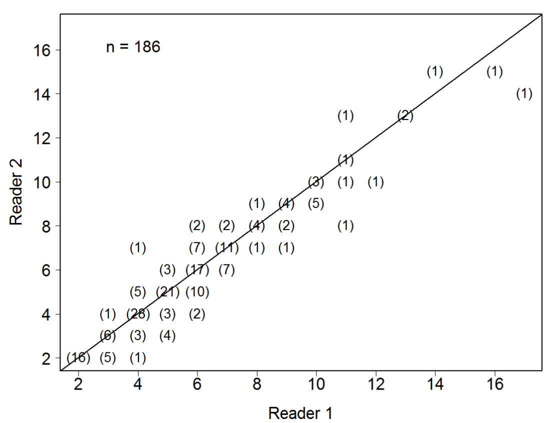


Figure 3. Between-reader comparison of operculum age estimates for tautog collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2010.

There is no time-series bias for both readers. The age readings of 72% fish by Reader 1 in 2010 had an agreement with those fish aged in 2003 with a CV of 4.1% (test of symmetry: $\chi^2 = 6$, df = 8, $P = 0.6472$). The age readings of 64% fish by Reader 2 in 2010 had an agreement with those fish aged in 2003 with a CV of 5.6% (test of symmetry: $\chi^2 = 11.47$, df = 8, $P = 0.1766$).

Of the 165 bay tautog aged with opercula, 10 age classes (from 2 to 11 years old) were represented (Table 3). The average age for the sample was 5.2 years. The standard deviation and standard error were 2.1 and 0.16, respectively. Year-class data indicates that recruitment into the fishery in Chesapeake Bay begins at age 2, which corresponds to the 2008 year-class for tautog caught in 2010. The year class of 2006 (24%) tautog was dominated in the sample in 2010, followed by 2004 (18%) and 2005 (17%) year-classes. The sex ratio of male to female was 1:1 for the bay fish (Figure 4).

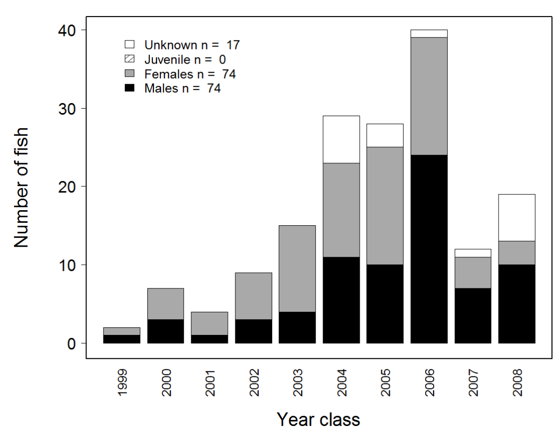


Figure 4. Year-class frequency distribution for tautog collected in Chesapeake Bay, Virginia for ageing in 2010. Distribution is broken down by sex and estimated using operculum ages. “Unknown” is for the fish gonads that were not available for examination or were not examined for sex during sampling.

Of the 21 ocean tautog aged with opercula, 13 age classes (from age 3 to 14, and 16) were represented (Table 4). The average age for the sample was 8.9 years. The standard deviation and standard error were 4 and 0.87, respectively. Year-class data indicates that recruitment into the fishery in Virginia waters of the Atlantic Ocean begins at age 3, which corresponds to the 2007 year-class for tautog caught in 2010. The sex ratio of male to female was 1:2.5 for the ocean fish (Figure 5).

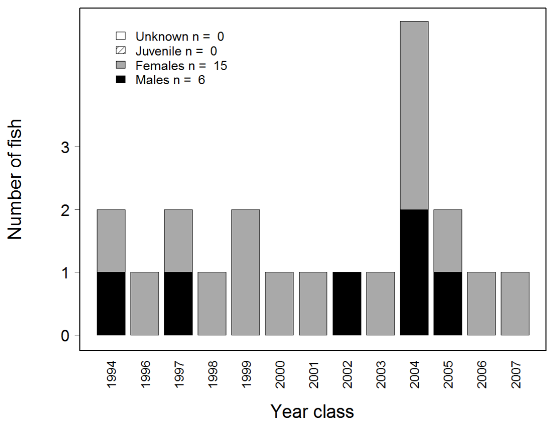


Figure 5. Year-class frequency distribution for tautog collected in Virginia waters of the Atlantic Ocean for ageing in 2010. Distribution is broken down by sex and estimated using operculum ages. “Unknown” is for the fish gonads that were not available for examination or were not examined for sex during sampling.

Otoliths — The measurement of reader self-precision was very good for both readers. There is no significant difference between the first and second readings for Reader 1 with a CV of 1.4% and an agreement of 90% (test of symmetry: $\chi^2 = 5$, df = 5, $P = 0.4159$). There is no significant difference between the first and second readings for Reader 2 with a CV of 1.2% and an agreement of 88% (test of symmetry: $\chi^2 = 6$, df = 5, $P = 0.3062$). There was no evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 88% and a CV of 1.3% (test of symmetry: $\chi^2 = 7.14$, df = 9, $P = 0.6222$) (Figure 6).

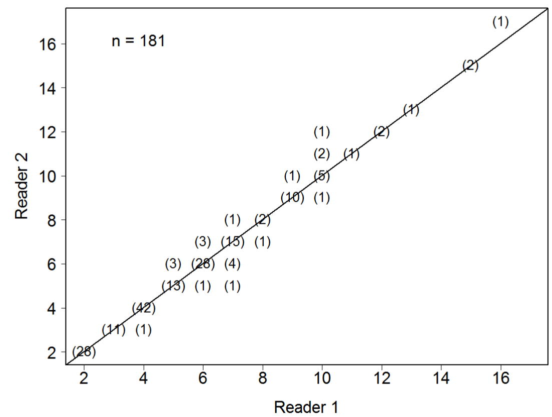


Figure 6. Between-reader comparison of otolith age estimates for tautog collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2010.

There is no time-series bias for both readers. Both Reader 1 and 2 had an agreement of 88% with the fish aged in 2003 with a CV of 1.3% (test of symmetry: $\chi^2 = 6$, df = 3, $P = 0.1116$).

Of 181 fish aged with otoliths, 14 age classes (2 to 13 and 15 to 17) were represented. The average age for the sample was 5.5 years. The standard deviation and standard error were 2.8 and 0.21, respectively.

Comparison of Operculum and Otolith Ages — We aged 180 tautog using both their opercula and otoliths (One fish had otoliths only and was removed from this comparison). There was no evidence of systematic disagreement between otolith and operculum ages in 2010 (CV = 7.7%, test of symmetry: $\chi^2 = 31.66$, df = 22, $P = 0.0834$), whereas there was evidence of systematic disagreement in 2009 (CV = 6.9%, test of symmetry: $\chi^2 = 49.05$, df = 24, $P = 0.0019$), indicating that we made improvement on ageing tautog opercula in 2010. There was an agreement of 56% between operculum and otolith ages whereas opercula were assigned a lower and higher age than otoliths for 19% and 25% of the fish, respectively (Figure 7). Although the symmetry test didn't find a systematic disagreement between operculum and otolith ages, an age bias plot shows that operculum ages tended to, more or less, over-estimate younger fish and under-estimate older fish (Figure 8).

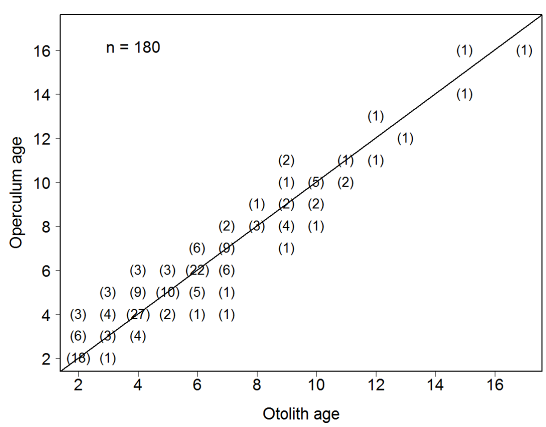


Figure 7. Comparison operculum and otolith age estimates for tautog collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2010.

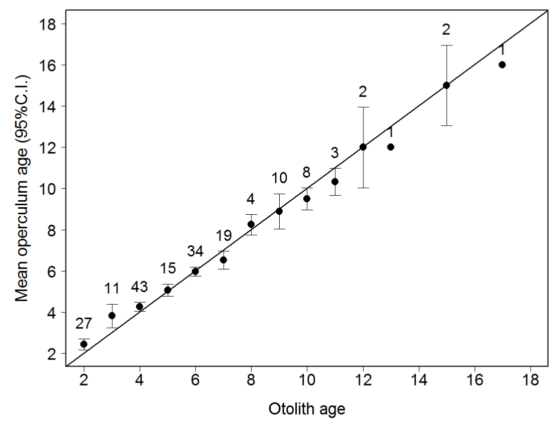


Figure 8. Age-bias plot for tautog operculum and otolith age estimates in 2010.

Age-Length-Key (ALK) — We developed an ALK for both bay (Table 5) and ocean fish (Table 6) using operculum ages, separately. Due to the small samples collected in 2010, we don't recommend to use the ALKs to do the conversion of numbers-at-length in the estimated catch to numbers-at-age.

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Table 1. Number of tautog collected in the Chesapeake Bay, Virginia in 2010 and operculum-aged in each 1-inch length interval. "Target" represents the sample size for ageing estimated for 2010, "Collected" represents number of fish with both total length and otoliths, and "Need" represents number of fish that were not obtained in each length interval compared to the optimum sample size for ageing and number of fish aged. One fish without opercula is not included.

Interval	Target	Collected	Aged	Need
9 - 9.99	5	0	0	5
10 - 10.99	6	0	0	6
11 - 11.99	10	0	0	10
12 - 12.99	7	1	1	6
13 - 13.99	50	18	18	32
14 - 14.99	89	41	41	48
15 - 15.99	71	20	20	51
16 - 16.99	50	29	29	21
17 - 17.99	42	21	21	21
18 - 18.99	24	15	15	9
19 - 19.99	14	13	13	1
20 - 20.99	9	3	3	6
21 - 21.99	5	1	1	4
22 - 22.99	5	2	2	3
23 - 23.99	5	1	1	4
24 - 24.99	5	0	0	5
Totals	397	165	165	232

Table 2. Number of tautog collected in Virginia waters of the Atlantic Ocean in 2010 and operculum-aged in each 1-inch length interval. "Target" represents the sample size for ageing estimated for 2010, "Collected" represents number of fish with both total length and otoliths, and "Need" represents number of fish that were not obtained in each length interval compared to the optimum sample size for ageing and number of fish aged.

Interval	Target	Collected	Aged	Need
8 - 8.99	5	0	0	5
9 - 9.99	8	0	0	8
10 - 10.99	8	0	0	8
11 - 11.99	27	0	0	27
12 - 12.99	16	0	0	16
13 - 13.99	41	0	0	41
14 - 14.99	41	0	0	41
15 - 15.99	58	2	2	56
16 - 16.99	49	3	3	46
17 - 17.99	39	0	0	39
18 - 18.99	43	1	1	42
19 - 19.99	33	4	4	29
20 - 20.99	14	2	2	12
21 - 21.99	16	0	0	16
22 - 22.99	21	4	4	17
23 - 23.99	5	2	2	3
24 - 24.99	5	0	0	5
25 - 25.99	8	0	0	8
26 - 26.99	5	3	3	2
27 - 27.99	5	0	0	5
30 - 30.99	5	0	0	5
Totals	452	21	21	431

Table 3. The number of tautog assigned to each total length-at-age category for 165 fish sampled for operculum age determination in Chesapeake Bay, Virginia during 2010.

	Age										
Interval	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**Totals**
12 - 12.99	0	0	0	0	1	0	0	0	0	0	1
13 - 13.99	8	3	4	3	0	0	0	0	0	0	18
14 - 14.99	10	7	13	7	3	1	0	0	0	0	41
15 - 15.99	1	2	7	4	5	0	1	0	0	0	20
16 - 16.99	0	0	9	5	8	5	1	0	0	1	29
17 - 17.99	0	0	6	8	5	1	0	0	1	0	21
18 - 18.99	0	0	1	1	4	5	2	1	1	0	15
19 - 19.99	0	0	0	0	2	3	4	2	2	0	13
20 - 20.99	0	0	0	0	0	0	1	1	1	0	3
21 - 21.99	0	0	0	0	1	0	0	0	0	0	1
22 - 22.99	0	0	0	0	0	0	0	0	1	1	2
23 - 23.99	0	0	0	0	0	0	0	0	1	0	1
Totals	19	12	40	28	29	15	9	4	7	2	165

Table 4. The number of tautog assigned to each total length-at-age category for 21 fish sampled for operculum age determination in Virginia waters of the Atlantic Ocean during 2010.

	Age													
Interval	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**16**	**Totals**
15 - 15.99	0	1	0	1	0	0	0	0	0	0	0	0	0	2
16 - 16.99	1	0	2	0	0	0	0	0	0	0	0	0	0	3
18 - 18.99	0	0	0	1	0	0	0	0	0	0	0	0	0	1
19 - 19.99	0	0	0	2	0	0	1	0	1	0	0	0	0	4
20 - 20.99	0	0	0	1	0	1	0	0	0	0	0	0	0	2
22 - 22.99	0	0	0	0	0	0	0	1	0	1	1	1	0	4
23 - 23.99	0	0	0	0	1	0	0	0	1	0	0	0	0	2
26 - 26.99	0	0	0	0	0	0	0	0	0	0	1	0	2	3
Totals	1	1	2	5	1	1	1	1	2	1	2	1	2	21

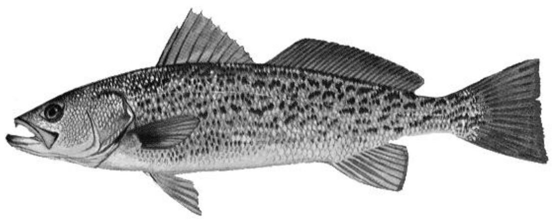
Table 5. Age-Length key, as proportion-at-age in each 1-inch length interval, based on operculum ages for tautog sampled in Chesapeake Bay, Virginia during 2010.

	Age									
Interval	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
12 - 12.99	0	0	0	0	1	0	0	0	0	0
13 - 13.99	0.444	0.167	0.222	0.167	0	0	0	0	0	0
14 - 14.99	0.244	0.171	0.317	0.171	0.073	0.024	0	0	0	0
15 - 15.99	0.05	0.1	0.35	0.2	0.25	0	0.05	0	0	0
16 - 16.99	0	0	0.31	0.172	0.276	0.172	0.034	0	0	0.034
17 - 17.99	0	0	0.286	0.381	0.238	0.048	0	0	0.048	0
18 - 18.99	0	0	0.067	0.067	0.267	0.333	0.133	0.067	0.067	0
19 - 19.99	0	0	0	0	0.154	0.231	0.308	0.154	0.154	0
20 - 20.99	0	0	0	0	0	0	0.333	0.333	0.333	0
21 - 21.99	0	0	0	0	1	0	0	0	0	0
22 - 22.99	0	0	0	0	0	0	0	0	0.5	0.5
23 - 23.99	0	0	0	0	0	0	0	0	1	0

Table 6. 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 2010.

	Age												
Interval	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**16**
15 - 15.99	0	0.5	0	0.5	0	0	0	0	0	0	0	0	0
16 - 16.99	0.333	0	0.667	0	0	0	0	0	0	0	0	0	0
18 - 18.99	0	0	0	1	0	0	0	0	0	0	0	0	0
19 - 19.99	0	0	0	0.5	0	0	0.25	0	0.25	0	0	0	0
20 - 20.99	0	0	0	0.5	0	0.5	0	0	0	0	0	0	0
22 - 22.99	0	0	0	0	0	0	0	0.25	0	0.25	0.25	0.25	0
23 - 23.99	0	0	0	0	0.5	0	0	0	0.5	0	0	0	0
26 - 26.99	0	0	0	0	0	0	0	0	0	0	0.333	0	0.667

Chapter 14
Weakfish



Cynoscion regalis

INTRODUCTION

We aged 260 weakfish, *Cynoscion regalis*, collected by the VMRC's Biological Sampling Program for age and growth analysis in 2010. The weakfish ages ranged from 0 to 14 years old with an average age of 2, and standard deviation of 1.8, and a standard error of 0.11. Eleven age classes (from 0 to 6, and 7, and 11 through 14) were represented, comprising fish from the 1996 to 1999, and 2003 , and 2005 through 2010 year-classes. Fish from the 2008 and 2009 year-class dominated the sample with 40% and 42%, respectively.

METHODS

Sample size for ageing — We estimated sample size for ageing weakfish in 2010 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:

$$A = \frac{V_a}{\theta_a^2 CV^2 - B_a / L}, \qquad (1)$$

where A is the sample size for ageing weakfish in 2010; 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 is a subsample from a catch and used to estimate length distribution in the catch. θ_a, V_a, B_a, and CV were calculated using pooled age-length data of weakfish collected from 2004 to 2008 and using equations in Quinn and Deriso (1999). For simplicity, the equations are not listed here. L was the total number of weakfish used by VMRC to estimate length distribution of the catches from 2004 to 2008. The equation (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 achieved by aging an additional 100 or more fish.

Handling of collection — Otoliths were received by the Age & Growth Laboratory in labeled coin envelopes. Once in our hands, they were sorted based on date of capture, their envelope labels were verified against VMRC's collection data, and assigned unique Age and Growth Laboratory sample numbers. All otoliths were stored dry inside of protective Axygen 2.0-ml microtubes within their original labeled coin envelopes.

Preparation — Sagittal otoliths (hereafter, referred to as "otoliths") were processed for age determination following our thin-sectioning method, as described in Chapter 1, 2, 5, and 8 for other sciaenids. The left or right sagittal otolith was randomly selected and attached 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 marked using a pencil across the otolith surface. At least one transverse cross-section (hereafter, referred to as "thin-section") was then removed from 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 0.4 mm (diameter 2.5"). The position of the marked core fell within the 0.3-mm space between the blades, such that the core was included in the transverse cross-section removed. Otolith thin-sections were placed on labeled glass slides and covered with a thin layer of Flo-texx mounting medium that not only adhered the sections to the slide, but more importantly, provided enhanced contrast and greater readability by increasing light transmission through the sections.

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

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 1).

Figure 1. Sectioned otolith of a female weakfish with 6 annuli.

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.

Comparison Tests — A symmetry test (Hoenig et al. 1995) and coefficient of variation (CV) analysis were used to detect any systematic difference and precision on age readings, respectively, for the following comparisons: 1) between the two readers in the current year, 2) within each reader in the current year, and 3) time-series bias between the current and previous years within each reader. The readings from the entire sample for the current year were used to examine the difference between two readers. A random sub-sample of 50 fish from the current year was selected for second readings to examine the difference within a reader. Fifty otoliths randomly selected from fish aged in 2000 were used to examine the time-series bias within each reader. A figure of 1:1 equivalence was used to illustrate those differences (Campana et al. 1995). All statistics analyses and figures were made using R (R Development Core Team 2009).

RESULTS

We estimated a sample size of 353 for ageing weakfish in 2010, ranging in length interval from 6 to 36 inches (Table 1). This sample size provided a range in CV for age composition approximately from the smallest CV of 6% for age 2 and the largest CV of 15% for age 1 and 4 fish. In 2010, we randomly selected and aged 260 fish from 379 weakfish collected by VMRC. We fell short in our over-all collections for this optimal length-class sampling estimate by 99 fish. However, these were primarily from the very large length intervals (Table 1), therefore, the precision for the estimates of major age groups (such as age 1 and 2) would not be influenced significantly.

The measurement of reader self-precision was high for both readers. Reader 1 had an agreement of 98% with a CV of 0.6% (test of symmetry: $\chi^2 = 1$, df = 1, $P = 0.3173$). Reader 2 had a 100% agreement. There was no evidence of systematic disagreement between Reader 1 and Reader 2 with an agreement of 100% (Figure 2).

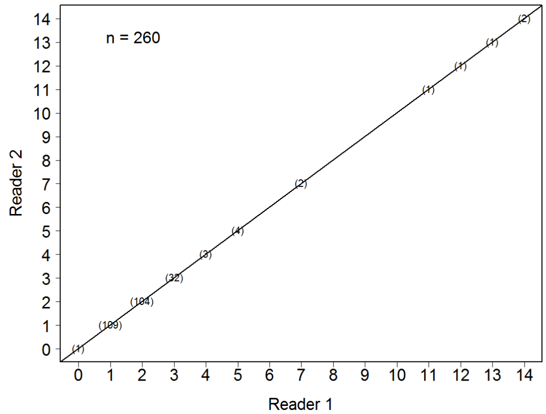


Figure 2. Between-reader comparison of otolith age estimates for weakfish collected in Chesapeake Bay and Virginia waters of the Atlantic Ocean in 2010.

There is no time-series bias for both readers. Reader 1 and Reader 2 had an agreement of 100% with ages of fish aged in 2000, respectively.

Of the 260 fish aged with otoliths, 11 age classes were represented (Table 2). The average age was 2 years old, and the standard deviation and standard error were 1.8 and 0.11, respectively.

Year-class data shows that the fishery was comprised of 11 year-classes, comprising fish from the 1996 to 1999, and 2003 through 2010 year-classes, with fish primarily from the 2009 (42%), followed by the 2008 (40%) year-classes. The females (80%) were highly dominant in the sample collected in 2010 (Figure 3).

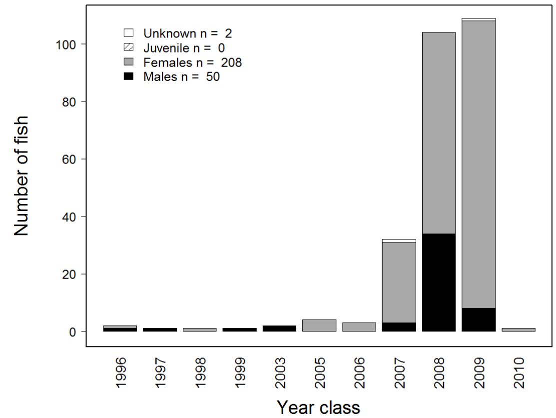


Figure 3. Year-class frequency distribution for weakfish collected for ageing in 2010. Distribution is broken down by sex. "Unknown" is for the fish gonads thatwere not available for examination or were not examined for sex during sampling.

Age-Length-Key — We present an age-length-key (Table 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. Number of weakfish collected and aged in each 1-inch length interval in 2010. "Target" represent the sample size for ageing estimated for 2010, "Collected" represents number of fish with both total length and otoliths, 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	1	1	4
7 - 7.99	5	6	6	0
8 - 8.99	5	25	6	0
9 - 9.99	30	64	30	0
10 - 10.99	67	117	68	0
11 - 11.99	47	61	47	0
12 - 12.99	29	27	27	2
13 - 13.99	16	16	16	0
14 - 14.99	12	15	12	0
15 - 15.99	13	16	16	0
16 - 16.99	13	6	6	7
17 - 17.99	9	4	4	5
18 - 18.99	9	5	5	4
19 - 19.99	7	0	0	7
20 - 20.99	6	1	1	5
21 - 21.99	5	0	0	5
22 - 22.99	5	0	0	5
23 - 23.99	5	1	1	4
24 - 24.99	5	1	1	4
25 - 25.99	5	1	1	4
26 - 26.99	5	1	1	4
27 - 27.99	5	1	1	4
28 - 28.99	5	2	2	3
29 - 29.99	5	2	2	3
30 - 30.99	5	0	0	5
31 - 31.99	5	1	1	4
32 - 32.99	5	0	0	5
33 - 33.99	5	0	0	5
34 - 34.99	5	3	3	2
35 - 35.99	5	2	2	3
36 - 36.99	5	0	0	5
Totals	353	379	260	99

Table 2. The number of weakfish assigned to each total length-at-age category for 260 fish sampled for otolith age determination in Virginia during 2010.

	Age											
Interval	**0**	**1**	**2**	**3**	**4**	**5**	**7**	**11**	**12**	**13**	**14**	**Totals**
6 - 6.99	1	0	0	0	0	0	0	0	0	0	0	1
7 - 7.99	0	6	0	0	0	0	0	0	0	0	0	6
8 - 8.99	0	4	2	0	0	0	0	0	0	0	0	6
9 - 9.99	0	15	15	0	0	0	0	0	0	0	0	30
10 - 10.99	0	28	37	3	0	0	0	0	0	0	0	68
11 - 11.99	0	16	29	2	0	0	0	0	0	0	0	47
12 - 12.99	0	10	11	6	0	0	0	0	0	0	0	27
13 - 13.99	0	12	3	1	0	0	0	0	0	0	0	16
14 - 14.99	0	8	2	2	0	0	0	0	0	0	0	12
15 - 15.99	0	8	2	6	0	0	0	0	0	0	0	16
16 - 16.99	0	2	1	2	1	0	0	0	0	0	0	6
17 - 17.99	0	0	0	3	1	0	0	0	0	0	0	4
18 - 18.99	0	0	1	3	1	0	0	0	0	0	0	5
20 - 20.99	0	0	0	1	0	0	0	0	0	0	0	1
23 - 23.99	0	0	0	1	0	0	0	0	0	0	0	1
24 - 24.99	0	0	1	0	0	0	0	0	0	0	0	1
25 - 25.99	0	0	0	0	0	1	0	0	0	0	0	1
26 - 26.99	0	0	0	1	0	0	0	0	0	0	0	1
27 - 27.99	0	0	0	0	0	1	0	0	0	0	0	1
28 - 28.99	0	0	0	0	0	2	0	0	0	0	0	2
29 - 29.99	0	0	0	1	0	0	1	0	0	0	0	2
31 - 31.99	0	0	0	0	0	0	1	0	0	0	0	1
34 - 34.99	0	0	0	0	0	0	0	1	0	1	1	3
35 - 35.99	0	0	0	0	0	0	0	0	1	0	1	2
Totals	1	109	104	32	3	4	2	1	1	1	2	260

Table 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 2010.

	Age										
Interval	**0**	**1**	**2**	**3**	**4**	**5**	**7**	**11**	**12**	**13**	**14**
6 - 6.99	1	0	0	0	0	0	0	0	0	0	0
7 - 7.99	0	1	0	0	0	0	0	0	0	0	0
8 - 8.99	0	0.667	0.333	0	0	0	0	0	0	0	0
9 - 9.99	0	0.5	0.5	0	0	0	0	0	0	0	0
10 - 10.99	0	0.412	0.544	0.044	0	0	0	0	0	0	0
11 - 11.99	0	0.34	0.617	0.043	0	0	0	0	0	0	0
12 - 12.99	0	0.37	0.407	0.222	0	0	0	0	0	0	0
13 - 13.99	0	0.75	0.188	0.062	0	0	0	0	0	0	0
14 - 14.99	0	0.667	0.167	0.167	0	0	0	0	0	0	0
15 - 15.99	0	0.5	0.125	0.375	0	0	0	0	0	0	0
16 - 16.99	0	0.333	0.167	0.333	0.167	0	0	0	0	0	0
17 - 17.99	0	0	0	0.75	0.25	0	0	0	0	0	0
18 - 18.99	0	0	0.2	0.6	0.2	0	0	0	0	0	0
20 - 20.99	0	0	0	1	0	0	0	0	0	0	0
23 - 23.99	0	0	0	1	0	0	0	0	0	0	0
24 - 24.99	0	0	1	0	0	0	0	0	0	0	0
25 - 25.99	0	0	0	0	0	1	0	0	0	0	0
26 - 26.99	0	0	0	1	0	0	0	0	0	0	0
27 - 27.99	0	0	0	0	0	1	0	0	0	0	0
28 - 28.99	0	0	0	0	0	1	0	0	0	0	0
29 - 29.99	0	0	0	0.5	0	0	0.5	0	0	0	0
31 - 31.99	0	0	0	0	0	0	1	0	0	0	0
34 - 34.99	0	0	0	0	0	0	0	0.333	0	0.333	0.333
35 - 35.99	0	0	0	0	0	0	0	0	0.5	0	0.5